Determine the prevalence of intestinal and soil-transmitted helminths using different copromicroscopic techniques in Krabi Province, Thailand

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ARTICLE INFO

ABSTRACT

Objective: To determine the prevalence rates of intestinal and soil-transmitted helminths (STHs) by cross sectional surveys in 3 districts (Lamthap, Khaophanom and Aoluek Districts) of Krabi Province, Southern Thailand.
Methods: Different copromicroscopic techniques including simple direct smear, modified formalin-ether concentration and modified Harada-Mori were used. The consent forms were filled and signed by the participants as requested.
Results: Between May and October 2012, the prevalence of intestinal helminth was 13.1% (29 out of 221 villagers) by simple direct smear and modified formalin-ether concentration technique. There were 7 helminths. Hookworm was found at the highest prevalence (8.1%) followed by *Ascaris lumbricoides*, *Trichuris trichiura* and *Opisthorchis viverrini* (1.4%), *Fasciolopsis/Fasciola/Echinostoma* (1 case and never reported in the south) and *Enterobius vermicularis* (0.5%). *Strongyloides stercolaris* was found only in modified Harada-Mori (3.2%). STH was found at 10.9%. No significant differences were found between genders and age groups ($P > 0.05$). The 3 copromicroscopic techniques from 159 cases raising the hookworm prevalence to 15.1% whereas a single method could only report the prevalence to be 6.9%–11.3%.
Conclusions: Using 3 different copromicroscopic methods to increase the prevalence of helminthic and STHs infections in Krabi Province might reflect the true prevalence.

1. Introduction

Intestinal parasitic infections are a common cause of gastrointestinal disorders, especially in developing countries. The role of parasitic infections in the world and their adverse effects on health, economic and social affairs of humans is of vital importance[1,2]. *Ascaris lumbricoides*, *Trichuris trichiura*, *Strongyloides stercoralis* (*S. stercoralis*) and hookworms are referred to as soil-transmitted helminths (STHs). It is estimated that over 1.4 billion people are infected with STHs globally[3].

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Southern Thailand, including Krabi Province, has a tropical and humid climate which is ideal for the survival of STH eggs/larvae in the environment[4]. The STH control strategy of the World Health Organization is, by 2020, to reduce morbidity from STH in school-aged children to a level which would not be considered a public health problem[5]. At present, there is a growing interest in investigating the transmission of STHs in all age groups, rather than focusing on young children[6,7]. STHs have also been classified as one of the most prevalent neglected tropical diseases as they persist exclusively in the poorest populations and thus are often forgotten[8]. In Thailand, the overall prevalence rate of intestinal helminthiases, determined by Kato's thick smear and formalin ether concentration techniques, decreased from 62.9% in 1957 to 22.5% by 2001[9] and continued to fall to 18.1% by 2002. Hookworm was the most prevalent (15.8%) in Southern Thailand[10]; however, this prevalence rate could be an underestimation.

There are no data on the prevalence of intestinal helminths in Krabi Province. Therefore, we conducted point prevalence surveys in 3 districts using 2 copromicroscopic techniques (direct smear and modified formalin-ether concentration methods) to screen the parasitic infections. We used the modified Harada-Mori (mHarada-Mori) culture[11,12] to increase the detection rate of both hookworm and *Strongyloides*.

2. Materials and methods

2.1. Study design, population and subjects

Cross-sectional surveys were carried out between May and October 2012. Stool samples were collected from people living in Lamthap, Khaophanom and Aoluek Districts, Krabi Province, Southern Thailand, where the main economic activities are centered on rubber and palm trees (rubber and palm oil production, respectively).

The study inclusion criteria were as follows: either sex aged more than 15 years old who gave written informed consent to give stool samples. A total sample size of 226 samples was required (according to $P = 0.18$[10]).

The study protocol (reference number: RSEC 08/54) was reviewed and approved by the Ethics Committee of Rangsit University. All participants signed informed consent forms prior to stool collection.

2.2. Stool samples and examination techniques

Clean plastic containers were labeled with a code number and distributed to the villagers one day before stool collection. Instructions were given by the research team on how to obtain the stool sample. On receipt of the stool sample by the research team, it was divided into three parts: (i) simple direct smear (screening parasitic infections in all samples), (ii) modified formalin-ether concentration, and (iii) Harada-Mori culture[13] and all were examined under light microscope.

