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Comparison between direct smear, formalin-ethyl acetate concentration, and Mini Parasep[®] Solvent-Free Concentrator for screening of intestinal parasitic infections among school-age children

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Comparison between direct smear, formalin-ethyl acetate concentration, and Mini Parasep[®] Solvent-Free Concentrator for screening of intestinal parasitic infections among school-age children. Chula Med J 2016 May – Jun;60(3): 255 - 69

Background : *Most patients infected with intestinal parasites are asymptomatic and usually undiagnosed. The sensitivity of stool examination by direct smear is quite low. Formalin-ethyl acetate concentration technique (FECT) is the method used to increase the sensitivity. However, FECT is considered to be a dangerous method because of the use of toxic solvent in the process. Mini Parasep[®] Solvent-Free (SF) Concentrator is a new diagnostic tool used as routine stool examination for parasitic infections in several countries worldwide.*

Objective : *To evaluate the efficacy of Mini Parasep[®] SF concentrator compared with the direct smear method and FECT for screening of intestinal parasitic infections in school-age children.*

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- Research design** : *Cross-sectional study.*
- Setting** : *Nakhon Pathom and Chanthaburi provinces, Thailand.*
- Participants** : *Students in Wat Don Yai-Hom School (N = 265), Wat Burapha Phitthaya Ram School (N = 32), Ban Patong School (N = 181), Ban Wang I-Aen School (N = 46), and Wat Nam Khun School (N = 50)*
- Methods** : *Stool samples (N = 574) were examined for intestinal parasites by simple smear, FECT, and Mini Parasep[®]SF concentrators.*
- Results** : *Mini Parasep[®]SF showed the highest sensitivity for overall detection of intestinal parasitic infections (56.38%), followed by direct smear method (40.43%), and FECT (37.23%). Using these three methods, the overall prevalence of intestinal parasitic infections among students was 16.38%. While the prevalence in Nakhon Pathom Province was 18.49%, the prevalence in Chanthaburi Province was 14.56%. The most common parasite found was Blastocystis hominis (8.01%), followed by Giardia intestinalis (2.61%) and Entamoeba histolytica (0.87%). Moreover, non-pathogenic protozoa, including Entamoeba coli (1.05%), Endolimax nana (5.57%), Iodamoeba butschlii (0.17%), were also detected. However, only 4 students (0.70%) in Chanthaburi Province were infected with helminth infections, including hookworm (0.35% prevalence) and Opisthorchis viverrini (0.35%).*
- Conclusion** : *As the advantages of Mini Parasep[®]SF which include high sensitivity, closed system, safety, and rapidness, this method is a useful diagnostic technique, especially for screening of intestinal parasitic infections.*
- Keywords** : *Intestinal parasites, diagnosis, direct smear, formalin-ethyl acetate concentration technique, Mini Parasep[®]SF concentrator.*

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วิวรรณ สรรประเสริฐ, สาทิธี จารุชัยบวร, อุทัยทิพย์ บุญเกษม, ลีริพร ศรีรุ่งเรือง, สุรางค์ นุชประยูร. การเปรียบเทียบเทคนิคการวินิจฉัยด้วยวิธีการตรวจอย่างง่าย วิธีการตรวจเข้มข้นโดย Formalin-ethyl acetate (FECT) และหลอดตรวจเข้มข้น Mini Parasep® Solvent-Free ในการสำรวจโรคปรสิตในลำไส้ในเด็กวัยเรียน. จุฬาลงกรณ์เวชสาร 2559 พ.ศ. - มิ.ย.; 60(3): 255 - 69

เหตุผลของการทำการวิจัย : ผู้ติดเชื้อปรสิตในลำไส้ส่วนมากไม่ปรากฏอาการ และมักไม่ได้รับการวินิจฉัย การตรวจอุจจาระโดยวิธีการตรวจอย่างง่ายมีความไวที่ต่ำมาก การตรวจโดยวิธีเข้มข้นโดย Formalin-ethyl acetate (FECT) เป็นวิธีที่ใช้เพื่อเพิ่มความไวในการวินิจฉัย อย่างไรก็ตาม วิธี FECT เป็นวิธีที่อันตรายเนื่องจากการใช้สารละลายอันตรายในขั้นตอนการตรวจวินิจฉัย หลอดตรวจเข้มข้น Mini Parasep® Solvent-Free (SF) เป็นเทคนิคใหม่ในการตรวจวินิจฉัยโรคปรสิตในลำไส้ซึ่งถูกใช้ในการตรวจอุจจาระในหลายประเทศทั่วโลก

