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RESEARCH ARTICLE

Higher efficacy of a single dosage albendazole and different soil-transmitted helminths re-infection profiles amongst indigenous Negritos from inland jungle versus those in resettlement at town peripheries

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ABSTRACT

Demarginalization through initiation of resettlement program since 1978 is an inevitable progress faced by the indigenous Orang Asli (OA) population in Peninsular Malaysia. As Malaysian hunter-gatherers, the Negrito has been exposed to various environmental-cultural variations. These changes may influence the pattern of soil-transmitted helminth (STH) infections, the common malady amongst OA. This study evaluated the deworming effects of single-dosage albendazole (400 mg) and STH-reinfection rate between Negritos who are still living in the inland jungle versus those living in resettlements at town peripheries (RPS). Stool samples from the consented participants were first examined using the direct faecal smear, formalin-ether sedimentation and Kato Katz techniques. Subsequently, stool collections were carried out in three time points following treatment (i.e., 21 days, 3 months and 6 months). In brief, a total number of 54 Negritos (inland: 24; RPS: 30) with a complete set of stool collection was included in this longitudinal study. This study revealed 72.2% cure rate against *T. trichiura* in the inland but only 15.0% in the RPS. Although the efficacy of albendazole against *T. trichiura* was ultimately low in the RPS, 62.6% egg reduction rate (ERR) (arithmetic mean) was noted (p = 0.001). For *A. lumbricoides* and hookworm, high cure rates were found in both communities (85.7–100.0%). Reinfection for *T. trichiura* was seen in less than 1 month with higher rate in the RPS (90.0%) as opposed to the inland (44.4%) at 21 days following treatment. This study found that the inland OA had better tolerability to single-dosage albendazole and experienced slower STH reinfection rates versus the RPS. Hence, the selection of albendazole dosage should be targeted and the use of single- dosage albendazole (biannually) would be more suitable for the inland OA. Conversely, we propose the use of 3-days albendazole regimens in the resettled RPS population.

Keywords: Negrito; soil-transmitted infection; albendazole; efficacy; re-infection.

INTRODUCTION

Soil-transmitted helminths (STH) ("helminth" means parasitic worm) refer to a group of nematodes which cause human intestinal infections. The medically important STH species are *Ascaris lumbricoides* (giant roundworm), *Trichuris trichiura* (whipworm), *Necator americanus* and *Ancylostoma duodenale* (hookworms). These infections are mainly transmitted by ova or eggs present in faeces of infected hosts which directly or indirectly pollute the soil especially in areas with improper sanitation (Jourdan *et al*., 2018). Humans acquire the infection by the ingestion of infective eggs in contaminated food and water, or through larval skin penetration in contaminated soil. Otherwise stated, the STH parasites require soil in their lifecycle for the development into infectious stages before they are capable to infect humans.

The most common associated symptoms are abdominal pain and diarrhoea especially in children (WHO, 2005). Although they seldom cause direct mortality, the STH infections are generally described as chronic and cause debilitating maladies of poverty due to their negative effects to nutritional status, human fitness and productivity, and cognitive and physical development of children (Bethony *et al*., 2006). Meanwhile in pregnant women, STH colonisation is commonly associated with anaemia and low birth weight of new-borns (Jourdan *et al*., 2018; Roach, 2020).

Over the past few years, Malaysia has witnessed a declining trend of intestinal parasitic infections due to economic development, advancement in medicine and improvement in healthcare services and environment. Studies have demonstrated that the prevalence rates of STH infections among estate workers (Kan *et al*., 1989), rural Malay villagers (Zulkifli *et al*., 2000) and slum areas (Kan *et al*., 1989) have undergone significant reductions over the years. However, STH infections remain unchanged and problematic in suburban and rural areas, especially among the underserved Orang Asli (OA) communities (Sinniah *et al*., 2014). Although the incidence

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of mortality associated with these diseases are low; STH infections continue to cause significant morbidity among OA despite vigorous implementation of socio-economic development programs following the resettlement initiatives ("Rancangan Penempatan Semula" or RPS) (Abdullah, 2018). In brief, the fraction of the OA communities that have been relocated under the RPS program was approximately 63.0% in 2011 (Idrus, 2011; SyedHussain *et al*., 2017). On the other hand, approximately 37.0% of OA tribes have remained within the forested inland jungle. Hunting and gathering activities are still prominent in these villages especially among the Negritos despite provision of basic infrastructure such as brick houses, community halls and public toilets in their villages (JAKOA, 2016; SyedHussain *et al*., 2017; Abdullah, 2018). These environmental-cultural variations may influence the different pattern of soil-transmitted helminth (STH) infections profiles and their consequences including preventive measure amongst the RPS and inland OA communities.

One of the major components of STH prevention and control strategies is periodic administration of anthelmintic treatment or chemotherapy (WHO, 2002). Anthelmintic is a drug used to destroy and facilitate the eviction of the helminths (worms) from infected individuals. There are many drugs available for the treatment of helminthic infections. However, only four anthelmintics are available and recommended to be used for the treatment of the STH infections: albendazole and mebendazole (both are benzimidazole carbamate types of anthelmintic agents), levamisole and pyrantel pamoate. Other than that, ivermectin (a common drug for the treatment of lymphatic filariasis and onchocerciasis) is also indicated as an anthelmintic drug for STH infections but is not listed as essential drug due to their suboptimal effect against the infections (WHO, 2006).

