

Establishment of An Animal Model for Use in Filariasis Studies

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OBJECTIVE :

To trap wild rodents of Genus *Rattus* infected with nematodes of Superfamily Filarioidea, to maintain these in the laboratory and to transmit the filarial infections from them, through mosquitoes, to laboratory albino rats.

BACKGROUND :

The need for a consistently reproducible laboratory infection with nematodes of Superfamily Filarioidea in a readily available, genetically controlled, laboratory animal has been expressed (1). *Litomosoides carinii* naturally found in the wild cotton rat can be experimentally transmitted to the laboratory albino rat and the Mongolian gerbil but not by a mosquito. A mosquito-transmitted filaria-laboratory rat system would more closely meet the requirements of the desired experimental model (2). Attempts to transmit *Brugia tupaia* through mosquitoes, to various laboratory animals were made at the SEATO Medical Research Laboratory (SMRL) in 1969 and 1970 (3, 4) but were unsuccessful. One mosquito-transmitted filarial parasite, *Brienlia booliati*, was recently reported in Malaysia and laboratory rats have been successfully infected with this nematode at the University of Singapore (5, 6).

A preliminary study in which wild rodents in Thailand were trapped and screened for microfilaria revealed the presence of unreported filarial nematodes in several species. This study was initiated to evaluate the model potential of some of these.

DESCRIPTION :

Small mammals were live-trapped, using bananas as bait, in 8 different locations in Southeastern and South Central Thailand from late August, 1975 through May, 1976 (Figure 1). The habitats trapped ranged from evergreen forests on mountain sides to the water front area of Bangkok. Traps were set in the evening and picked up in the morning. Blood was drawn from the rats and examined for microfilariae in late morning and early afternoon, generally between 1130 and 1530 hours.

The animals were anesthetized with ether and 1/4 to 1 1/2 cc of blood was withdrawn by cardiac puncture with a heparin-wet syringe. The blood was mixed with 20 to 30 cc of normal saline and passed through a 3 or 5 micron Millipore filter. The filter was placed on a clean glass slide with one or two drops of saline, a coverslip was added, and the filter was examined at a magnification of 100 x. Movement of the microfilariae was readily discernible and an estimate of size could be made from these live mounts. By using this procedure, relatively large numbers of animals could be examined quickly and the animals determined to be positive or negative within 10 minutes of bleeding. All negative animals were released within 3 to 4 hours of bleeding and positive animals were transferred to laboratory rat cages.

Although no attempts were made to study periodicity of microfilaria, different bleeding times were used occasionally to see if the percentage of positive animals varied. Animals were bled as early as 0900 hours and as late as 2400 hours and no significant difference was noted.

Any positive animals that died as a result of handling or bleeding were necropsied and examined for adult filariae. If any were found, they were placed in 10% glycerin-alcohol fixative. Microfilaremic animals in which the adult filariae were not found were placed in 10% neutral buffered formalin and returned to the laboratory for histopathological examination. Live positive animals were returned to the laboratory for mosquito feedings and as a source of adult filariae for taxonomic study.

For mosquito feedings, donor rats were restrained in wire cloth cylinders and placed in a cage containing laboratory reared *Aedes togoi*, *Anopheles balabacensis*, and *Armigeres subalbatus*, respectively. Mosquitoes that fed on donor rats were dissected after 2 to 3 weeks to detect filarial infections. If larval stages of filaria were found, mosquitoes from that group were fed on albino laboratory rats by the procedure described above. After 3 months thick blood films were made from the laboratory rats and examined for microfilaria.

To study the morphology of the microfilariae, smears from wild caught rats were made at the laboratory, dried overnight at room temperature, dehemoglobinized for two minutes in tap water, fixed for 30 seconds in methyl alcohol, and stained in Giemsa and/or Field's stain.