วัตถุประสงค์ : เพื่อประเมินประสิทธิภาพของหลอดตรวจเข้มข้น Mini Parasep® SF เปรียบเทียบกับวิธีการตรวจอย่างง่ายและการตรวจโดยวิธีเข้มข้น FECT

รูปแบบการวิจัย : การศึกษาแบบตัดขวาง

สถานที่ทำการศึกษา : จังหวัดนครปฐมและจังหวัดจันทบุรี ประเทศไทย

ตัวอย่าง : เด็กนักเรียนโรงเรียนบ้านดอนยายหอม (จำนวน 265 คน) โรงเรียนวัดบูรพาพิทยาราม (จำนวน 32 คน) โรงเรียนบ้านปะตง (จำนวน 181 คน) โรงเรียนบ้านวังอีแอ่น (จำนวน 46 คน) และโรงเรียนวัดน้ำขุ่น (จำนวน 50 คน)

วิธีการศึกษา : ตรวจหาปรสิตในลำไส้ในตัวอย่างอุจจาระโดยวิธีการตรวจอย่างง่าย วิธี FECT และหลอดตรวจเข้มข้น Mini Parasep® SF ในตัวอย่างอุจจาระจำนวน 574 ตัวอย่าง

- ผลการศึกษา** : หลอดตรวจเข็มชั้น Mini Parasep[®] SF ให้ความไวในการตรวจหาปรสิตในลำไส้ได้ดีที่สุด (56.38%) ตามด้วยวิธีการตรวจอย่างง่าย (40.43%) และวิธีเข็มชั้น FECT (37.23%) จากการตรวจด้วยวิธีทั้งสามนี้ ความชุกของปรสิตในลำไส้โดยรวมในเด็กนักเรียนคือ 16.38% โดยความชุกในจังหวัดนครปฐมคือ 18.49% ในขณะที่ความชุกในจังหวัดจันทบุรีคือ 14.56% ปรสิตที่ตรวจพบมากที่สุดคือ *Blastocystis hominis* (8.01%) ตามด้วย *Giardia intestinalis* (2.61%) และ *Entamoeba histolytica* (0.87%) นอกจากนี้ยังตรวจพบโปรโตซัวไม่ก่อโรค ได้แก่ *Entamoeba coli* (1.05%), *Endolimax nana* (5.57%), *Iodamoeba butschlii* (0.17%) อย่างไรก็ตาม มีนักเรียนเพียง 4 คนในจังหวัดจันทบุรีตรวจพบการติดเชื้อหนอนพยาธิ ได้แก่ พยาธิปากขอ (0.35%) และพยาธิใบไม้ในตับ *Opisthorchis viverrini* (0.35%)
- สรุป** : จากข้อดีของหลอดตรวจเข็มชั้น Mini Parasep[®] SF ได้แก่ ความไวสูง ระบบปิด ปลอดภัยและรวดเร็ว วิธีนี้จึงเป็นวิธีการวินิจฉัยที่มีประโยชน์ โดยเฉพาะในการสำรวจโรคปรสิตในลำไส้
- คำสำคัญ** : ปรสิตในลำไส้, การวินิจฉัย, วิธีการตรวจอย่างง่าย, การตรวจโดยวิธีเข็มชั้นโดย Formalin-ethyl acetate (FECT), หลอดตรวจเข็มชั้น Mini Parasep[®] SF.

