

RESEARCH NOTES

MALAYAN FILARIASIS IN BANGKOK?

The "Weekly Epidemiological Surveillance Report" Vol. 17, no. 50 published in Thai by the Division of Epidemiology, Ministry of Public Health, Bangkok, Thailand, on December 19, 1986, reported the finding of *Brugia malayi* microfilariae (mf) in a lymph node removed from under the chin of a Thai woman age 20 years. She moved to the Ladkrabang District, an area on the outskirts of Eastern Bangkok, two years ago from Chachoengsao, a province adjoining and to the east of Bangkok, and has not been to any other province since then. The relationship between Bangkok and the areas of Thailand where filariasis is endemic is shown in Fig. 1. The woman came to Phramonggutklao Hospital with the chief complaint of lymphadenitis beneath her chin. Laboratory investigation revealed no abnormalities except slight eosinophilia (9%). The excised lymph nodes were imprinted and impressed and stained with Wright and Giemsa stain and mf of the parasite were reported.

Epidemiological studies were carried out in 2 villages in Ladkrabang District and Bangnampreo District in Chachoengsao Province which is the birth place of the patient. The studies included a survey of the blood of people and cats as well as an entomological survey. 60 μ l of blood obtained from both humans and cats was smeared and stained with Giemsa. The results are shown in Table 1. The 589 human subjects surveyed were all mf negative and no one was found with clinical symptoms of the disease. 8 of 20 cats from Ladkrabang District were found positive with *Brugia* sp. No *Brugia* larvae were found in any mosquito. Third

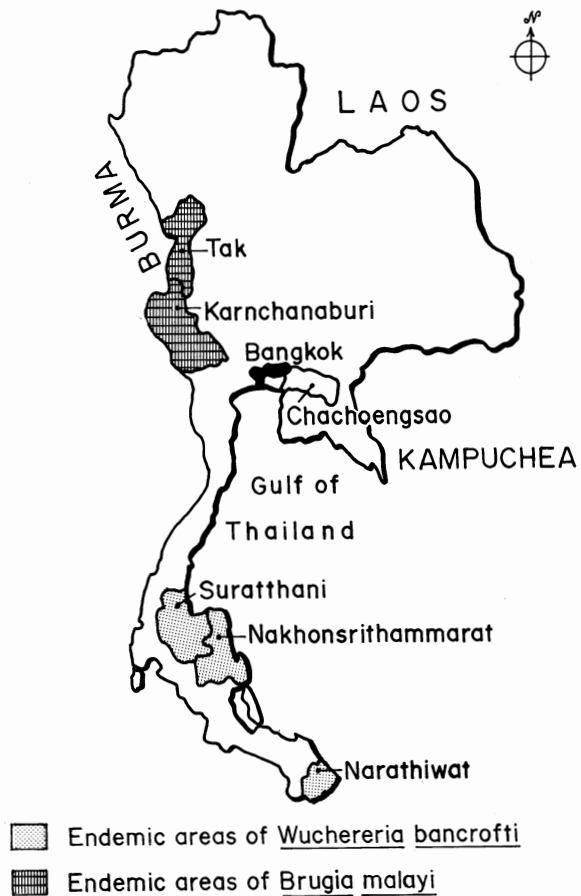


Fig. 1—Map of Thailand showing endemic areas of filariasis.

stage larvae of *Dirofilaria* sp. were found in 2 *Mansonia uniformis*. The mosquitoes from Bangnampreo District were all negative except for one *M. uniformis* which harboured second stage larvae of unknown filarial worm.

Three infected cats from Ladkrabang District were brought back to the Filariasis Research Unit of the Department of Microbiology, Faculty of Science, Mahidol Uni-

Table 1
Results of epidemiological studies in 2 villages in Ladkrabang and Bangnampreo District.