2.3. The modified formalin-ether concentration technique (mFECT)

We modified the Ritchie technique of 1948[14-16]. Preserved stool was filtered through two layers of wet gauze into a centrifuge tube. The volume was adjusted to 10 mL with 10% formalin and centrifuged at 2 500 r/min for 3 min. The supernatant was discarded and 3 mL of ether and 7 mL of 10% formalin were added to the sediment and further centrifuged at 2 500 r/min for 3 min. The supernatant was discarded, leaving only the sediment to which 3 mL of 10% formalin (as preservative) was added. The sediment was mixed before examination.

2.4. The mHarada-Mori method (to detect hookworm and Strongyloides)

Two grams of fresh faeces were placed on a strip of filter paper, leaving the edges free. The strip was then placed in a plastic bag tube (size 30 mm × 200 mm) containing 5 mL sterile distilled water and incubated at room temperature (25–35 ℃) for 7 days; 0.5 mL formaldehyde was then added and the tube was centrifuged at 2 000 r/min for 5 min. The sediment was examined under microscope. All larvae were identified according to World Health Organization criteria (1981)[17] and the third stage larvae were identified by species.

2.5. Anti-helminth treatment

Individuals who were positive for intestinal helminths were informed of the result and referred for treatment, following national guidelines. Study participants were given instructions on how to prevent intestinal helminths.

2.6. Data analysis

Descriptive statistics were used to to summarize general information. Percentages were used to present the categorical data, the prevalence of intestinal helminths and STHs.

A *Chi*-squared test was used to examine the association between the age groups, genders, districts and STH infections. A *P*-value < 0.05 was considered statistically significant.

3. Results

3.1. The prevalence of intestinal helminths and STHs in Krabi Province by simple direct smear method (n = 240)

A total of 240 stool samples were examined by simple direct smear method at the first screening for intestinal parasites. The prevalence of helminth infection was 5.8% (14 out of 240). Three species of STHs were found but *Strongyloides* was not detected by this method and also no other helminths were detected (Table 1).

Table 1

The prevalence of intestinal helminths in Krabi Province by simple direct smear (*n* = 240).

Helminths		Infections [*n* (%)]
STH	Hookworm	11 (4.6)
	S. stercoralis	0 (0.0)
	Trichuris trichiura	2 (0.8)
	Ascaris lumbricoides	1 (0.4)
	Total STH	14 (5.8)
Other helminths and parasites	*Opisthorchis viverrini*	0 (0.0)
	Enterobius vermicularis	0 (0.0)
	Fasciola/Fasciolopsis/Echinostoma spp.	0 (0.0)
	Mixed 2 spp.	0 (0.0)
	Mixed 3 spp	0 (0.0)
	Total helminths	14 (5.8)

3.2. The prevalence of intestinal helminths and STHs using simple direct smear and mFECT (n = 221)

A total of 221 of the 240 stool samples were sufficient to process by 2 methods (direct smear and mFECT) and to compare the prevalence of helminth infections. Modified formalin-ether showed higher rate (10.9%) of detection than simple direct smear (5.9%) whereas 2 copromicroscopic techniques provided the highest prevalence of helminthic infections (13.1%) and STHs (10.9%), higher than single technique (Table 2).

Table 2
Prevalence of intestinal helminths by simple direct smear and modified formalin-ether concentration (n = 221) [n (%)].

Helminths		Direct smear	mFECT	Total
STHs	Hookworm	10 (4.5)	15 (6.8)	18 (8.1)
	S. stercoralis	0 (0.0)	0 (0.0)	0 (0.0)
	Trichuris trichiura	2 (0.9)	1 (0.5)	3 (1.4)
	Ascaris lumbricoides	1 (0.5)	3 (1.4)	3 (1.4)
	Total STHs	13 (5.9)	19 (8.6)	24 (10.9)
Other intestinal helminths	*Opisthorchis viverrini*	0 (0.0)	3 (1.4)	3 (1.4)
	Fasciolopsis buski/Fasciola/ Echinostoma	0 (0.0)	1 (0.5)	1 (0.5)
	Enterobius vermicularis	0 (0.0)	1 (0.5)	1 (0.5)
	Mixed 2 species	0 (0.0)	3 (1.3)	3 (1.9)
	Mixed 3 species	0 (0.0)	1 (0.5)	1 (0.5)
	Total	13 (5.9)	24 (10.9)	29 (13.1)