Of the four drugs, the benzimidazoles (i.e., albendazole and mebendazole) have been widely used due to their broad-spectrum activity against intestinal parasites, better efficacy, low cost of production and good safety profiles with minimal adverse effects reported (Horton, 2000; McCarthy & Moore, 2015). Patients tolerate these drugs with minimal side effects. Very few patients (around 1.0%) report nausea, vomiting, and headache, and allergic reactions with fever are rare (Horton, 2000). In single dosage administration, albendazole was reported to be superior than mebendazole in the treatment of hookworm infection (Bennett & Guyatt, 2000). On the other hand, mebendazole has better efficacy against *T. trichiura* infections. Nevertheless, both drugs have very high curative effect against *A. lumbricoides* (Keiser & Utzinger, 2008). Hence, justification in choosing between both drugs must be based on the prevalence and intensity of every STH species in the population.

Albendazole was first introduced in Malaysia in the 1980s as the major component in national prevention and control of the STH infections. The first evaluation study on the efficacy of the single dosage (400 mg) albendazole among 91 individuals revealed 100% cure rate in both *A. lumbricoides* and hookworm infections, but approximately 27.3- 31.2% in *T. trichiura* infection. The effectiveness of the drug towards *T. trichiura* infections improved up to 60.9 - 85.1% in increased dosage of 600mg and found to be superior to other drugs. Moreover, neither side effect nor biochemical changes in the blood and urine were observed (Ramalingam *et al*., 1983). Based on that, albendazole became the drug of choice for Malaysia mass deworming program since 1982 replacing pyrantel pamoate which was discontinued due to its ineffectiveness (Pene *et al*., 1982; Ramalingam *et al*., 1983; Nasr *et al*., 2013). The efficacy of albendazole was further observed to be greatly improved if given in a 3-days regimen (Penggabean *et al*., 1998). However, it must be weighed against cost, logistical issues and the possibility of a less than perfect compliance rate (Nasr *et al*., 2013).

Although the practice of three-day courses of single dosage albendazole is highly recommended for the treatment of STH infections, the implementation is not feasible considering the remoteness of the indigenous area of the Negritos, especially those who still live in the inland jungle. Therefore, it would be useful to know whether a single dose of albendazole is sufficient for control and prevention within this population. Since there was no information describing the efficacy of albendazole treatment among the Negritos, we aimed to investigate the efficacy of single dose albendazole (400 mg) and the STH reinfection rates following treatment among the Negritos from the inland and resettled RPS communities at 3 time points (i.e., 21 days, 3 months and 6 months) post-treatment.

The study would be essential for the customization of effective STH treatment regime and will assist in the modification of deworming policies in terms of frequency of mass drug administration and cost-effectiveness of the deworming program, based on the type of modalities observed in the inland and RPS Orang Asli communities. Importantly, the outcome scenarios (whether there is a significant reduction and interruption of the cycle and an understanding of how rapid the reinfection rate in follow-up assessments) of the STH infections following the mass drug administration must be identified and any action plans must be integrated with the National Orang Asli Development Plan being formulated by the Department of Orang Asli Development (JAKOA).

MATERIALS AND METHODS

Ethical approval

This study was approved by the National Medical Research Register (NMRR), Ministry of Health, Malaysia [NMRR-17-3055-37252 (IIR)] and Ethics Committee of the Universiti Teknologi MARA [reference no: 600-IRMI (5/1/6)]. Permission to conduct the study among the Orang Asli Negritos was permitted by the Department of Orang Asli Development (JAKOA) [Reference no: JAKOA/pp.30.052J1d9 (29)]. A short briefing was held before each sampling session, during which the purpose and methods of the study were clearly informed to prospective participants. Those who agreed to participate either signed or thumb-printed the informed consent forms, witnessed by the accompanying JAKOA officer(s). Permission and consent via signature from legal representatives were obtained for participants below 12 years old. Participants were informed of their rights to withdraw from the study at any time without prior notice.

Study design, study participants and study area

This longitudinal follow-up study was nested within the previous STH cross-sectional study amongst the Negrito population in Peninsular Malaysia (Muslim *et al*., 2019). Pregnant women and individuals who had a recent history of anthelmintic treatment or antibiotics for at least three months prior to sample collection and examination were excluded from the study. In brief, a total of 416 Negrito participants (inland: n= 149; RPS: n= 222) was first examined for STH infections. A supervised mass albendazole treatment (brand name: Zentel, single dosage, 400 mg, GlaxoSmithKline) was later administered to 235 consented participants (inland: n= 79; RPS: n=156). Participants were invited for STH re-examination of faecal samples after 21 days, 3 months and 6 months of receiving the albendazole treatment using similar approaches (direct wet smear, formalin-ether concentration technique and Kato-Katz thick smear) as described in previous study (Muslim *et al*., 2019).

Re-sampling was conducted in two Negrito villages: Kg. RPS Banun (representing the RPS community) and Kg. Bukit Asu (representing the Inland OA village). Both villages are located at the Northern part of Perak but separated approximately 93.4km from each other. They share almost similar climate, rainfall of 2243 - 2308 mm and annual temperature averages from 25.9 - 26.9°C. In total, during the third visit (first follow-up sampling), 67 faecal samples were collected, of which 54 were from individuals (inland: 24; RPS: 30) who had received the previous treatment, while another 13 samples were from individuals who missed the treatment during the previous intervention visit. The summary of dropout participants, and numbers who missed or refused the treatment is shown in Figure 1.

