J Trop Med Parasitol. 2008;31:77-84.

ORIGINAL ARTICLE

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Reexamination of Parasitic Infections in Karen Children on the Western Border of Thailand: Two-year Follow-up

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Abstract

In 2005, helminth and protozoan infections were found to infect over half the Karen children in Ratchaburi Province, on the western border of Thailand with Myanmar. The current study aimed to reexamine infections in the same area 2 years later. Intestinal-helminth infection remained unchanged, and whipworm still predominated. Whipworm, hookworm, and roundworm, were the top 3 helminths found in this area. On the other hand, intestinal protozoan infections increased from 26.4 to 43.0%, with more cases infected by both non-pathogenic (*Entamoeba coli* and *Endolimax nana*) and pathogenic (*Giardia lamblia* and *E. histolytica*) organisms. Despite drug treatment and rigorous preventive control, infection rates remained high. The number of students whose infections persisted for 2 years roughly equaled the number of new infections in the present study. The numbers of those who remained uninfected throughout the 2 periods were disappointingly lower. This evidence shows that urgent improvements to the control program are needed, to tackle these neglected infectious tropical diseases more effectively.

Keywords: NTD, intestinal helminths, intestinal protozoa, Karen children, prevalence

Introduction

Intestinal-helminth and protozoan infections have long been major global public-health problems. However, in some areas, especially in developing countries, these diseases have been left untreated. Such diseases have been named “neglected tropical diseases” (NTD), enabling them to attract more interest and help boost several programs to deal with them. Some remote areas of Thailand also suffer from these NTD. Areas along the border between Thailand and Myanmar have a reportedly high prevalence of intestinal-helminth and protozoan infections [1]. Pinworm, hookworm, whipworm and roundworm were the most common helminths found, whereas non-pathogenic *Entamoeba coli* was the main infective protozoan. These helminths, and other pathogenic protozoa, cause mild to severe symptoms in patients, especially children [2]. Infected children normally exhibit malnutrition, anemia, diarrhea, underweight, and retarded growth. Poor sanitation and lack of education have been major factors

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influencing the persistence of intestinal parasitic diseases [3].

In 2005, Maneeboonyang and colleagues used simple smear and Kato techniques to show a high prevalence of intestinal parasites among Karen children aged 4-16 years in 3 schools [4]. The most common organisms found were soil-transmitted nematodes, whereas protozoa were found in significantly lower prevalence rates. As the eradication of intestinal helminths was the priority, a single dose of albendazole (400 mg) was given to every infected individual and an educational program delivered to students and their families. The current study re-examines parasite prevalence in the same area, post-treatment.

Materials and methods

This cross-sectional study was conducted to explore the intestinal-parasite infection rate among Karen children aged 5-13 years, studying in Ban Suan Phueng, Ban Tha Makham, and Ban Huai Phak primary schools, in Ratchaburi Province, on the western border of Thailand with Myanmar. The area is mountainous, with creeks and rivers, and the inhabitants mostly poor, uneducated Karen. Those with better educational opportunities reach their maximum level at elementary school, and live by farming and collecting wood (Fig 1).

In July 2007, students registered at 3 primary schools (total: 850) were requested to collect feces after obtaining informed consent from their parents. Each sample was processed as previously described [4]. Briefly, feces samples were examined when fresh by wet smear and Kato-Katz techniques [5]. Infection-rate data were collected and compared with those of the 2005 survey. The comparison was calculated statistically for significance at P-value < 0.05 using Chi-square.

To measure stool diagnostic efficiency, the remaining portion of each sample, after diagnosis by wet smear and Kato-Katz techniques, was stored in 10% formalin and transported to the laboratory for modified formalin ethyl acetate concentration examination, as described by Cheesbrough [6].