3.3. The prevalence of hookworm and Strongyloides using simple direct smear, mFECT and mHarada-Mori (n = 159)

A total of 159 of the 240 stool samples were sufficient to process by 3 methods (direct smear, mFECT and mHarada-Mori) and compare the efficacy. Hookworm infections examined using copromicroscopic method demonstrated a prevalence of 15.1% (6.9%–11.3% by any one method) (Figure 1 and Table 3). The mHarada-Mori culture detected 18 of 24 subjects with hookworm (75.0% detection rate), which was higher than other techniques and all 18 hookworm infections were identified as *Necator americanus* (*N. americanus*). Nevertheless, 3 combined methods gave the highest hookworm prevalence of 15.1%. *S. stercoralis* was only detected by mHarada-Mori method at a prevalence rate of 3.1%.

Table 3
Detection rates of hookworm and *S. stercoralis* by 3 copromicroscopic techniques (n = 159).

Total positive		No. of detection (%)		
		Direct smear	mFECT	mHarada-Mori
Hookworm	24 (15.1)	11 (6.9)	16 (10.1)	18 (11.3)
Detection rate		45.8%	66.7%	75.0%
S. stercoralis	5 (3.2)	0	0	5 (3.1)
Detection rate		0	0	100%

There were 7 species of helminths found in Krabi Province

(Tables 1–3). STHs showed the highest prevalence in these areas. The STHs infection was found in male (19.6%) more than female (10.5%) with a statistical difference (*P* = 0.047). The infections of STHs had no significant differences (*P* > 0.05) between age groups (Figure 2). STHs prevalence rates by districts varied between 8.4%–15.1% with no significant differences (Table 4).
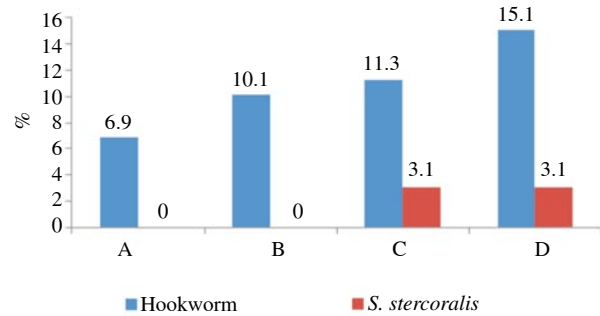

Figure 1. Infection rates of hookworm and *S. stercoralis* by different detection methods.
A: Direct smear; B: Modified formalin-ether; C: mHarada-Mori method; D: Combined 3 methods.
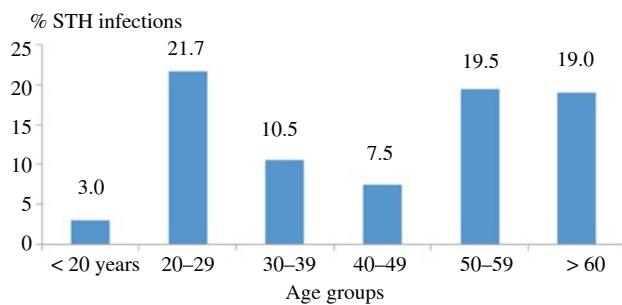

Figure 2. The infection rate of STHs by age groups.

Table 4
Prevalence rates of STHs in three districts of Krabi Province, Southern Thailand.

STHs	No. of infection (%)			Total
	Khaophanom (n = 83)	Lamthap (n = 71)	Aoluek (n = 86)	
Hookworm	6 (2.5)	12 (5.0)	9 (3.8)	27 (11.3)
Ascaris lumbricoides	2 (0.8)	1 (0.4)	0 (0.0)	3 (1.3)
S. stercoralis	1 (0.4)	2 (0.8)	2 (0.8)	5 (2.1)
Trichuris trichiura	0 (0.0)	0 (0.0)	3 (1.3)	3 (1.3)
Total	7 (8.4)	12 (16.9)	13 (15.1)	32 (13.3)

$\chi^2 = 2.743$, *df* = 2, *P* = 0.254.

