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A Cross-Sectional Study on Intestinal Parasitic Infections in Children in Suburban Public Primary Schools, Saraburi, the Central Region of Thailand

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Abstract. Intestinal parasitic infection rate among school-aged children in Thailand has been decreasing. However, certain intestinal parasites remain problematic in some regions. This cross-sectional study was conducted between February and September 2016 in three suburban government primary schools (KK, BR, and HK), Saraburi, Thailand. Stool was collected from 263 asymptomatic subjects (4–15 years old), using simple direct smear, formalin–ether concentration, Boeck and Drbohlav's Locke–Egg–Serum (LES) medium culture, and agar plate culture. A self-administered questionnaire was used to collect data about lifestyle and socioeconomic status. The overall rate of intestinal parasites was 22.1% (15.6% single infection and 6.5% multiple infections). The helminths involving the digestive system found were *Strongyloides stercoralis* (1.5%) and *Opisthorchis viverrini* (0.4%). For protozoan infection, the major cause was *Blastocystis hominis* (17.5%). The other protozoa included *Endolimax nana* (4.6%), *Entamoeba coli* (3.4%), *Entamoeba histolytica/Entamoeba dispar* (1.1%), and *Giardia intestinalis* (0.8%). The sensitivity for the detection of *B. hominis* increased with the LES culture technique. The infection rate of each organism was not significantly different among the three schools except for *B. hominis* which showed the highest prevalence in the HK school ($P = 0.001$). This was correlated with the questionnaire results in which the HK school showed the highest risk of drinking contaminated water ($P = 0.004$). The present study emphasized the persistent problems of protozoan infections among suburban school-aged children. Lifestyle was still an important factor for intestinal parasitic infections among suburban school-aged Thai children in this study. Health education as well as routine surveillance was necessary to control the infections.

INTRODUCTION

Helminth infections of the digestive system which cause chronic disability throughout childhood and adolescence are still a burden in many developing countries. An estimated 268.8 million preschool-aged children and 571.4 million school-aged children require regular and periodic deworming in endemic countries.[1] Preschool- and school-aged children are at the highest risk of morbidity. Growth retardation and intellectual developmental delay can be results of chronic helminth infection.[2] In Southeast Asia, including Thailand, parasitic infections have been a public health problem. The prevalence of parasitic infection has been decreasing for decades. However, this type of infection still remains a problem, especially in a rural community where people still have cultural beliefs in diet, poor personal hygiene, and sanitation.[3] Food-borne parasites can be found in people who frequently consume contaminated fresh vegetables and raw meat.[4] Walking barefoot on contaminated soil increases the risk of soil-transmitted helminths such as hookworms and *Strongyloides*.[5] Previous reports of parasitic infection in the central region of Thailand among preschool- and school-aged children showed an overall prevalence of 4.2–13.9% during the past decade.[6–8] Despite effective anthelmintics and prevention programs of parasitic infections, some intestinal parasites remain a problem.

This study presented a cross-sectional survey of the prevalence of parasitic infections involving the digestive system among school-aged children in the central region of Thailand. We determined the rate of parasitic infections, socioeconomic data, and lifestyles of children in this area.

METHODS

Data collection. A cross-sectional study was conducted in Kaeng Khoi district located in the northeastern part of Saraburi province, from February to September 2016. Saraburi is on the east side of the Chao Phraya River valley and has a tropical savanna climate. The location of Saraburi province is shown in Figure 1.[9] We made a simple random sampling for 3 from 39 suburban government primary schools. The schools namely KK, BR, and HK were included for this study. Inclusion criteria included all the asymptomatic healthy students in all three schools. Students who were unavailable or unwilling to participate in the project were excluded. Fecal samples were requested from all students (4–15 years old) of the three schools. As all the subjects were less than 18 years of age, their parents were asked to sign a consent form before the subjects were enrolled in the study. A written informed consent about the purpose, procedures, risks, and potential benefits of this study was obtained from each participant. A self-administered questionnaire concerning socioeconomic status, infrastructure, and lifestyles was asked to be completed by subjects aged more than 7 years. For subjects aged less than 7 years, parents helped complete the questionnaire. All protocols were approved by the Human Ethics Committee of Chulalongkorn University, Bangkok, Thailand.

