Asian Pacific Journal of Tropical Disease

journal homepage: http://www.apjtcm.com

Original article https://doi.org/10.12980/apjtd.7.2017D7-156

Using Mini Parasep® SF to determine intestinal parasitic infections comparing to conventional methods in gardener of Chanthaburi Province, Thailand

Sirima Kitvatanachai[1*], Pochong Rhongbutsri[2]

[1]*Faculty of Medical Technology, Rangsit University, Pathumthani, 12000, Thailand*

[2]*Department of Preclinical science, Faculty of Medicine, Thammasat University, Pathumthani , 12120, Thailand*

ARTICLE INFO

Article history:
Received 3 Jul 2017
Received in revised form 14 Jul 2017
Accepted 15 Aug 2017
Available online 25 Sep 2017

Keywords:
Mini Parasep® SF kit
Intestinal parasites
Gardener
Chanthaburi Province

ABSTRACT

Objective: To determine the effectiveness of the Mini Parasep SF kit in detecting intestinal parasites compared with two other methods, whilst also report on the prevalence and species of these parasites in gardeners of Soi Dao, Kaenghangmeao and Khao Khitchakut Districts, Chanthaburi Province, Thailand.
Methods: Simple direct smear technique, modified formalin-ether concentration and the Mini Parasep SF kit were used to determine intestinal parasitic infections.
Results: A total of 226 fecal samples were used to compare the efficacy of the three methods. The simple direct smear technique showed the highest efficacy with a detection rate of 74.2%, followed by the modified formalin-ether concentration technique (M-FECT) (65.67%) and Mini Parasep SF kit (55.22%) with a statistical significance ($P < 0.05$). However, M-FECT showed three cases of *Opisthorchis viverrini* (*O. viverrini*) infections (100%), while Mini Parasep SF kit returned three cases of hookworm infection (100%). A total of 253 samples were submitted and reported prevalence. The mean intestinal parasitic infection in Chanthaburi Province was 29.6%. There was no significant difference in infections between genders and age groups ($P > 0.05$). The prevalence of protozoa was found to be 23.7%. The highest protozoa prevalence was *Blastocystis hominis* (9.1%). Three helminthic infections were *Strongyloides stercolaris* (6.3%), *O. viverrini* (1.2%) and hookworm egg (1.2%).
Conclusions: In the area of protozoa prevalence, simple direct smear is recommended as it's easy to process, convenient, safe and also economical. Mini Parasep® SF has proved to be good for hookworm but not *O. viverrini*. M-FECT is still needed for diagnosis of *O. viverrini* infection.

1. Introduction

A long term helminth control program has reduced the level of parasites from a high or moderate to moderate or low prevalence with light intensity[1-3] whilst protozoa has increased in prevalence[4,5]. A gold standard to diagnose parasites is lacking due to a variance in egg output per day. Single stool examination fails to detect parasites in up to 70% of cases, whilst repeat stool examination increases diagnostic sensitivity to 50% (3 times serial stool samples) and can reach 100% if 7 stool samples are examined[6-8]. However, it is impractical in a field survey as a false negative diagnosis is high when the intensity of infection is light[9]. Mini Parasep® SF faecal parasite concentrator has been routinely used in hospitals in Thailand since 2012, although the sensitivity or efficacy of this method is still contradictory (controversial). Zeeshan *et al.* found that Parasep filters enhanced the ability to detect intestinal parasites in stool; 100 samples were negative for parasites using direct microscopy whereas 13 of them were positive with Parasep[10]. Funk *et al.* reported that the Kato-Katz technique gave the best overall diagnostic performance of soil-transmitted helminths in India, with the highest results in all measures (prevalence, faecal egg count, sensitivity) followed by the conventional ethyl acetate and then the Midi Parasep(®) technique[11]. A lightly detailed study conducted in Thailand used Parasep® faecal parasite concentration where Suwanvattana reported the diagnostic yield for parasites, which was higher than that of simple smear, formalin-ethyl acetate concentration (10.5%, 8.0% and 4.0%, respectively) in HIV patients[12]. Recently, Mini Parasep® SF showed the highest sensitivity (56.38%) for detection

*Corresponding author: Sirima Kitvatanachai, Faculty of Medical Technology, Rangsit University, Pathumthani, 12000, Thailand.
Tel: +66229972222 ext 1517, 1437
Fax: 6629972222 ext 1451
E-mails: kitvatanachai@yahoo.com, sirima@rsu.ac.th

The current study was reviewed and approved by the Ethics Committee of Rangsit University (reference number: RSEC 19/2556). Informed consent was obtained from study participants.

