PARAGONIMIASIS PREVALENCES IN SARABURI PROVINCE, THAILAND, MEASURED 20 YEARS APART

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Abstract. Saraburi Province, Central Thailand has been a paragonimiasis-endemic area since 1956. This study compared the prevalences of human paragonimiasis in two villages near Chet Khot Waterfall, Kaeng Khoi District, investigated in 1984-1985 and 2005. The results from the 1980s showed 6.3% and 1% of villagers were positive for *Paragonimus* eggs in sputum and stool, respectively. In 2005, *Paragonimus* eggs were not found in feces or sputum. An IgG-ELISA for paragonimiasis was conducted on 33 serum samples collected in the 1980s, 23 collected in 2005 and 25 diagnosed with other parasitic infections. Ninety percent of the samples from the eighties were positive for paragoimiasis, and 43% from 2005 were positive, equivalent to 10.9% and 4.9% of the total population examined in the 1980s and 2005, respectively. Serodiagnosis is currently the best method for detecting paragonimiasis. The positive cases in the 1980s were age 10-60 years and in 2005 were age 34-67 years old. The prevalence and intensity of *Paragonimus* metacercariae in fresh waterfall crabs collected from Chet Khot Waterfall were significantly lower in the 1980s than in 2005. The prevalence of paragonimiasis in this endemic area has decreased to the level that no egg-producing cases were detected. No infections were found in villagers age <30 years, despite the high density of metacercariae in the crabs, indicating a change in the habit of eating raw food among the younger people.

INTRODUCTION

Paragonimiasis is an important parasitic zoonosis in the tropics. Humans become infected by eating fresh- or brackish-water crustaceans containing live metacercariae, or by ingesting raw meat from mammals acting as paratenic hosts (Miyazaki, 1991). *Paragonimus westermani* is the most common species and is widely distributed in Asia, including Japan, China, Taiwan, Korea, India, Russia, Indonesia, The Philippines, Vietnam, Cambodia, Lao PDR, Nepal, Pakistan, and Thailand; the species exhibits much biological variation. *Paragonimus heterotremus* is distributed in China, Vietnam, Lao PDR, and Thailand (Blair *et al*, 1999). Both paragonimiasis and tuberculosis cause pulmonary disease with similar clinical manifestations, especially hemoptysis, which frequently confuses diagnosis (Toscano *et al*, 1995). ELISA has been used widely in the immunodiagnosis of paragonimiasis, especially to detect specific antibodies (Cho *et al*, 1981; Lee and Choi, 1983; Waikagul, 1989; Maleewong *et al*, 1997; Mukae *et al*, 2001).

The infective metacercarial stages of *Paragonimus westermani* and *P. heterotremus*, two human species, are found in Thailand.

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P. heterotremus is the only species proven to cause human paragonimiasis as confirmed by worms recovered from patients (Vanijanonta *et al*, 1981). In Central Thailand, Saraburi and Nakhon Nayok Provinces have been recognized as paragonimiasis-endemic areas. During 1957-1965, several paragonimiasis cases were reported, and epidemiological surveys using sputum and stool examinations showed that the prevalence varied in these areas (1.3-6.6%) (Vajrasthira *et al*, 1959; Sirisumpun, 1963).

During 1984-1985, members of our group investigated paragonimiasis in Cha-om District, Saraburi Province, and positive cases were treated. Disease-preventive education was provided at schools, and was also given to all villagers (unpublished data). Recently, we received two serum samples from residents of Cham Phak Phaeo Subdistrict, Cha-om District, a paragonimiasis-endemic area previously studied, for discriminatory diagnosis between paragonimiasis and tuberculosis; immunoblot confirmed paragonimiasis.

To study the trend of the paragonimiasis problem in the Chet Khot Waterfall vicinity, we compared the paragonimiasis prevalence of communities closest to Chet Khot Waterfall: Bung Mai in 1984-1985 and Pong Kon Sao in 2005. The prevalence and intensity of *Paragonimus* metacercariae in crabs collected from Chet Khot Waterfall in 1984-1985 and 2005 were also compared.

MATERIALS AND METHODS

Study sites

The study site in June 1984-March 1985 was Bung Mai Village, Cha-om Subdistrict, Kaeng Khoi District, Saraburi Province, the nearest village to Chet Khot Waterfall at that time. The study site in 2005 was Pong Kon Sao Village, Tha Maprang Subdistrict, Kaeng Khoi District, adjacent to the previous study site, and the nearest village to Chet Khot Waterfall at that time. These two villages are about 134 km north of Bangkok (Fig 1). At that time, Bung Mai village had 150 households; 145 houses were investigated for paragonimiasis. Bung Mai Village has now become Hin Tang and Chet Khot villages. Pong Kon Sao Village was established after 1985, and has 148 households, with 212 males and 211 females.

