



Available online at www.ptat.thaigov.net

House Flies: Potential Transmitters of Soil-Transmitted-Helminth Infections in an Unsanitary Community

**Wanna Maipanich¹, Surapol Sa-nguankiat¹, Somchit Pubampen¹,
Teera Kusolsuk¹, Wichtit Rojekittikhun¹, Francesco Castelli²**

¹Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Thailand;

²University of Brescia Italy, Italy

Abstract

This study aimed to determine an appropriate technique for isolating helminthic objects from the exteriors of the bodies of flies, and to investigate helminth transmission rates among flies in an unsanitary community. The study area was Ban Nam Khem Village, Takua Pa District, Phang-nga Province, Thailand. In 2006, the prevalence of soil-transmitted-helminth (STH) infections in the community was 34.9%. Soil contamination in the swamp areas, where human feces were observed, ranged between 41.2-100% in the period February 2005-May 2006. Flies were abundant in defecation areas and around houses.

One year after treatment and health education, the prevalence decreased to 22.5%. While the infection rate among the schoolchildren decreased, the rate among the villagers increased to 50.0%. In June 2007, the soil contamination rate was 13.3%. The 567 houseflies in the study were all *Chrysomya megacephala*. Hookworm and *Trichuris trichiura* eggs on the body surfaces of the flies were isolated using an ultrasonic cleaner. The helminth transmission rate for flies in the defecation area was 25.9%, and in the household surroundings 11.8%. The average number of eggs on the body surfaces of flies in the defecation area was 0.4. After feeding on human excreta, 508 resting flies left 0.5 g of feces with pathogens in the surroundings. Anthelminthic treatment and health education were repeated to improve the helminth infection situation in the community.

Manual shaking and ultrasonic-cleaner techniques provided equal detection rates (80%), but ultrasonic cleaning retrieved more eggs.

Keywords: houseflies, helminth transmission rate, Phang-nga Province

Introduction

Soil transmitted helminths (STH) are relatively common parasites in the slum and rural areas of many countries [1-9], the high prevalence of which

is closely related to poverty, poor environmental hygiene, and impoverished health services [10]. The main source of transmission is defecation outside latrines by heavily infected persons [11]. While contaminated water, carriers, and food handlers might be the major transmission mode, indirect transmission by non-biting flies cannot be excluded [12,13]. Many authors have indicated

Correspondence:

Wanna Maipanich,
E-mail: <tmwmp@mahidol.ac.th>

that primary-school children are an ideal target group for STH [14], as they frequently defecate indiscriminately around their houses, particularly in the courtyards, sitting rooms, drains and bushes, even where every household has a latrine [15,16]. Furthermore, some adults habitually excrete in the woods/groves not far from their houses [15,17,18]. In unsanitary communities, garbage, dead animal carcasses, and piles of feces, are often scattered around the houses. Flies are commonly found both indoors and outdoors. They persist on excrement, dead animal bodies, and contaminated areas where fecal matter, large amounts of organic waste, and piles of garbage are left exposed and unattended [19]. Theoretically, flies can transmit helminths through mechanical or biological means [20]. When infected persons excrete in open areas, there is an increased risk of contact between flies and pathogen-positive fecal matter [13]. Several studies have shown that eggs of *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm, *Enterobius vermicularis*, *Capillaria hepatica*, *Taenia* sp, *Hymenolepis nana*, *Toxocara canis*, hookworm larvae, and *Strongyloides stercoralis* larvae, are carried by many species of houseflies [13,19,21-24].

The aims of this study were to determine a practical method for isolating helminth objects from the exterior body surfaces of flies, and to investigate helminth transmission rates among flies in a contaminated area.

The study was carried out in an unsanitary community where helminth infections and soil contamination with helminth ova were high, and where flies were abundant in defecation areas and household environs.

The Ethics Committee of the Faculty of Tropical Medicine provided ethical clearance for this study protocol: Approval Number: MUTM 2006-054.