Intestinal parasitic infections, caused by intestinal helminths and protozoa, are common health problem worldwide, mainly in the tropical and sub-tropical countries. In addition, parasitic infections are related to health-system factors, biological, behavioral, environmental, and socioeconomic.⁽¹⁾ About 3.5 billion people worldwide are infected by intestinal parasites, and approximately 450 million develop clinical symptoms. The transmissions of these parasites include the fecal-oral route, food contamination, or by skin penetration. Therefore, the parasites can be easily transmitted, especially in children. Infections by these parasites result in malabsorption, malnutrition, and long-term effects on retardation of physical growth and impaired cognitive development in children.⁽¹⁾ The most common intestinal parasites that infect children are soil-transmitted helminths (STHs), and intestinal protozoan parasites, including *Giardia intestinalis*, *Entamoeba histolytica*, and *Blastocystishominis*.

The major problem in the control of intestinal parasitic infections is the difficulty of diagnosis. There are a lot of methods for diagnosis of intestinal parasitic infections, including parasitological methods by stool examination, molecular methods, and immunological methods. Molecular methods, such as polymerase chain reaction (PCR) and real time PCR, are the effective methods with high sensitivity and useful for differential diagnosis. However, they need expensive and complex equipment, as well as the technical expertise.⁽²⁾ Immunological methods for antigen and/or antibody detections are alternative methods for the diagnosis of parasitic infections. But the limitations of these methods are the cross-reactivity to other parasites, and inability to distinguish between current and past

infections.^(3, 4)

Currently, the routine laboratory diagnostic methods for parasitic infections (detection of eggs, larvae or cysts) are direct smear and formalin-ethyl acetate concentration technique (FECT). These techniques are simple, rapid, and inexpensive. However, using direct smear method alone is insufficient to diagnose the diseases, particularly in cases that have low infections because of the intermittent releases of diagnostic stages of parasites into stool samples and using very small amount of stool samples.⁽⁵⁾ FECT is therefore a very beneficial method. It has been used to increase the sensitivity of stool examination. However, FECT is too complicated to prepare its materials, reagents, and special instruments. Moreover, trophozoites or cysts of some protozoa could be destroyed or distorted during the process of FECT. Therefore, these parasites are usually missed out or difficult to be identified by FECT. Moreover, this method is considered to be dangerous for the technician because of the use of hazardous reagents. Formalin is used in FECT as the fixative agent. However, formalin is irritant to the eye, skin, and respiratory tract. It is also suspected as carcinogen, and may cause sensitization by inhalation or skin contact. Ether or ethyl acetate used in FECT method is an extractor of fat from stool samples, float the debris, and leave parasites to sediment into the tube bottom. However, ether is an explosive agent and has potential toxicity. Ethyl acetate, a less flammable agent, has been used in FECT instead of ether with the same efficacy.⁽⁶⁾ However, ethyl acetate is also an irritant to the mucous membrane that causes the eye and upper respiratory tract irritation in the laboratory personnels.

Mini Parasep[®] Solvent-Free Fecal Parasite Concentrator (DiaSys Ltd, Berkshire, England) is a new diagnostic tool for parasitic infections.⁽⁷⁾ It is a useful method for concentration of eggs, larvae and cysts of parasites without exposure of harm reagents to laboratory personnel. The concentrator of Mini Parasep[®] SF (Figure 1) is composed of patented 'filter matrix' to reject large particles in stool samples, the large particles will be trapped in 'debris trap' to prevent extrusion into the 'sedimentation cone' during centrifugation. Then, fat contents in stool samples will be separated and small debris will be

also removed in the 'fat dispersion chamber'. Therefore, the sedimentation of parasites could be performed without the use of ether or ethyl acetate in Mini Parasep[®] SF. Hence forth, we evaluated the efficacy of three diagnostic methods including direct smear, FECT, and Mini Parasep[®] SF for detections of intestinal parasite infections among school-age children. The considered criteria include recovery of the parasites, ease of handling, and health and safety aspects. Moreover, the prevalence of intestinal parasitic infections among the students was also observed.