The journal implements double-blind peer review practiced by specially invited international editorial board members.

of intestinal parasites in school-age children in Thailand, followed by direct smear (40.43%), and formalin-ethyl acetate concentration (37.23%)[13].

Mini Parasep® faecal parasite concentration method (DiaSys Ltd, Berkshire, England) was designed to replace the traditional formalin-ether concentration due to the toxicity of formalin and ether carrying significant health hazards[14-16] and being time consuming. The modification of the kit employed a 2 stage filtration matrix to remove the debris and fat in a closed, solvent free system without exposure to harmful reagents for laboratory personnel. The kit additionally takes only 6 min to process. However, the cost of this concentrator kit (USD 0.9/sample) is still 3 times higher than the conventional direct smear (USD 0.3/sample)[10]. Detection of parasites is usually easier in cases of moderate to heavy infection, because of large numbers of worms being involved. The concentration technique will be needed in light infections or in asymptomatic persons and will also be valuable in surveys due to a low number of eggs or larvae production. Gardeners are also at risk of parasitic infections, and are also a group of interest to focus on as they could be asymptomatic or assumed as having light infection. Chanthaburi Province is an area located in Eastern Thailand, famous for tropical fruit production in Thailand, with various kinds of gardens in the area and also no publication in the prevalence of parasitic infection in gardeners in this area.

Therefore, the goal of this study was to determine the efficacy of the Mini Parasep® SF kit against formalin-ether concentration and simple direct smear whilst also evaluate the cost and processing time in asymptomatic gardeners in Chanthaburi Province as a model of light infections. We also provided the prevalence of parasitic infections in the area to keep as baseline data for future research.

2. Materials and methods

2.1. Study site

This study was conducted in Chanthaburi Province in the east of Thailand, on the border with Battambang and Pailin of Cambodia and on the shore of the Gulf of Thailand. Neighbouring provinces are Trat in the east and Rayong, Chonburi, Chachoengsao, and Sa Kaeo to the west and north. The province is quite mountainous, with the Chanthaburi mountain range in the north having the highest elevation of the province[17]. Tropical fruits are among the main products of the province, such as durian, rambutan, longan and sugar apple *etc*. Three districts in the north part of Chanthaburi were included in this study: Soi Dao (n = 67), Kaenghangmaeo (n = 100) and Khao Khitchakut (n = 86). Occupational hazards associated with soil and water transmitted parasites might be a possible risk.

2.2. Study design

A cross-sectional survey was carried out during June 2014 with stool samples collected from gardeners. Socio-demographic characteristics of the gardeners, including age, sex, location, religion, education, income and risk factors for parasitic infections were recorded via questionnaires. No participant complained of any gastrointestinal symptoms such as abdominal pain or diarrhea. Gardeners aged more than 15 years old (both men and women) and supplying fecal samples were enrolled in this study. Only a sufficient amount of stool samples for diagnosis by 3 tests were used to compare the detection rate of 3 methods.

2.3. Stool samples and examination

Clean plastic containers were labeled with a code number and distributed to the villagers to obtain suitable and appropriate samples one day before collection. Instructions were given regarding how to take the sample. A single stool sample was obtained from each participant. Each stool sample was divided into 3 parts and examined as follows: simple direct smear[18], modified formalin-ether concentration technique (M-FECT) and Mini Parasep® SF (DiaSys Ltd, Berkshire, England).