Stool collection and examination. Participants were informed about the process of stool collection with plain plastic containers. One subject received one container marked with the participant identification number. We re-collected the containers with stools 48 hours after distributing. All samples were then transferred to the Parasitology Laboratory, Faculty of Medicine, Chulalongkorn University, within a 2-hour drive

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FIGURE 1. Location of Saraburi province in the central region of Thailand. This figure appears in color at www.ajtmh.org.

from Kaeng Khoi district, after complete collection. Subsequently, each specimen was examined under alight microscope by simple smear (wet mount) (one slide) and formalin–ether concentration method (another slide). Each slide was observed by two parasitologists who reported the final result after making an agreement between them. A stool sample was considered positive if at least one parasite was detected in a slide and an organism type was identified. Other parts of the stool samples were used for culture methods including Boeck and Drbohlav's Locke–Egg–Serum (LES) medium culture and agar plate culture.

The questionnaire structure. Highest education and occupation of each subject's parent was asked. The data concerning lifestyles and infrastructure that can be considered as risks of parasitic infection were categorized as three domains: risk of contact with soil-transmitted helminths, risk of drinking contaminated water, and risk of eating contaminated food as shown in Table 1. The response for each question was classified into two groups ("with the risk" or "without risk" for parasitic infection); then the percentage of the "with the risk" group was summed up by average weight from each question in each domain.

Data analysis. The prevalence of overall and separate species of intestinal parasites was calculated. The numerical data were shown as mean ± standard deviation. The categorical data such as socioeconomic status and lifestyles were reported by percentage. Difference of infection rate among the three schools and the association between infection rate and gender were analyzed by using χ^2 test. Upon analyzing the results of the questionnaire, the response from "with the risk" for parasitic infection group was presented as percentage; the higher the percentage, the higher was the risk for infection. The percentages of the "with the risk" group were compared among those of the schools by using χ^2 test. Statistical analyses were performed using SPSS statistical package version 22.0 (SPSS Inc., Chicago, IL). P values < 0.05 were considered statistically significant.

RESULTS

Demographic and socioeconomic data. Of all the 539 students from the three schools, 263 asymptomatic subjects (48.8%) were recruited for this study and 276 students were excluded because of unavailability and unwillingness. One hundred and forty-four samples were collected from males (54.8%), 116 from females (44.1%), and three samples with unknown gender status. The mean age of the subjects was 7.9 ± 2.5 years (ranged from 4 to 15 years). Majority of the subjects' parents (36.1%) had completed pre-high school, whereas 29.2% of the parents' education was less than the compulsory requirement (i.e., less than pre-high school level). The occupations of parents were laborer (68.5%), employee (17.8%), merchant (8.2%), and agriculturist (1.4%).

Prevalence of parasitic infection. Of the 263 participants, the overall prevalence of digestive parasitic infection was 22.1% (58 cases). The rate of intestinal protozoan infection accounted for 20.5% (54 cases). *Blastocystis hominis* was the

TABLE 1
Three domains and sample responses with the risk of parasitic infection

Domains	Area of interest and sample responses that are considered as a risk ("with the risk" group)
Risk of contact with soil-transmitted helminths (*Strongyloides* and hookworms)	Walking barefoot, type of toilet (e.g., pit toilet, dump into any place outside a toilet)
Risk of drinking contaminated water	Source of drinking water (e.g., other than tap water), no manipulation (e.g., boil, filter) before drinking water other than tap or bottled water, method for discarding used water (e.g., drain to soil or natural water source, no pipe drainage)
Risk of eating contaminated food	Not cleaning hand before meal or after pet contact, not washing fruit before eating, eating raw or undercook food

TABLE 2
Prevalence (%) and infection rate (%) of each organism among three schools

Organism	Number of overall infections (%) (*N* = 263)	Number of infections by school (%)			*P* value*
		KK (*N* = 147)	BR (*N* = 53)	HK (*N* = 63)	
Blastocystis hominis	46 (17.5)	19 (12.9)	6 (11.3)	21 (33.3)	0.001
Endolimax nana	12 (4.6)	5 (3.4)	4 (7.5)	3 (4.7)	0.462
Entamoeba coli	9 (3.4)	7 (4.8)	2 (3.8)	0 (0.0)	0.218
Entamoeba histolytica/Entamoeba dispar	3 (1.1)	1 (0.7)	1 (1.9)	1 (1.6)	0.723
Giardia intestinalis	2 (0.8)	1 (0.7)	1 (1.9)	0 (0.0)	0.500
Strongyloides stercoralis	4 (1.5)	2 (1.4)	1 (1.9)	1 (1.6)	0.963
Opisthorchis viverrini	1 (0.4)	1 (0.7)	0 (0.0)	0 (0.0)	0.673