Materials and methods

Study area

The study area was Ban Nam Khem Village, a fishing village in Takua Pa District, Phang-nga Province. This area was chosen because, when

we conducted a study of helminth infections in a tsunami-affected area, many piles of fecal matter were observed in the nearby mangrove swamp. That study [18] found that the prevalence of STH among the population was quite high (34.9%), while the soil in the mangrove swamp was highly positive (41.2-100%) for *A. lumbricoides* and *T. trichiura* eggs. We found *A. lumbricoides* and *T. trichiura* eggs in every fecal sample from the swamp. Numerous flies were feeding on the excrement and many were resting on plants (sea-holly) near the excreta. Near the swamp was an area for making dried cuttlefish; during the process, a lot of flies fed and rested on the cuttlefish. A community of poorly constructed domiciles resided near the mangrove swamp, crowded together in an unhygienic environment, with dirty house floors, scattered garbage, and damp soil around the houses. Flies were seen everywhere in the village—on food during meals, around children eating confectionery, sleeping children, dropped food, baby toys, and garbage. The abundance of flies around the household was attributable to the stock of rotten fish, a byproduct of the cuttlefish harvest.

Prevalence of STH infection

Stool samples were collected from schoolchildren and villagers in the community, and examined by Katz's modified thick-smear technique [25]. Stool samples from the swamp area were also examined to assess any relationship between contamination in human hosts, soil, and vectors.

Soil examination

Surface soil samples were collected in individual plastic bags from various sites in the mangrove swamp and examined by sugar flotation method [26].

Fly collection

Flies were collected from the mangrove swamp and the nearby community, since many flies were seen on every pile of fecal matter in the swamp. The best method for collecting them was

by stool-bait trap. A stool sample with a mass of flies on its surface was chosen as the bait; this was placed in a flytrap left in the area and left for 1 hour. To attract the attention of the flies, other piles of fecal matter were covered with sand, soil, leaves or wood during fly collection. Due to the strong sunlight and high temperatures during the daytime in summer (June), most flies became somnolent and were easily collected in the collecting chamber and transferred to collecting tubes.

In the community, rotten fish was used to lure and trap the flies.

Fly examination

The flies were divided into 3 groups, as follows: flies from the mangrove swamp represented a homogeneous contamination of the fly population, since they were exposed to the same sources of infection.

Group I: comparison of detection rates for helminth ova on the body surfaces using 2 techniques—manual shaking and ultrasonic cleaner. Ten flies were pooled and stored in a test tube with formalin detergent solution (FD solution) [27]; 200 flies were processed per test.

Group II: study of transmission rates of flies in the mangrove swamp; 108 flies were kept in individual microcentrifuge tubes with FD solution.

Group III: investigation of transmission rates of flies in the community (120 meters from the mangrove swamp); 68 flies were trapped. Each was kept in a microcentrifuge tube with FD solution.

All specimens were brought to the laboratory for processing.

Laboratory processing

The collected flies in group I were divided into 2 sets:

Set 1: washing by manual shaking

Each test tube containing 10 flies in FD solution was shaken for 1 minute. Detergent frees helminth objects from the body surfaces of flies, as it does fecal debris. The flies were removed by clean forceps and kept for identification. The

remaining preparation was centrifuged at 2,000g for 2 minutes. All sediments were examined under a light microscope for helminthic objects.

Set 2: ultrasonic cleaner technique

The tested samples were placed in an ultrasound (Elna Transsonic Digital) at power No. 5 for 15 minutes, temperature off, and the flies removed for identification. The supernatant was centrifuged at 2,000g for 2 minutes and the sediment examined for helminthic objects.

In groups II and III, the ultrasonic cleaner was the method of choice, since our trials showed that the ultrasonic-cleaner washing process recovered more helminth eggs than the manual shaking technique.

The fecal matter observed on the inner surfaces of the collection chambers was washed with FD solution and collected for examination. The delicate sediment after sedimentation was checked for helminth objects.

Results

Prevalence of STH in the population

In June 2007, the prevalence of STH infection in the population residing near the mangrove swamp was 22.5%. *Trichuris* gave the highest infection rate of 14.8%, while *Ascaris* and hookworm infections were 9.2 and 4.9%, respectively. All hookworm- and *Trichuris*-infected cases were rated as light, but 30.8% (4/13 cases) of ascariasis were moderate. The present study showed the result for the tsunami project at 1 year post-treatment. The prevalence among schoolchildren decreased from 27.8 to 14.5%, in contrast with the increasing prevalence among the villagers, from 41.3 to 50.0% (Table 1).