Some positive animals were later sacrificed in the laboratory to obtain adult filariae. These nematodes and thick blood films from some of the animals in which they were sent to the Filariasis Research Division of the Institute for Medical Research (IMR), Kuala Lumpur, Malaysia for identification.

PROGRESS :

One thousand six hundred and ninety four (1694) animals of 15 different species were trapped, 235 of which were microfilaremic. The trapping locations, the number and species of animals trapped and the number and percent of those that were microfilaremic are given in Table 1. Six different types of microfilariae were evident on the live mounts and this was confirmed when stained thick films were studied. *Brienlia booliati*, *Brugia tupaia*, and *Dunnifilaria ramachandri* have tentatively been identified from the microfilariae, the host species, and the location and size of the adult filariae. Adult filariae of five of the six species have been submitted to the IMR for identification but these studies are incomplete. Unfortunately, the six positive *Hylomys suilis* all died shortly after trapping, and the adult worms were not found at necropsy.

Mosquitoes were infected with *B. booliati* which was found in *Rattus rattus*, *Rattus neilli*, and *Rattus koratensis*. *Aedes togoi*, which fed on infected *Rattus rattus* became infected and when dissected later third stage larvae were found in these mosquitoes. These *Aedes togoi* were allowed to feed on laboratory rats. There were no microfilariae on thick smears made from these rats 3 months later. Unsuccessful attempts were made to infect mosquitoes with an unidentified filaria from *R. rattus*. Mosquito transmission was not attempted with animals infected with *Brugia tupaia* or *Dunnifilaria ramachandri*.

Screening histopathology sections proved to be an unrewarding method of locating adult filariae in the animals and was discontinued.

SUMMARY :

One thousand six hundred and ninety four (1694) small wild mammals were trapped in Thailand and screened for microfilaremia. Two hundred and thirty five were found to be positive. Identification of the species of filariae found and mosquito transmission studies of the filariae to laboratory rats are incomplete.

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SPECIES	LOCATION		Bangkok		Chantaburi		Nakorn Rajsima		Prachinburi		Sai Yok		Sa Kaeo		Sakaret		Sangkha Buri		Total By Species		
	#	+	#	+	#	+	#	+	#	+	#	+	#	+	#	+	Total	Pos	% Pos		
<i>Bandicota indica</i>	0	0	14	0	0	0	0	0	0	0	0	0	5	0	0	0	19	0	0		
<i>Hylomys suillus</i>	0	0	5	0	0	0	0	0	0	0	0	0	0	0	46	6	51	6	11.8		
<i>Menetes berdmorei</i>	0	0	12	2	0	0	0	0	0	0	0	0	0	0	5	1	69	7	10		
<i>Rattus berdmorei</i>	0	0	31	1	0	0	0	0	0	0	0	0	0	0	0	0	35	1	2.9		
<i>Rattus exulans</i>	17	0	0	0	3	0	26	0	0	0	0	0	15	0	0	0	100	0	0		
<i>Rattus koratensis</i>	0	0	80	20	0	0	0	0	0	0	0	0	0	0	9	0	95	29	30.5		
<i>Rattus losea sakeratensis</i>	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	0	0			
<i>Rattus neilli</i>	0	0	0	0	0	0	0	0	0	0	5	2	0	0	0	5	2	40			
<i>Rattus bukit</i>	0	0	5	0	0	0	0	0	0	0	0	0	0	0	40	4	47	4	8.5		
<i>Rattus norvegicus</i>	106	0	0	0	5	0	77	0	0	0	0	0	0	0	0	188	0	0			
<i>Rattus rattus</i>	0	0	63	2	0	0	0	0	0	1	0	0	0	0	117	46	280	49	17.5		
<i>Rattus sabanus</i>	0	0	1	1	0	0	0	0	0	0	0	0	0	0	49	34	51	36	70.6		
<i>Rattus surifer</i>	0	0	146	1	0	0	0	0	0	3	0	0	0	0	386	15	544	16	2.9		
<i>Suncus murinus</i>	11	0	0	0	0	0	22	0	0	0	0	0	0	0	0	33	0	0			
<i>Tupaia glis</i>	0	0	46	23	0	0	0	0	0	4	4	0	0	0	78	38	175	85	48.6		
Total By Location	134	0	403	50	8	0	125	0	13	6	22	0	736	154	253	25					

Total + Positive
Table 1. Results of Trapping and Screening for Microfilaria by Location and Species of Animal Trapped.