Efficacy of a single dosage of albendazole treatment, STH re-infection and statistical analyses

Cure rate $= 100\% \times \left(1 - \frac{\text{number of subjects excreting eggs at follow-up}}{\text{number of subjects excreting eggs at baseline}}\right)$

Egg reduction rates based on arithmetic mean $= 100\% \times \left(1 - \frac{\text{arithmetic mean of group FEC at follow-up}}{\text{arithmetic mean of group FEC at baseline}}\right) = 100 \% \times \left(1 - \frac{\frac{\sum_{i=1}^{n} \text{FEC at follow-up}_i}{n}}{\frac{\sum_{i=1}^{n} \text{FEC at baseline}_i}{n}}\right)$

Egg reduction rate based on geometric mean $= 100\% \times \left(1 - \frac{\text{geometric mean of group FEC at follow-up}}{\text{geometric mean of group FEC at baseline}}\right) = 100 \% \times \left(1 - \frac{e^{\frac{\sum_{i=1}^{n} \ln(\text{FEC at follow-up}_i + 1)}{n}} - 1}{e^{\frac{\sum_{i=1}^{n} \ln(\text{FEC at baseline}_i + 1)}{n}} - 1}\right)$

*FEC= Faecal egg count

Statistical analysis was conducted using excel and SPSS version 21. Paired t-tests (for difference in geometric mean) and Wilcoxon analyses (for difference in arithmetic mean) were performed to compare the mean epg of STH during pre-treatment and 21 days post-treatment. Bootstrap resampling technique with 1,000 iterations was employed to calculate the 95% CI for the egg reduction rate. A p value of less than 0.05 (p< 0.05) was taken as significant.

The prevalence and intensity of STH infections for each species (i.e., *T. trichiura*, *A. lumbricoides* and hookworms) at 3 post-treatment time points were later evaluated for reinfection status. Reinfection rates were represented by Negritos who were positive at baseline, negative at 21 days following treatment but positive after 3 months and 6 months. New infections were represented by individuals negative at the baseline and 21 days post- treatment but positive at least during 3 months post-treatment. The formula for reinfection rate (Olsen *et al.*, 2003) calculation is shown as below:

$$\text{Reinfection rate} = \frac{\%\ \text{Prevalence after treatment}}{\%\ \text{Prevalence before treatment}}\ 100$$

RESULTS

Characteristic of the participants

The participants in this study were a subset of participants described in previous study (Muslim *et al.*, 2019). Almost half of the participants (43.5%) refused or missed the treatment, giving a total of 235 (inland: 79; RPS: 156) who had the albendazole administration. However, from that number, only 67 Negritos (inland: 27; RPS: 40) from two Negrito villages participated during the stool resampling at day 21, post-treatment. For efficacy analyses of the albendazole, only participants that received previous albendazole treatment and participated in re-examination of the stool samples were included (N= 54: inland: 24; RPS: 30). Overall, they consisted of 28 females (51.9%) and 26 males (48.1%). Majority of them aged between 2 - 12 y/o (N = 44; 81.4%).

Efficacy of single dosage albendazole against STH infections among the Negrito (Pre-treatment vs 21-days post-treatment)

In general, the cure rate was found to be higher in the inland (16/24; 66.7%) as compared to the RPS (3/20; 15.0%). Specifically, the cure rate of *T. trichiura* was 72.2% in the inland but 15.0% in the RPS. Although the efficacy of albendazole against *T. trichiura* was ultimately low in the RPS, the egg reduction rate was still