Soil contamination

Trichuris and hookworm eggs were detected from 4 soil samples in the mangrove swamp area, with a detection rate of 13.3%. *Trichuris* eggs from 3 samples were in multicellular (20%) and embryonated (80%) stages. One multicellular-stage hookworm egg was found in a positive sample (Table 2). The average number of eggs per gram of soil was 2.75.

Contamination of the environment with human excreta can be determined by detecting helminth eggs in soil samples. In our study, piles of fecal matter were consistently observed in the swamp. If the feces contained helminth eggs, there was no doubt of soil contamination. We could estimate the number of persons defecating in this area by the kinds of parasites and the degree of infestation of the collected fecal samples. Examinations indicated that, in the recent past, at least 4 persons had defecated in this area. After treatment and health education in the community, only one person still excreted there. The stool samples collected from the swamp contained *Trichuris* and hookworm eggs, and the number of eggs indicated light infections. It was possible that they were passed from the same host. The average numbers of eggs per gram of feces were 808 for *Trichuris* and 552 for hookworm. This was supported by the decreasing contamination rates for soil samples in June 2007 (Table 2).

Flies with helminthic objects on the body surface

A total of 576 houseflies (195 males and 381 females) were studied; all were identified as *Chrysomya megacephala*.

Trials of the efficiency of the two washing techniques showed more helminth eggs were detected by ultrasonic cleaner (Table 3); however, the detection rates did not differ.

To detect the transmission rates in the flies, 108 flies from the swamp area and 68 houseflies in the community were studied by ultrasonic cleaner technique. The results are shown in Table 4. Among 28 flies from the swamp area, 17 hookworm eggs, 17 *Trichuris*, and 1 *Ascaris* were detected (25.9%). The average egg count per positive fly was 1.3. Most flies carried only one egg on the body surface, while 17.9 and 7.1% had 2 and 3 eggs, respectively. In the community, 8 houseflies (11.8%) were contaminated with hookworm and *Trichuris* eggs. The average egg

Table 1 Soil-transmitted-helminth infection rates in Ban Nam Khem Village, Takua Pa District, Phang-nga Province.

	No. of examinations			No. positive (%)		
	Schoolchildren	Villagers	Total	Schoolchildren	Villagers	Total
May 2006*	133	150	283	37 (27.8)	62 (41.3)	99 (34.9)
June 2007	110	32	142	16 (14.5)	16 (50.0)	32 (22.5)

* All infected cases and schoolchildren were treated

Table 2 Soil-contamination rates in mangrove swamp near Ban Nam Khem Village, Takua Pa District, Phang-nga Province (February 2005 - June 2007).

	No. of samples	+ve sample (%)	Helminth eggs
February 2005	17	7 (41.2)	<i>A.l</i> and <i>T.t</i>
January 2006	7	7 (100.0)	<i>A.l</i> and <i>T.t</i>
May 2006	20	12 (60.0)	<i>A.l</i> and <i>T.t</i>
June 2007	30	4 (13.3)	Hw and <i>T.t</i>

A.l = *Ascaris lumbricoides*, *T.t* = *Trichuris trichiura*, Hw = hookworm

count per positive fly was 1.0.

Fecal dots attached to the inner surfaces of the collection chamber were washed with FD solution and examined for helminth objects. This contamination occurred when the flies were trapped in the chamber. In the laboratory, the

washing was processed by sedimentation method. 0.5 ml of sediment was obtained, containing 27 *Trichuris* and 27 hookworm eggs.

The total number of eggs carried by the 508 flies in the mangrove swamp was derived from the pooled eggs from the sediment, eggs in the two

Table 3 Number of eggs detected from houseflies (*C. megacephala*) by manual shaking and ultrasonic cleaner techniques.

Technique	No. of samples (no. of flies)	+ve sample (detection rate)	No. of helminth eggs			
			Hw	T.t	A.I	Total
Manual shaking	20 (200)	16 (80.0)	38	17	0	55
Ultrasonic cleaner	20 (200)	16 (80.0)	48	17	1	66

Hw = hookworm, T.t = *Trichuris trichiura*, A.I = *Ascaris lumbricoides*

Table 4 Transmission rate of flies in the study areas at Ban Nam Khem Village, June 2007.

Area	No. exam	+ve flies (%)	Helminth eggs & larvae		
			Hw	T.t	A.I
Mangrove swamp	108	28 (25.9)	17	17	1
Community	68	8 (11.8)	5	3	0

Hw = hookworm, T.t = *Trichuris trichiura*, A.I = *Ascaris lumbricoides*

Table 5 Number of STH eggs on the body surfaces of flies in the swamp area of Ban Nam Khem Village, June 2007.

