INTESTINAL PARASITES IN SCHOOL-AGED CHILDREN IN VILLAGES BORDERING TONLE SAP LAKE, CAMBODIA

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Abstract. The objective of this study was to study *Schistosoma mekongi* and other intestinal parasitic infections, and intestinal symptoms and related complaints among school-age children and adolescents living around Tonle Sap Lake. Villages were selected where there were potential signs of schistosomiasis (hepatomegaly), and where subjects complained of intestinal symptoms. Stool samples were collected from 1,616 children and were examined by Kato-Katz, SAF concentration, and Baermann technique; short clinical examinations were also performed. No *S. mekongi* infection was detected, although a high level of intense human water contacts was reported. Helminth infection such as *Ascaris lumbricoides* (27.7%) and hookworms (29.7%) were common. *Trichuris trichiura* 4.4%), *Hymenolepis nana* (6.2%), *Giardia lamblia* (4.2%), and *Entamoeba* spp (14.4%) were also recorded. *Strongyloides stercoralis* was frequently diagnosed (20.2%). It was concluded that it is unlikely that *S. mekongi* is transmitted in Tonle Sap Lake. However, other intestinal parasitic infections are widespread. In particular, *S. stercoralis* should be considered an important etiologic agent in children and adolescents with abdominal complaints.

INTRODUCTION

Tonle Sap Lake, also known as "Great Lake", is located in central Cambodia. It is the largest freshwater lake in Southeast Asia. It is situated within the floodplain of the Mekong and represents a vast natural resource area. Fish, water plants, and the mineral-rich soil of the annually flooded shoreline attract human settlements. Water resources are intensively used, therefore, promoting the spread of water-borne infections, potentially also *Schistosoma mekongi*.

Schistosomiasis mekongi has been described in Kratie and Stung Treng Provinces in Cambodia, (Biays *et al*, 1999; Stich *et al*, 1999) and in Champassack Province of southern Lao PDR (Urbani *et al*, 2002). Although control efforts targeted against this severe parasitic infection have been undertaken in Lao PDR since 1983 and since 1994 in Cambodia, transmission continues (Ohmae *et al*, 2004) and patients with active infections of *S. mekongi* are still diagnosed in both countries. *Neotricula aperta*, the intermediate host snail, is found in the Mekong River but also in its tributaries, such as the Xe Kong River in southern Lao PDR and northern Cambodia (Attwood *et al*, 2004).

There has been considerable discussion whether the transmission of *S. mekongi* occurs in Tonle Sap Lake. On several occasions, patients with an *S. mekongi* infection were reported among refugees originating from the provinces bordering Tonle Sap Lake, in particular Battambang Province (Keittivuti *et al*, 1982a, 1982b, 1983). Furthermore, for health impact assessments of large-scale water resources development projects of the Mekong river system such as dams and lakes, an in-

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depth understanding of the suitable transmission area of *S. mekongi*, and therefore its presence in Tonle Sap Lake, is of great importance.

The objective of the present study was to investigate *S. mekongi* and other intestinal parasitic infections, and intestinal symptoms and related complaints among school-age children and adolescents.

MATERIALS AND METHODS

Study area and population surveyed

The study was conducted between December 1998 and June 1999. Districts of all provinces around Tonle Sap Lake were visited, except those of Siem Reap Province. School-age children were selected for the study according to the following procedure: first, health authorities at the district level were asked about villages where schistosomiasis-related morbidity was suspected (reports of hepatomegaly, or blood or mucus in stool). Second, in the identified villages, teachers and health staff were interviewed about children who had suspected symptoms of schistosomiasis. Third, in each village children with suspected symptoms were included; and/or a sample of schoolchildren and adolescents were randomly selected at the local school ($50 < n < 150$, depending on the size of the school) and enrolled in the study.

Field and laboratory procedures

A single stool sample was obtained from each participant and examined for intestinal parasites using the Kato-Katz thick smear technique (Katz *et al*, 1972). One slide was examined per stool. A random sub-sample (50%) of the stools was fixed in sodium acetate-acetic acid-formalin (SAF) and examined at the National Malaria Center, Phnom Penh, for the presence of helminthic and protozoal intestinal parasites using a concentration technique (Yang and Scholten, 1977). A further random sub-sample (10%) was examined on the survey day for the presence of *S. stercoralis* larvae using the modified Baermann technique (Watson and Al Hafidh, 1957).

Children were clinically examined for the presence of hepatomegaly and splenomegaly, and screened for the presence of blood and mucus in stool, intestinal complaints, and risk factors for intestinal parasitic infections (water contacts such as bathing, and fishing; wearing of shoes, and utilization of latrines).

Data management and statistical analysis

Data was entered in EpiInfo (version 6.0) and analyzed with STATA (Stata Cooperation, College Station, TX, USA). Rates and means were calculated for categorical and continuous data, respectively. Standard statistical procedures (95% confidence interval, χ^2 test, Student's *t*-test) were applied where appropriate.

The study was approved by the Ministry of Health, and provincial and district health departments. Oral consent was obtained from head of the villages and school teachers. Diagnosed infections were treated appropriately.

