PREVALENCE OF INTESTINAL PROTOZOAN INFECTIONS AMONG CHILDREN IN THAILAND: A LARGE-SCALE SCREENING AND COMPARATIVE STUDY OF THREE STANDARD DETECTION METHODS

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Abstract. A significant impact of intestinal parasitic infections on public health has mostly been neglected. Parasitic infections are one of risk factors for malnutrition in children. In this study, a large-scale screening of intestinal parasitic infections among children in 16 schools in 6 regions of Thailand was performed. In addition, we compared sensitivity of methods currently employed for detection of intestinal parasitic infection. Fecal samples collected from 1,909 students were examined for intestinal parasites by simple smear, formalin-ethyl acetate concentration (FECT), and Locke-egg-serum (LES) medium culture methods. Seven hundred and thirteen samples were infected with at least one intestinal parasite. The highest prevalence (82.8%) was found in Kanchanaburi Province, western Thailand. *Blastocystis* spp was the most common (32.8%) parasite, followed by *Giardia duodenalis* (4.2%), *Ascaris lumbricoides* (3.6%), hookworms (1.6%), *Entamoeba histolytica* (0.7%), *Trichuris trichiura* (0.5%), *Enterobius vermicularis* (0.5%), *Strongyloides stercoralis* (0.4%), minute intestinal flukes (0.2%), and *Taenia* spp (0.1%). Mixed parasitic infections were found in 121 students. In a comparative study, we found that FECT was more sensitive (74.0%) than simple smear (55.0%) method for detecting helminths. However, sensitivity of these two methods is not significantly different for protozoan detection (31.2% by simple smear and 33.5% by FECT). LES culture technique was the most sensitive method (77.5%) for detecting *Blastocystis* spp. Our results indicate a high prevalence of intestinal parasite infection among Thai students. More sensitive methods should be developed for a large-scale screening of intestinal protozoan infection.

Keywords: *Blastocystis* spp, intestinal parasite, school-age student, Thailand

INTRODUCTION

Parasitic infection remains a major public health problem worldwide, particularly in developing countries. However, the impact of these infections on public health has been mostly ignored. To date, a national prevalence of intestinal parasites

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in Thailand has not been reported. Most studies report prevalence in specific areas. In 2009, the national survey of helminthiasis in Thailand showed high prevalence of parasitic infection (18.1%) in Thai people (Wongsaroj *et al*, 2012). Prevalence of intestinal parasitic infections varies from one area to another depending on the degree of personal and community hygiene, sanitation and climatic factors (Soriano *et al*, 2001; Manganelli *et al*, 2012; Wongsaroj *et al*, 2012; Boonjaraspinyo *et al*, 2013). Prevalence of intestinal parasitic infections among Thai patients in urban areas who visited the outpatient unit of King Chulalongkorn Memorial Hospital, Bangkok is 6.1-8.94% (Nuchprayoon *et al*, 2002; Saksirisampant *et al*, 2002). This prevalence is lower than the average prevalence (34-72%) in remote areas of Thailand (Triteeraprapab *et al*, 1997; Triteeraprapab and Nuchprayoon, 1998; Triteeraprapab *et al*, 1999; Nuchprayoon *et al*, 2009). This may be due to the fact that people in remote areas usually have cultural food beliefs, poor dietary habit, poor sanitation and hygiene as well as difficulty in gaining access to good health information and healthcare services.

Parasitic infection in children are still common, even in developed countries. Globally, over 1 billion people are estimated to be infected with at least one type of intestinal helminths, with the highest prevalence in school-age children (de Silva *et al*, 2003). Parasitic infection can lead to malabsorption and chronic blood loss in children, with long-term effects on their physical (height and weight) and cognitive development. Malnutrition is common in school-age children in developing countries. Parasitic infection is one of risk factors for malnutrition, poor psychomotor development, growth retardation, and stunting in children (Casapia *et al*, 2006; Walker *et al*, 2007; Jardim-Botelho *et al*, 2008; Yentur Doni *et al*, 2015). In Thailand, the studies of intestinal parasite infections usually report only the prevalence of certain parasites (*eg*, soil-transmitted helminths) (Anantaphruti *et al*, 2008), or in a selected group of children in certain areas (*eg*, in orphanages and in students in central region of the country) (Saksirisampant *et al*, 2003, 2006; Ngrenngarmlert *et al*, 2007).