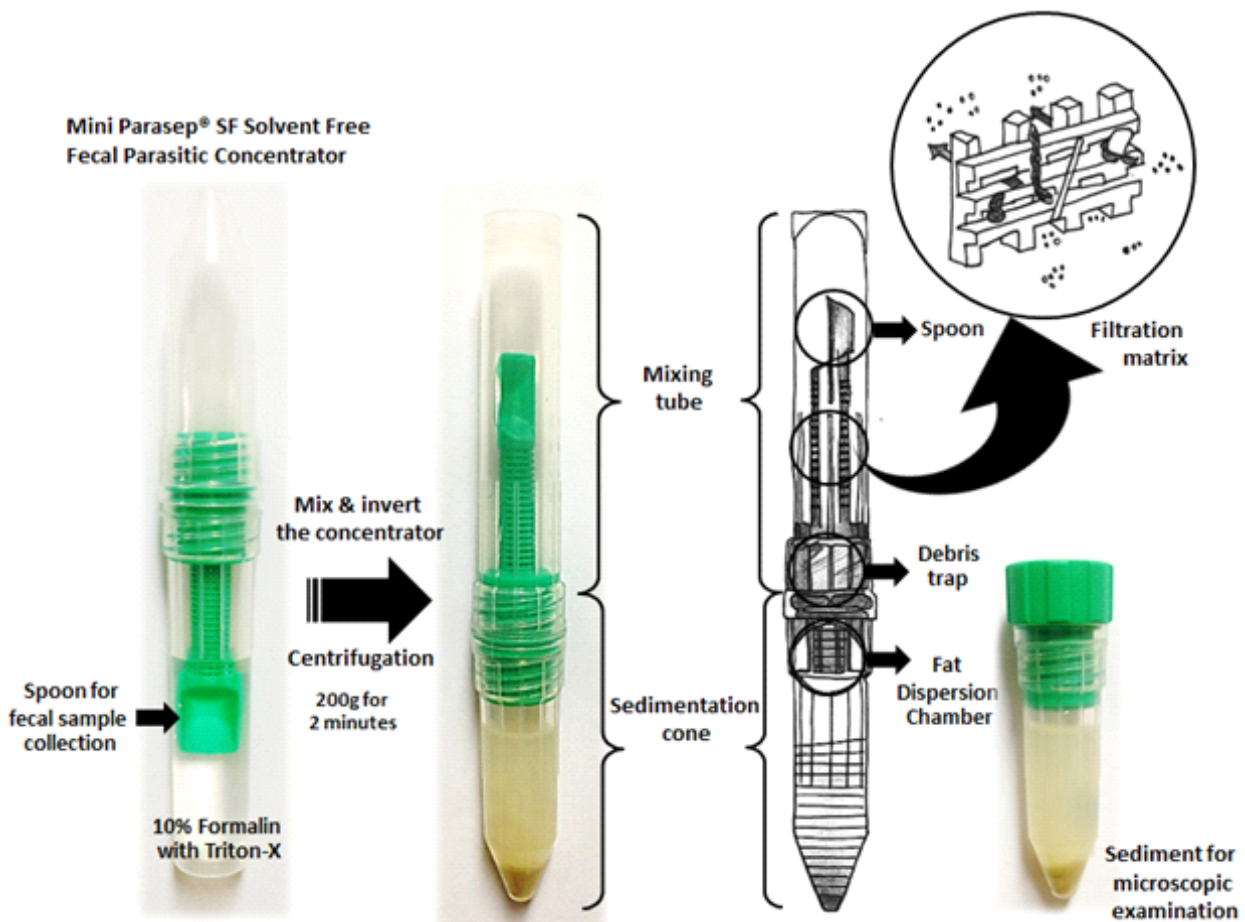


Figure 1. Diagram of Mini Parasep[®] Solvent-Free (SF) Concentrator.

Methods

Study areas and population

The cross-sectional survey study was conducted in 5 schools in the provinces of Nakhon Pathom (N = 265) and Chanthaburi (N = 309), Thailand between February - April, 2015 (Figure 2). The importance of parasitic diseases, modes of transmission, prevention methods, as well as instructions for collecting and handling of stool samples were explained to all students. Each student was provided with a clean plastic container, marked with their name and identification number two days prior to the sample collection. The participants were classified according to their classes. Socio-demographic characteristics of the students, including age, sex, parental education, parental occupation, and personal hygiene behaviors were recorded via questionnaires.