RESULTS

We visited 23 villages in all districts of four of the five provinces bordering the lake (approximately equivalent to two-thirds of its shoreline) (Table 1). A total of 1,616 children were enrolled from whom stool samples were obtained and examined using the Kato-Katz technique. Forty-nine percent (789) and 12% (188) of the stools were examined by SAF concentration and Baermann technique, respectively. Ninety-eight percent (1,584) were examined clinically.

Fifty-four percent (876) of the enrolled children were male. The sex ratio of the subsamples for SAF concentration technique (57% male, $p = 0.2$), Baermann technique (56% male, $p = 0.6$), and clinical examination (54% male, $p = 0.9$) did not differ significantly

Table 1
Sample distribution of the survey.

Province	Village	n / Village	n / Province
Kampong Thom	Kumrou	50	
	Kompong Chertoul	65	
	Tang Krasav	71	
	Koung Meac	65	
	Tang Kroung	60	
	Kompong Chean	61	
	Pol Hounl	64	
	Pobakor	57	
	Preak Sbouv	63	
	Techomarc	63	619
Kampong Chnang	Chnok Trou	108	
	Koh Ressey	84	
	Koh Thom	107	
	Koh Tkuv	75	
	Stung Chrouv	104	
	Ta Koh	79	557
Pursat	Kan Chor	103	
	Koh Chom	57	
	Kompong Loung	48	
	Snar Ansar	49	257
Battambang	Bak Prea	54	
	Koh Chivanh	69	
	Prey Chas	60	183
Total			1,616

from the overall sex ratio. The overall mean age was 11.2 years (95% CI: 11.1-11.4). The mean ages for the sub-samples were 11.3 (95% CI: 11.0-11.4), 11.5 years (95% CI: 11.2-11.8), 11.3 years (95% CI: 11.1-11.4) for the SAF concentration technique, Baermann technique, and clinical examination sub-samples, respectively. There were no significant differences between the mean ages of the overall sample and the sub-samples (SAF concentration technique: $p = 0.93$; Baermann technique: $p = 0.06$; clinical examination $p = 0.97$).

Table 2 summarizes the results of the parasitological examinations. We found no child with a *S. mekongi* infection. Furthermore, none of the health staff at provincial, district or village levels reported a child or adult with the typical signs and symptoms of advanced schistosomiasis.

The clinical examination of the children revealed only very low prevalence rates of hepatomegaly (4.0%) and splenomegaly (0.1%) (Table 3). Enlargement of the liver and spleen, when present, was modest.

Behavior risk factors for potential transmission of schistosomiasis were reported in high frequencies (Table 3). For example, 68.1% of the children reported that they do not have access to any latrine or toilet at home, and 34.5% reported regular recreational swimming and bathing in the lake. In addition, 7.0% mentioned regular fishing activities.

Table 2
Parasitological results.

	Kato Katz technique		SAF concentration		Baermann technique	
	n=1,616	(%)	n=789	(%)	n=188	(%)
Schistosoma mekongi	0		0		NA	
Ascaris lumbricoides	448	(27.7)	215	(27.1)	NA	
Trichuris trichiura	71	(4.4)	0		NA	
Hookworm	480	(29.7)	187	(23.6)	NA	
Strongyloides stercoralis	NA		21	(2.7)[a]	38	(20.2)
Hymenolepis nana	81	(5.0)	49	(6.2)	NA	
Enterobius vermicularis	12	(0.7)	2	(0.3)	NA	
Taenia sp	1	(0.1)	1	(0.1)	NA	
Entamoeba sp	NA		114	(14.4)	NA	
Giardia lamblia	NA		33	(4.2)	NA	

NA = not applicable, [a]larvae observed

Table 3
Results of the clinical examination, reported symptoms and risk factors (n = 1,584).

	Number	(%)
Clinical signs		
Hepatomegaly	63	(4.0)
Splenomegaly	2	(0.1)
Reported symptoms		
Diarrhea	703	(44.3)
Abdominal pain	872	(55.1)
Blood in stool	317	(20.0)
Mucus in stool	247	(15.6)
Risk factor		
No toilet/latrine at home	1,078	(68.1)
Recreational swimming	561	(35.4)
Fishing	125	(8.0)
Treatment with anthelminths	52	(3.3)
Wearing shoes (observed)	515	(32.6)
Shoes during defecation	988	(62.5)

However, other intestinal parasites were frequently encountered, such as *Ascaris lumbricoides* (Kato-Katz: 27.7%, SAF: 27.1%) and hookworms (Kato-Katz: 29.7%, SAF: 23.6%). *Hymenolepis nana* and *Entamoeba* cysts were also diagnosed with considerable frequencies. Others identified were *Enterobius vermicularis*, *Taenia* spp and *Giardia lamblia*. Twenty point two percent of the Baermann technique analysis yielded a positive result for *S. stercoralis* infection.