	Ladkrabang District	Bangnampreo District
Total population in the village	367	411
No. of people examined (6 pm–11 pm)	314	275
No. of positive cases	0	0
No. of cats examined	20	25
No. of positive cases	8	4
Genus of filaria found in cats	<i>Brugia</i> sp.	<i>Dirofilaria</i> sp.
No. of mosquitoes caught	1,018	171
Species of mosquitoes		
<i>Mansonia uniformis</i>	96.76%	85.38%
<i>Mansonia annulifera</i>	1.87%	0.58%
<i>Armigeres subalbatus</i>	1.38%	7.60%
<i>Anopheles campertris</i>	–	6.43%

versity, for further investigation. 767, 782, and 50 mf were found in 60 µl of ear blood. Blood smears were stained with Giemsa for Innenkörper measurement (Sivanandam and Fredericks, 1966). The average length of the Innenkörper of 20 mf from each of the three cats and those of mf of *Brugia pahangi* and *B. malayi* reported by Sivanandam and Fredericks (1966. *Med. J. Malaya*, 20 : 337) are shown in Table 2. The morphology of mf was characteristic of the genus *Brugia* except for the length of the Innenkörper of mf which in all 3 cats was found to lie between those of *B. pahangi* and *B. malayi*. Some mf were stained for acid phosphatase activity distribution (Yen and Mak, 1978. *Ann. Trop. Med. Parasitol.*, 72 : 157). Sites of intense acid phosphatase activity were found at the amphids, excretory vesicle, anal vesicle and phasmids and the remainder of the body showed very diffuse activity of the enzyme similar to the pattern found in *B. malayi* (Redington *et al.*, 1975. *Ann. Trop. Med. Parasitol.*, 69 : 489; Yen and Mak, 1978.

Ann. Trop. Med. Parasitol., 72 : 157).

The three cats were anaesthetized and *Aedes aegypti* (Black-eyed Liverpool strain) allowed to feed on them. The infective larvae obtained 10-12 days later were injected separately into the peritoneal cavity of jirds. Adult worms recovered 60 days later were examined and were all found to possess the morphological characteristics of *B. malayi* as described by Buckley and Edeson (1956. *J.*

Table 2
Innenkörper measurements

	Length in micrometers	
	Range	Average
Cat no. 1	30.7–46.6	36.6 (±5.0)
Cat no. 2	29.3–44.0	37.7 (± 3.9)
Cat no. 3	30.7–46.6	40.3 (±5.2)
<i>Brugia pahangi</i> [*]	44–63	53.1
<i>Brugia malayi</i> [*]	24–34	30.7

* Data of Sivanandam and Fredericks (1966).

Helminthol., 30 : 1). However, as the mf from the same animals are slightly different from those of *B. malayi*, this led to the suspicion that the filarial parasite in our cats could be a new species. However, before any conclusion could be drawn, further studies on the adult worms have to be made. Blood meal identification of the potential mosquito vectors in the endemic area have to be performed to ensure the possibility of the transmission of the parasite from animal to man. Besides, hybridization potential between our isolate and *B. pahangi* and *B. malayi* using the method of Suswillo *et al.*, (1978. *Parasitology*, 77 : 153) have to be investigated to prove that our worms are true species.

Although 40% of the cats surveyed were found positive with mf typical of *Brugia* sp., no mf were found in any human surveyed and the only evidence of the infection was the finding of mf in lymph nodes under the patient's chin which is a very unusual site for the parasite. If the parasite found in our cats

were the normal *B. malayi*, at least some normal human infections should have been found.

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TRANSIENT RISE IN *OPISTHORCHIS* EGG COUNT AFTER PRAZIQUANTEL TREATMENT

The intensity of infection of *Opisthorchis viverrini* is based on the quantity of faecal egg count; the evaluation of the efficacy of anthelmintic is also based on the egg reduction. We observed that in patients treated with praziquantel the egg count increased greatly during the first two days after drug administration then declined sharply to very low level on Day 3 and 4, and disappeared within Day 5–10. The increase in egg output may be due to stimulation of the egg production, expulsion of eggs before the worms died or massive eggs released from the destroyed flukes. A case of opisthorchiasis

was studied by duodenal intubation to assess the parasitological findings pre and post treatment with praziquantel.

A 46-year-old woman, weight 39 kg, a resident of northeastern Thailand, an endemic area of opisthorchiasis, was admitted to the Bangkok Hospital for Tropical Diseases, for treatment of opisthorchiasis. Praziquantel 50 mg/kg body wt was given at bed time. Six hours later 45 ml of saturated magnesium sulfate solution was given with a glass of warm water. Duodenal juice was obtained by intubation before and 12 hours after praziquantel administration. The intestinal juice

was allowed to drip freely from the tube into a small container. The examination of the contents showed 180 and 3,486 eggs/ml of duodenal juice pre and post treatment respectively. Five *Opisthorchis* flukes were recovered from the post treatment juice. The flukes were examined under the microscope. Most of the flukes had disintegrated, their integuments destroyed, the internal organs including uterus ruptured. Large amount of eggs were released from the fluke (Figs. 1 and 2).

