# ECOLOGY AND INFECTION RATES OF NATURAL VECTORS OF FILARIASIS IN TANAH INTAN, SOUTH KALIMANTAN (BORNEO), INDONESIA

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#### ABSTRAK

Data ekologi nyamuk vektor dan tingkat infeksi filaria secara alami dan secara buatan telah diperoleh dari perkebunan karet di Kalimantan Selatan, Indonesia. Berbagai macam cara penangkapan dalam kondisi ekologi yang berbeda telah dipakai dalam pengumpulan 51 jenis nyamuk (N = 95.735). Pembedahan nyamuk, infeksi buatan dan identifikasi larva filaria mengikuti prosedur dan kunci yang sudah baku. Infeksi filaria Brugia, Breinlia dan Cardiofilaria secara alami ditemukan pada nyamuk Coquillettidia crassipes. Dari penelitian ini dapat dijelaskan hasil infeksi buatan, kepadatan populasi nyamuk secara musiman dan perbandingan cara penangkapan nyamuk.

#### INTRODUCTION

In September 1978, a collaborative study was begun in Tanah Intan, South Kalimantan (Borneo), Indonesia by the Indonesian Ministry of Health and the U.S. Naval Medical Research Unit No. 2, Detachment, Jakarta, in order to define the role of feral and domestic animals as reservoir hosts for human filarial pathogens. (Fig. 1) The results of this study revealed that the domestic cat (Felis catus) and the Silvered leaf monkey (Presbytis cristata) were hosts for both Brugia malayi and Brugia pahangi. (1,2,3,4) Additionally, it was also found that P. cristata harbored three other species of filariid nematodes, namely, Wuchereria kalimantani, a Cardiofilaria sp. and Dirofilaria magnilar $vatum^{(1,2)}$  (Table 1).

The study area consisted of a rubber estate (1,680 hectares) (3° 20' S, 115° 02' E,) surrounded by small rice fields and secondary

forest at approximately 25 meters elevation (Fig. 2). The majority of the human population on the estate (245 persons), comprised of approximately 60% resettled Javanese and 40% indigenous Banjarnese, derived their livelihood from tapping rubber. Before initial mass treatment with diethylcarbamazine citrate (Filarzan<sup>R</sup>), the infection rate for Brugia malayi in this human population was approximately 29.0%. Wuchereria bancrofti was not detected in the blood sampled. At eight months posttreatment the infection prevalence decreased to 2.4% (20 ul fingerprick blood, 250 individuals) and decreased further at 14 months post-treatment to 1.6% (N = 191). At 24 months post-treatment the infection rate had increased to 4.9% (N = 314), presumably because of a resurgance in transmission.

This report includes information on the adult ecology and infection rates of vector mosquitoes in the defined study areas.

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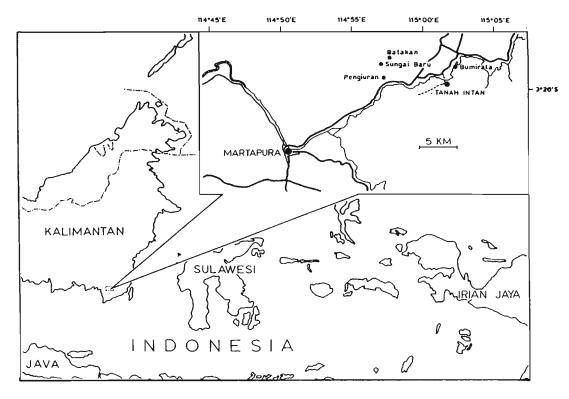


Figure 1. Location of study site (Tanah Intan) in South Kalimantan, Indonesia.

Tabel 1. Infection rate of animal hosts for filariid nematodes in South Kalimantan (Borneo), Indonesia.

Number (%) positive for									
Host and Location	Number Examined	Brugia malayi	Brugia pahangi	Wuchereria kalimantani	Cardiofilaria	Dirofilaria magnilarvatum			
Presbytis cristata									
Tanah Intan	106	22 (20.8)	5 (4.7)	36 (33.9)	1 (0.9)	1 (0.9)			
Felis catus L.									
Barabai	144	2 (1.4)	23 (16.0)	_		-			
Pengiuran	18	-	3 (16.7)	_	-				
Sungai Siepai	23	_	11 (47.9)	_	_				
Tanah Intan	102	2 (1.9)	8 (7:8)	_	_	_			
TOTAL	287	26 (9.1)	50 (17.4)	36 (12.5)	1 (0.4)	1 (0.4)			

from Palmieri et. al (2,4)

## MATERIALS AND METHODS

#### Collection

Mosquitoes were captured in various ecological settings using battery-operated CDC light traps and modified Trinidad-type traps baited with either monkey, cat, or CO2 (dry ice). Baited Trinidad-type traps were suspended from large trees using a rope and pulley system at either 1-3 meters, 9-12 meters or 15-21 meters for 12-hour periods.