As expected, a high level of intestinal symptoms was reported. Within the previous three days, 44.3, 55.1, 20.0, and 15.6% of the children reported having had diarrhea, abdominal pain, and blood and mucus in their stool, respectively.

The risk factors for intestinal helminths were highly prevalent, thus assuring the transmission of soil-transmitted helminths. More than two-thirds of the children (68.1%) reported not having a latrine or toilet in the household. Only one-third of the children (32.6%) were wearing shoes on the survey day, and only two-thirds of them (62.5%) reported wearing shoes when defecating.

DISCUSSION

No *S. mekongi* infection has been detected in any of the more than one thousand persons examined by the two diagnostic tech-

niques. Neither the Kato-Katz technique (the standard diagnostic field approach) nor the SAF concentration technique (used to screen light infections) obtained any infection with this parasite. Furthermore, typical clinical signs, such as hepatosplenomegaly, were rare and of low degree only. This was not comparable to the clinical burden detected in the schistosomiasis endemic province of Kratie where, in 1995, more than half of the population had hepatomegaly (Biays *et al*, 1999).

The Mekong River contributes heavily to the annual flooding of Tonle Sap Lake via the Tonle Sap River. During this event the intermediate snail host, *N. aperta*, can easily be transported into the lake. However, the main ecological features of the shoreline of the Grant Lake are distinctly different from those of the Mekong river shores. The lake's water is stagnant; its'bed is mainly clay, which produces turbid waters, and this is likely less favorable for sustaining *N. aperta* snails, which require predominantly clear waters, and sandy or rocky substrates (Urbani *et al*, 2002). Snail surveys should be conducted in the future to confirm these observations. Finally, additional studies carried out in the province of Kampong Chnang confirmed the absence of *S. mekongi* in schoolchildren (Sinuon *et al*, 2003).

The present study confirmed the high level of soil-transmitted helminths and associated risk-factors for transmission that have been largely documented in Southeast Asian countries. However, more importantly, it showed that *S. stercoralis* may reach substantial prevalence rates. This parasite is rarely diagnosed in surveys because it requires a specific concentration technique (Baermann technique). Clinically, this infection may lead to skin (larva currents) or to gastro-intestinal diseases. In immune-depressed subjects, *S. stercoralis* may lead to systemic disease due to its ability for autoinfection. Therefore, infection with *S. stercoralis* requires more attention.

Our study demonstrated that schistosomiasis mekongi is not present in the studied villages along the Tonle Sap Lake, and that it is very unlikely that it is transmitted in Tonle Sap Lake. This conclusion is of importance to water development projects that create artificial lakes featuring similar ecological settings.

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REFERENCES

Attwood SW, Campbell I, Upatham ES, *et al*. Schistosomes in the Xe Kong river of Cambodia: the detection of *Schistosoma mekongi* in a natural population of snails and observations on the intermediate host's distribution. *Ann Trop Med Parasitol* 2004; 98: 221-30.

Biays S, Stich AH, Odermatt P, *et al*. [A foci of Schistosomiasis mekongi rediscovered in Northeast Cambodia: cultural perception of the illness; description and clinical observation of 20 severe cases]. *Trop Med Int Health* 1999; 4: 662-73.

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.

Keittivuti B, D'Agnes T, Keittivuti A, *et al*. Prevalence of schistosomiasis and other parasitic diseases among Cambodian refugees residing in Bang-Kaeng holding center, Prachinburi Province, Thailand. *Am J Trop Med Hyg* 1982a; 31: 988-90.

Keittivuti B, Keittivuti A, D'Agnes T. Schistosomiasis in Cambodian refugees at Ban-Kaeng Holding Center, Prachinburi Province, Thailand. *Southeast Asian J Trop Med Public Health* 1982b; 13: 216-9.

Keittivuti B, Keittivuti A, O'Rourke TF. Parasitic diseases with emphasis on schistosomiasis in Cambodian refugees, in Prachinburi Province Thailand. *Southeast Asian J Trop Med Public Health* 1983; 14: 491-4.

Ohmae H, Sinuon M, Kirinoki M, *et al*. Schistosomiasis mekongi: from discovery to control. *Parasitol Int* 2004; 53: 135-42.

Sinuon M, Anantaphruti MT, Socheat D. Intestinal helminthic infections in schoolchildren in Cambodia. *Southeast Asian J Trop Med Public Health* 2003; 34: 254-8.

Stich AH, Biays S, Odermatt P, *et al*. Foci of Schistosomiasis mekongi, Northern Cambodia: II. Distribution of infection and morbidity. *Trop Med Int Health* 1999; 4: 674-85.

Urbani C, Sinoun M, Socheat D, *et al*. Epidemiology and control of mekongi schistosomiasis. *Acta Trop* 2002; 82: 157-68.

Watson JM, Al Hafidh R. A modification of the Baermann funnel technique, and its use in establishing the infection potential of human hookworm-carriers. *Ann Trop Med Parasitol* 1957; 51: 15-6.

Yang J, Scholten T. A fixative for intestinal parasites permitting the use of concentration and permanent staining procedures. *Am J Clin Pathol* 1977; 67: 300-4.