Effective diagnosis will assist in a better surveillance of intestinal parasitic infections. Early diagnosis is needed to reduce and prevent transmission. There are many alternative methods to detect parasitic infections, including molecular and immunological assays. However, these techniques are time-consuming and require expensive reagents, instruments, as well as technical expertise. Thus, microscopic examination remains the routine method for laboratory diagnosis of intestinal parasitic infection. The current routine techniques for screening intestinal parasitic infection are simple smear and formalin-ethyl acetate concentration (FECT). The simple smear technique is easy to perform but its sensitivity is low, leading to misdiagnosis. Sensitivity of FECT is higher than simple smear technique for diagnosis of intestinal helminth infection (Saksirisampant *et al*, 2003; Nuchprayoon *et al*, 2009; Hailu and Abera, 2015).

In this study, a large-scale survey of the prevalence of intestinal parasitic infection in school-age children was performed in 6 regions of Thailand. In addition, a comparative study was conducted among different techniques for parasite identification.

MATERIALS AND METHODS

Study areas and test population

Cross-sectional surveys were per-

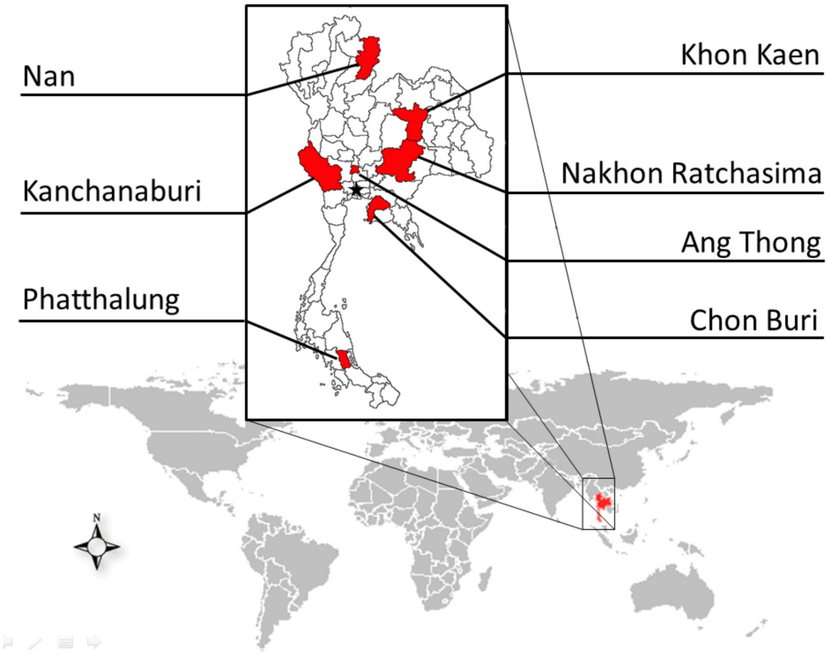

Fig 1 - Location of the study areas in the six regions of Thailand.

formed in 16 schools in 7 provinces (Ang Thong, Nakhon Ratchasima, Khon Kaen, Nan, Chon Buri, Kanchanaburi and Phatthalung) located in central, northeastern, northern, eastern, western, and southern Thailand (Fig 1). All schools, except those in Nakhon Ratchasima Province, are in rural areas. Schools in Nan and Kanchanaburi Provinces are located in mountain villages and most of students are from hill tribes. A number of students in Chon Buri and Kanchanaburi Provinces are migrants from Myanmar. In cooperation with school directors, 1,909 students aged between 1 to 23 years (mean ± range, 9.9 ± 4.9 years) were recruited to the study.

The study was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University, Bangkok (IRB no. 396/56). All subjects were well informed about the health effects and the danger from intestinal parasitic infection. Prevention methods against common parasitic diseases (*eg*, consumption of well-cooked food, hygienic defecation and no bare-feet behavior) also were explained to the participants. Written informed consent was obtained from legal guardian of each individual to participate in the study.

Stool examination

Stool collection containers were distributed to all participants. In order to avoid contamination, specific precaution and guideline for specimen collection were explained to all students. Stool samples were stored at room temperature prior to cultivation and stool examinations on the following day at Lymphatic Filariasis and Tropical Medicine Research Unit, Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok. Simple smear, FECT and Boeck and Drbohlav's Locke-egg-serum (LES) medium culture techniques for detection of intestinal parasite eggs or larvae were performed as described previously (Nuchprayoon *et al*, 2009). In brief, stool samples were examined microscopically of simple smears using normal saline and iodine preparation. About 2-5 mg of each stool specimen was processed by FECT method. LES medium culture was conducted for identification of *Blastocystis* sp and *Entamoeba histolytica*. The presence of intestinal parasite eggs, larvae or cysts was determined microscopically. Samples were independently examined by two

Table 1
Prevalence of intestinal parasitic infections among surveyed students in Thailand classified by gender and age groups.