* Chi-square test for the infection rate by schools.

most common protozoa found in this survey (17.5%). The rate of digestive helminthic infection was 1.9%, which included four cases of *Strongyloides stercoralis* (1.5%) and one case of *Opisthorchis viverrini* (0.4%). Forty-one individuals (15.6%) were infected with a single parasite species, whereas 17 individuals (6.5%) were infected with more than one parasite species. Most of the cases with mixed parasitic infection were the combination of *B. hominis* and *Endolimax nana* (seven cases) and *B. hominis* and *Entamoeba coli* (six cases). Table 2 shows the prevalence of each parasite and the comparison of the infection rate of each parasite in each school. Only the infection rate of *B. hominis* showed a significant difference among the three schools ($P = 0.001$), of which HK school had the highest rate of infection. Table 3 compares the overall infection rate among the three schools. There was a significant difference in the overall infection rate in which HK school had a higher infection rate than the other two schools ($P = 0.006$). There was no significant difference in the overall infection rate between males (20.1%) and females (25.0%) ($P = 0.349$).

Using simple direct smear alone, the prevalence of digestive parasitic infection was 9.5%, whereas the prevalence was 14.1% with the combination of simple direct smear and modified formalin–ether concentration. However, the overall prevalence using all methods was 22.1%. *Blastocystis hominis* detection using LES technique was 17.1%, compared with the 4.9% from the combination of simple smear and modified formalin–ether concentration. Table 4 compares the prevalence of each parasite detected by simple direct smear, formalin–ether concentration, Boeck and Drbohlav's LES medium culture, and agar plate culture.

Community lifestyle and infrastructure. Overall from the three schools, the subjects frequently washed their hands before meals (72.6%) and mostly washed fruit before eating (94.5%). Raw food such as pork/beef, fish, and fresh-water crab was sometimes consumed by the subjects (57.5%). Subjects generally drank tap water (51.4%), although some drank rainwater (11.1%). They mostly filtered or boiled water from each source before consuming (70.8%). Tap water was the largest source (95.8%) of water supply. After use, the water was normally drained into the pipe system (79.5%), whereas some contaminated natural water reservoirs such as rivers and ponds (6.9%). Among the subjects, 84.9% wore shoes every time when going outside the house. A lavatory with a septic tank was the most common method for urination or defecation (76.4%). A flush toilet and a pit toilet were less popular with 19.4% and 4.2%, respectively. All participants denied urinating or defecating into natural water reservoirs or soil. Ninety-three percent of the subjects had domestic animals or pets. Most subjects (82.9%) washed their hands after touching animals or pets. To summarize the risk for parasitic infection into three domains, the percentage of lifestyles and infrastructure "with the risk" of the parasitic infection from three schools is shown in Table 5.

DISCUSSION

Intestinal protozoa were the predominant parasites involving the digestive system (20.5%) in this suburban area where the infection rate of intestinal helminths was low (1.9%). This finding was consistent with previous studies in the central region of Thailand.[7,8] Our findings reflected the wide distribution of intestinal pathogenic and nonpathogenic protozoa in this area. Multiple infections were common and accounted for nearly one-third of those who got the parasitic infection. Species and rates of infection of most protozoa in the present study were approximated to those of the prior studies in the same areas except for *B. hominis* which showed much higher prevalence in our study (17.5% versus 0.2–6.2%).[6–8] The use of the LES culture technique increased the sensitivity to detect *B. hominis* in the present study. There were 33 cases in which *B. hominis* was positive by LES culture but negative by the combination of simple smear and formalin–ether concentration method, which increased the prevalence of the infection from 4.9% to 17.5%. Therefore, we have recommended applying LES techniques in future surveys to maximize the detection rate. Moreover, the infection of *B. hominis* accounted for the different rates of infection among the three schools, of which HK school had a significantly higher prevalence of *B. hominis* infection than the other two. According to a previous study, *B. hominis* infection was shown to be related to drinking water.[10] As per our expectation, HK school had the highest percentage of risk for drinking contaminated water as shown in Table 5. The contaminated water may be the source of transmission in this school. Therefore, a control measure for the prevention of these protozoa should be emphasized in all the three schools, especially HK school before an outbreak

TABLE 3
Overall infection rate among three schools by gender

School (*N*)	Number of infections/total sample (%)*		Total infection (%)†
	Male	Female	
KK (147)	12/85 (14.1)	13/61 (21.3)	25 (17.0)
BR (53)	7/28 (25.0)	3/24 (12.5)	10 (18.9)
HK (63)	10/31 (32.3)	13/31 (41.9)	23 (36.5)
Total (263)	29/144 (20.1)‡	29/116 (25.0)‡	58 (22.1)

* Missing gender for three cases.
† Chi-square test for total infection of three schools ($P = 0.006$).
‡ Chi-square test for total infection by sex ($P = 0.349$).