Stool examinations

Stool examinations were performed at the Department of Parasitology, Faculty of Medicine, Chulalongkorn University. To evaluate and compare the efficacy of the three different diagnostic methods, i.e., direct smear, FECT, and Mini Parasep® SF, stool samples were taken from participating students and divided into two parts. First, stool samples were examined microscopically by direct smear. The remaining samples were examined by FECT as previously described.⁽⁶⁾ Briefly, about two grams of stool samples were mixed in normal saline (0.85% NaCl). The suspension was filtered through two layers of gauze into 15 milliliters centrifuge tube. Three milliliters of ethyl acetate was added into the tube. The volume was adjusted to 10 milliliters with 7 milliliters of 10% formalin and then was centrifuged at 700 xg for 5 minutes. The supernatant were discarded

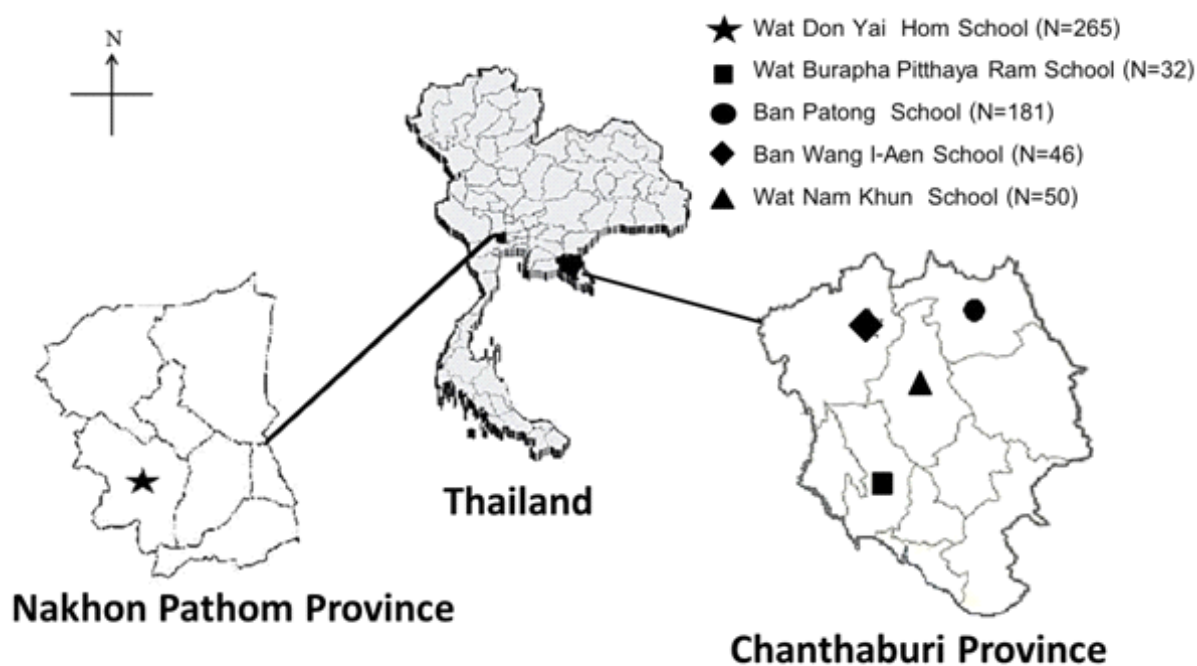


Figure 2. The study areas in Nakhon Pathom and Chanthaburi Province, Thailand

and the sediment was examined under a microscope for the presence of eggs, larvae, or cysts of intestinal parasites. Second part of the stool samples were examined by Mini Parasep[®] SF. Briefly, the stool samples were introduced to the concentrator using a spoon on the end of the filter. They were then mixed with prefilled 10% formalin and Triton-X in the mixing tube by vortex. Then, Mini Parasep[®] SF concentrator were centrifuged at 200 xg for 2 minutes. The suspensions in the sedimentation cone were discarded and the sediments were microscopically examined. All samples were independently examined by two individuals. Participants who showed positive for any intestinal parasites were treated by standard treatments.

Data analysis

The recorded data were analyzed using the Graph Pad Prism version 5 for Windows. Sensitivity of each test was then determined. Numerical data were summarized as mean \pm SD. Differences of data were analyzed by chi-square test for trend. *P* values < 0.05 were considered statistically significant.