Age (years)	Number (male, female, unspecified)	Number of positives (male, female, unspecified)	Percent prevalence (male, female, unspecified)
1-6	278 (145, 133, 0)	79 (43, 36, 0)	28.4 (29.7, 27.1, 0)
7-12	1,193 (551, 642,0)	555 (261, 294, 0)	46.5[a] (47.4, 45.8, 0)
13-23	403 (207, 196, 0)	71 (29, 42, 0)	17.6 (14.0, 21.4, 0)
Not available	35 (0, 0, 35)	8 (0, 0, 8)	22.9 (0, 0, 22.9)
Total	1,909 (903, 971, 35)	713 (333, 372, 8)	37.3 (36.9, 38.3, 22.9)

[a]Significantly higher than other age groups ($p < 0.005$).

examiners. Individuals infected with pathogenic parasites were treated with standard drug regimens.

Data analysis

Data were analyzed using Microsoft Excel 2010 program and GraphPad Prism version 5.01 for Windows. Numerical data are presented as mean ± SD. Association between population groups was analyzed using chi-square or Fisher exact test. A p-value < 0.05 is considered statistically significant.

RESULTS

Prevalence of intestinal parasitic infection in school-age students

Out of 1,909 participants, 713 (37.3%) students (333 males, 372 females and 8 unspecified) were infected with at least one kind of intestinal parasite (Table 1). No statistically significant difference between prevalence of intestinal parasitic infection was found among genders (36.9% in males, 38.3% in females and 22.9% in unspecified subjects) ($p = 0.164$) (Table 1). The highest prevalence of intestinal parasitic infection was found in students aged 7-12 years old (46.5%) ($p < 0.001$).

The lowest prevalence of intestinal parasitic infection was found among students in Nakhon Ratchasima (0.5%) and the highest in Kanchanaburi (82.8%) ($p < 0.001$) (Table 2). Prevalence in Ang Thong, Chon Buri, Phattalung, Khon Kaen, and Nan Province was 14.5%, 23.9%, 41.0%, 43.6%, and 54.6%, respectively.

The most commonly identified parasite was *Blastocystis* spp (32.8%) (Table 2). Other protozoan infections were *Giardia duodenalis* (4.2%) and *E. histolytica* (0.7%). Nematode infections were *Ascaris lumbricoides* (3.6%), hookworm (1.6%), *Strongyloides stercoralis* (0.4%), *Trichuris trichiura* (0.5%), and *Enterobius vermicularis* (0.5%). A trematode (minute intestinal fluke) (0.2%) and a cestode (*Taenia* spp) (0.1%) also were detected.

Classified according to route of transmission, the majority (88.1%) of infected students had parasites transmitted by fecal-oral route (*ie*, *Blastocystis* spp, *G. duodenalis*, *E. histolytica*, and *E. vermicularis*) (Table 2). Soil-transmitted helminthes (*ie*, *A. lumbricoides*, *T. trichiura*, *S. stercoralis*, and hookworms) were found in 16.3% of students, while food-borne parasites (*ie*, minute intestinal fluke and *Taenia* spp) were present in 0.7% of these students.

Non-pathogenic protozoa, *Entamoeba*

Table 2
Prevalence of intestinal parasitic infections among surveyed students classified by parasites and province in Thailand.

Province in Thailand	Number	Number of positives (percent prevalence)	Pathogenic parasite (prevalence)									
			Bh	Gd	Eh	Al	Hw	Ss	Tt	Ev	Mn	Tn
Nakhon Ratchasima	187	1 (0.5)	1	0	0	0	0	0	0	0	0	0
Khon Kaen	188	82 (43.6)	80	2	0	0	0	1	0	0	0	0
Nan	240	131 (54.6)	105	7	2	63	2	1	0	2	0	0
Kanchanaburi	268	222 (82.8)[a]	215	45	6	4	7	2	4	2	0	0
Ang Thong	330	48 (14.5)	26	14	6	1	8	0	0	0	0	0
Phatthalung	366	150 (41.0)	145	5	0	0	4	1	2	1	0	0
Chon Buri	330	79 (23.9)	54	8	0	1	9	2	4	5	4	1
Total	1,909	713 (37.3)	626 (32.8)	81 (4.2)	14 (0.7)	69 (3.6)	30 (1.6)	7 (0.4)	10 (0.5)	10 (0.5)	4 (0.2)	1 (0.1)