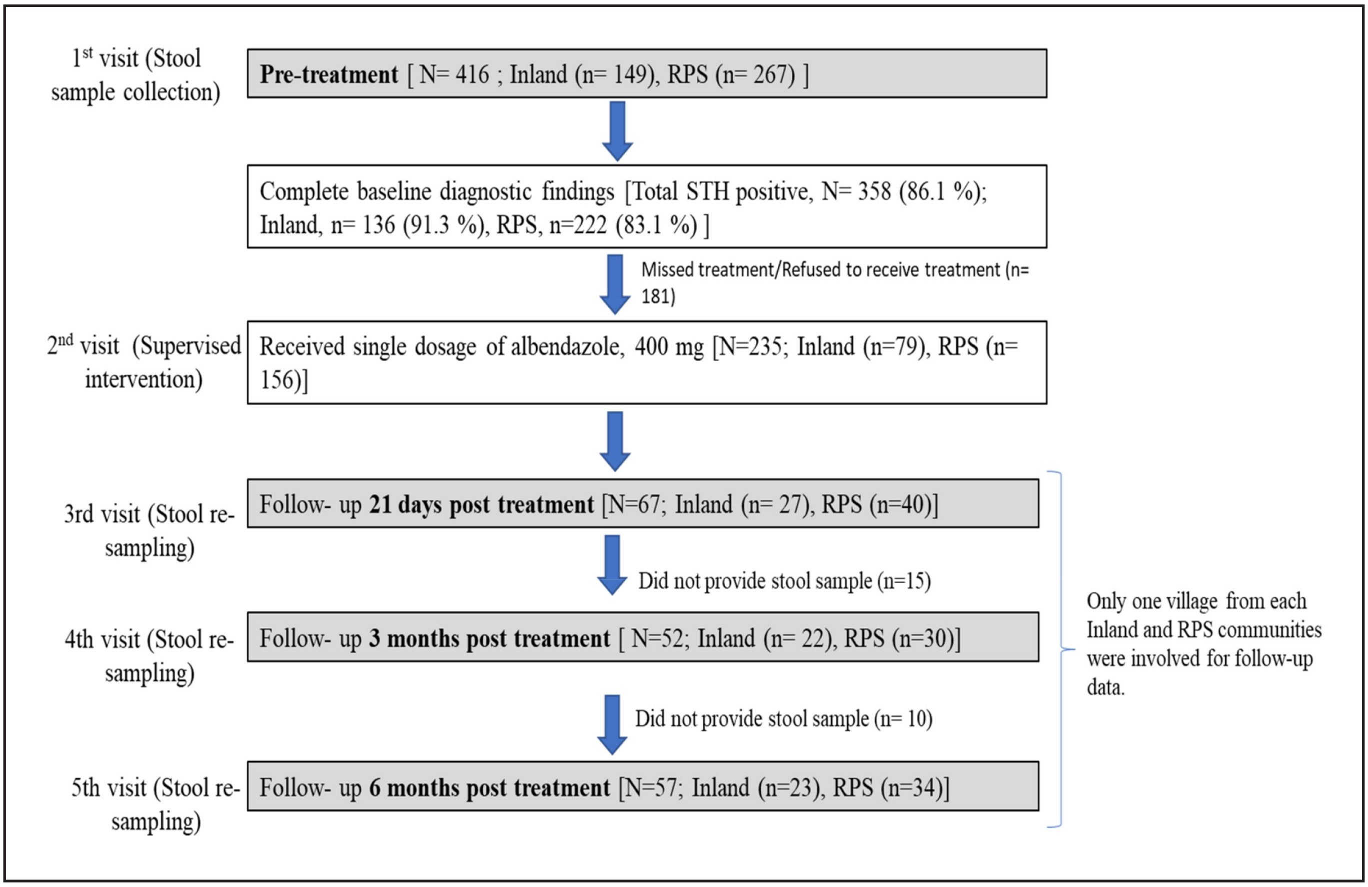

Figure 1. Number of participants, dropouts, missed/refused to receive treatment.

prominent (62.0% and 57.7% for both arithmetic and geometric means, respectively) (Table 1).

In the case of *A. lumbricoides*, a high cure rate was observed after 21 days post-treatment in both communities (inland: 100.0%; RPS: 87.5%). The finding was further supported by a very significant overall egg reduction rate following treatment by both arithmetic ($p < 0.001$) and geometric means ($p = 0.001$) as shown in Table 2.

Similar to the findings in the *A. lumbricoides* infection, the efficacy of the albendazole against hookworm infection was high in both inland and RPS communities. Both cure rate and egg reduction rate analyses supported these findings. Nevertheless, the inland had slightly lower cure rate (85.7%) as compared to the RPS (100.0%) (Table 3).

STH new infection, reinfection and intensity at pre-treatment and 3 post-treatment time points (i.e., at 21 days, 3 months and 6 months)

From a total of 54 participants, only 10 were not infected with any STH infections at pre-treatment. Of the 10, only 2 (20.0%) were found to be positive after 21 days of treatment indicating the possibility of them having acquired a new infection. Only one participant from the RPS was observed to be consistently free from any of the STH infection, before treatment and all post-treatment time points.

Reinfection was prominent in both communities (Table 4). In the inland community, the prevalence at baseline, 21 days, 3 months and 6 months post-treatment for each helminth infections were as follows: *T. trichiura* (75.0%; 33.3%; 70.0%; 95.0%); *A. lumbricoides*

Table 1. Cure rate and egg reduction rate by both arithmetic and geometric means of *T. trichiura* infection after albendazole treatment

Cure rate status in *T. trichiura* infection 21 days following albendazole treatment						
	Pre-treatment infection status [no. of positive participant, n (%)]			Cure rate – 21 days post-treatment [no. of cured participant, n (%)]		
Overall (N=54)	38 (70.4)			16 (42.1)		
Inland (N=24)	18 (75.0)			13 (72.2)		
RPS (N=30)	20 (66.7%)			3 (15.0)		
Egg reduction rate of *T. trichiura* infection following treatment						
	Arithmetic, mean epg (95% CI)			**Geometric, mean epg log 10 (epg+1) (95% CI)**		
	Overall	**Inland**	**RPS**	**Overall**	**Inland**	**RPS**
Pre-treatment	5,534.5 (4,053.2-6,982.7)	3,928.0 (2,525.1-5,489.9)	4,653.6 (2,672.8-6,557.3)	2.4 (2.0-2.9)	2.6 (2.0-3.2)	2.4 (1.8-3.0)
21 days post-treatment	1,575.79 (5,87.8-2,180.8)	422.67 (107.6-837.2)	1,784.8 (873.6-2,882.2)	1.6 (1.1-2.0)	1.1 (0.5-1.7)	2.0 (1.5-2.6)
Egg reduction rate	71.5%	89.2%	62.0%	55.1%	59.0%	57.7%
***p* value**	< 0.001[a*]	< 0.001[a*]	< 0.001[a*]	0.004[b*]	0.005[b*]	0.13[b]

[a] *p* value for the difference between the findings during pre- and 21 days post-treatment in arithmetic analysis was derived from Wilcoxon-Rank test (Monte Carlo with 1000 iteration for 95% CI was employed).
[b] *p* value for the difference between the findings during pre- and 21 days post-treatment in geometric analysis was derived from Paired-t test (bootstrap with 1000 iteration for 95% CI was employed).
* Significant difference $p \leq 0.05$.