Sample collection

Plastic bags with applicators were provided to the villagers, to collect about 5 grams of stool specimens. A total of 304 and 203 samples were submitted in the 1980s and 2005, respectively. Sputum and serum samples

Fig 1–Map of Thailand with study location.

obtained from villagers with signs and symptoms of paragonimiasis, and having a history of eating improperly cooked waterfall crab, were collected. Thirty-three and 23 villagers submitted samples in 1984-1985 and 2005, respectively.

The crabs, *Larnaudia larnaudii*, were collected from under rocks in the tributary streams of Chet Khot Waterfall in Saraburi Province, from June 1984 to March 1985, and January-February 2005.

The study in 2005 was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, and all subjects provided informed consent.

Parasitological examination

The following methods apply to both the 1980s and 2005 investigations. Feces were examined by cellophane thick-smear technique (Kato and Miura, 1954). Sputum samples were dissolved in 1% NaOH for a few minutes and centrifuged at 2,500 rpm for 2-3 minutes; the sediment was then examined under a light microscope for *Paragonimus* eggs (Suzuki, 1981).

Crabs were identified by species and sorted by size. The carapaces were removed and the gills, hearts, viscera, and muscles examined separately by compression between large glass plates under a stereomicroscope. The legs were ground by blender in physiological saline. The separation was poured into a sedimentation flask and allowed to stand for 30 minutes; this stage was repeated until the supernatant became clear, then, the sediment was examined for metacercariae under a stereomicroscope. The species and numbers of metacercariae were recorded.

Serological examination

Indirect enzyme-linked immunosorbent assay (ELISA). Optimal coating-antigen concentration conditions, test serum and conjugate dilutions were used after checkerboard titration. Briefly, *P. heterotremus* crude antigen (Dekumyoy *et al*, 1995) was diluted to a concentration of 2 μg/ml. Each microELISA well (Nunc) was sensitized with the diluted antigen and incubated at 37ºC following overnight coating. After blocking the unbinding sites of the wells, the diluted serum samples (1:800) reacted with the antigens. The immune complex was then combined with diluted rabbit anti-human immunoglobulin G peroxidase conjugate (1:1,000) (Dakopatt). The reactions were visualized with substrate, azino diethylbenzothiazoline sulfonic acid (ABTS, Sigma) [the substrate in 1988 was p-phenylenediamine (PPD)] and optical density values were measured at 405 nm.

The normal sera and the samples from 25 diseases were tested in the study namely: opisthorchiasis, clonorchiasis, fascioliasis, schistosomiasis, echinococcosis, neurocysticercosis, taeniasis, hymenolepiasis nana, sparganosis, hookworm infection, strongyloidiasis, ascariasis, toxocariasis, enterobiasis, trichuriasis, angiostrongyliasis, trichinellosis, capillariasis, gnathostomiasis, Bancroftian filariasis, Malayan filariasis, giardiasis, amebiasis, blastocystosis, and toxoplasmosis.

Patients with either sample positive for paragonimiasis were treated with 10-15 doses of bithionol, at 40 mg/kg on alternate days (Kim, 1970) in the 1980s, and in 2005 with praziquantel at 25 mg/kg three times daily after meals for 3 days (Srisont, 1983).

RESULTS

The investigation in the 1980s showed a paragonimiasis prevalence of 1% in the stool and 6.3% in the sputum (Table 1). The IgG ELISA system resulted in a sensitivity of 90% and a specificity of 96.6%, at cut-off OD of 1.040. The paragonimiasis sera ODs ranged from 0.821 to 1.426 (1.203 ± 0.141) (Fig 2). The ages of the positive cases ranged from 10 to 60 years.