The M-FECT was modified from Ritchie in 1948[19,20]. Preserved stool was filtered through two layers of wet gauze into a centrifuge tube. The volume was adjusted to 10 mL with 10% formalin, centrifuged at 2 500 r/min for 5 min until the supernatant was clear then the supernatant was discarded. Three milliliters of ether and 7 mL of 10% formalin were added to the sediment then centrifuged at 2 500 r/min for 5 min. The plug of debris along with ether and 10% formalin was discarded, leaving only the sediment to which 3 mL of 10% formalin was added as a preservative until diagnosis. The sediment was mixed before examination under microscope.

Briefly, the stool samples filled the spoon at the end of the filter. They were then mixed with 10% formalin and Triton X in the mixing tube by vortex. Mini Parasep® SF concentrator was centrifuged at 1 500 r/min for 2 min. The mixing chamber and filter were then unscrewed and the suspension was discarded. The sediments were microscopically examined by 2 independent parasitologists. The cost of 3 techniques was calculated and processing times were recorded.

2.4. Ethics and consent form

Informed consent was obtained from study participants. All infected gardeners were informed and reported to hospital in each district, where arrangements were made for the villagers to meet a doctor and give anti-helminth treatment; study participants were also given instructions about how to prevent and avoid helminth infections. The current study was reviewed and approved by the Ethics Committee of Rangsit University (reference number: RSEC 19/2556).

2.5. Data analysis

Descriptive statistics were used to to describe the prevalence. The percentage was used to report the prevalence, age, gender, distribution and socio-demographic characteristics of the gardeners. The detection rate (dividing the total number of cases of intestinal parasitic infections detected by the number of cases proving positive by each technique) was determined and reported in percentage to ascertain whether there were any differences between the techniques' effectiveness.

Chi-square test was performed to compare age groups, genders and areas with intestinal parasitic infections. P values < 0.05 were considered statistically significant. The efficacy was calculated for each of 3 methods, considering the combined results from the individual methods as a total positive. The efficacy of method (%) = (No. of positive in each method/Total No. of positive) × 100

3. Results

3.1. The prevalence of intestinal parasitic infections in Chanthaburi Province

A total of 253 individuals provided a stool to analyze by at least one of three methods. The mean prevalence of intestinal parasitic

infection was 29.6% from 3 districts in Chanthaburi (Table 1) with no statistical difference among the areas ($\chi^2 > 0.05$).

Table 1

Prevalence of intestinal parasitic infections in 3 districts of Chanthaburi Province.

Districts	*n*	No. of infection	% of infection
Soi Dao	67	25	37.3
Kaenghangmaeo	100	27	27.0
Khao Khitchakut	86	23	26.7
Total	253	75	29.6

$\chi^2 = 2.571$, $df = 2$, $P = 0.28$.

The prevalence rate of protozoan infections was found in 35 cases (13.8%). There were 4 species of non-pathogenic protozoa (9.9%) and 3 pathogenic protozoa (5.1%) found in Chanthaburi Province (Table 2). Six different species of protozoa were identified.

Table 2

Prevalence of protozoan infections in Chanthaburi Province ($n = 253$).

Protozoa		No. of infection	% of infection
Non-pathogenic protozoa	*Blastocystis hominis* < 5 cells/HPF	15	5.9
	Chilomastix mesnili	1	0.4
	Entamoeba coli	5	2.0
	Endolimax nana	4	1.6
	Total	25	9.9
Pathogenic protozoa	*Blastocystis hominis* > 5 cells/HPF	8	3.2
	Entamoeba histolytica-like	4	1.6
	Giardia lamblia	1	0.4
	Total	13	5.1
Mixed infection		22	8.7
Total		60	23.7

Mixed protozoan infection: 1–4 species of protozoa in 1 person. HPF: High power field (40×).