Table 2. Cure rate and egg reduction rate by both arithmetic and geometric means of *A. lumbricoides* infection after albendazole treatment

Cure rate status in *A. lumbricoides* infection 21 days following albendazole treatment						
	Pre-treatment infection status [no. of positive participant, n (%)]			Cure rate -21 days post-treatment), [no. of cured participant, n (%)]		
Overall (N=54)	27 (50.0)			26 (96.3)		
Inland (N= 24)	19 (79.2)			19 (100.0)		
RPS (N=30)	8 (26.7)			7 (87.5)		
Egg reduction rate of *A. lumbricoides* following treatment						
	Arithmetic, mean epg (95% CI)			**Geometric, mean epg log 10 (epg+1) (95% CI)**		
	Overall	**Inland**	**RPS**	**Overall**	**Inland**	**RPS**
Pre-treatment	27,675.3 (20,440-35349.7)	22,778.53 (14,117.3-32,586.9)	39,305.0 (26,659.0-5,0629.6)	2.1 (1.5- 2.7)	3.3 (2.5- 3.9)	1.2 (0.4- 1.9)
21 days post-treatment	94.22 (94.2-376.9)	0	318.0 (212.0-1017.6)	0.1 (0.0, 0.2)	0.0	0.1 (0.0- 0.3)
Egg reduction rate	99.6%	100.0%	99.2%	95.2%	100%	91.7%
***p* value**	< 0.001[a*]	nc	0.003[a*]	0.001[b*]	nc	0.002[b*]

*Significant difference $p \leq 0.05$; nc: not computed due to 100% reduction.

Table 3. Cure rate and egg reduction rate by both arithmetic and geometric means of hookworm infection after albendazole treatment

Cure rate status in hookworm infection 21 days following albendazole treatment						
	Pre-treatment infection status [no. of positive participant, n (%)]			Cure rate -21 days post- treatment, [no. of cured participant, n (%)]		
Overall (N=54)	10 (18.5)			9 (90.0)		
Inland (N= 24)	7 (29.2)			6 (85.7)		
RPS (N=30)	3 (10)			3 (100.0)		
Egg reduction rate of hookworm following treatment						
	Arithmetic, mean epg (95% CI)			Geometric, mean epg [log 10 (epg+1)] (95% CI)		
	Overall	Inland	RPS	Overall	Inland	RPS
Pre-treatment	1,538.4 (1,092.1-2,008.7)	1,422.86 (1,000.0-1,928.0)	1,808.0 (907.5-2,592.0)	3.2 (2.9-3.3)	3.1 (2.9-3.3)	3.2 (2.7-3.4)
21 days post-treatment	43.2 (0.0-129.6)	61.7 (48.0-216.0)	0	0.3 (0-0.8)	0.4 (0-1.1)	0
Egg reduction rate	99.5%	99.3%	100%	90.6%	87.1%	100%
***p* value**	< 0.005[a*]	< 0.02[a*]	nc	0.01[b*]	0.001[b*]	nc

*Significant difference $p \leq 0.05$; nc: not computed due to 100% reduction.

Table 4. Prevalence and reinfection rate (RR) dynamics of STH infections among the Negritos at pre-treatment and 3 post-treatment time points (i.e., 21 days, 3 months and 6 months)

Overall (N=54)						
	T. trichiura infection		*A. lumbricoides* infection		Hookworm infection	
Sampling points	Prevalence n (%)	RR (%)	Prevalence n (%)	RR (%)	Prevalence n (%)	RR (%)
Pre-treatment	38 (70.4)	-	27 (50.0)	-	10 (18.5)	-
21 days post-treatment	26 (48.1)	68.3	1 (1.9)	3.8	1 (1.9)	10.3
3 months post-treatment	33 (76.7)	108.9	9 (21.4)	42.8	1 (2.4)	13.0
6 months post-treatment	41 (95.3)	135.3	21 (48.8)	97.6	5 (11.6)	62.7
Inland (N= 24)						
	Prevalence n (%)	RR (%)	Prevalence n (%)	RR (%)	Prevalence n (%)	RR (%)
Pre-treatment	18 (75.0)	-	19 (79.2)	-	7 (29.2)	-
21 days post-treatment	8 (33.3)	44.4	0 (0.0)	0.0	1 (4.2)	14.4
3 months post-treatment	14 (70.0)	93.3	5 (25.0)	31.6	1 (5.0)	17.1
6 months post-treatment	19 (95.0)	126.7	12 (60.0)	75.8	5 (25.0)	85.6
RPS (N=30)						
	Prevalence n (%)	RR (%)	Prevalence n (%)	RR (%)	Prevalence n (%)	RR (%)
Pre-treatment	20 (66.7)	-	8 (26.7)	-	3 (10.0)	-
21 days post-treatment	18 (60.0)	90.0	1 (3.3)	12.4	0 (0.0)	0.0
3 months post-treatment	19 (82.6)	123.8	4 (18.2)	68.2	0 (0.0)	0.0
6 months post-treatment	22 (95.7)	143.5	9 (39.1)	146.4	0 (0.0)	0.0

N: Number of total participants; n: number infected; RR: reinfection rate derived from % prevalence after treatment (i.e., 21 days-, 3- and 6 months post-treatment) divided by % prevalence before treatment.