TABLE 4
Comparison of parasite prevalence by simple direct smear, formalin–ether concentration, Boeck and Drbohlav's Locke–Egg–Serum (LES) medium culture, and agar plate culture

Parasitic infection	Number of positive (%) from total 263 samples			
	Simple smear	Formalin–ether concentration	LES technique	Agar plate
Helminth				
Strongyroides stercoralis	1 (0.4)	2 (0.8)	0 (0.0)	4 (1.5)
Opisthorchis viverrini	0 (0.0)	1 (0.4)	0 (0.0)	NA
Protozoa				
Blastocystis hominis	6 (2.3)	11 (4.2)	46 (17.5)	NA
Giardia lamblia	1 (0.4)	2 (0.8)	0 (0.0)	NA
Entamoeba coli	7 (2.7)	8 (3.0)	6 (2.3)	NA
Endolimax nana	8 (3.0)	8 (3.0)	9 (3.4)	NA
Entamoeba histolytica/dispar	2 (0.8)	2 (0.8)	2 (0.8)	NA
Total	25 (9.5)	34 (12.9)	63 (24.0)	4 (1.5)

NA = not applicable.

can start. Health education should also be implemented for students and their families.

For helminthic infection, the rate of the infection was 1.9% in our study, whereas previous reports showed lower prevalence among school-aged children in the central region of Thailand with 0.5–0.7%.[6–8] In the present study, *S. stercoralis* was the helminth with the highest prevalence (1.5%) which was higher than those in the previous studies. This could be explained by the application of agar plate culture compared with formalin–ether concentration technique which was the only method used in previous studies (1.5% versus 0–0.1%).[6–8] In addition, *O. viverrini* was the other helminth found in only one case with prevalence of 0.4%. However, the previous surveys from the central region of Thailand also found *Trichuris trichiura*, hookworm, and *Taenia* spp. (0.2–0.5%).[6–8] The decrease in prevalence in our study might be explained by the active control programs for helminthic infection especially for *O. viverrini* and hookworm by the Ministry of Public Health which resulted in the decreasing infection rate over decades.[11] Preschool- and school-aged children in the central region of Thailand had much lower rates of helminthic infection compared with the northern region (29.8–60.0%)[12–15] and the northeastern region (57.0%).[16] The low prevalence of intestinal helminths might be because of the urbanization of basic public sanitation and general living conditions in the central region compared with the rural areas in the north and northeast regions. Moreover, consumption of raw fish still persists especially in the northeast regions which relates to poverty and the ease of food preparations.

TABLE 5
Comparison of the percent of lifestyles and infrastructures "with the risk" for parasitic infection among three schools

Domain	KK (N = 147)	BR (N = 53)	HK (N = 63)	P value*
Risk of contact with soil-transmitted helminths (*Strongyloides* and hookworms)	13.8	29.1	15.1	0.049
Risk of drinking contaminated water	30.7	14.1	42.2	0.004
Risk of eating contaminated food	27.2	34.8	28.3	0.648

* χ^2 test for the percent of lifestyles "with the risk" by schools.

This study certainly had limitations. First, the report with *Entamoeba histolytica/Entamoeba dispar* was needed as the distinction between the two species is difficult because some *E. dispar* strains may grow in the LES medium[17] and some carrier of *E. histolytica* are asymptomatic.[18] To differentiate these two, molecular diagnosis such as polymerase chain reaction may be required.[10] Second, the number of subjects was limited and it was not meant to be representative of the all central regions of Thailand because the rural areas were not included. Therefore, the rate of parasitic infection might be underestimated with regard to the true infection rate of the whole region. Third, the direct association between the lifestyle with the risk of the parasitic infection and the infected subjects was not presented in this article as the risk of infection of the specific organisms presented in this study is already well established. However, we emphasized the analysis based on different schools to tackle the problem directly to the site with a high prevalence of infection.

CONCLUSION

Our results concerning suburban government primary school children in Saraburi should not be considered as a representative of the whole child population. However, our survey provided essential data for developing a control program to decrease intestinal parasitic infection in these local areas. High standard hygienic conditions especially sanitized water supply and health educations given to the families and school directors should be implemented.

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