Collection of human and monkey biting mosquitoes were made during the day (06.00-18.00) and night (18.00-06.00) from either temporary huts built of bamboo and banana leaves at ground level or from wooden platforms built in the canopy at 15-25 meters above ground level (Fig. 2). Biting and landing mosquitoes were collected from human volunteers or caged silvered leaf monkeys (Presbytis cristata) using mouth aspirators. Within the central village electricity was supplied to the estate director's home, from 19.00 to 22.30, allowing the viewing of a television, made available to the villagers. During this period, landing and biting mosquitoes were collected using mouth aspirators from the T.V. viewing audience.

Daily rainfall data was collected on site and tabulated to monthly totals. No attempt was made to measure temperature or humidity changes at the macro or micro-environmental levels. Based on local meterological data, monthly temperature extremes are slight and are principally a reflection of amounts and frequency of seasonal precipitation.

# **Ecology**

The general ecological settings in the area of study have been described elsewhere. (1,3) Mosquitoes were trapped at 7 routine surveillance collecting sites within the study area. Four areas used wooden platforms built in trees at 15 and 25 meters above ground level with pulley systems between trees for suspending and retriving traps (Fig. 2). These collecting sites included the following environmental interfaces: ricefield/forest, swamp/forest; riverine/forest; rubber forest; dense secondary forest; riverine/village; rubber village; and open-village.

## Specimen Preparation

Collected mosquitoes were transferred to paper holding cups for identification and dissection within the following day. Mosquitoes were anesthetized with chloroform and identified to species based upon morphological characters. Because of the close similarity in morphology, Mansonia bonneae and Mansonia dives were pooled as one group. Mosquitoes were dissected for filarial infections, (6) and semi-permanent mount preparations of a representative sample of early stage and infective larvae were made. (7) Initially, grouped species were dissected in pools of 15-50 in saline solution (0.85%) and microscopically examined for all developing forms of filaria. Subsequent capture of species previously found positive for filariid Iarvae, were individually dissected and examined for infection rates.

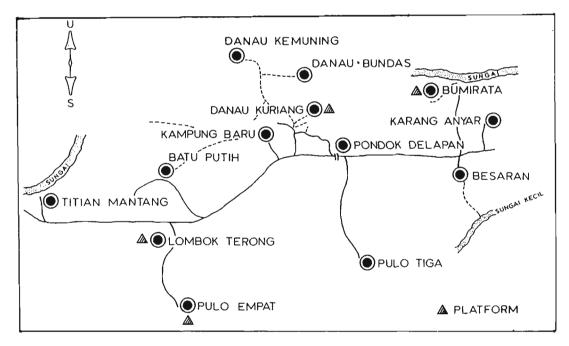


Figure 2. Study Site depicting villages and platform locations in Tanah Intan.

# **Experimental Infections**

A sample of the infective larvae recovered from individually dissected mosquitoes were either injected intraperitoneally or subcutaneously into domestic cats (Felis catus) and mongolian jirds (Meriones unquiculatus) for observations on experimental development. (6,8,9)

Laboratory-bred adult female mosquitoes, 2-3 days after emergence, were fed on hosts with known filarial infections. Female mosquitoes were allowed to sugar-feed 2 days before blood feeding and thereafter as desired. Fully fed mosquitoes were grouped and dissected every 24-48 hours to recover first, second and third-stage developing filaria larvae (Table 5). Larval forms were morphologically

compared with those larvae recovered form natural infections with the exception of Wucherena kalimantani where the natural vector was not yet known.

#### Filariid Larvae Identification

Experimentally developed filariid larvae were examined in accordance with the technique described. (10) Identification of various larval stages to genus or species were based upon established taxonomic keys. (6,11,12)

## Microfilaria

Microfilariae (mff) were recovered from blood and/or intraperitoneal fluid of experimentally infected jirds. Blood and intraperitoneal fluid was either smeared on glass slides, air dried, dehemoglobinized, air dried, fixed with absolute methanol and stained in Giemsa (pH 7.2) or placed in 2% buffered formalin (modified Knott technique) and stained with methylene blue. (6,13) Identification was made using available taxonomic keys.