Trials	No. of flies	Number of eggs found			
		Hw	T.t	A.I	Total
Group I					
- manual shaking	200	38	17	0	55
- ultrasonic cleaner	200	48	17	1	66
Group II (contamination rate)	108	17	17	1	35
washing sediment	-	27	27	0	54
Total	508	130	78	2	210

trials (groups I and II) and the eggs on the body surfaces of 108 flies (Table 5). The average number of eggs on the body surface of a fly was 0.4.

Mites, the ectoparasites of flies, were also isolated from the body surfaces of the houseflies; the number of mites per fly ranged between 1-40.

Discussion

One year after health education and treatment, the overall prevalence of STH in the community decreased from 34.9 to 22.5%. In the previous year, mass treatment had been provided to all school children; thence, the infection rate decreased from 27.8 to 14.5%. In the village, people did not consider STH infection a serious problem; most did not submit stools for examination. Some infected cases refused to take anthelmintic drugs, although we tried to treat all infected cases and inhabitants with a habit of indiscriminate fecal disposal. These factors resulted in unsuccessful treatment of the villagers; thus, one year later, the infection rate increased from 41.3 to 50.0%. However, at the end of the study, both groups were re-treated.

Examination of the stool samples in the swamp area revealed that the number of people defecating in the area had decreased after community health education. Since only one person still defecated there, soil contamination in the swamp area had decreased from 100 to 13.3%. The decreased contamination rate also indicated partial success of the health education program in the community.

To extract helminth objects from the carriers, manual shaking and ultrasonic cleaner techniques were tested. The two methods provided equal detection rates, but more eggs were isolated using the ultrasonic cleaner. With this washing technique, *A. lumbricoides* eggs were discovered, although none was detected from feces in the defecation area. This finding supported the higher efficacy of the ultrasonic cleaner, and also incriminated flies as potential carriers of helminth infections. The flies carried *A. lumbricoides* eggs from contaminated sites outside the swamp area. The result also correlated with the finding of STH infection in the community.

Although house flies, bush flies, and blow flies were common around the households, in garbage, and on human and animal excreta, the dominant fly species was *C. megacephala* [13,19,21,22]. *C. megacephala* is a proven mechanical transmitter of pathogens to humans, since they feed on both human feces and human food [28]. Ten intestinal helminth eggs and larvae have been isolated from flies collected around households, in an urban slum area, on an open defecation area, garbage heaps, a small open-air market, and meat butchers near human dwellings [13,19,21-24]. *C. megacephala* was the most prevalent carrier, exhibiting a significantly higher average egg load than *Musca domestica* [21]. In the present study, *C. megacephala* was the only species trapped in the study area. Because the mangrove swamp was the post-tsunami defecating area for the villagers, piles of fecal matter attracted flies. After feeding and resting, the flies traveled into the community, about 120 meters from the feeding site.

Flies that had direct contact with helminth-positive feces were efficient carriers, because at least 25.9% were contaminated with pathogens. In the defecation area, every 2-3 flies carried at least 1 helminthic object on the body surface (average number of eggs per fly = 0.4). After feeding, they rested in the area and contaminated the environment with the pathogens on their footpads, hairs, bristles, and external mouthparts. An almost invisible dot of feces in the environment might contain eggs or larvae that develop further and then transmit to humans. In the mangrove swamp, sea-holly, a medical herb that grows in damp soil, was common. Numerous flies were seen on the leaves and stems of this plant. Infection could occur when these herbs were collected for medication, with unintended contact by humans or during woodcutting. In this community, 11.8% of flies had eggs on their body surfaces, and could transmit them to human food and household surroundings. 25% of infected flies had 2-3 eggs adhering to their body surfaces. The study found that 508 flies could leave 0.5 ml of fecal sediment in the collection chamber. This was considered to be the amount of pathogenic fecal matter

distributed into the environment by 508 flies; thus, a fly carried 0.001 g fecal mass on the body surface after feeding on human waste.

We did not investigate the presence of helminth objects in the flies' guts. Nevertheless, many researchers have reported higher parasite-detection rates in the gastrointestinal lumen than on body surfaces [13,22,23]. Sulaiman *et al* [19] found hookworm eggs and larvae in the guts of flies, but found none on the external surfaces.