The prevalence rate of helminth infection was found in 21 cases (8.3%). Three different helminth species were detected in Chanthaburi Province: *Strongyloides stercolaris* (*S. stercolaris*) (6.3%), hookworm (1.2%), *Opisthorchis viverrini* (*O. viverrini*) (1.2%) and 7 out of 253 were of mixed infection between helminth and other protozoa (Table 3). The distribution of intestinal parasites was found in all age groups above 21 years old with no statistical difference ($\chi^2 > 0.05$) as shown in Figure 1 and also no significant difference between male (30.8%) and female (28.9%) ($\chi^2 > 0.05$).

Table 3

Prevalence of helminth infections in Chanthaburi Province ($n = 253$).

Helminths	No. of infection	% of infection
S. stercolaris	14	5.5
O. viverrini	1	0.4
Hookworm and protozoa	3	1.2
S. stercolaris and protozoa	2	0.8
O. viverrini and protozoa	2	0.8
Total	21	8.3

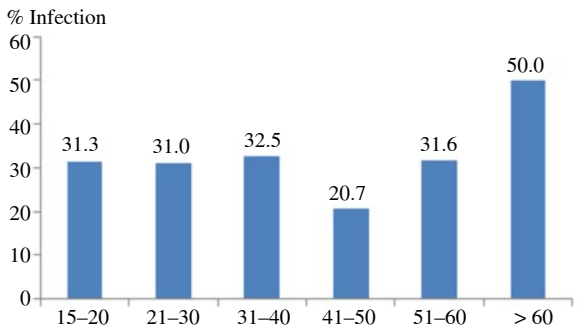

Figure 1. Intestinal parasitic infections by age groups in Chanthaburi. $\chi^2 = 8.35$, $df = 5$, $P = 0.213$.

3.2. The comparison of 3 diagnostic methods to detect intestinal parasitic infections

A total of 226 samples were individually examined by 3 methods to evaluate the efficacy of simple direct smear, M-FECT and Mini Parasep® SF. In Table 4, simple direct smear showed the highest efficacy in detection of intestinal parasites (74.62%), followed by M-FECT (65.67%) and Mini Parasep® SF (55.22%) with the same efficacy in detecting protozoa (66.67%, 50.00% and 44.44%) and *S. stercolaris* (71.43%, 57.14% and 35.71%), respectively. However, Mini Parasep® SF showed superior efficacy in detecting hookworm infection (100.00%) where simple direct smear was unable to detect any case. M-FECT was able to detect all cases of *O. viverrini* (100.00%) whilst simple direct smear was unable to detect any case.

Table 4

The efficacy of 3 methods to detect parasitic infections ($n = 226$).

Parasites	*n*	No. of positive (%)		
		Direct smear	M-FECT	Mini Parasep® SF
Blastocystis hominis (< 5 cells/HPF)	18	12 (66.67)	12 (66.67)	7 (38.89)
Chilomastix mesnili	1	0 (0.00)	1 (100.00)	0 (0.00)
Entamoeba coli	6	2 (33.33)	4 (66.67)	2 (33.33)
Endolimax nana	9	2 (22.22)	3 (33.33)	5 (55.56)
Blastocystis hominis (> 5 cells/HPF)	9	9 (100.00)	5 (55.56)	7 (77.78)
Entamoeba histolytica-like	8	8 (100.00)	1 (12.50)	2 (25.00)
Giardia lamblia	3	3 (100.00)	1 (33.33)	1 (33.33)
Single protozoa infection	54	36 (66.67)	27 (50.00)	24 (44.44)
Hookworm	3	0 (0.00)	1 (33.33)	3 (100.00)
S. stercolaris	14	10 (71.43)	8 (57.14)	5 (35.71)
O. viverrini	3	0 (0.00)	3 (100.00)	0 (0.00)
Total	67	50 (74.62)	44 (65.67)	37 (55.22)

Mixed infection was not counted in this table to compare the methods. HPF: High power field (40×).

Direct smear has a significantly greater efficacy in detecting intestinal parasites than M-FECT and Mini Parasep® SF ($P = 0.000$). Positive detection by M-FECT was higher than Mini Parasep® SF ($P = 0.000$) as shown in Tables 5–7.

Table 5

Comparison between direct smear and M-FECT.