Results

Study population and socio-demographic characteristics

In total, 574 students returned stool samples which were recruited into this study. Of these 574 students, 279 were male (48.6%) and 295 were female (51.4%). The age of the participants ranged from 4 to 15 years old. The majority of students were primary school students (5 - 10 years old). Socio-demographic characteristics of the students were summarized in Table 1. No statistically significant difference between the socio-demographic

characteristics was found among the students from both provinces (*P* > 0.05).

Prevalence of intestinal parasitic infections

Using the 3 methods to detect parasites in stool samples, 94 students were found infected with at least one intestinal parasite (Table 2). The overall prevalence of intestinal parasitic infections was 16.38% (94 of 574). No statistically significant difference between the prevalence among males (18.28%; 51 of 279) and females (14.58%; 43 of 295) was observed (*P* > 0.05) (data not shown). While the prevalence in Nakhon Pathom Province was 18.49% (49 of 265), the prevalence in Chanthaburi Province was 14.56% (45 of 309). The highest prevalence of intestinal parasites was found in students of Wat Nam Khun School, Chanthaburi Province (28%) with statistical significance (*P* = 0.02) (Table 2).

Most of the infected students were infected with intestinal protozoa (95.74% of infected students) (Table 2). The most common parasite found among the students was *B. hominis* (48.9% of infected students) (Table 2). However, students in Nakhon Pathom Province were infected with *B. hominis* with the significantly higher prevalence than those in Chanthaburi Province (12% prevalence in Nakhon Pathom Province and 4.5% prevalence in Chanthaburi Province) (*P* = 0.001). *G. intestinalis* (2.61% prevalence) and *E. histolytica* (0.87% prevalence), pathogenic protozoa, were also found in students from both provinces with no significant difference (Table 2). Moreover, non-pathogenic protozoa, including *Entamoeba coli* (1.05% prevalence), *Endolimax nana* (5.57% prevalence), *Iodamoeba butschlii* (0.17% prevalence), were also detected.

Table 1. Socio-demographic characteristics of children at school from 5 schools in Nakhon Pathom Province and Chanthaburi Province in February-April, 2015

Characteristics	Nakhon Pathom (N = 265)	Chanthaburi (N = 309)	Total (N = 574)
Sex			
- Male	127	152	279
- Female	138	157	295
Class (Age)			
- Kindergarten (<5yr)	59	62	121
- Primary school (5 - 10ry)	191	238	429
- Junior high school (11 - 15yr)	15	9	24
Parent's education			
- Primary school and lower	45.8%	57.1%	51.5%
- Secondary school and diploma	49.4%	38.8%	44.1%
- Bachelor degree and over	4.8%	4.1%	4.4%
Parent's occupation			
- Agriculturist	9.0%	19.3%	14.2%
- Labor	57.5%	58.9%	58.2%
- Merchant	12.1%	12.1%	12.1%
- Officer	17.2%	4.6%	10.9%
- Others	4.2%	5.1%	4.6%
Drinking water consumption			
- Rain drinking	42.9%	12.5%	27.7%
- Tap water	32.7%	35.3%	34.0%
- Natural sources	0.5%	19.9%	10.2%
- Others	23.9%	32.3%	28.1%
Hand washing before meals			
- Usually	38.4%	38.0%	38.2%
- Sometimes	61.6%	62.0%	61.8%
Shoe wearing when going to outside			
- Usually	61.9%	81.5%	71.7%
- Sometimes	38.1%	18.5%	28.3%
Half-cooked food consumption			
- Usually	2.9%	3.1%	3.0%
- Sometimes	30.9%	43.8%	37.4%
- Never	66.2%	53.1%	59.6%

Table 2. Prevalence of intestinal parasitic infections among children classified by schools.