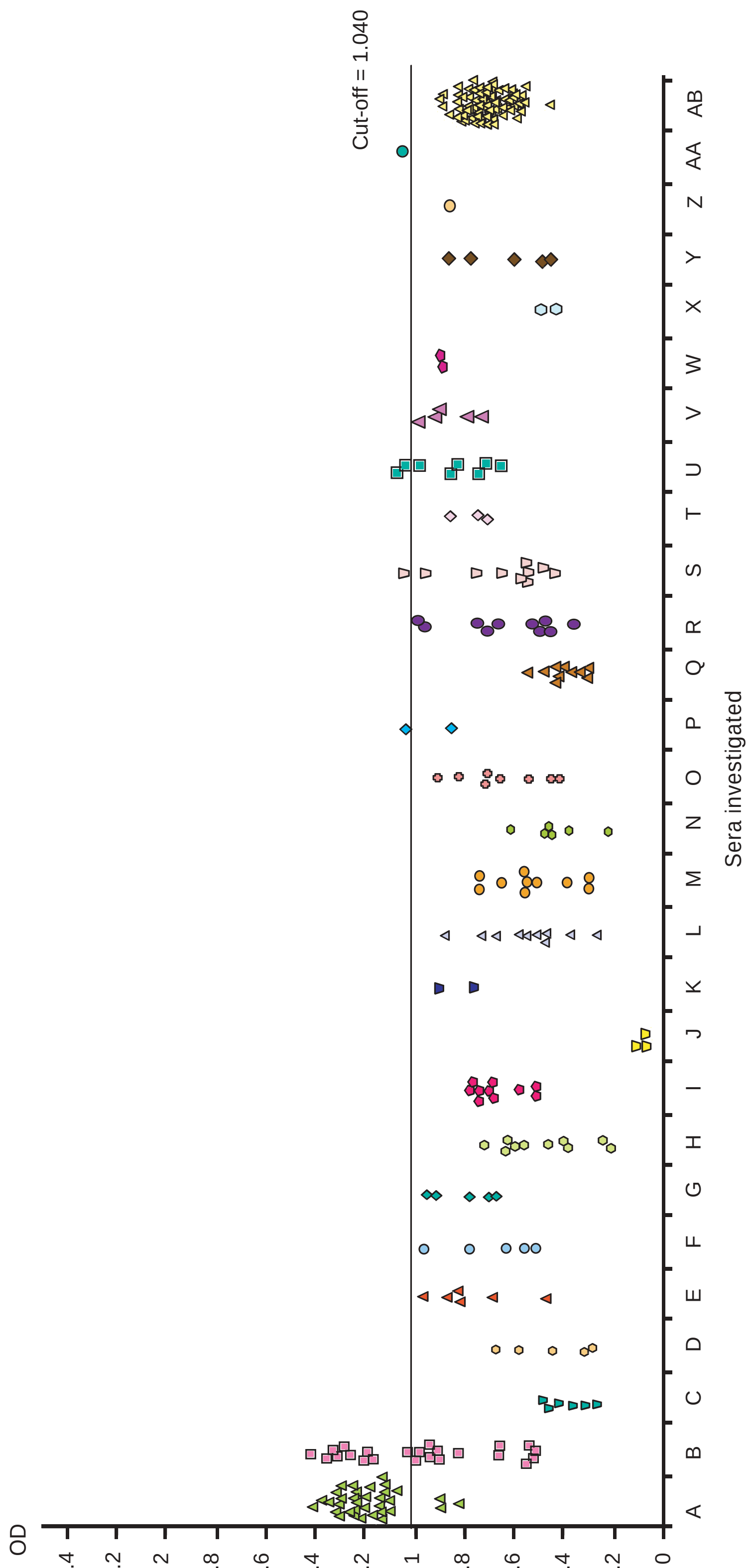

Fig 2–Scattering of ODs-ELISA produced from delipidized antigen of *P. heterotremus* adult worms against 33 and 23 serum samples in the 1980s and in 2005, respectively, 25 different helminthic infections, and healthy controls, (A = Collected serum samples in the 1980s, B = Collected serum samples in 2005, C = opisthorchiasis, D = clonorchiasis, E = fascioliasis, F = schistosomiasis, G = echinococcosis, H = neurocysticercosis, I = taeniasis, J = hymenolepiasis nana, K = sparganosis, L = hookworm infection, M = strongyloidiasis, N = ascariasis, O = toxocariasis, P = enterobiasis, Q = trichuriasis, R = angiostrongyliasis, S = trichinellosis, T = capillariasis, U = gnathostomiasis, V = Bancroftian filariasis, W = Malayan filariasis, X = giardiasis, Y = amebiasis, Z = blastocystosis, AA = toxoplasmosis, AB = normal sera).

Table 1
Paragonimus egg-positive cases (feces and sputum), serum-positive paragonimiasis cases, Bung Mai Village, Cha-om Subdistrict, Kaeng Khoi District, Saraburi Province, June 1984-March 1985, and Pong Kon Sao Village, Tha Maprang Subdistrict, Kaeng Khoi District, Saraburi Province, May 2005.