		M-FECT		Total
		Negative	Positive	
Direct smear	Negative	165	11	176
	Positive	16	34	50
Total		181	45	226

$\chi^2 = 93.105$, $df = 1$, $P = 0.000$.

Table 6

Comparison between direct smear and Mini Parasep® SF.

		Mini Parasep® SF		Total
		Negative	Positive	
Direct smear	Negative	166	10	176
	Positive	22	28	50
Total		188	38	226

$\chi^2 = 70.485$, $df = 1$, $P = 0.000$.

Table 7

Comparison between M-FECT and Mini Parasep® SF,

		Mini Parasep® SF		Total
		Negative	Positive	
M-FECT	Negative	170	11	181
	Positive	18	27	45
Total		188	38	226

$\chi^2 = 74.920$, $df = 1$, $P = 0.000$.

3.3. Cost and processing time of 3 techniques

Cost per test was calculated in 3 techniques. The cheapest test was conventional direct smear technique (USD 0.1/sample) and M-FECT (USD 0.4/sample) cost less than Mini Parasep® SF (USD 1.4/sample). Modified formalin-ether was more time consuming (15 min in processing time) than Mini Parasep® SF (3 min).

4. Discussion

This study is the first to report the prevalence of intestinal parasitic infections in asymptomatic gardeners of Chanthaburi Province, using 3 combined techniques, which gave a higher prevalence than Nuchprayoon *et al.* who reported parasitic infections among Thai patients at the King Chulalongkorn Memorial Hospital, Bangkok to be 8.90% in the eastern part[21]. Using 3 techniques could yield the prevalence of 29.6% from 3 districts in Chanthaburi Province, which was higher than Sanprasert *et al.* who reported 14.56% in school-age children in the same province[13]. This may be because the gardeners are at higher risk from their carrier than the children. The reports of soil-transmitted helminths in Southern Thailand were found to be the highest in fishermen (72.8%), while in farmers and gardeners the infection rates were 33.8% and 31.9%, respectively[22].

Our study found protozoan infection at a higher distribution in the area than helminthic infections, in agreement with Sanprasert *et al.* who performed a study in the same province[13] and other provinces in Thailand, such as in Pathum Thani[23], Nakhon Pathom[24] and Nakhon Ratchasima[25]. *Blastocystis* spp. was the most common in this study (approximately 9%) and a half of protozoan infections were non-pathogenic. Three different helminth species were detected in Chanthaburi Province, unlike Sanprasert *et al.*, which did not report *S. stercolaris*[13]. In this study, *S. stercolaris* (6.3%) show more prevalence than hookworm and *O. viverrini* which was found at 1.2%. It may be that this study was carried out in gardeners who might come into contact with soil more than children. Not many species of helminth were found in the eastern part; however, in Northeastern Thailand recently, 6 species were reported in Khon Kaen Province[26].

There was no statistical difference in either age group or gender (χ^2 > 0.05). The Ministry of Public Health (2008) revealed that intestinal parasites were found in males in greater numbers than females and were prevalent in all age groups[2]. This area might be endemic for intestinal protozoa in that everyone is at risk of parasitic infections.