Praziquantel destroys the flukes (Mehlhorn *et al.*, 1983). Ultrastructural investigations on the effects of praziquantel on human trematodes from Asia: *Clonorchis sinensis*, *Metagonimus yokogawi*, *Opisthorchis viverrini*, *Paragonimus westermani* and *Schistosoma japonicum*. *Drug Res.*, 33(1) 1:91). The uterus ruptures and the eggs are expelled, (like in this case) along with evacuation of the bile into the duodenum and comes out in the faeces. Thus, the faecal egg count increases greatly during the first 48 hours. Some eggs may remain in the gall bladder and are slowly



Fig. 1—A section of *Opisthorchis viverrini* showing ruptured fluke and eggs expelled.

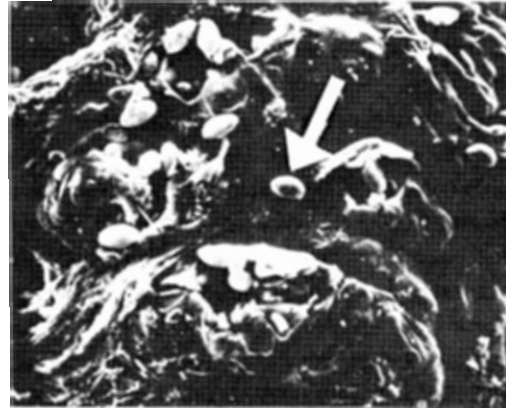


Fig. 2—Electron micrograph showing ovum of *Opisthorchis viverrini* (arrow) expelled from the ruptured uterus (15 KV \times 240).

expelled in the bile. Therefore, a very low egg count was observed for 5 to 10 days before it became negative.

Quantitation of faecal eggs in the first two days after praziquantel treatment will lead to misinterpretation of the efficacy of the drug. The evaluation of praziquantel in the treatment of liver or intestinal flukes based of faecal egg counts should be carried out after 10 days post drug administration. Also by duodenoscopy, cholecystography and duodenal intubation, the viability of the flukes could be examined before and after treatment.

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SUSCEPTIBILITY OF *TRICULA APERTA* (β RACE) TO *PARAGONIMUS HETEROTREMUS*

In Thailand, paragonimiasis is acquired by the ingestion of crabs of *Tiwaripotamon* sp. infected with *Paragonimus heterotremus* metacercariae. The first natural intermediate host of this fluke is still unknown, although *Oncomelania* sp. has been reported as experimental intermediate hosts (Asavisanu, 1985. *Southeast Asian J. Trop. Med. Pub. Hlth.*, 16:345). Studies are being carried out to find natural and experimental hosts of the lung fluke.

Experimental infection of snails using *Paragonimus heterotremus* miracidia maintained in the laboratory of the Department of Helminthology were carried out in October, 1987. Thirty snails of each species, *Melanoides tuberculata*, *Filopaludina (Siamopaludina) martensi martensi* (Frauenfeld), *Bithynia* sp. and *Tricula aperta* (β-race) were infected with the miracidia. The snails are reared in the laboratory aquarium on diatoms, snail food and lettuce. Six weeks post-infection, the snails were crushed and searched for rediae and cercariae. Twenty-six (86.7%) of *Tricula aperta* snails were found

to be infected with *Paragonimus heterotremus* cercariae. Ellipsoidal shaped redia, size 710 μ × 216 μ, each with 1–10 cercaria was obtained from freshly crushed snails. Actively moving cercariae measured average size 275 microns by 88 microns, elongated in shape, oral sucker armed with 33 microns stylet, and short tail average 22 microns in width and 27.5 microns in length. The other species of snails were not infected. Thus, *Tricula aperta* is a susceptible host for *Paragonimus heterotremus*.

Tricula aperta, β race, Family Pomatiopsidae, also the vector of Mekong schistosomiasis is found distributed in the Mun River, a tributary of the Mekong River at Pibun District, Ubon Ratchatani Province, Thailand (Lohachit, 1980. *Malacol. Rev.*, 2 : 47–51).