Students and their parents were immediately

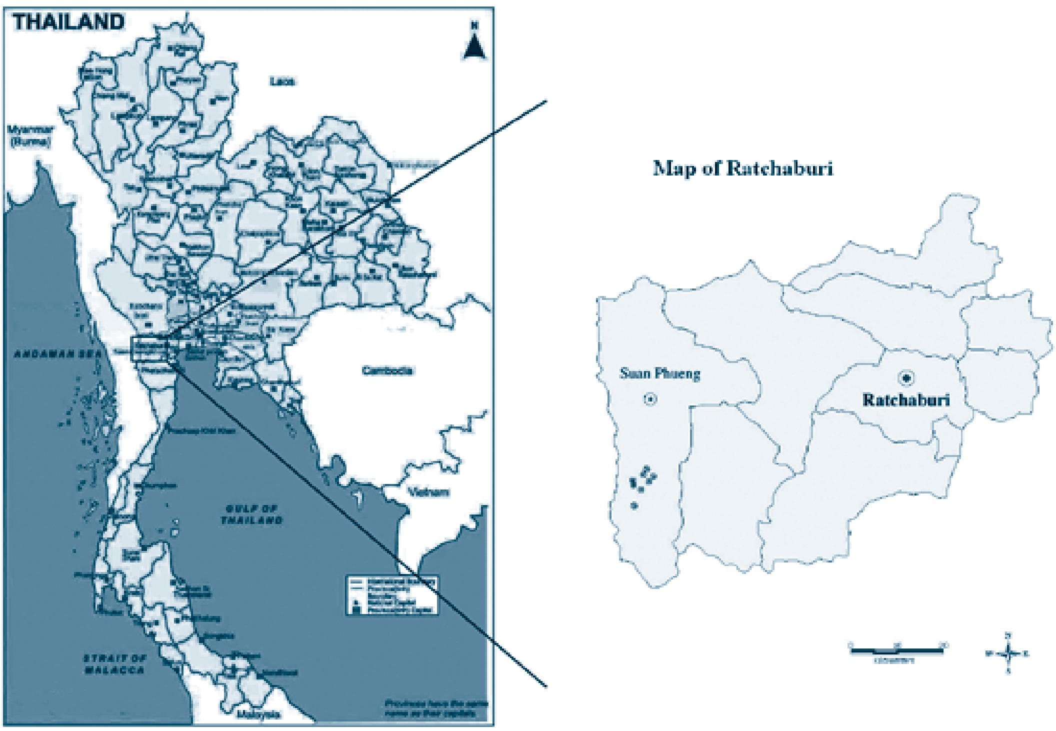

Fig 1 Ratchaburi Province, on the western border of Thailand.

informed if any intestinal-parasite infection was found. Each case was treated individually, according to the infection. A single dose of 500 mg mebendazole was prescribed for helminths, except for *Strongyloides stercoralis,* for which a dose of 400 mg albendazole was given for 3 consecutive days. Metronidazole (15 mg/kg/day for 7 days) was administered for protozoan infections.

Results

Infection rate

About 76% of students (646/850) were successfully enrolled in this study by delivering their stool samples appropriately. The details are shown in Table 1. There were 395 (61.1%) cases infected with at least one organism by combining the 2 detection techniques (wet smear and Kato-Katz). A comparatively high rate of infection was found in all schools, with the highest in Grade 1 (72.3%) and the lowest in Grade 5 (52.0%). When the modified formalin ethyl acetate concentration method was added, the overall infection rate increased slightly; however, the pattern of infection remained the same in each academic level. The details are shown in Table 2.

Comparative prevalence between the years 2005 and 2007

Using the same diagnostic methods, the prevalence increased significantly from 55.1% (2005) to 61.1% (2007). Intestinal-helminth infections, however, reduced slightly from 40.8 to 40.4%, whereas intestinal protozoan infections increased markedly, from 26.4 to 43.0%. The helminths found in the present study were of the same soil-transmitted types found previously, *ie*, hookworm, whipworm, *A. lumbricoides* and *E. vermicularis*. *Opisthorchis viverrini* were not detected, whereas more *S. stercoralis* were diagnosed in 4 cases.

