

# Skin Test for Paragonimiasis among Schoolchildren and Villagers in Namback District, Luangprabang Province, Lao PDR

Hyun-Ouk Song<sup>1</sup>, Duk-Young Min<sup>1,\*</sup>, Han-Jong Rim<sup>2</sup>, Vonghachack Youthanavanh<sup>3</sup>, Bouakhasith Daluny<sup>3</sup>, Vongsouvan Sengdara<sup>3</sup>, Banouvong Virasack<sup>4</sup> and Phommasak Bounlay<sup>5</sup>

<sup>1</sup>Department of Parasitology, Hanyang University College of Medicine, Seoul 133-791, Korea; <sup>2</sup>Korea Association of Health Promotion, Seoul 157-704, Korea; <sup>3</sup>Department of Parasitology, Faculty of Medical Sciences, National University of Laos, Vientiane, Lao PDR; <sup>4</sup>Malaria Center, Luangprabang, Lao PDR; <sup>5</sup>Department of Hygiene and Prevention, Ministry of Health, Vientiane, Lao PDR

**Abstract:** As a part of a broader effort to determine the status of *Paragonimus* species infection in Lao PDR, an epidemiological survey was conducted on villagers and schoolchildren in Namback District between 2003 and 2005. Among 308 villagers and 633 primary and secondary schoolchildren, 156 villagers and 92 children evidenced a positive reaction on a *Paragonimus* skin test. Only 4 schoolchildren out of 128 skin test-positive cases had *Paragonimus* sp. eggs in their sputum, all of which was collected on 1 day. Several types of crabs, which were identified as the second intermediate host of the *Paragonimus* species, were collected from markets and streams in a paragonimiasis endemic area for the inspection of metacercariae. Among the examined crabs, only "rock crabs" (*Indochinamon ou*) harbored *Paragonimus* sp. metacercariae, and it is speculated that the life cycle of *Paragonimus* sp. was maintained via rock crabs in Namback District, Lao PDR.

**Key words:** *Paragonimus*, paragonimiasis, skin test, crab, Lao PDR

Human paragonimiasis, which is caused by the lung fluke, *Paragonimus* species, is a globally distributed parasitic infection, and occurs frequently in Far East Asia, Southeast Asia, Central America, and West Africa [1-3]. Since the first description of human paragonimiasis in hill tribes men in San Neua Province, Laos (Lao People's Democratic Republic; Lao PDR) in 1947 [4], several clinical reports have been published. According to Soh [5], Pathammavong reported 14 *Paragonimus* egg-positive cases out of 1,939 examined for tubercle bacilli in 1967, and Miyazaki and Fontan [6] confirmed a case of *Paragonimus heterotremus* infection after the autopsy of a Laotian man in Vientiane. During the Vietnam War, Kirkely [4] detected 46 pulmonary paragonimiasis patients in Ban Xong refugee camps, and treated them with a combination of emetin hydrochloride and chloroquine phosphate, as well as with bithionol. The patients among the refugees belonged primarily to 3 hill tribes, the Meo, the Lao Theung, and the Laos. They moved from Sam Neua Province along the north Vietnamese border to southern Xieng Khuang and northern Vientiane province. At the same time in 1973, a nationwide epidemiological survey of paragonimiasis was con-

ducted by Soh [5].

Despite the definite cases of clinical paragonimiasis detected among Indochinese refugees in the United States, Australia, and other countries after 1975, little attention has been paid to the relationship between host and *Paragonimus* sp. in Laos [7]. For the purpose of preparing the basic information on paragonimiasis, field investigations were conducted in Laos between February 2003 and November 2005 under the auspices of the Korea-Lao Parasite Control Project.