#### RESULTS

## **Mosquito Collections**

Of the 95,735 mosquitoes collected and examined from September 1978 through November 1979, 9 genera (Coquillettidia, 47.1%; Mansonia, 24.8%; Culex 24.5%; Anopheles, 2.4%; Aedes, 0.8%; Uranotaenia, Aedeomyia, Armigeres, and Ficalbia, 0.4%) and 51 different species were identified (Table 2). Only Mansonia uniformis, Ma. bonneae, Ma. dives, and Coquillettidia crassipes were found harboring developing filariid larvae. Only these four species will be described herein.

## Coquillettidia crassipes Van der Wulp 1881

Coquillettidia crassipes was the most commonly trapped mosquito, representing 29.1% of the overall collections, irrespective of bait used or season trapped.

Habitat, Cq. crassipes was most often collected throughout the day and night in forested areas where the canopy provided either partial or full coverage. This included rubber forest, secondary forest, village forest fringe, and forested and village areas bordering rice

fields. In areas where the canopy provided partial covering, *Cq. crassipes* could be collected using sweep nets in the heavy bush undergrowth. This species was seldom collected in unprotected open village areas.

Elevation. The majority of *Cq. crassipes* were collected from the mid-to-upper-level collection sites of the canopy (9-21 meters) except in cases where monkey bait was used (Table 3). Nearly 70% of the *Cq. crassipes* attracted to monkeys were at the 1-3 meter level.

Bait Preference. During January to November 1979, human and cat baited traps were placed together in a variety of locations at various elevation. Of the *Cq. crassipes* collected, 58.6% preferred cat bait while 41.4% were attracted to the human bait. In collections made between November 1978 and June 1979, a comparison between human and monkey bait revealed that 65.6% of the *Cq. crassipes* were attracted to monkeys while only 34.4% preferred humans.

Infections Rates. Of the 27,883 Cq. crassipes examined, 193 (0.7%) were positive for 873 infective stage larva of Cardiofilaria sp. (4.6 larvae/infective mosquito). No other filariid species were recovered from Cq. crassipes.

Rainfall and Seasonal Fluctuation. From October 1978 through November 1979 the percent population of *Cq. crassipes* trapped, relative to all species collected varied by 10.9 to 40%. In general, the overall percentage trapped dropped following periods of highest rainfall and increased during periods of lowest precipitation. (Table 4)

## Tabel 2. Mosquitoes from Tanah Intan, South Kalimantan.

#### **AEDES**

- 1. Ae. albopictus
- 2. Ae. lineatopennis
- 3. Ae. novoniveus
- 4. Ae (par) spp.
- 5. Ae. vexans
- 6. Ae (fin) spp
- 7. Ae (par) spp

## **AEDEOMYIA**

Ae. catastica

## **COQUILLETITIDIA**

- 1. Cq. ochracea
- 2. Cq. crassipes
- 3. Cq. nigrosignata
- 4. Cq. hdgkini
- 5. Cq. aureosquamata

## URANOTAENIA SP.

## FICALBIA SP.

## **ANOPHELES**

- 1. An. aconitus
- 2. An. barbirostris
- 3. An. nigerrimus
- 4. An. peditaeniatus
- 5. An. maculatus
- 6. An. vagus
- 7. An. umbrosus

## **CULEX**

- 1. Cx. gelidus
- 2. Cx. quinquefasciatus
- 3. Cx. (pipiens) spp.
- 4. Cx. fuscocephala
- 5. Cx. sinensis
- 6. Cx. vishnui
- 7. Cx. pseudovishnui
- 8. Cx. ochracea
- 9. Cx. nigropuctatus
- 10. Cx. (sitiens) spp
- 11. Cx. mimulus
- 12. Cx. hutchinsoni
- 13. Cx. whitmorei

## **MANSONIA**

- 1. Ma. annulata
- 2. Ma. annulifera
- 3. Ma. uniformis
- 4. Ma. indiana
- 5. Ma. bonneae
- 6. Ma. dives

## **ARMIGERES**

- 1. Ar. kuchingensis
- 2. Armigeres sp.
- 8. An. kochi
- 9. An. tessellatus
- 10. An. pujutensis
- 11. An. separatus
- 12. An. campestris
- 13. An. minimus
- 14. An. letifer

Tabel 3. Comparison of percentages of Coquillettidia crassipes, Mansonia bonneae/dives and Ma. uniformis collected at various elevations using various bait attractants.