Samples	1980s		2005	
	No. examined	No. positive (%)	No. examined	No. positive (%)
Feces	304	3 (1.0)[a]	203	0
Sputum	95	6 (6.3)[b]	23	0
Serum	33	30 (90.9)[a]	23	10 (43.5) [a]

[a] % of total studied population

Table 2
Infection rates of *Paragonimus heterotremus* metacercariae in mountain crabs (*Larnaudia larnaudii*) from Chet Khot Waterfall.

Dates of collection	No. crabs		No. metacercariae recovered
	Examined	Positive(%)	
Jun 1984-Mar 1985	671	141 (21.0)	287
Jan-March 2005	729	262 (35.9)	856

In the 2005 study, microscopic examination showed no *Paragonimus* eggs in the sputum or stool samples (Table 1). ELISA results for the 23 serum samples collected in 2005 showed 10 cases (43.5%) had OD values greater than the cut-off OD of 1.040. Cross-reactivity occurred with 4 diseases: 2 cases of gnathostomiasis (ODs, 1.11 and 1.087), and 1 case each of enterobiasis (1.050), trichinellosis (1.077), and toxoplasmosis (1.065) (Fig 2). The paragonimiasis prevalence in 2005 was lower than in the 1980s. Among the 3 methods examined, antibody detection gave the highest result.

The average size of the male crabs was 3.6x2.9 cm, while the females were 3.4x2.7 cm. The overall infection rate was 35.9%. Of these, 183 (25.1%) males and 79 (10.8%) females were positive. Female crabs had a significantly lower infection rate than males (χ^2 = 7.55, p ≤ 0.01). A total of 856 *Paragonimus* metacercariae were found in the crabs examined. About 50% of the metacercariae recovered were *P. heterotremus*. The results from the June 1984-March 1985 study were compared with the survey in January-March 2005 (Table 2). The prevalence of metacercariae was significantly higher in 2005 (χ^2= 37.2, p≤0.01).

DISCUSSION

During the past 20 years, the population of Kaeng Khoi District has increased and the administrative structure has changed. In 1985, Cha-om Subdistrict had 7 villages, but now there are 11. The study site in the 1980s, Bung Mai Village, was the largest and parts were included in the National Forest Reserve; it has now become Hin Tang and Chet Khot villages. Pong Kon Sao Village, in Tha Maprang Subdistrict, was separated from Cham Phak Phaeo Subdistrict, and adjoins Chet Khot Village. Chet Khot-Pong Kon Sao Nature and Eco-Tourism Study Center is situated in these areas. At the time of each investigation, the two villages were closest to Chet Khot Waterfall, where the crabs were heavily infected with *Paragonimus* metacercariae.

In the 1980s investigation, *Paragonimus* eggs were found in both sputum (6.3%) and feces (1%), but none were found in 2005, indicating no active, egg-producing cases in Chet Khot Village. Surprisingly, 10 of 23 serum samples were positive by IgG-ELISA. Although all 23 cases had chronic cough and a history of eating improperly cooked waterfall crab, only 10 were positive. The positive cases carried antibodies against *Paragonimus* antigens, but their sputum samples were negative for *Paragonimus* eggs, and may have been extra-pulmonary infections, immature worms, or previous *Paragonimus* infections.

The previous ELISA evaluation in 1988, recorded in a master-degree thesis, resulted in 80% sensitivity and 97% specificity; cross-reactivity was observed with only 1 schistosomiasis japonicum serum specimen (from 6 disease sera) (unpublished data).

Our results show the recent IgG-ELISA is more sensitive than the previous test, which may be due to a higher-quality substrate (ABTS) than the PPD, and also higher ODs than in 1988. The use of delipidized antigen may produce better results, as demonstrated with *Taenia solium* metacestodes (Dekumyoy *et al*, 1998). The specificities of the ELISA tests in 19Sera investigatedSera investigated88 and 2005 were similar (97% and 96.6%, respectively), but the recent study indicates better discrimination between paragonimiasis and 25 different diseases, with only six with cross-reactivity.

The most sensitive method to detect paragonimiasis is by antibody detection, followed by egg detection in sputum, then feces. A definitive diagnosis of paragonimiasis is the demonstration of lung-fluke eggs in sputum, feces, or thoracic fluid. In ectopic parasitism, such as cerebral paragonimiasis, it is not possible to detect eggs. Therefore, serodiagnosis should serve as a reference in the diagnosis of paragonimiasis.