During Korea-Laos project of the Korea Association of Health Promotion (KAHP) and the Korea International Cooperation Agency (KOICA) until 2003, Namback District in Luangprabang province was recommended for a survey area of paragonimiasis by Prof. V. Sengdara (Faculty of Medical Sciences, National University of Laos, Vientiane, Laos). An epidemiological study was conducted in the Phonsavanh and Phonkeo villages, as well as nearby schoolchildren to provide basic information about paragonimiasis. A total of 308 villagers (166 females and 142 males), and 633 schoolchildren (302 females and 331 males) were subjected to intradermal skin tests. Veronal buffered saline-extracted whole worm antigen of *Paragonimus westermani* (Shinpoong Pharm. Co. Ltd., Seoul, Korea) was inoculated into the forearm, and the wheal size was measured 15 min later. A

• Received 24 May 2008, accepted after revision 22 August 2008.

\* Corresponding author (dymin@hanyang.ac.kr)

wheel measuring in excess of 60 mm<sup>2</sup> was considered as a positive reaction according to the manufacturer's instructions.

Among the 308 villagers, 156 (62 females and 94 males) evidenced a positive reaction, and the male villagers exhibited a higher positive rate (66.2%) than was seen in the female villagers (37.3%). The higher positive age groups were the 45-49 (68.4%), 55-59 (62.5%), and 25-29 (62.2%) yr age groups, respectively. The most prevalent positive age group was males aged 45-49 (88.9%), in which 8 of 9 tested village men had positive reactions on the skin test (Table 1).

The skin test positive reaction rate in schoolchildren was 14.5% and with the exception of class 4 in the primary school, the positive rate of the higher classes was approximately twice as high as that detected in class 3 in the primary school. Unlike what was observed with the villagers, no difference in positive reactivity was seen between boys and girls in the tested schoolchildren (Table 2). Additionally, the sputum of 128 skin test-positive reactors was examined for the presence of *Paragonimus* sp. eggs. Unlike the relative high rate of *Paragonimus* skin test results, eggs were observed in the sputum of only 4 of 128 total

positive cases, and 3 of these egg-positive samples were from school boys (2 were 13-yr-old and 1 was 14-yr-old) (data not shown). These dissonances of positive rate between skin and sputum test of paragonimiasis may be speculated due to incorrect collection of sputum by young children.

The majority of schoolchildren reported numerous experiences of eating roast crabs in the field. On the other hand, the adult villagers reported frequent consumption of seasoned crabs (Tan Cheoy Koun) and papaya salad (Tammack Koun) with crushed raw crab. In addition to this characteristic feature of the villagers' food culture, the denizens of this area drink fresh crab juice as a traditional cure for measles, and this was also thought to constitute a route for infection. Some of the positive reactors, both the schoolchildren and the villagers, complained of chronic cough, bloody sputum, mild chest pain, fish smell, and lassitude. These symptoms disappeared after treatment with praziquantel (25 mg/kg, 3 times a day for 2 days).

Crab was the only second intermediate host for *Paragonimus* sp. in Namback District. In order to identify the second intermediate host of *Paragonimus* sp. in Namback District, several types of crabs, i.e., the rock, rice-field crab, and iron crab in common names, were collected from streams, rice fields, and the Namback market, all of which are located near the residences of the villagers and schoolchildren.

In our examination for *Paragonimus* sp. metacercariae, the collected crabs were crushed individually or as a batch, then digested with artificial gastric juice (0.6% pepsin in 0.85% NaCl)

**Table 1.** Results of the skin test for paragonimiasis by age and sex in inhabitants of Phonkeo and Phonsavanh villages, Namback District, Luangprabang province, Lao PDR

Age	No. examined	Male		No. of positive (%)	Total (%)
		Female	Male		
15-19	28	14	7 (50.0)	9 (32.1)	
		14	2 (14.3)		
-24	43	16	12 (75.0)	23 (53.5)	
		27	11 (40.7)		
-29	37	14	8 (57.1)	23 (62.2)	
		23	15 (65.2)		
-34	35	20	14 (70.0)	18 (51.4)	
		15	4 (26.7)		
-39	31	10	8 (80.0)	16 (51.6)	
		21	8 (38.1)		
-44	46	22	15 (68.2)	26 (56.5)	
		24	11 (45.8)		
-49	19	9	8 (88.9)	13 (68.4)	
		10	5 (50.0)		
-54	24	13	5 (38.5)	7 (29.2)	
		11	2 (18.2)		
-59	16	11	9 (81.8)	10 (62.5)	
		5	1 (20.0)		
-64	10	4	2 (50.0)	3 (30.0)	
		6	1 (33.3)		
≥ 65	19	9	6 (66.7)	8 (42.1)	
		10	2 (20.0)		
Total	308	142	94 (66.2)	156 (50.6)	
		166	62 (37.3)		