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DISTRIBUTION OF *PARAGONIMUS HETEROTREMUS* METACERCARIAE IN FRESH WATER CRAB, *TIWARIPOTAMON* *BEUSEKOMAE*—BOTT 1970

Paragonimiasis caused by *Paragonimus heterotremus* is an endemic problem, especially in Saraburi and Nakhon-Nayok Provinces of Central Thailand (Vajrasthira, 1959. *Jap J. Exp. Med.*, 29 : 159; Vajrasthira, 1966, *Abstr. 2nd Med. Conf. Parasit. Dis.*, Bangkok, p. 57). *Paragonimus heterotremus* is the only human species infecting

man (Miyazaki and Harinasuta, 1966. *Ann. Trop. Med. Parasit.*, 60 : 509., Miyazaki and Fontan, 1970, *Jap. J. Parasit.*, 19 : 109; Vanijanonta *et al.*, 1981. *Southeast Asian J. Trop. Med. Pub. Hlth.*, 12 : 104) and the important second intermediate host a fresh water crab, *Tiwaripotamon beusekomae* (*Potamon smithianus*) harbour the infective

stage or metacercariae of *P. heterotremus* (Vajrasthira, 1969. *J. Med. Ass. Thailand*, 49 : 965). Studies were done to determine the distribution of *P. heterotremus* metacercariae in various parts of the crab.

The fresh water mountain crabs, *Tiwaripotamon beusekoma*e—Bott 1970 were collected from a stream at Ban Tadan, Tambon Hin-Tung, Amphoe (district) Muang in Nakhon-Nayok Province during June 1984 to March 1987. Measurement of each crab was recorded. The carapace was dissected out. The gills, liver, viscera, abdominal muscle, leg muscle and claw were compressed between the two thick glass plates (4 × 6 inches) to search for metacercariae under a dissecting microscope. Location of the metacercariae obtained from each crab was recorded.

Six hundred and seventy-seven out of 1,766 crabs were found positive for *P. heterotremus* metacercariae, with the infection rate 38.3% Table 1. According to the size, crab size 50–60 mm gave the highest percentage (52.0%) followed by 40–50 mm (42.9%), 30–40 mm, (36.7%) and 20–30 mm (27.3%). The organs of preference for lodgement of *P. heterotremus* metacercariae are

shown in Table 2. Average number of metacercariae per crab was 1.88 + 1.75. The maximum number of metacercariae was 25 which obtained from crab size 40–50 mm.

The present study indicated that the metacercariae of *P. heterotremus* were found most frequently and abundantly in the leg muscles (52.0%) and a higher infection rate could be observed in the larger crabs than in the smaller ones. It might be possible that the larger crabs or older ones have stayed in the endemic area longer. Therefore they have more chance to be exposed to the infection than the younger ones. Nevertheless, the intensity of *P. heterotremus* metacercariae per crab found in any size were not different. The infection rate between both sexes of the crabs was not significantly different ($p > 0.05$).

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Table 1

Infection rates of *P. heterotremus* metacercariae in fresh water crabs (*Tiwaripotamon beusekoma*e) according to sex.

Sex of crab	No. examined	No. infected	Percent
Male	646	245	37.9
Female	456	204	44.7
Total	1,102*	449	40.7

* The sex of 664 out of 1,766 collected crabs were not identified.

Table 2
The distribution of *P. heterotremus metacercariae* by size and organ in the natural infected crabs (*Tiwaripatamon beusekomae*).

Size of the carapace (mm.)	No. of crab examined	No. of crab infected (%)	No. of metacercariae found (%)						Total	Mean No. metacer per crab \pm S.D.	Max. No. metacer. per crab
			Gill	Liver	Viscera	Abd.m.	Leg.m.	Claws			
20-30	355	97 (27.3)	9 (5.0)	25 (13.9)	6 (3.3)	34 (18.9)	99 (55.0)	7 (3.9)	180	1.85 \pm 1.32	8
30-40	634	233 (36.7)	31 (7.4)	20 (4.8)	8 (1.9)	53 (12.6)	242 (57.6)	66 (15.7)	420	1.80 \pm 1.22	5
40-50	627	269 (42.9)	66 (12.3)	23 (4.3)	4 (0.7)	41 (7.7)	284 (53.1)	117 (21.9)	535	1.98 \pm 2.20	25
50-60	150	78 (52.0)	32 (22.4)	9 (6.3)	4 (2.8)	18 (12.6)	39 (27.3)	41 (28.6)	143	1.83 \pm 1.02	4
Total	1,766	677 (38.3)	138 (10.8)	77 (6.0)	22 (1.7)	146 (11.4)	664 (52.0)	231 (18.1)	1,278	1.88 \pm 1.75	25

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