Microscopic examination of feces is a standard laboratory method for diagnosing intestinal parasite infections; however, laboratory techniques need to be standardized as different hospitals may adopt slightly different procedures for examining stools for ova and parasites. Formalin-ether concentration technique is commonly used in laboratory diagnosis and epidemiological studies due to its high efficacy in detecting parasites. However, formalin and ether carry significant health hazards for laboratory workers and also are environmentally detrimental and fiscally detrimental in terms of the impact of proper disposal. Mini Parasep® SF faecal concentrator allows collection and concentration within a single collection vial. This technique was designed to replace the conventional method and distributed routinely in laboratories in Thailand. There is a lack of reports here proving the efficacy or sensitivity of the kit and are still questions to be answered regarding its use instead of conventional concentration method. This evaluation provides more information for laboratories to consider if it is appropriate to use this method to detect intestinal parasites over concentration method. Highly accurate diagnostic tools are needed to accurately identify specific parasites before giving an appropriate treatment. In this study, the simple direct smear technique gave the highest efficacy in detecting intestinal parasites (74.62%), followed by M-FECT (65.67%) and Mini Parasep® SF kit (55.22%), which is similar to a previous study by Funk *et al.*, which reported that the Kato-Katz technique gave the best overall diagnostic followed by ethyl acetate and then the Midi Parasep®[11]. Saez *et al.* showed the Midi Parasep SF faecal parasite system recovered significantly fewer ova and cysts and resulted in a notably larger deposit than the one with ethyl acetate[27]. Maybe this area, protozoa (*Blastocystis* spp.), is the most common and therefore wet smear is more efficient since it requires fresh fecal samples which might be distinguished in morphology better than preserved specimens. *Blastocystis* spp. is the most sensitive to preservation in our experience. Many laboratories sample the unconcentrated stool to detect *Blastocystis hominis* due to concerns that concentration will change the morphology of parasite or lyse the fragile trophozoites[10]. In contrast to our study, Parasep® faecal parasite concentrator detected parasites and opportunistic protozoa in stool samples from HIV patients (10.5%), simple smear was 8% and formalin ethyl acetate was 4%. However, the kit showed a detection rate of 10.0%, similar to simple direct smear (10.5%) after being stained with modified Ziehl-Neelsen[12]. Sanprasert *et al.* showed Mini Parasep® SF is the most sensitive (56.38%) in the detection of intestinal parasites in school-age children, followed by direct smear (40.43%) and M-FECT (37.32%)[13]. The sensitivity of Mini-Parasep with ethyl acetate for diagnosing *Schistosoma mansoni* in Kenya was 77.5%, Kato-Katz was 56.1% and modified Mini-FLOTAC FS7 was 33.8%[28]. In our study, M-FECT showed 3 cases of *O. viverrini* infection (100%) while Mini Parasep® SF and direct smear could not report any, in accordance with Laoprom *et al.*, which showed M-FECT detection of *O. viverrini* was significantly higher than the kit[26]. This contrasts with Kaewpitoon *et al.*, where Mini Parasep® SF revealed superior detection of *O. viverrini* than direct wet smear[29]. A gold standard is lacking to diagnose helminths, including *O. viverrini* and the chance for a false negative result is high when the intensity of infection is light[9]. Only hookworm eggs were detected by Mini Parasep® SF kit (3 cases of infections or 100%). Of interest, the large pore size of filtration (425 μm) in Mini Parasep® SF, which allows the sediment to contain a contaminating substance, was equally problematic with a large piece of artifact contaminated in sediment causing misdiagnosis with small size parasites such as protozoa or *O. viverrini* eggs. It might benefit to use this method for a large size of ova or if the faster concentration speed would allow the accuracy enumeration. Concentration at a centrifugation speed of 400 × *g* produced a yield of parasites comparable to that achieved by centrifugation at 200 × *g* (recommended centrifugation speed) with no loss of parasite morphology[30].

In the area where protozoa is prevalent, simple wet smear is still needed for diagnosis. Mini Parasep® SF will be beneficial in detecting a large group of ova parasites since the kit is easy to use, taking less time to process and concentrating stool without the need of volatile solvents (ether or ethyl acetate), which reduces health hazard. Formalin-ether techniques showed advantage for *O. viverrini* and should be considered in the endemic area when the intensity of infection is light because this parasite is the carcinogenic liver fluke.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This study was partially supported by annual research fund of Rangsit University and Mini Parasep® SF kits were supplied by Bangkok Inter Product Ltd. We are grateful to Ms. Karaporn Seebursod, Ms. Nithinant wootthiphongkullayos, Ms. Panisara Charoenchon, Ms. Sasitorn Sittichotirat and Ms. Sirada Phonsod for their kind assistance in this project. Appreciation is also extended to Soi Dao, Kaenghangmaeo and Khao Khitchakut Districts health officers for specimen collections.

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