The total number of infected individuals detected by each technique is not equal to the sum of infected individuals with each parasite because some subjects have mixed infections with more than one parasite. AL, *Ascaris lumbricoides*; Bh, *Blastocystis* spp; Eh, *Entamoeba histolytica*; Ev, *Enterobius vermicularis*; Gd, *Giardia duodenalis*; Hw, hookworm; Mn, minute intestinal fluke; Ss, *Strongyloides stercoralis*; Tn, *Taenia* spp; Tt, *Trichuris trichiura*. [a]Significantly higher than other provinces ($p < 0.005$).

coli, *Entamoeba nana*, *Trichomonas hominis* and *Iodamoeba butschlii*, also were detected with prevalence of 6.3%, 3.9%, 0.2%, and 0.1%, respectively (Table 3).

Of the 713 infected individuals, 121 (17.0%) students had mixed parasitic infections: 86.8% with two species of intestinal parasites, 11.5% with three species and 1.7% with 4 species (Table 4). *Blastocystis* spp and *G. duodenalis* constituted the most common mixed infections (38.8%), followed by *Blastocystis* spp and *A. lumbricoides* (29.0%) (data not shown).

Comparative study of the different techniques for parasite detection

A comparison between simple smear and FECT methods revealed no significant difference in sensitivity of parasite detection (41.7% *vs* 46.4%) (Table 5). However, for detection of helminth infection FECT method was significantly more sensitive (74.0%) than simple smear (55.0%) ($p < 0.001$). Moreover, FECT recovered considerably more eggs and larvae of helminths than simple smear method (data not shown). Interestingly, simple smear was more sensitive than FECT technique in detecting *A. lumbricoides* eggs (87% *vs* 62%) (Table 5). For protozoan detection, no significant difference in sensitivity was discerned between overall simple smear (31.2%) and FECT (33.5%) method ($p = 0.19$). However, FECT technique was superior to simple smear method for detecting *G. duodenalis* (61.7% *vs* 46.9%).

Table 3
Prevalence of non-pathogenic protozoan infections among surveyed students classified by parasite and province in Thailand.

Province	Number	Number of positives (percent prevalence)	Non-pathogenic parasite (percent prevalence)			
			Ec	En	Th	Ib
Nakhon Ratchasima	187	1 (0.5)	1	0	0	0
Khon Kaen	188	10 (5.3)	4	5	2	0
Nan	240	22 (9.2)	22	0	0	0
Kanchanaburi	268	70 (26.1)	54	31	1	1
Ang Thong	330	17 (5.2)	13	4	0	0
Phatthalung	366	21 (5.7)	11	9	1	0
Chon Buri	330	35 (10.6)	15	25	0	0
Total	1,909	176 (9.2)	120 (6.3)	74 (3.9)	4 (0.2)	1 (0.1)

EC, *Entamoeba coli*; En, *Endolimax nana*; Ib, *Iodamoeba butschlii*; Th, *Trichomonas hominis*.

Table 4
Mixed infections of the intestinal parasites among surveyed students in Thailand.

Parasitic infection	Number of positives (%) (N = 1,909)
Single infection	592 (83.0)
Mixed infections	121 (17.0)
2 types of parasites	105 (86.8)[a]
3 types of parasites	14 (11.5)
4 types of parasites	2 (1.7)
Total	713 (37.3)

[a]Significantly higher than other mixed parasite infections ($p < 0.05$).

To compare the sensitivity of sample smear and FECT procedures with that of concentration techniques with LES culture, only *Blastocystis* spp and *E. histolytica* were cultivable. Only 1,001/1,909 stool samples were of sufficient quantity to be used for all three techniques. LES culture has the highest sensitivity for protozoan detection (82.9%), compared with 28.9% by simple smear and 27.7% by FECT techniques. Only 49 (8.4%) students were positive by all three tests. Interestingly, 53.5% of infected individuals could only be detected by LES culture. Comparison of the sensitivity of the three techniques employed in this study is summarized by a Venn diagram (Fig 2).