There were mixed infections in 7 cases: 2 cases of mixed infection between hookworm and *S. stercoralis*, another 2 cases mixed infection between hookworm and *Ascaris lumbricoides*, 3 cases mixed infections, always involving hookworm with other helminths.

One case of helminth infection was *Fasciolopsis buski/Fasciola/ Echinostoma* (Figure 3).
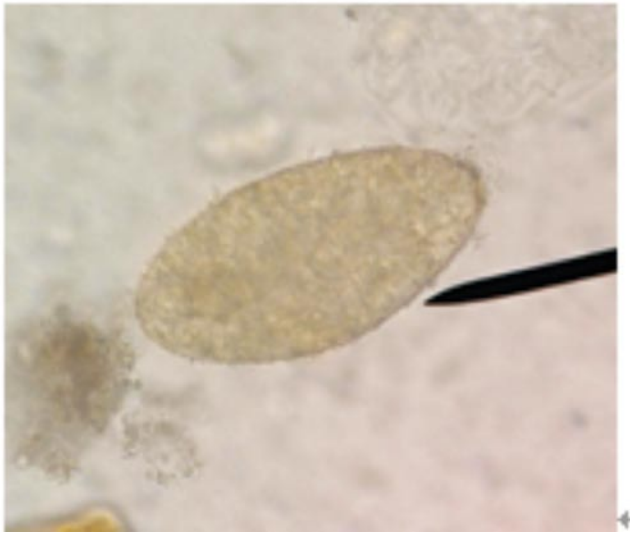
Figure 3. *Fasciolopsis buski/Echinostoma/Fasciola* egg.

4. Discussion

The prevalence of helminthic infection in Krabi Province was shown to be 5.8% by simple direct smear method and 2 copromicroscope methods presented the prevalence of 13.1%, a somewhat lower rate than the reported 18.1% in Southern Thailand by Ministry of Public Health (MOPH), Thailand (2008). However, this area has never been the subject of published data before and the MOPH use different techniques: semiquantitative Kato's thick smear and formalin-ether concentration technique[10]. It is worth noting that one case harbored *Fasciolopsis buski/Fasciola/Echinostoma* which has never been reported before in Southern Thailand. Unfortunately, we were unable to investigate the source of infections and the morphology of the eggs cannot establish the species of this helminth.

Using 2 copromicroscopic methods, STHs were found 10.9% in 3 areas of Krabi Province which is higher than that by single technique. The age distribution of STHs peaked in the 20–29 age groups and remained high across the older age groups, as previously reported[16]. Data on the STH prevalence rates in Krabi Province are limited. Wongsaroj *et al.* reported *S. stercoralis* rates of 9.5% in Lamthap and 20.0% in Khaophanom, by agar plate culture[18] which is higher than our study reported, which used the mHarada-Mori culture. One agar plate method study reported a detection rate of 96% sensitivity[19] which was 4.4 times more efficient than the direct smear[20]. However, in our study *S. stercoralis* was detected only in mHarada-Mori culture.

Hookworm was the most common STH in this area (15.1%), a similar figure to that reported by the MOPH in 2008. We only detected *N. americanus*, but it should be noted that most studies report the hookworm species because the eggs of the two species are similar and not readily distinguishable from one another by classical parasitological methods[21]. Although there are established morphological differences between the adult worms[22-24], the adult stages are rarely available for routine examination. Thus, species

identification has traditionally been done by using morphological characteristics to differentiate the third stage larvae which have been cultured from eggs in coprocultures[17]. We modified the Harada-Mori culture using a simple and inexpensive plastic bag that was readily available in the local market. This method is sensitive for detecting hookworm, economical (0.06 USD/test), noninvasive and the easiest to carry out. However, it requires fresh stool and is time consuming (5–10 days) and can't be performed with diarrheal stool[25]. In areas with non STH data, simple direct smears are used to screen populations and are easy to carry out, although this method might underestimate the true prevalence. The mFECT is used to increase the detection rate of intestinal helminths[10,26] but formalin and ether are hazardous chemicals[27-29] and this method is difficult to process.