In our study, paragonimiasis patients were generally older than in the previous study. The age range of patients in 1984-1985 was 10-60 years. In another study in 1984, infected patients age 20-50 years old were found in Cha-om and Cham Phak Phaeo Subdistricts (Benjapong *et al*, 1984). This is similar to the findings of another endemic area, the Wihan Daeng District, where the cases were age 20-45 years old (Vajrasthira *et al*, 1959). However, in 2005, the cases were age 34-67 years old. Thus, the age group was older than the previous investigation. Of these, 2 had been infected with lung flukes 10 years previously and had been treated, but were still antibody-positive. Infections occurred in the elderly, not younger individuals, reflecting improvements in the knowledge and practices of the younger generation.

Another conclusion from our study was that the prevalence of paragonimiasis in Thailand has decreased. This agrees with another report of paragonimiasis in another paragonimiasis endemic area, Noen Maprang District, Phitsanulok Province, with a prevalence of 15.8% among the 303 villagers who underwent sputum examinations (Pannarunothai *et al*, 1988). A follow-up study in 2000 showed a significant change, with only 0.51% of villagers (2/391) positive for *Paragonimus* eggs on sputum examination (Waree *et al*, 2001).

During August-September 1987, Kawashima *et al* (1989) found 643 *P. heterotremus* and 5 *P. harinasutai* metacercariae in waterfall crabs at Chet Khot Waterfall, Kaeng Khoi District, Saraburi Province; they were more prevalent in the leg muscles than other organs. In the present study, metacercariae were obtained from *P. heterotremus*, *P. westermani*, *P. bangkokensis* and *P. harinasutai*. From August to September 1987, crab surveys found > 600 *P. heterotremus* metacercariae in 312 crabs collected from Chet Khot Waterfall, and at the same time, about 100 *P. heterotremus* metacercariae were found in 169 crabs from Hin Tang, Nakhon Nayok Province (Kawashima *et al*, 1989). This indicates the presence of reservoir hosts, such as tigers, leopards, and opossums, in endemic areas, high mountains and dense forests.

The prevalence of paragonimiasis in Thailand has tended to decrease. In Japan, many parasitic diseases have been successfully eradicated, however, some have re-emerged, especially food-borne parasitic zoonoses. Since the 1980s, paragonimiasis has been re-emerging and has now become a major parasitic disease in southern Kyushu (Nawa, 2000). Among 134 Japanese cases of paragonimiasis in 1998-2002, middle-aged men with a history of eating raw wild-boar meat were the main foci of infection (Obara *et al*, 2004).

In Japan, the causes of infection are eating raw second intermediate hosts (crabs), or paratenic hosts (wild boar). Wild-boar meat is the main source of infection. Paragonimiasis infection occurs at home in the family setting. The hunters consumed wild-boar meat along with their family members and friends (Nishida and Shibahara, 2003). Although people living near the habitat of *P. ohirai* might not have eaten them, they may still become infected. A recent report on paragonimiasis among immigrants in Japan found they had become infected by eating freshwater crab, while the Japanese became infected by eating wild-boar meat (Obara *et al*, 2004). Wild boar is also a paratenic host for *P. ohirai* in Japan, similar to *P. westermani* and *P. miyazakii.* A paragonimiasis ohirai patient who ate raw, wild-boar meat has been reported (Nishida and Shibahara, 2003). The larvae of the lung fluke may migrate and reside in the muscle tissues. In Thailand, there have been no reports of wild boar paratenic hosts for *Paragonimus* species.

Paragonimus mexicanus is the main species infecting humans in Peru; the prevalence rates of infection among crabs in endemic areas have been reported as high in previous studies. Cornejo *et al* (2000) found a low rate of *Paragonimus* infection among the children of the Condebamba Valley. However, based on the small number of crabs collected from the local streams, the prevalence of infection among crabs was high. While infection rates among crabs still represent a significant transmission risk, changes in habitat may have reduced transmission rates. The reduction may also be due to health-education campaigns to reduce the consumption of raw or undercooked crab.

In conclusion, the trend of paragonimiasis has decreased in Thailand, although the prevalence of the infectious stage, the metacercaria, in the 2^{nd} intermediate host remains high. Since eating raw waterfall crab has only caused paragonimiasis infection in Thailand, health education in the community and primary school are effective strategies for eradicating this disease.

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