**Table 2.** Results of the skin test for paragonimiasis in primary and secondary schools in Namback District, Luangprabang province, Lao PDR

		Class		No. examined	No. of positive (%)
Primary school	3	Male	82	5 (6.1)	
		Female	86	6 (6.8)	
		Subtotal	170	11 (6.5)	
	4	Male	116	25 (21.6)	
		Female	115	25 (21.7)	
		Subtotal	231	50 (21.6)	
	5	Male	85	12 (14.1)	
		Female	73	10 (13.7)	
		Subtotal	158	22 (13.9)	
Secondary school	2	Male	48	8 (16.7)	
		Female	26	1 (3.8)	
		Subtotal	74	9 (12.2)	
		Male	331	50 (15.1)	
		Female	302	42 (13.9)	
		Total	633	92 (14.5)	

for 2 hr, and washed 3 times with normal saline. After filtration with a fine sieve, the digested crab materials were examined with a stereomicroscope. Metacercariae of *Paragonimus* sp. were isolated from rock crabs only, and not from any other kinds of crabs, and the rock crabs were identified as *Indochinamon ou* by its morphology [8,9]. Both the infection rate and density of metacercariae in the crabs were generally low.

Paragonimiasis still occurs all over the world, including Laos. In the present study, human paragonimiasis was confirmed in Nambak District of Luangprabang province, Lao PDR via skin tests, sputum studies, and identification of *Paragonimus* sp. metacercariae. After the Vietnam War, paragonimiasis was frequently reported in Laotian refugees throughout the world. In Chicago, 3 Laotian refugee children with chronic pulmonary complaints were diagnosed as paragonimiasis via the identification of *P. westermani* eggs in stool, sputum, and bronchoscopic examinations [10]. In California State, 4 pulmonary paragonimiasis cases were reported in refugees from Southeast Asia, and 3 of these were Laotians [3]. A 25-yr-old Laotian farmer living in Australia demonstrated numerous *Paragonimus* eggs upon a cytological examination of bronchial washing, which was undertaken in the process of tuberculosis treatment [11]. At least 2 *Paragonimus* sp. (*P. heterotremus* and *P. westermani*) have been identified as causative agents of human paragonimiasis in Laos [12,13]. Moreover, due to the fact that *Paragonimus* infection is frequently accompanied by tuberculosis or pulmonary complaints, and the annual risk of tuberculosis infection in Luangprabang has been estimated to be 0.6%, further study into *Paragonimus* sp. in Luangprabang, Laos is still necessary [14,15].

Until the present, several studies of *Paragonimus* infection have been conducted in Vientiane province. Among several species of crabs, freshwater snails, and prawns in the Nam Set River near the Vientiane province, 4 types of *Paragonimus* metacercariae (*P. westermani*, *P. heterotremus*, *P. harinasutai*, and *P. bangkokensis*) have been identified [16]. Moreover, Yahiro et al. [13] collected *Paragonimus* eggs from the sputum of 6 paragonimiasis patients and identified them as *P. heterotremus* [13].

In our study, we confirmed the presence of human paragonimiasis in Luangprabang province, Lao PDR, and the life cycle of *Paragonimus* sp. was shown to be perpetuated through the rock crab (*Indochinamon ou*). Although this study provides some basic information regarding paragonimiasis in Luangprabang province, the ecology and roles played by snail and crab intermediate hosts remain to be clearly elucidated. Furthermore, the

clinical characteristics of human infection with this parasite remain unclear. For this reason, an integrated study of *Paragonimus* infection in this region should be undertaken in order to better understand the host-parasite relationships.