In our study, 159 stool samples were examined by all three methods. The mHarada-Mori gave the best result, even though it was able to detect only hookworm and *S. stercoralis*. The detection rate of the 3 methods was highest for detecting hookworm (~15%) compared with single methods (~7%–11%). The *S. stercoralis* detection rate in this study was low (~3%), and was only detected by mHarada-Mori. mFECT contained the same amount of 2 g faeces as mHarada-Mori, although the filtration process in mFECT might allow a limited number of eggs and larvae pass through the gauze, resulting in less efficacy in detection of parasites than mHarada-Mori. It may also be that the larvae were excreted intermittently in minimal amount by the host, therefore requiring more sensitive detection techniques such as agar plate culture to determine the true prevalence rate[10,30]. The larvae of helminths are often difficult to find in a single stool sample because of the irregular larval output and low intensity of parasites.

It has been reported that STHs were found in older adolescents and adults. *N. americanus* was the dominant species in 3 districts of Krabi Province. We recommend using combined methods to more accurately define the STH prevalence rate. The mHarada-Mori culture is a suitable method because it is cheap and requires very little technical expertise.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

References

[1] Gonçalves AQ, Junqueira AC, Abellana R, Barrio PC, Terrazas WC, Sodré FC, et al. Prevalence of intestinal parasites and risk factors for specific and multiple helminth infections in a remote city of the Brazilian Amazon. *Rev Soc Bras Med Trop* 2016; **49**: 119-24.

[2] Hicks JH, Kremer M, Miguel E. The case for mass treatment of intestinal helminths in endemic areas. *PLoS Negl Trop Dis* 2015; **9**: e0004214.

[3] Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. *Parasit Vectors* 2014; **7**: 37.

[4] Brooker S, Singhasivanon P, Waikagul J, Supavej S, Kojima S, Takeuchi T, et al. Mapping soil-transmitted helminths in Southeast Asia and implications for parasite control. *Southeast Asian J Trop Med Public Health* 2003; **34**(1): 24-36.

[5] World Health Organization. Eliminating soil-transmitted helminthiases as a public health problem in children: progress report 2001–2010 and strategic plan 2011–2020. Geneva: World Health Organization; 2012. [Online] Available from: http://apps.who.int/iris/bitstream/10665/44804/1/9789241503129_eng.pdf [Accessed on 8th September, 2017]

[6] Brooker SJ, Nikolay B, Balabanova D, Pullan RL. Global feasibility assessment of interrupting the transmission of soil-transmitted helminths: a statistical modelling study. *Lancet Infect Dis* 2015; **15**(8): 941-50.

[7] Lo NC, Bogoch II, Blackburn BG, Raso G, N'Goran EK, Coulibaly JT, et al. Comparison of community-wide, integrated mass drug administration strategies for schistosomiasis and soil-transmitted helminthiasis: a cost-effectiveness modeling study. *Lancet Glob Health* 2015; **3**(10): e629-38.

[8] Liese B, Rosenberg M, Schratz A. Programmes, partnerships and governance for elimination and control of neglected tropical diseases. *Lancet* 2010; **375**: 67-76.

[9] Jongsuksantigul P. Soil-transmitted helminthiases control in Thailand. Lecture note presented at ACIPAC International Training Course on School-based Malaria and Soil-transmitted Helminthiases Control for Programme Managers at the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand on September 6, 2002.

[10] Wongsaroj T, Nithikathkul C, Rojkitikul W, Nakai W, Royal L, Rammasut P. Nation survey of helminthiasis in Thailand. *Asian Biomed* 2014; **8**: 779-83.

[11] Kitvatanachai S, Pipitgool V. Efficacy of three methods in the detection of hookworm and *Strongyloides stercoralis* infections. *J Trop Med Parasitol* 1999; **22**: 80-1.

[12] Inês Ede J, Souza JN, Santos RC, Souza ES, Santos FL, Silva ML, et al. Efficacy of parasitological methods for the diagnosis of *Strongyloides stercoralis* and hookworm in faecal specimens. *Acta Trop* 2011; **120**(3): 206-10.