·		Cq. crassipes	·		
1 - 3	CO2	Cat	Human	Monkey 68.2	
	23.7	37.3	32.4		
	41.9				
15 - 21	34.4	62.7	67.6	31.8	
		Ma. bonneae/dives			
1 - 3	11.7	32.5	46.2	37.0	
9 - 12	46.4	-	-	-	
15 - 21	41.9	67.5	53.8	63.0	
		Ma. uniformi	s		
1 - 3	38.5	66.8	66.1	63.8	
9 - 12	27.7	-			
15 - 21	33.8	33.2	33.9	36.2	

# Mansonia bonneae Edwards 1930 Mansonia dives (Schiner) 1868

Adult *Ma bonneae* and *Ma dives* are morphologically very similar and were grouped together for practical and statistical purposes. They were the least common of the filaria vector mosquitoes trapped but the fourth most common species collected, representing 9.2% of the overall collections. *Ma. bonneae/dives* were collected throughout the year at all elevations.

Habitat. Both species were collected both day and night most often in forested areas heavily or partially covered by canopy. In forest/village areas, Ma. bonneae/dives were trapped near human and animal dwellings. At night they would be found feeding in open village areas unprotected by the canopy as well as inside dwellings. These species were seldom found in open rice fields or swamps.

Elevation. This species group showed no strong preference, although the majority trapped while using animal bait (53.8%-68.5%) were found in the upper levels of the canopy (12-21 meters). Between 32.5% to 46.2% of Ma. bonneae/dives were collected at ground level (1-3 meters) while using animal bait (Table 3).

Bait Preference. During January to November 1979 collections from human and cat bait, placed together in a variety of ecological settings at various elevations, showed that 70.9% of Ma. bonneae/dives were attracted to human bait while only 29.1% were attracted to cat bait. During November 1978 and June 1979, when human and monkey bait preferences were compared, it was found that 91.5% of Ma. bonneae/dives were attracted to humans while only 8.5% were attracted to monkey bait.

Table 4. Comparison of Coquillettidia crassipes, Mansonia bonneae/dives and Ma. uniformis with overall collections due to influence of seasonal variation and rainfall fluctuation (September 1978 - November 1979).

	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Rainfall (mm)	375	240	160	628	631	410	325	213	90	110	40	35	75	100	250
Cq. crassipes				•											
(% of overall collection)	-	21.7	20.1	18.7	14.0	19.7	39.2	32.1	32.9	35.9	35.5	17.4	31.6	10.9	40.0
% of total															
Cq. crassipes collected/month	-	3.6	6.2	12.6	5.7	7.0	6.0	12.4	7.7	15.9	7.9	5.9	7.4	0.2	1.5
Ma. bonneae/dives															
(% of overall collection)	-	3.9	14.4	6.7	17.4	13.9	9.8	9.0	6.6	1.8	0.9	1.9	6.8	1.1	15.7
% of total															
Ma. bonneae/dives collected/month	-	2.2	12.7	14.3	22.5	15.6	4.5	11.0	4.9	2.4	0.7	2.1	5.1	0.1	1.9
Ma. uniformis															
(% of overall collection)	-	4.9	14.7	19.7	12.3	12.9	13.1	25.6	21.4	19.9	13.0	7.4	1.5	0.0	0.3
% of total					,										
Ma. uniformis collected/month	-	1.3	7.4	22.3	8.4	7.7	3.4	16.6	8.5	15.0	4.5	4.3	0.6	0.0	0.0

<u>Infection Rates</u>. A total of 8,785 Ma. bonneae/dives were examined of which 3 were found positive for 5 larvae (L3) of Brugia sp. during January 1979. No other filariid species were recovered from this species group.

Rainfall and Seasonal Fluctuation. From December to February (rainy season) populations of *Ma. bonneae/dives* were at their maximum. The overall percentage of populations trapped (14.4%-22.5%) increased following periods of increased rain and during peak rains (410-630 mm/month). Conversely, populations decreased to 4.5% in March and 0.1% in October during the dry season (35-325 mm/month) (Table 4).

# Mansonia uniformis Theobald 1901

Mansonia uniformis was most often trapped during the wet seasons, and accounted for 17.2% of the overall collections. It was the third most commonly trapped mosquito and the most commonly trapped vector of Brugian filariasis. This species showed the highest preference for human and/or animal bait.