## ACKNOWLEDGEMENTS

The authors wish to thank Dr. Hironori Komatsu, National Museum of Nature and Science, and Professor Takeshi Agatsuma, Kochi Medical School, Japan, for their help with the identification of the crabs examined in this study. Additionally, the authors also wish to thank Mr. Han-Gyoo Choi, Department of Parasitology, Hanyang University College of Medicine for his technical assistance during this study.

## REFERENCES

1. Pachucki CT, Levandowski RA, Brown VA, Sonnenkalb BH, Vruno MJ. American paragonimiasis treated with praziquantel. *N Engl J Med* 1984; 311: 582-583.
2. Hillyer GV, Apt W. Food-borne trematode infections in the Americas. *Parasitol Today* 1997; 13: 87-88.
3. Yee B, Hsu JI, Favour CB, Lohne E. Pulmonary paragonimiasis in Southeast Asians living in the central San Joaquin Valley. *West J Med* 1992; 156: 423-425.
4. Kirkley SE. Treatment of active paragonimiasis in north central Laos. *Yonsei Rep Trop Med* 1973; 4: 78-87.
5. Soh CT. Epidemiological investigation of *Paragonimus* infection in Laos. *Yonsei Rep Trop Med* 1973; 4: 65-77.
6. Miyazaki I, Fontan R. Mature *Paragonimus heterotremus* found from a man in Laos. *Japanese Journal of Parasitology* 1970; 19: 109-113 (in Japanese).
7. Johnson RJ, Johnson JR. Paragonimiasis in Indochinese refugees. Roentgenographic findings with clinical correlations. *Am Rev Respir Dis* 1983; 128: 534-538.
8. Yeo DCJ, Ng PKL. Freshwater crabs of the *Potamon tannanti* species group (Crustacea: Decapoda: Brachyura: Potamidae) from northern Indochina. *Raffles Bull Zool* 1998; 46: 627-650.
9. Yeo DCJ, Ng PKL. On the genus "Potamon" and allies in Indochina (Crustacea: Decapoda: Brachyura: Potamidae). *Raffles Bull Zool* 2007; 16 (suppl1): 273-308.
10. Burton K, Yegor R, London N, Boyer K, Shulman ST. Pulmonary paragonimiasis in Laotian refugee children. *Pediatrics* 1982; 70: 246-248.
11. Brown RW, Clarke RJ, Denham I, Trembath PW. Pulmonary paragonimiasis in an immigrant from Laos. *Med J Aust* 1983; 2: 668-669.
12. Tran DS, Nanthapone S, Odermatt P, Strobel M. A village cluster of paragonimiasis in Vientiane Province, Lao PDR. *Southeast Asian J Trop Med Public Health* 2004; 35: 323-326.

13. Yahiro S, Habe S, Duong V, Odermatt P, Barennes H, Strobel M, Nakamura S. Identification of Human paragonimiasis causative agents In Lao PDR. *J Parasitol* 2008; DOI: 10.1645/GE-1457.1 (in press).
14. Arnadottir TH, Soukaseum H, Vangvichit P, Bounmala S, Vos E. Prevalence and annual risk of tuberculosis infection in Laos. *Int J Tuberc Lung Dis* 2001; 5: 391-399.
15. Strobel M, Veasna D, Saykham M, Wei Z, Tran DS, Valy K, Odermatt P, Dreyfus G. Pleuro-pulmonary paragonimiasis. *Med Mal Infect* 2005; 35: 476-481 (in French).
16. Odermatt P, Habe S, Manichanh S, Tran DS, Duong V, Zhang W, Phommathet K, Nakamura S, Barennes H, Strobel M, Dreyfuss G. Paragonimiasis and its intermediate hosts in a transmission focus in Lao People's Democratic Republic. *Acta Trop* 2007; 103: 108-115.