DISCUSSION

In this study, we performed a large-scale screening of intestinal parasitic infection among 1,909 students in 6 regions of Thailand. We found a high (37.3%) prevalence of infection among the students. Our data agreed with several studies, which

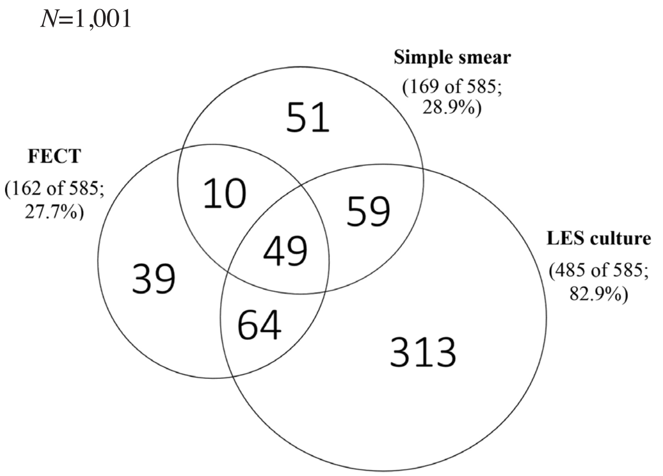

Fig 2–Venn diagram showing patterns of detection of protozoa by simple smear (Simple), formalin-ethyl acetate concentration (FECT) and Boeck and Drbohlav's Locke-egg-serum (LES) medium culture methods. Number in circle indicate number of positive samples. Number in parenthesis indicate sensitivity of each technique for protozoan identification. *N* = total number of samples examined.

showed that intestinal parasitic infections are common (4.2-48.9%) among children in Thailand (Piangjai *et al*, 2003; Saksirisampant *et al*, 2006; Ngrenngarmlert *et al*, 2007; Kitvatanachai and Rhongbutsri, 2013). The prevalence in children is much higher than in adults (6.1%) (Saksirisampant *et al*, 2002), but still lower than in immigrant (81.1%) and Thai children (71.0%) in orphanages (Saksirisampant *et al*, 2003; Sagnuankiat *et al*, 2016). This may be due to the overcrowded environment and poor sanitation in orphanages.

The common parasites identified in children in Thailand are *T. trichiura* (0.19-50.8%), *E. vermicularis* (0.19-25.2%), hookworms (0.19-11.6%), *A. lumbricoides* (9.8-15.3%), *S. stercoralis* (0.92%), *Blastocystis* spp (0.19-0.5%), and *G. duodenalis* (1.25-10.2%) (Saksirisampant *et al*, 2004, 2006; Sagnuankiat *et al*, 2016). On the other hand, we found a higher prevalence of protozoan than helminth infection. This may be due to the annual treatment of helminth infection by mebendazole in some families and mass treatment in some villages. However, the diagnosis and treatments of protozoan infection have usually been ignored. There is no report of clinical manifestations of these affected students. However, intestinal protozoan and helminth infections have been shown to have significant association with growth retardation and poor psychomotor development of children (Yentur Doni *et al*, 2015). Therefore, surveillance of intestinal protozoan infection should be emphasized in the country's public health programs.

The most common parasites found in this study were *Blastocystis* spp and *G. duodenalis*. Less than 10% of students were infected with non-pathogenic protozoa (*E. nana*, *E. coli*, *T. hominis*, and *I. butschlii*). These findings imply that poor sanitation exists among school-age children in rural areas. *Blastocystis* spp and *G. duodenalis* are commonly found in water supplies, especially ponds and canals (Khalifa *et al*, 2014). These parasites were detected with high prevalence in Nan and Kanchanaburi Provinces. Schools we studied are located in mountain villages. Water supplies used in these areas are from mountain sources. Thus, contaminated water supplies might be the importance source of these pro-

Table 5
Comparison of sensitivity of two techniques for parasite identification.

Parasite	Number (%)	Number of positives (percent sensitivity)	
		Simple smear method	Formalin-ethyl acetate concentration method
Helminth			
Taenia spp	1 (0.1)	0	1 (100)
E. vermicularis	10 (1.4)	5 (50.0)	7 (70.0)
T. trichiura	10 (1.4)	1 (10.0)	9 (90.0)
Hookworms	30 (4.2)	3 (10.0)	26 (86.7)
A. lumbricoides	69 (9.7)	60 (87.0)	43 (62.3)
S. stercoralis	7 (1.0)	3 (42.9)	7 (100)
Minute intestinal flukes	4 (0.6)	0	4 (100)
Total	131	72 (55.0)	97 (74.0)
Protozoa			
G. duodenalis	81 (11.4)	38 (46.9)	50 (61.7)
E. histolytica	14 (2.0)	9 (64.3)	7 (50.0)
Blastocystis spp	626 (87.8)	178 (28.4)	177 (28.3)
Total	721	225 (31.2)	234 (33.5)
Total	713	297 (41.7)	331 (46.4)

The total number of infected individuals detected by each technique is not equal to the sum of infected individuals with each parasite because some subjects have mixed infections with more than one parasite.

tozoa infections. Water treatment was reported to be associated with reduction of some protozoan infections, including *Blastocystis* spp and *G. duodenalis* (Speich *et al*, 2016).