The increase in protozoa infections consisted of both pathogenic and non-pathogenic types, similar to the previous report, with the addition of *Blastocystis hominis* and *Trichomonas hominis*. *E. coli* was the most common organism found in this study (24.5%), followed by *B. hominis* (17.5%). Pathogenic *G. lamblia* and *E. histolytica* were found at rates of 10.1 and 1.5%, respectively.

When modified formalin ethyl acetate concentration was added to the diagnostic set, an additional 5.3% infection rate was obtained.

Table 1 Enrolled samples, classified by academic grades and schools.

	Ban Tha Makham		Ban Huai Phak		Ban Suan Phueng		Total		
	Enrolled	Total	Enrolled	Total	Enrolled	Total	Enrolled	Total	%
Sex									
Male	121	159	111	146	93	122	325	427	76.1
Female	102	136	108	150	111	137	321	423	75.9
Level									
Nursery	53	77	44	65	52	57	149	199	74.9
Grade 1	28	45	30	39	25	39	83	123	67.5
Grade 2	21	26	29	39	32	39	82	104	78.8
Grade 3	27	32	41	49	24	33	92	114	80.7
Grade 4	32	39	12	19	29	34	73	92	79.3
Grade 5	33	44	39	51	26	32	98	127	77.2
Grade 6	29	32	24	34	16	25	69	91	75.8
Total	223	295	219	296	204	259	646	850	76.0

Table 2 Infection rates classified by academic grades.

	Number of samples	Infected cases by WS+ K n (%)	Infected cases by WS + K + F n (%)
Nursery	149	90 (60.4)	92 (61.7)
Grade 1	83	60 (72.3) **	64 (77.1) **
Grade 2	82	54 (65.8)	61 (74.4)
Grade 3	92	55 (59.8)	60 (65.2)
Grade 4	73	43 (58.9)	45 (61.6)
Grade 5	98	51 (52.0) **	58 (59.2) **
Grade 6	69	42 (60.9)	49 (71.0)
total	646	395 (61.1)	429 (66.4)

Abbreviations: wet smear (WS), Kato-Katz (K), modified formalin ethyl acetate concentration (F)
** $p \leq 0.01$

Table 3 Prevalence of intestinal parasites.

	2005 WS + K n (%)	2007 WS + K n (%)	2007 WS + K + F n (%)
Number of samples examined	701	646	646
Number of infected cases	386 (55.1)	395 (61.1)*	429 (66.4)*
Helminths	286 (40.8)	261 (40.4)	294 (45.5)
Hookworm	172 (24.5)	91 (14.1)***	118 (18.3)**
Whipworm	157 (22.5)	155 (24.0)	192 (29.7)**
Ascaris lumbricoides	55 (7.9)	112 (17.3)***	124 (19.2)***
Enterobius vermicularis	17 (2.4)	9 (1.4)	10 (1.5)
Opisthorchis viverrini	6 (0.9)	0 (0)	0 (0)
Strongyloides stercoralis	0 (0)	3 (0.5)	4 (0.6)
Protozoa	185 (26.4)	278 (43.0)*	356 (55.1)*
Entamoeba coli	99 (14.1)	158 (24.5)***	274 (42.4)***
Entamoeba histolytica	18 (2.6)	10 (1.5)	18 (2.8)
Giardia lamblia	59 (8.4)	65 (10.1)	79 (12.2)*
Endolimax nana	34 (4.9)	55 (8.5)**	92 (14.2)***
Cyclospora cayetanensis	1 (0.1)	0 (0)	0 (0)
Blastocystis hominis	0 (0)	113 (17.5)***	129 (19.9)***
Trichomonas hominis	0 (0)	8 (1.2)**	8 (1.2)**

Abbreviations: wet smear (WS), Kato-Katz (K), modified formalin ethyl acetate concentration (F)
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Helminth infections increased by 5.1% and protozoan infections increased significantly, by 12.1%. However, the overall pattern of infection, both helminths and protozoa, remained unchanged. The details are shown in Table 3.