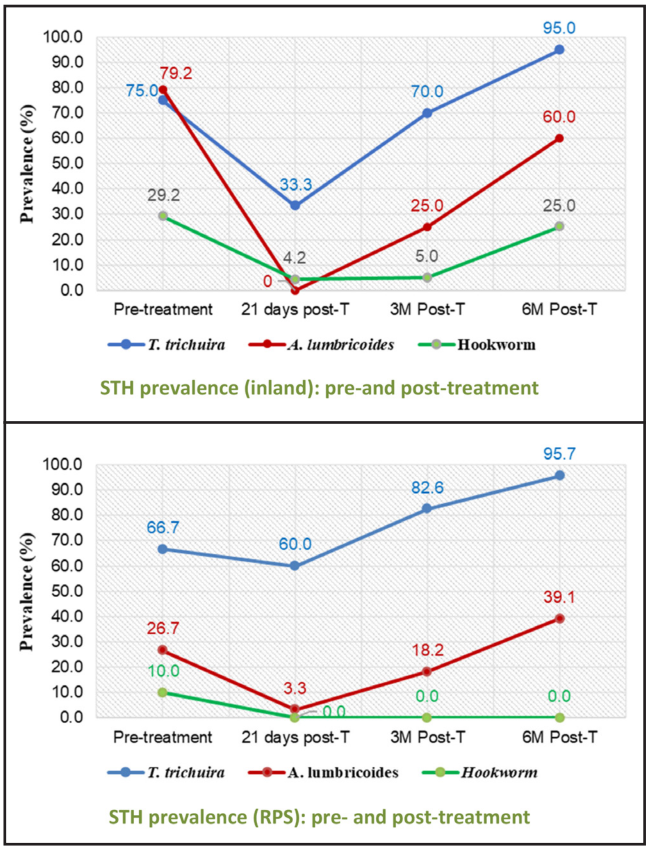

Figure 2. A comparative prevalence of STH infection among the inland and RPS Negritos at pre-treatment and 3 post-treatment time points (i.e., 21 days, 3 months and 6 months).

(79.2%; 4.2%; 25.0%; 60.0%), and hookworm (29.2%; 4.2%; 5.0%; 25.0%). In the RPS, the prevalence at baseline, 21 days, 3 months and 6 months post-treatment for each helminth infections were as follows: *T. trichiura* (66.7%; 60.0%; 82.6%; 95.7%), *A. lumbricoides* (26.7%; 3.33%; 18.2%; 39.1%), and hookworm (10.0%; 0.0%; 0.0%; 0.0%) (Table 4 and Figure 2).

Based on Table 4, the reinfection rate of the *T. trichiura* infection was highly observed in the RPS (90.0%) as opposed to the inland (44.4%) at 21 days following treatment. Crucially, the reinfection rate increased to 93.3% (inland) and 100% (RPS) within 3 months post-treatment and eventually to 100% within 6 months of post-treatment in the inland. Nevertheless, none of the inland Negritos with severe *T. trichiura* was observed within 6 months post-treatment. In contrast, despite a decline in severity, a few cases of severe *T. trichiura* infection (13.0%) were observed in the RPS community.

In the RPS, reinfection of the *A. lumbricoides* was also observed as early as at 21 days following treatment (12.4%). The rate of reinfection increased to 68.2% within 3 months and 100% at 6 months post-treatment. Nevertheless, none had as severe an *A. lumbricoides* infection following treatment (Table 5). On the other hand, in the inland, the reinfection of *A. lumbricoides* was only observed at 3 months post-treatment but reached up to 75.8% by 6 months. Similarly, no severe infection was observed. For hookworm, the reinfection rate was 4.2%, 5.0% and 25.0% at 21 days, 3 months and 6 months post-treatments, respectively in the inland. Conversely, no hookworm infection was seen in the RPS within 6 months post-treatment.

DISCUSSION

This study aimed to compare the efficacy of single dosage albendazole (400mg) and reinfection status following treatment between the inland and RPS Orang Asli Negritos. The findings from this study are important to identify the best strategy in controlling the STH infections especially when the 3-days course of albendazole is not feasible among indigenous people living in the inland jungle. At baseline, the most prevalent STH infection among the examined participants was *T. trichiura*, followed by *A. lumbricoides* and hookworm. Severe infections were also prominent at baseline especially in the RPS. The overall cure rate of the albendazole against STH infections was significantly higher in the inland (66.7%) as compared to the RPS community (15.0%).

We highlighted better efficacy of single dosage albendazole in the inland as characterized by 72.2% cure rate against the *T. trichiura* infection as compared to the 15.0% cure rate in the RPS. Despite huge disparities in cure rate status between both communities, the egg reduction rate (> 50.0%) was still prominent in the RPS and relatively comparable with those in the inland as shown by arithmetic and geometric means. All participants which remained positive following treatment had only mild infections of *T. trichiura.*

High variations and inconsistency in the efficacy of single dosage albendazole against *T. trichiura* have been reported in previous studies. Most studies including a few conducted in Malaysia revealed low curative effect (< 50.0%) of the albendazole against *T. trichiura*, which reportedly ranged between 4.9- 50.0% (Ramalingam *et al*., 1983; Chien *et al*., 1989; Sinniah *et al*., 1990; Albonico *et al*., 1994; Norhayati *et al*., 1997; Bennett & Guyatt, 2000; Keiser & Utzinger, 2008). On the other hand, a few studies claimed > 50% cure rate, for instance: 68.9% cure rate in the Philippine (Ovedoff, 1984), 83.9% in Kenya (Bwibo & Pamba, 1984), 52.7% in Haiti (Beach *et al*., 1999), 76.0% in France and West Africa (Pene *et al*., 1982) and 81.7% in Indonesia (Anto & Nugraha, 2019). The reason behind these huge disparities is still unknown and many factors have been suggested which includes the possibility of anthelminthic resistance or reduced efficacy of the drugs by underdosing (Vercruysse *et al*., 2011; Shalaby, 2013).