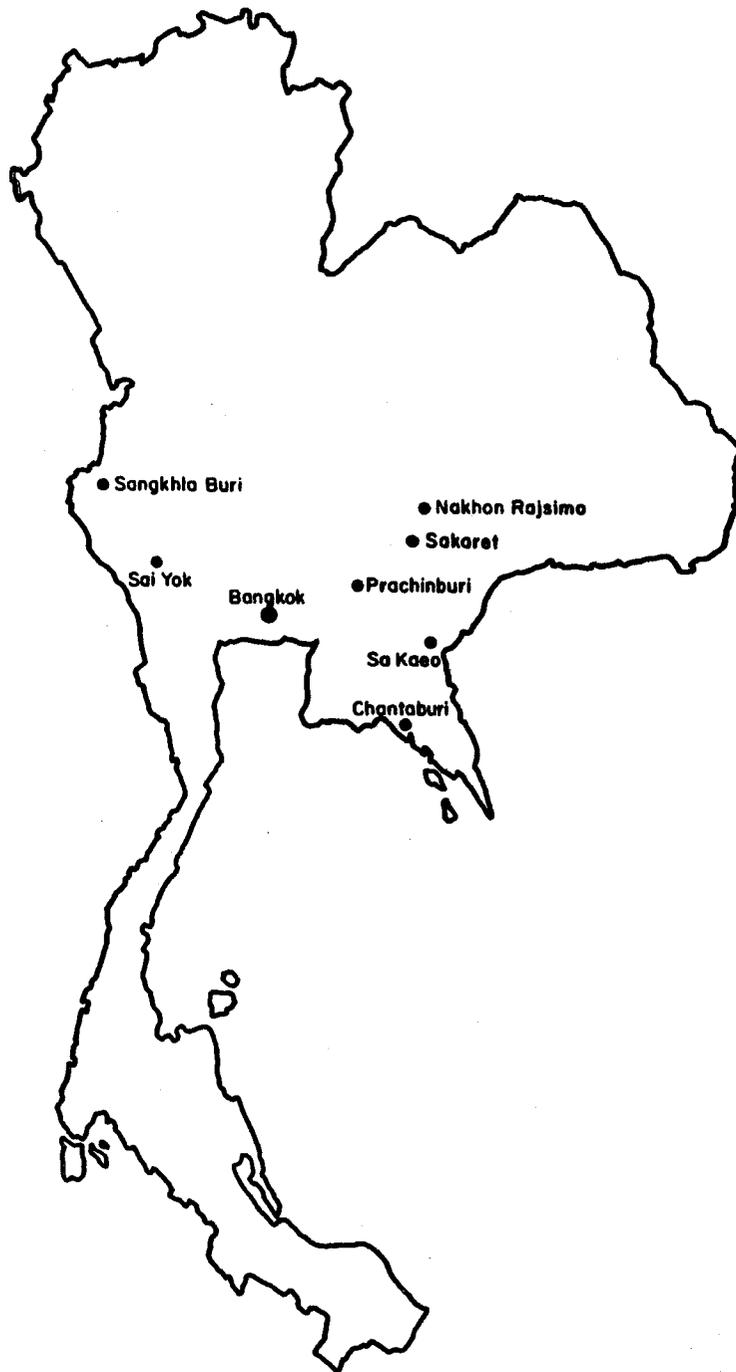


Figure 1. Sites where animals were trapped in South-eastern and South-central Thailand