Habitat. This species was most often collected from early evening through mid-morning in villages with open canopy and with heavy bush and banana groves. During the evening, Ma. uniformis was commonly found in the open village areas where it accounted for over fifty percent of all mosquito species collected.

Elevation. 63.8% to 66.8% of all Ma. uniformis were collected at 1-3 meters in elevation, regardless of the animal bait used (Table 3). This species was also present at 15-21 meter elevations. (33.2%-36.2%).

Bait Preference. Ma. uniformis was equally attracted to human, cat and monkey

bait (Table 3). This affinity for animal and human bait was independent of the elevation of capture.

Infection Rates. Of the nearly 16,500 Ma. uniformis examined, 7 were found positive for 13 L3 larvae of Brugia sp. In addition 1 specimen was found infected with a single L3 of the genus Breinlia.

Rainfall and Seasonal Fluctuation. In general, the overall percentage of population of *Ma. uniformis* trapped dropped following periods of highest rainfall and increased when rainfall was below 300 mm/month (Table 4). During October and November of 1979, following periods when rainfall fell below 100 mm/month, no *Ma. uniformis* were collected.

## Experimental Infections in Mosquitoes

Table (5) summarizes results from laboratory reared mosquitoes allowed to feed on infected hosts of *Brugia malayi*, *Brugia pahangi* and *Wuchereria kalimantani*. All mosquitoes were fed once on infected blood and maintained under controlled laboratory conditions. Mosquitoes were dissected 10-12 days post-infection.

Mansonia uniformis was shown to support B. pahangi and B. malayi, whereas, Aedes aegypti was completely refractory to both. Four species of Anopheles were tested, and with the exception of An. vagus, all were found susceptible to a limited degree to Brugia and/or W. kalimantani. The role these anophelines play in the transmission of these parasites to natural and human hosts is unknown.

All results reported herein should be considered preliminary in scope because in most cases the number of mosquitoes tested was small. Conclusive observations can only be reported after repeated experimentation and further field work.

Tabel 5. Results of mosquitoes experimentally exposed to hosts with known filariid infections.

Mosquito Species	Number mosquitoes exposed	Source Host/ infection (filariid)	Results		
Ma. uniformis	7	Jird (B. malayi)	1 + L2, L3		
An. nigerrimus	5	Jird (B. malayi)	2 + L3		
An. nigerrimus	15	Jird (B. pahangi)	5 + L3		
An. subpictus	117	P. cristata (W. kalimantani)	13 + L1, L2, L3		
Ar. subalbatus	12	Cat (B. pahangi)	1 + L3		
Ar. subalbatus	25	Cat (B. pahangi)	L1 - L3		
An. vagus	6	P. cristata (W. kalimantani)			
Ma. uniformis	20	Cat (B. pahangi)	5 + L1, L2, L3		
Ma. uniformis	29	Jird (Cardiofilaria)	_		
Ae. aegypti	22	Cat (B. pahangi)	_		
Cx. quinquefasciatus	200	P. cristata (W. kalimantani)	2 + L1, L2		
An. barbirostris	12	Cat (B. pahangi)	2 + L1, L3		

#### DISCUSSION

Previous Vector Studies. Over the past 100 years, many detailed studies and comprehensive reviews concerning the vectors of human and animal filariasis in Malaysia and Indonesia have been reported. (14,15,16,17) The majority of the reports concerning vectors of Brugian and Bancroftian filariasis originated from Peninsular Malaysia (18,19) and East Malaysia<sup>(20,21)</sup>. Several reports have combined reviews of past vector studies with original vector data from Southeast Asia<sup>(14)</sup>, Malaysia<sup>(22,23,24)</sup> and Indonesia.<sup>(25,26,27)</sup> A comprehensive bibliography of medical parasitology in Indonesia provides a large number of early reports of human and animal filariasis from the Dutch East Indies and the Malay archipelago. (28)