Nevertheless, the concern on the possibility of anthelmintic resistance was largely based on the experience in the veterinary field. In livestock, resistance was not just reported in benzimidazole class of drugs but also in levamisole and the other nicotinic agonists (Shalaby, 2013). In Australia, anthelmintic resistance was reported to affect the entire sheep industry (Besier & Love, 2003). High frequency of treatment has been clearly shown as one of the possible factors contributing to the development of AR and reduction in efficacy as shown by a study in goats in Malaysia (Dorny *et al*., 1994). It is practically common in livestock that anthelmintic was usually given five times or up to ten treatments a year. To compare with, the frequency of anthelmintic treatment in human is limited to 1 - 3 times per year (Warren *et al*., 1993) hence the factor is less likely to be the cause of anthelmintic resistance in human STH infections. However, selective anthelmintic resistance has also been reported in STH infections of sheep and goats with lower treatment frequencies (Coles *et al*., 2006). It was suggested that the prolonged use of the same anthelmintic might be the cause of AR in particular cases (Reinemeyer *et al*., 1992).

In human infection, few studies have highlighted the possibility of efficacy reduction based on measurement of efficacy from the initial implementation of mass deworming program and after few years of its commencement. For example, the efficacy of mebendazole against hookworm have fallen from 82.4% to 52.1% over a period of five years in a population study in Zanzibar (Albonico

Table 5. Percentages of infected Negritos in each class of STH intensity during pre-treatment and 3 post-treatment time points (i.e., 21 days, 3 months and 6 months)

Overall Negritos												
	Intensity of *T. trichiura* infection, n (%)				Intensity of *A. lumbricoides severity*, n (%)				Intensity of hookworm infection, n (%)			
Sampling points	**Negative**	**Mild**	**Moderate**	**Severe**	**Negative**	**Mild**	**Moderate**	**Severe**	**Negative**	**Mild**	**Moderate**	**Severe**
Pre-T (N=54)	16 (29.6)	7 (13.0)	15 (27.8)	16 (29.6)	27 (50.0)	4 (7.4)	14 (25.9)	9 (16.7)	44 (81.5)	7 (13.0)	3 (5.6)	0
21-days post-T (N=54)	27 (50.0)	11 (20.4)	15 (27.8)	1 (1.9)	53 (98.1)	1 (1.9)	0	0	53 (98.1)	1 (1.9)	0	0
3-months post-T (N=43)	10 (23.3)	6 (14.0)	25 (58.1)	2 (4.7)	33 (78.6)	9 (21.4)	0	0	41 (97.6)	1 (2.4)	0	0
6-months post-T (N=43)	2 (4.7)	3 (7.0)	35 (81.4)	3 (7.0)	22 (51.2)	9 (20.9)	12 (27.9)	0	38 (88.4)	5 (11.6)	0	0
Inland												
	Negative	**Mild**	**Moderate**	**Severe**	**Negative**	**Mild**	**Moderate**	**Severe**	**Negative**	**Mild**	**Moderate**	**Severe**
Pre-treatment (N=24)	6 (25.0)	3 (12.5)	10 (41.7)	5 (20.8)	5 (20.8)	4 (16.7)	10 (41.7)	5 (20.8)	17 (70.8)	6 (25.0)	1 (4.2)	0
21-days post-T (N=24)	16 (66.7)	4 (16.7)	4 (16.7)	0	24 (100.0)	0	0	0	23 (95.8)	1 (4.2)	0	0
3-months post-T (N=20)	6 (30.0)	3 (15.0)	11 (55.0)	0	15 (75.0)	5 (25.0)	0	0	19 (95.0)	1 (5.0)	0	0
6-months post-T (N=20)	1 (4.3)	1 (5.0)	18 (90.0)	0	8 (40.0)	8 (40.0)	4 (20.0)	0	15 (75.0)	5 (25.0)	0	0
RPS												
	Negative	**Mild**	**Moderate**	**Severe**	**Negative**	**Mild**	**Moderate**	**Severe**	**Negative**	**Mild**	**Moderate**	**Severe**
Pre-treatment (N=30)	10 (33.3)	4 (13.3)	5 (16.7)	11 (36.7)	22 (73.3)	0	4 (13.3)	4 (13.3)	27 (90.0)	1 (3.3)	2 (6.7)	0
21-days post-T (N=30)	11(36.7)	7 (23.3)	11 (36.7)	1 (3.3)	29 (96.7)	1 (3.3)	0	0	30 (100.0)	0	0	0
3-months post-T (N=23)	4 (17.4)	3 (13.0)	14 (60.9)	2 (8.7)	18 (81.8)	4 (18.2)	0	0	22 (100.0)	0	0	0
6-months post-T (N=23)	1 (4.3)	2 (8.7)	17 (73.9)	3 (13.0)	14 (60.9)	1 (4.3)	8 (34.8)	0	23 (100.0)	0	0	0

T: Treatment; Class of STH intensity was based on egg per gram (epg) found in the stool [*T. trichiura* (mild: 1-999; moderate: 1000-9999; severe: $\geq$10 000), *A. lumbricoides* (mild: 1-5000; moderate: 5000-49999; severe: $\geq$50000), hookworm (mild: 1-1999; moderate: 2000-4000; severe: >40000).

et al., 2003) and Australia (Reynoldson *et al*., 1997). Also, studies conducted in Haiti, Kenya and Panama have discovered increased frequencies of single nucleotide polymorphism (SNPs) which relate to benzimidazole resistance in codon 167, 198, 200 of the ꞵ-tubulin gene of *T. trichiura* following anthelmintic intervention (Diawara *et al*., 2009; Diawara *et al*., 2013).