From this investigation, it may be concluded that only one person with a light STH infection can contaminate both defecation areas and disease vectors. Over 25.9% of the fly population was contaminated. After resting and contaminating the environment with infective matter carried on the body surface, they transmitted the infection to the community, at a rate of 11.8%. The discovery of *A. lumbricoides* eggs on the flies supported the supposition that *C. megacephala* was a potential STH transmitter, which could carry and spread pathogens to other places, since they are able to travel up to 20 miles to unsanitary sites [29].

The current study showed that the housefly is a potential mechanical vector for STH infection, and therefore its role in disease transmission should not be underrated. In high-risk areas, health education targeting the elderly should emphasize personal and environmental hygiene. In areas where open-air defecation is common, food must be strictly protected from houseflies, since in this study one in every 11 flies around the house was found positive for helminth eggs. Other microorganisms causing protozoan and bacterial infections have also been reported [13,24]. The control or eradication of houseflies should be attempted, to stop intestinal-parasite transmission in the community, in addition to drug administration.

Acknowledgements

This project was funded by the University of Brescia, Medicus Mundi Italy, and the Faculty of Tropical Medicine, Mahidol University, Thailand. The authors wish to thank all of the subjects in the study, and the schoolteachers from Ban Nam Khem

School for their kind cooperation throughout the study. Our thanks are extended to Mr Yudthana Samung, Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, for identifying fly species. Special thanks are also to Dr Francesco Castelli, University of Brescia, for his guidance and kind advice.

References

1. Che Ghani BM, Oothuman P, Hashim BB, Rusli BI. Patterns of hookworm infections in traditional Malay villages with and without JOICFP Integrated Project in Peninsular Malaysia-1989. In: Yokogawa M, *et al*, editors. Collected papers on the control of soil-transmitted helminthiases, Vol. V. Tokyo: APCO; 1993. p. 14-21.
2. Kan SP. Environmental and socioeconomic factors affecting endemicity of soil-transmitted helminthiases within urban slum dwellers. In: Yokogawa M, *et al*, editors. Collected papers on the control of soil-transmitted helminthiases, Vol. IV. Tokyo: APCO; 1989. p. 176-83.
3. Ismid IS, Margono SS, Sasongko A. Treatment of *Ascaris lumbricoides* infections, sanitation activities and contamination of soil with *Ascaris lumbricoides* eggs (1988-1990). In: Yokogawa M, *et al*, editors. Collected papers on the control of soil-transmitted helminthiases, Vol. V. Tokyo: APCO; 1993. p. 211-6.
4. Sornman S, Harinasuta C, Bunnag T, Vivatanasesth P, Thirachandra S, Phatihatakorn W. A preliminary report on integrated family planning and parasite control programme in a developing slum area. In: Yokogawa M, *et al*, editors. Collected papers on the control of soil-transmitted helminthiases, Vol. I. Tokyo: APCO; 1980. p. 99-107.
5. Toan ND. Effect of periodic half year deworming on prevalence of soil-transmitted helminthiases. In: Yokogawa M, *et al*, editors. Collected papers on the control of soil-transmitted helminthiases, Vol. V. Tokyo: APCO; 1993. p. 244-50.
6. Chhetri MK. An effect of rural latrine on parasite control in Panchkhal village region of

Nepal. In: Yokogawa M, *et al*, editors. Collected papers on the control of soil-transmitted helminthiases, Vol. V. Tokyo: APCO; 1993. p. 265-70.