The prevalence of intestinal parasite infection in each region of Thailand was significantly different. The reasons for this may be directly related to specific geographic characteristics, as well as to ecological, sanitary, socioeconomic, and cultural factors (Soriano *et al*, 2001; Sinniah *et al*, 2014). The highest prevalence (82.8%) of intestinal parasitic infection was found in Kanchanaburi Province located in the western region of Thailand. Common parasites found in this region were *Blastocystis* spp (80.2%) and *G. duodenalis* (16.8%). The major risk factor for infection with these parasites is drinking water from mountain sources contaminated with infective stages of the parasites.

In the northern region, prevalence of parasitic infection was 54.6%, not different from previous reports (37-72%) (Triteeraprapab *et al*, 1997; Triteeraprapab and Nuchprayoon, 1998; Triteeraprapab *et al*, 1999; Piangjai *et al*, 2003; Nuchprayoon *et al*, 2009). The high prevalence of *Blastocystis* spp (43.8%) and *A. lumbricoides* (26.3%) were similar to those of Kanchanaburi Province. The majority of infected students in Nan were from hill tribes, where sanitation is poor and water supplies are from mountain sources. Moreover, hill tribes in northern regions usually live in house raised on stilts and keep domestic animals, such as pig and chicken, under their houses. Cesspools are usually outside the house. Manure made from human and animal waste is used in the fields. It is not unexpected that we found a high prevalence of *A. lumbricoides* infection in this region. In one of the schools surveyed 90% of stool samples contained *A. lumbricoides* (data not shown). Zoonotic *A. suum* should also be of concern. Although the majority of students did not wear shoes, we found low prevalence (0.8%) of hookworms.

In northeastern region, we surveyed Nakhon Ratchasima and Khon Kaen Provinces and found lower prevalence of intestinal parasites (22.1%) compared to other previous studies (6.22-37.2%) (Kitvatanachai *et al*, 2008; Boonjaraspinyo *et al*, 2013; Kaewpitoon *et al*, 2015). The prevalence rate of intestinal parasitic infections was significantly higher in Khon Kaen (43.6%) compared to Nakhon Ratchasima Province (0.5%). Only one student in Nakhon Ratchasima Province was infected with *Blastocystis* spp. Reasons for this difference might be difference in sanitary conditions and socioeconomic factors. In contrast to students in Khon Kaen Province, the school we surveyed in Nakhon Ratchasima Province is a primary school in a barrack in an urban area. The environment in the school is clean. From the questionnaire, we observed that family status of all students in Nakhon Ratchasima Province is quite high.

Interestingly, we found *O. viverrini* infection (normally found in northeastern region) in four students, 6-9 years old, in Chon Buri Province located in western region (data not shown). The prevalence of *O. viverrini* infection among this age group was 1.5%, slightly higher than a previous report (1.2%) of the World Health Organization for northeastern Thailand (WHO, 1997).

In the comparative study of standard detection techniques, we found sensitivity of FECT not different from simple smear method for diagnosis of intestinal parasitic infection. However, FECT was more sensitive than simple smear method for detection of helminth infection. For protozoan detection, no significant difference was found between overall sensitivity of simple smear and FECT methods. However, FECT had better sensitivity for detecting *G. duodenalis* than simple smear method. LES culture had the highest sensitivity for detecting *Blastocystis* spp and *E. histolytica*. Although cultivation of luminal protozoa is usually performed in research laboratories (Clark and Diamond, 2002), cultivation of protozoa might be useful for the detection of intestinal protozoan infection. Our results showed that *Blastocystis* spp and *E. histolytica* infections were detected only by the LES medium culture. Our data suggest that the simple smear technique is insufficiently sensitive to be used alone for screening of parasitic infections.

Improvements in sanitation, personal hygiene, quality of water supplies, as well as basic health education are needed if intestinal parasitic infections are to be reduced among children in Thailand. Moreover, surveillance of the sources of protozoan infection particular to each area, such as protozoa in water supplies and in animals, should be conducted with appropriate methods with higher sensitivity.

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