[13] Harada T, Mori O. A new method for culturing hookworm. *Yonago Acta Med* 1995; **1**(3): 177-9.

[14] Elkins DB, Haswell-Elkins M, Anderson RM. The epidemiology and control of intestinal helminths in the Pulicat Lake region of Southern India. I. Study design and pre- and post-treatment observations on *Ascaris lumbricoides* infection. *Trans R Soc Trop Med Hyg* 1986; **80**: 774-92.

[15] Ritchie LS. An ether sedimentation technique for routine stool examinations. *Bull U S Army Med Dep* 1948; **8**(4): 326.

[16] Preuksaraj S, Jeradit C, Sathitayathai A, Kijvannee S, Seedonrusmi T. Studies on prevalence and intensity of intestinal helminthic infections in the rural population of Thailand 1980–1981. Collected papers on the control of soil-transmitted helminthiases 1983; **2**: 54-8.

[17] World Health Organization. Intestinal protozoan and helminthic infections. Geneva: World Health Organization; 1981. [Online] Available from: http://apps.who.int/iris/bitstream/10665/41519/1/WHO_TRS_666.pdf [Accessed on 8th September, 2017]

[18] Wongsaroj T, Phatisatakorn W, Ramasoota P, Anamnart W, Kaewpoonsri N, Chiewchanyon B. Epidemiological study of strongyloidiasis in Southern Thailand, 2007. *J Trop Med Parasitol* 2008; **31**: 6-13.

[19] Sato Y, Kobayashi J, Toma H, Shiroma Y. Efficacy of stool examination for detection of *Strongyloides* infection. *Am J Trop Med Hyg* 1995; **53**: 248-50.

[20] de Kaminsky RG. Evaluation of three methods for laboratory diagnosis of *Strongyloides stercoralis* infection. *J Parasitol* 1993; **79**: 277-80.

[21] Hawdon JM. Differentiation between the human hookworms *Ancylostoma duodenale* and *Necator americanus* using PCR-RFLP. *J Parasitol* 1996; **82**(4): 642-7.

[22] Yoshida Y, Matsuo K, Kondo K, Arizona N, Ogino K. Scanning electron microscopy of hookworms. 1. Adults and infective-stage larvae of *Necator americanus* (Stiles, 1902). *Southeast Asian J Trop Med Public Health* 1974; **5**: 510-4.

[23] Yoshida Y, Matsuo K, Kondo K, Arizona N, Ogino K. Scanning electron microscopy of hookworms. 2. Adults and infective-stage larvae of *Ancylostoma duodenale* (Dubini, 1843). *Southeast Asian J Trop Med Public Health* 1974; **5**: 515-8.

[24] Pawlowski ZS, Schad GA, Stott GJ. Hookworm infection and anaemia: approaches to prevention and control. Geneva: World Health Organization; 1991.

[25] Sa-nguankiat S, Maipanich W, Yoonuan T, Kusolsuk T, Puangsa-art S, Outchareon W, et al. Modification of polyethylene tube culture technique in cultivation of hookworm larvae. *J Trop Med Parasitol* 2011; **34**: 30-5.

[26] Moore CE, Nget P, Saroeun M, Kuong S, Chanthou S, Kumar V, et al. Intestinal parasite infections in symptomatic children attending hospital in Siem Reap, Cambodia. *PLoS One* 2015; **10**: e0123719.

[27] Kulle JT, Sauder LR, Hebel JR, Green D, Chatham MD. Formaldehyde dose-response in healthy nonsmokers. *JAPCA* 1987; **37**: 919-24.

[28] Weber-Tschopp A, Fisher T, Granjean E. [Irritating effects of formaldehyde on man (author's transl)]. *Int Arch Occup Environ Health* 1977; **39**: 207-18.

[29] The University of Edinburgh. Hazardous substances. Edinburgh: The University of Edinburgh; 2016. [Online] Available from: http://www.edinburgh.ac.uk/health-safety/guidance/hazardous-substances [Accessed on 8th September, 2017]

[30] van der Feltz M, Slee PH, van Hees PA, Tersmette M. *Strongyloides stercoralis* infection: how to diagnose best? *Neth J Med* 1999; **55**(3): 128-31.