Follow-up result

Two hundred and fifty-two (252) students examined in 2005 were successfully recruited from the 3 schools for reexamination, as shown in Table 4.

Wet-smear and Kato-Katz techniques showed that the infection rate increased significantly, from 119 (2005) to 148 (2007). Students infected with helminths alone evidently reduced by more than half. However, more students became infected with protozoa and both types of organisms. This increment was more than quadruple (4x).

When all 3 methods were used in diagnosis, more infected students were revealed. The number of students infected with helminths alone reduced to 24. On the other hand, protozoan infections increased (57), while mixed protozoan/helminthic infections increased dramatically (84). The details are shown in Table 5.

Re-infection was noted in 82 students, with 66 new infections with ≥ 1 parasite. Ban Huai Pak had the most persisting infections (42), whereas Ban Tha Makham had the most new cases (30). Only 35 students had positive-turn-negative

Table 4 Follow-up samples classified by school.

Number	Ban Suan Phueng			Ban Tha Makham			Ban Huai Phak		
tested	Total	Boy	Girl	Total	Boy	Girl	Total	Boy	Girl
252	73	32	41	78	41	37	101	45	56

Table 5 Infection rate comparison between year 2005 and 2007.

	2005 WS + K	2007 WS + K	2007 WS + K + F
Helminths	95	42 ***	24 ***
Protozoa	12	51 ***	57 ***
Both	12	55 ***	84 ***
Number infected	119	148 **	165 ***

** $p < 0.01$, *** $p < 0.001$

Table 6 Comparison of infection status for follow-up cases, 2005 and 2007, by wet-smear and Kato-Katz techniques.

Result 2005	Result 2007	Ban Suan Phueng 73	Ban Tha Makham 78	Ban Huai Phak 101	Total 252
+	+	23	17	42	82
+	-	8	12	15	35
-	+	18	30	18	66
-	-	24	19	26	69

results, whereas 69 remained negative from both diagnoses (Table 6). Details of the infecting organisms are described in Table 7.

Discussion

The current study explored intestinal parasitic infections among Karen children who attended schools in Ratchaburi Province, on the western border of Thailand with Myanmar, following up on the report of the year 2005. The infection rate increased significantly from 55.1 to 61.1%, mostly from protozoan infections. The intestinal-helminth infection rate remained unchanged. Nonetheless, high rates of infection were still found in this area, despite drug administration and a preventive program of health education. This suggests that the control program being in place may not be sufficiently effective to eradicate infection. This perhaps is due to difficulties in changing the habits of people in the area, such as the local diet and unhygienic lifestyle, which influence parasite infections, like the population of north-east Thailand [7].

A change in rare parasite infection was found. For the helminths, *Opisthorchis* infection was not detected in the current study, while *S. stercoralis,* which was not found in 2005, was noted in a few samples. For the protozoa, numerous specimens were infected with *B. hominis* and *T. hominis,* neither of which was detected before, but *C. cayetanensis* infection was no longer evident.

Whipworm remained the leading cause of infection among the helminths, and the infection rate for *A. lumbricoides* increased twice as much. However, hookworm infection reduced significantly. In contrast, protozoan infections increased significantly in almost all types, both pathogenic and non-pathogenic organisms. *E. coli* and *B. hominis* were diagnosed most frequently (Table 7).

High infection rates were clearly demonstrated in the follow-up study. It showed that high numbers of students were still being infected with at least one parasitic organism. The numbers of those who had been found negative, and later became infected, were alarmingly high. On the

Table 7 Helminth and protozoa intensity in the follow-up group (2005; 2007).