Province	No. of examined	No. of positive (%)	Identified parasites (% prevalence)							
			HW	Ov	Bh	Gi	Eh	En	Ec	Ib
Nakhon Pathom										
- Wat Don Yai Hom School	265	49 (18.49)	0	0	32	6	2	17	0	1
Chanthaburi	309	45 (14.56)								
- Wat Burapha Pitthaya Ram School	32	4 (12.50)	1	1	0	1	0	1	0	0
- Ban Patong School	181	18 (9.94)	0	0	5	5	1	5	2	0
- Ban Wang I-Aen School	46	9 (19.57)	0	0	4	0	0	4	1	0
- Wat Nam Khun School	50	14* (28.0)	1	1	5	3	2	5	3	0
Total	574	94 (16.38)	2 (0.35)	2 (0.35)	46 (8.01)	15 (2.61)	5 (0.87)	32 (5.57)	6 (1.05)	1 (0.17)

HW = Hookworms, Ov = *Opisthorchis viverrini*, Bh = *Blastocystis hominis*, Gi = *Giardia intestinalis*, Eh = *Entamoeba histolytica/dispar*, En = *Endolimax nana*, Ec = *Entamoeba coli*, Ib = *Iodamoeba butschlii*

* significantly higher than other schools ($P < 0.05$)

Helminth infections, including hookworm (0.35% prevalence) and *Opisthorchis viverrini* (0.35% prevalence), were found only in 4 students in Chanthaburi Province. No helminth infection was found among students in Nakhon Pathom Province. Mixed infections were also found only among intestinal protozoan infections, and not among the helminth infections. Mixed infections found were *B. hominis* with *E. nana*, *B. hominis* with *G. intestinalis*, *B. hominis* with *E. histolytica/dispar*, *B. hominis* with *E. coli*, *E. nana* with *E. coli*, *E. nana* with *E. histolytica/dispar*, and *E. nana* with *G. intestinalis*. (data not shown).

Comparative study of diagnostic methods for screening of intestinal parasitic infections

A comparison between the direct smear, FECT, and Mini Parasep[®] SF showed a significant difference in the number of parasites recovered. The Mini Parasep[®] SF recovered considerably more protozoa than direct smear and FECT did ($P < 0.05$). Out of 94 infected students, only 38 cases were identified by direct smear technique, while FECT could detect 36 cases (Table 3). No significant difference was found between the sensitivity of direct smear (40.43%) and FECT (37.23%) for diagnosis of intestinal parasites. Moreover, our results showed that

the Mini Parasep®SF had the highest sensitivity (56.38%) for diagnosis of intestinal parasitic infections. The Mini Parasep®SF could detect significantly higher infected cases (53 cases) than that direct smear and FECT did ($P<0.05$). However, this method could not detect eggs of hookworm, and *O. viverrini* (Table 3).

Discussion

Since the intestinal parasitic infections are still causing a morbidity and mortality around the world. To prevent the transmission and to treat the parasites, the high accurate diagnostic tools are needed to ensure the species of parasites and give the suitable treatment. In the present study, we assessed the prevalence of intestinal parasitic infections and compared the efficacy of three different diagnostic methods among in school students from 5 schools in the provinces of Nakhon Pathom and Chanthaburi, Thailand. Our results confirmed the data from

several studies that report the high prevalence of intestinal parasitic infection among the school-age children.^(9 - 11) Our study showed the higher prevalence than the parasitic prevalence in adults in Thailand.^(12 - 16) Although we reported the higher prevalence than school-age children in other studies,^(10, 11) we found the lower prevalence than the prevalence among children in orphanage in Pathum Thani Province (81.1%).⁽¹⁷⁾ This may be due to the overcrowded environment and poor sanitation in the orphanage.

In this study, we found the protozoan infections with the higher prevalence than helminths infections, consistent with previous study in other province in Thailand, such as in Pathum Thani,^(10, 17) Nakhon Pathom⁽¹⁸⁾ and Nakhon Ratchasima Province.⁽¹⁹⁾ The parasite commonly found among the students in both provinces was *B. hominis*. Other protozoa found among these students were *E. nana*,

Table 3. Sensitivity of each method for diagnosis of intestinal parasitic infections