To attribute with the given postulation, the prolonged used of albendazole, coupled with higher frequency of treatment among the RPS as compared to the inland might have contributed to the high variations of cure rate against *T. trichiura* infection between these communities. This is probably due to the characteristic of the inland which could be considered as anthelmintic-naïve population due to their lower exposure to the modern medicine which leads to better efficacy of the albendazole. However, to answer the question whether environmental variations between the inland and RPS communities play a role in the disparity of the findings, the answer remains inconclusive.

Other multifactorial factors which relate to intensity of the parasites before treatment (Bennett & Guyatt, 2000), albendazole underdosing (Smith *et al*., 1999) or possibly refugia might as well influence the findings. Refugia is the term used to explain the proportion of the parasites in population that is not exposed to the treatment (Van Wyk, 2001), hence escaping the resistance selection. Other simple explanations to poor performance of anthelmintic treatment against the *T. trichiura* are related to the location of the adult parasite in the large intestine and the fact that its anterior part is embedded into the intestinal mucosa. Hence the drugs need to pass through the gastro-intestinal system before reaching the parasite (Betson *et al*., 2015).

In the case of sub-optimal dosage, the use of 3 days regimens of albendazole have been reported to tremendously increase the efficacy of the treatment against the *T. trichiura* (Penggabean *et al*., 1998; Keiser & Utzinger, 2008; Steinmann *et al*., 2011). However, the implementation is not feasible for mass-scale intervention weighted with logistical issues, costs and good compliance rates. Meanwhile, the cure rate and egg reduction rate of single dosage albendazole against the hookworm and *A. lumbricoides* were very high (85.7 - 100.0%) and comparable in both communities. Unlike *T. trichiura*, the high curative effect on the hookworm and *A. lumbricoides* was expected and consistently reported in most of the previous findings in Malaysia and other countries (Penggabean *et al*., 1998; Keiser & Utzinger, 2008; Steinmann *et al*., 2011). Population study amongst the indigenous Temuan subtribe of Proto-Malay revealed 92.0% cure rate of the single dosage albendazole against hookworm (Norhayati *et al*., 1995).

Generally, the rate of STH reinfection was considered rapid especially in the case of *T. trichiura* in both communities. Comparatively, the situation of *T. trichiura* infection and reinfection rate following treatment were relatively more precarious in the RPS indicated by 100.0% reinfection rate at 3 months post-treatment. The problem was further aggravated by the existence of severe intensity in small number of Negritos at that time point. Although the result was expected based on the low curative effect of the albendazole, this finding supports the observation made in previous study (Muslim *et al*., 2019) which revealed higher intensity of *T. trichiura* possibly due to the massive contamination of the parasitic eggs in their soil environments.

In the inland, 100.0% reinfection rate of *T. trichiura* was observed at 6 months post-treatment, but unlike in the RPS, none of the inland Negritos was found with severe *T. trichiura* at that time point. It is also essential to highlight the fact that albendazole is relatively insoluble in water making the drug poorly absorbed from the gastro-intestinal system resulting in limited systemic effect. Therefore, the drug will not be able to kill the immature worm and later it becomes adult, excretes eggs and this increase environmental contamination leading to high reinfection rate especially in highly STH endemic population.

Meanwhile slower reinfection rate of *A. lumbricoides* and hookworm infections showed promising result in controlling both infections. Although reinfection could be seen as early as 3 months, no severe infection was recorded even 6 months following treatment. Our findings on the status of reinfection of *A. lumbricoides* and hookworm were more promising compared to previous studies conducted on other indigenous tribes of OA which reported intensity at 6 months close to the figures at baseline (Norhayati *et al*., 1995; Norhayati *et al*., 1997; Al-Mekhlafi *et al*., 2008).

Another interesting finding was that no hookworm infection was recorded among participants in the RPS. This observation corresponds with the decline in numbers of cases of hookworm infections as seen consistently among the indigenous group during their progression towards modernity. However, in the inland, hookworm infections were observed after 6-months of drug administration. These findings, explained high exposure of the inland Negritos towards hookworm. The behaviour of walking barefooted among the inland Negritos is one of the most probable explanations for this scenario.

There were limitations in this study to be considered while interpreting the present findings. First, the number of participants was small. During the albendazole administration, most of the Negritos refused to participate since most of them had no symptoms. Apart from poor cooperation rate, it was difficult to reach the Negrito population for follow-up study especially in acquiring complete samples at four time points of duration. Therefore, further downstream analyses based on demographic factors such as gender and age groups could not be done due to statistically irrelevant. However, this direction can be utilised in future studies with more respondents or participants.

In summary, this study suggests that the indigenous OA living in the inland jungle had better tolerability on albendazole and experienced slower STH reinfection rates as compared to the resettled RPS. Whether cultural-environmental factors between these two communities played a role in the variations of findings remained inconclusive. Hence, the use of single dosage albendazole (twice a year) would be more suitable for indigenous living in the inland. Moreover, the 3-days albendazole course is deemed not feasible due to logistical challenges and low compliance rate. On the other hand, the single dosage of albendazole administration (twice a year) did not seem sufficient as a treatment for OA living in the resettled RPS based on the lower cure rate as well as rapid escalation of individuals with severe infections over time. After weighing the trade-off between effort and cost with better treatment efficacy, the use of 3-days albendazole regimens amongst the RPS community is strongly suggested. Since the location of the RPS is closer to the mainstream community and healthcare facilities, and together with the full cooperation from the OA people, the compliance to the treatment could probably be achieved. Otherwise, by the assumption of the probability of anthelmintic resistance in the future caused by prolonged usage of the same medication and when the use of 3-days regimens is not possible, alternation between albendazole and mebendazole treatments may need to be considered.

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Conflict of interest

The authors declare that they have no conflict of interest.

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