7. 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. In: Yokogawa M, *et al*, editors. Collected papers on the control of soil-transmitted helminthiases, Vol. II. Tokyo: APCO; 1983. p. 54-8.
8. Kan SP. Soil-transmitted helminthiasis in Selangor, Malaysia. In: Yokogawa M, *et al*, editors. Collected papers on the control of soil-transmitted helminthiases, Vol. II. Tokyo: APCO; 1983. p. 72-84.
9. Sornmani S, Vivatanareseth P, Harinasuta C, Potha U, Thirachantra S. The control of Ascariasis in a slum community of Bangkok. In: Yokogawa M, *et al*, editors. Collected papers on the control of soil-transmitted helminthiases, Vol. II. Tokyo: APCO; 1983. p. 290.
10. Montresor A, Crompton DWT, Hall A, Bundy DAP, Savioli L. Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level. WHO/CTD/SIP/98.1
11. Mott KE. The World Health Organization and the control of intestinal helminths. In: Yokogawa M, *et al*, editors. Collected papers on the control of soil-transmitted helminthiases, Vol. IV. Tokyo: APCO; 1989. p. 189-200.
12. Chandler AC, Read CP. Introduction to parasitology with special references to the parasites of man. 10th ed. New York: John Wiley & Sons; 1962.
13. Getachew S, Gebre-Michael T, Erko B, Balkew M, Medhin G. Non-biting cyclorrhaphan flies (Diptera) as carriers of intestinal human parasites in slum areas of Addis Ababa, Ethiopia. *Acta Tropica*. 2007;103:186-94.
14. Bundy DAP, Hall A, Medley GF, Savioli L. Evaluation measures to control intestinal parasitic infections. *World Health Stat Q* 1992;45:168-79.
15. Lai KPF, Ow Yang CK. Soil-transmitted helminthiasis in a rubber and oil-palm estate in Selangor, Peninsular Malaysia. In: Yokogawa M, *et al*, editors. Collected papers on the control of soil-transmitted helminthiases, Vol. V. Tokyo: APCO; 1993. p. 72-7.
16. Yu S, Xu L, Jiang Z, Chai Q, Zhou C, Fang Y, *et al*. Environmental and human behavioral factors in propagation of soil-transmitted helminth infections. In: Yokogawa M, *et al*, editors. Collected papers on the control of soil-transmitted helminthiases, Vol. V. Tokyo: APCO; 1993. p. 83-8.
17. Anantaphruti MT, Waikagul J, Maipanich W, Nuamtanong S, Pubampen S. Soil-transmitted helminthiases and health behaviors among schoolchildren and community members in a west-central border areas of Thailand. *Southeast Asian J Trop Med Public Health*. 2004;35:260-6.
18. Maipanich W, Sanguankiat S, Pubampen S, Kusolsuk T, Rojekittikhun W, Castelli F. Helminthic infections in a tsunami-affected area: soil contamination and infection rates in the population. *J Trop Med Parasitol*. 2007;30:5-11.
19. Sualiman S, Mohammed CG, Marwi MA, Oothuman P. Study on the role of flies in transmitting helminths in a community. In: Yokogawa M, *et al*, editors. Collected papers on the control of soil-transmitted helminthiases, Vol. IV. Tokyo: APCO; 1989. p. 59-62.
20. Harwood RF, James MT. Entomology in human health, 7th ed. New York: Macmillan Publishing; 1979.
21. Monzon RB, Sanchez AR, Tadiaman BM, Najos OA, Valencia EG, de Rueda RR. A comparison of the role of *Musca domestica* (Linnaeus) and *Chrysomya megacephala* (Fabricius) as mechanical vectors of helminthic parasites in a typical slum area of Metropolitan Manila. *Southeast Asian J Trop Med Public Health*. 1991;22:222-8.
22. Sulaiman S, Sohadi AR, Yunus H, Iberahim R. The role of some cyclorrhaphan flies as carriers

of human helminths in Malaysia. Med Vet Entomol. 1988;2:1-6.

23. Umeche N, Mandah LE. *Musca domestica* as a carrier of intestinal helminths in Calabar, Nigeria. East Afr Med J. 1989;66:349-52.

24. Khan AR, Huq F. Disease agents carried by flies in Dacca city. Bangladesh Med Res Counc Bull. 1978;4:86-93.

25. Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. Rev Inst Med Trop Sao Paulo. 1972;14:397-400.

26. Maipanich W, Waikagul J, Visessuk K. Efficacy of three flotation media in separating *Ascaris* eggs from soil. Mahidol Univ J. 1996;3:157-60.

27. Waikagul J, Anantaphruti MT, Nuamtanong S, Sanguankiat S. In: Hayashi S, *et al*, editors. Collected papers on the control of soil-transmitted helminthiases, Vol. VI. Tokyo: APCO; 1997. p. 5-11.

28. Beaver PC, Jung RC, Cupp EW. Clinical Parasitology. 9th ed. Philadelphia: Lea & Febiger; 1984.

29. Greenberg B. Flies and disease. I. Ecology, classification and biotic association. Vol. I. New Jersey: Princeton University Press; 1973.