Parasites	Total	No. of positive (%sensitivity)		
		Direct smear	FECT	Mini Parasep® SF
Protozoa				
<i>B. hominis</i>	46	14 (30.43)	13 (28.26)	34 (73.91)
<i>G. intestinalis</i>	15	6 (40.00)	4 (26.67)	5 (33.33)
<i>E. histolytica/dispar</i>	5	2 (40.0)	2 (40.00)	2 (40.0)
<i>E. nana</i>	32	12 (37.50)	11 (34.37)	10 (31.25)
<i>E. coli</i>	6	2 (33.33)	2 (33.33)	2 (33.33)
<i>I. butschlii</i>	1	1 (100.00)	0 (0.00)	0 (0.00)
Helminthes				
Hookworms	2	0 (0.00)	2 (100.00)	0 (0.00)
<i>O. viverrini</i>	2	1 (50.00)	1 (50.00)	0 (0.00)
Total	94	38 (40.43)	35 (37.23)	53 (56.38)*

* significantly higher than other methods ($P<0.05$)

G. intestinalis, *E. histolytica*, *E. coli*, and *I. butschlii*. These protozoan infections among the students imply the poor sanitation in students in rural areas. Only one species of nematode (hookworm) and trematode (*O. viverrini*) were found in these regions, while in other regions nematode could be identified with high prevalence.^(5, 20, 21) We hypothesized that different environment sanitation, climatic conditions, physical properties of soil, education and economic status could affect the biology of the parasites. In addition, mixed infections were found only among intestinal protozoan infections, not among the helminth infections. The mixed infection patterns in our study were similar to results from a previous study.⁽¹¹⁾

The Mini Parasep[®] SF is reported as a useful and good efficient technique.^(7, 22, 23) In this study, we compared the efficacy of different diagnostic methods for screening of intestinal parasitic infections, including the Mini Parasep[®] SF along the FECT and direct smear. Our results showed that assessment of the efficacy of routine techniques did not show a significant overall difference in parasite recovery between direct smear and FECT ($P > 0.05$). These results were different from other studies that reported the higher sensitivity of FECT than direct smear for diagnosis of intestinal parasitic infections.^(5, 17) Similar to a previous study,⁽⁷⁾ we found that Mini Parasep[®] SF was the most efficient diagnostic tool for detection of intestinal parasites (56.38% sensitivity). There was a statistically significant different from two other methods, FECT (37.23% sensitivity) and direct smear (40.43% sensitivity) ($P < 0.05$). Although some previous studies showed that FECT was more efficient

than Mini Parasep[®] SF, there was no statistically significant.^(22, 23) Even though Mini Parasep[®] SF showed the highest sensitivity, this method could not detect eggs of hookworm and *O. viverrini* and some species of protozoan that could be detected by FECT or direct smear (Table 3). These results were similar to a previous study that reported failure of Mini Parasep[®] SF to detect eggs of hookworm, *Taenia* sp., and *Trichuris trichiura*.⁽²⁵⁾

The dual-filters in Mini Parasep[®] SF are claimed to enhance the clarity of sediment and reduce human error during the process of FECT. However, from our study, we found the larger size and denser of fecal debris concentrated in Mini Parasep[®] SF than that concentrated in FECT. Moreover, the morphology of the parasites found in Mini Parasep[®] SF was distorted. Therefore, it was more difficult to identify the parasites in the sediment in Mini Parasep[®] SF. In contrast, the smaller size and clearer of sediment was found in FECT. This may be due to the higher efficacy on the removal more fecal debris by ethyl acetate in FECT. This finding was similar to the previous study that compared the size of the final sediment from the Parasep[®] SF with Parasep[®] using ethyl acetate.⁽²⁵⁾ They reported the smaller size and less dense of the sediment from Parasep[®] using ethyl acetate.⁽²⁵⁾ Although the larger size of sediment and the lower sample size used in the Mini Parasep[®] SF, this method still the most sensitivity method. Moreover, Mini Parasep[®] SF tube is closed concentration system with the disposable device. Therefore, the advantages of the Mini Parasep[®] SF are solvent-free method that reduces the risk of hazardous solvent exposure. The closed system of the method can also eliminate cross-contamination

between samples. However, this system still requires adding formalin into the tube. Even the concentrator has a seal lock, we still need to perform the procedure in a safety cabinet.

In conclusion, because of the high sensitivity and safety benefit of Mini Parasep® SF, we suggested that the Mini Parasep® SF is a useful technique for stool examination of intestinal parasitic infections, especially for screening of intestinal parasitic infections that have to examine a high numbers of stool samples in limited time.

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