	2005 WS + K	2007 WS + K	2007 WS + K + F
Helminths			
Ascaris lumbricoides	32	37	43
Whipworm	61	64	78
Hookworm	57	32 **	38 **
Enterobius vermicularis	6	5	5
Opisthorchis species	2	0	0
Strongyloides stercoralis	0	1	1
Protozoa			
Giardia lamblia	7	22 **	27 ***
Entamoeba histolytica	1	5	8 **
Entomoeba coli	12	54 ***	105 ***
Endolimax nana	5	18 **	33 ***
Blastocystis hominis	0	49 ***	53 ***
Trichomonas hominis	0	1	1

Abbreviations: wet smear (WS), Kato-Katz (K), modified formalin ethyl acetate concentration (F)
** $p < 0.01$, *** $p < 0.001$

other hand, the numbers of students who were infected in 2005 and showed negative results in 2007 were rather low.

A combination of diagnostic methods proved beneficial in identifying true-negative samples, as described elsewhere [8]. Using a combination of wet smear and Kato-Katz, detection sensitivity was acceptably high. The Kato-Katz method is best for the diagnosis of helminths, but not protozoa, which can be compensated by wet smear [9]. However, by adding the modified formalin acetate method, diagnostic sensitivity increased significantly, especially for protozoa.

The sustained high infection rate may derive from several possible factors: (1) the unavoidable tropical climate provides a perfect niche for organisms to breed, resulting in dense numbers of parasites; (2) even though the schoolchildren had been treated with the appropriate dose of anti-parasitic drugs, not all infected children could be treated. Those who could not attend school regularly might have been missed by the program, not receive treatment, and remain infected, so becoming carriers of parasite infections; (3) the parents of the Karen schoolchildren were mostly uneducated or inadequately schooled, and this lack of education, combined with their habit of eating uncooked meat and inadequately washed fresh vegetables, was causing re-infection; (4) reservoir hosts are a significant target for parasites to reside. In this study, people lived in remote forested areas, where feral animals and domestic pets harbored parasites and acted as reservoir hosts, spreading organisms to humans. Both helminths (*eg T. trichiura* and *E. vermicularis*) and protozoa (*eg E. coli, E. histolytica, G. intestinalis* and *Blastocystis* species) can often be found in non-human primates [10,11] and companion animals, like dogs [12-14]. These animals may be another key factor in the zoonotic transmission of the gastro-intestinal parasitic diseases; (5) the anthelminthic drugs used nowadays cannot absolutely cure infection. Single-dose albendazole showed a high, but not complete, cure rate of 88% for *A. lumbricoides*, a rather low rate (28%) for *T. trichiura*, and 72% for hookworm [15]; and (6) drug resistance might be another reason. By using massive anti-helminth drugs worldwide, some people in remote areas may misuse or take inadequate amounts of the drug, causing helminths to survive the action of the chemical or develop drug-resistance mechanisms, as found in several nematodes [16,17].

In conclusion, intestinal parasitic infections persist among Karen children living in Suan Peung District, Ratchaburi Province , on the Thai-Myanmar border. A single dose of albendazole (400 mg), prescribed for infected children in 2005, failed completely to eradicate soil-transmitted helminth infections in this group of students. Moreover, the increase in protozoan infections was a remarkable discovery. New drugs are, therefore, needed–mebendazole and metronidazole–for helminth and protozoan infections, respectively. To control parasitic infections successfully, different strategies and concepts of treatment may help improve therapy. For example, students might be monitored with directly observed treatment (DOTS) more frequently, perhaps every trimester. Students' parents, guardians and their relatives living under the same roof must be included in the diagnosis and receive treatment simultaneously; this includes pets and wild animals in neighboring areas. Finally, multiple doses or combinations of drugs may be used for better cure rates, as reviewed and reported elsewhere [18,19].

Acknowledgements

Thanks to the staff, teachers, and the directors of Ban Suan Phueng, Ban Huai Phak and Ban Tha Makham schools for their kindness and diligent assistance. We also thank all staff from the Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, for the successful completion of this study. Special thanks to Mr Sompong Sripochang from the Department of Parasitology, Faculty of Medical Technology, Mahidol University, for enormous help in the laboratory. This work was partly supported by funds from the Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University.

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