Errors in Filariid Identification and Vector Incrimination. All to commonly these authors have encountered situations where L2 and/or infective L3 filariid larvae have been ascribed to a human infecting filariid species without previous specific morphological characterization. Bird, amphibian, reptile and mammal infecting filariids are much more widespread than commonly realized (29,30,31,32) and can be confused with developing larvae of either Brugia or Wuchereria. For example, Table 6 (NAMRU-2, unpublished data) summarizes the variety of filarial worms and vectors from various locations in South Kalimantan found between 1977 and 1979. In this study, 193 Cq. crassipes were found positive for 873 filariid larvae, all identified as Cardiofilaria sp. on direct examination or confirmed by experimental feeding. This

mosquito vector was not observed to harbor other species of filariae. Piessens et al. (33) listed Coquillettidia sp (presumably Cq. crassipes as an important vector of Brugia malayi from the same areas as the present study. Partono et al. (34) also lists Ma. crassipes (= Cq. crassipes) as a vector of B. malayi in two areas of West Kalimantan. Unfortunately, in neither case do the authors report how species identification was determined. However, this is not to say that this species can not play a role in Brugia transmission. Based on laboratory and field observations Chiang et al. (35,36) concluded that in an endemic area with high densities of Cq. crassipes, it could act as a secondary vector of Brugian filariasis in Peninsular Malaysia.

In this study we found no significant morphological difference between third-stage larvae of *B. malayi* and *B. pahangi* and would find it little more than speculation to place a species determination on these larvae. During this investigation, careful indentification of *Brugia* microfilaria and developing larvae were made regarding the possible presence of *Brugia* 

timori. (37,38) This and other unpublished surveys have so far failed to detect B. timori as a natural parasite in Kalimantan. Without detailed morphologic descriptions and experimental studies of sympatric third-stage developing larvae common to animals and man in a given area, attempts at specific identification will yield, at best, suspect results.

The Natural Vectors. Because of the recent changes and refinements in mosquito taxonomy, accuracy of earlier vector identifications are questionable, especially when original specimens are no longer available for examination. (29)

Most authors agree that the principal natural vectors of Brugia pahangi and B. malayi within the Southeast Asian area are either Mansonia uniformis, Ma. bonneae, or Ma. dives although Ma. indiana, Ma. annulata, Anopheles barbirostris An. nigerrimus and An. campestris have also been implicated in Indonesia. (17)

From this investigation it can be concluded that: 1) man, cat, and silvered leaf monkey are

Tabel 6.	Mosquito host-range for filariid larvae (natural infection) in South
	Kalimantan, 1977 - 1979.

Genus/Species	Breinlia	Brugia	Cardio- filaria	Diro- filaria	Pele- citus	Setaria	Unknown spp
Ma. annulata Ma. annulifera Ma. bonneae/dives Ma. indiana Ma. uniformis Cq. crassipes An. nigerrimus An. peditaeniatus	+	+ + + + + +	+	+ + + + + +	+	+	+

the primary definitive hosts for Brugia infections. The silvered leaf monkey also serves as host for Wuchereria kalimantani and Dirofilaria magnilarvatum, and possibly Cardiofilaria sp., 2) Coquillettidia crassipes was the most commonly caught mosquito from the area studied, being found in areas partially to fully covered by canopy, at all elevations, and was attracted to cat, monkey, and human bait. The population density of Cq. crassipes increased during periods of low rainfall, and captures harbored only Cardiofilaria sp. 3) Mansonia bonneae and Ma. dives were the least commonly trapped vector species but was found at all elevations, in areas both heavily and partially covered by canopy and in forest-village localities unprotected by the canopy. The population density of Ma. bonneae/dives increased during periods of high rainfall. Both species, as a group, were preferentially attracted to humans and harbored species of Brugia. 4) Mansonia was the third most commonly uniformis trapped mosquito and the most commonly trapped vector of Brugia. It was most often collected in village areas during the night at 1-3 meter elevations. Ma. uniformis was equally attracted to animal and human bait all elevations. The population density of Ma. uniformis increased during periods of low rainfall but decreased when rainfall fell below 100 mm/month. Ma. uniformis harbored species of Brugia and Breinlia. 5) Preliminary observations on experimental intermediate hosts for the following filariae included: BRUGIA sp.: Aedes togoi, Anopheles nigernmus, An. barbirostris, Armigeres subalbatus;

WUCHERERIA KALIMANTANI: Aedes togoi, Anopheles subpictus and Culex quinquefasciatus (L1, L2).

As illustrated in table (6) much work remains to be done in order to unravel the complex zoonotic and epidemiologic relationships between parasite, vector and humans. Much of the work so far described is preliminary and thus requires more intensive investigations and follow up. As new and existing filarial parasites of animals and humans are studied, efforts must continue in elucidating their life cycle, vectors and possible epidemiological implications.

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In conducting research using animals, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" of the Institute of Laboratory Animal Resources, National Research Council (revision, 1978).

#### REFERENCES

- Palmieri, J.R., Purnomo, Lee, V.H., Dennis, D.T., Marwoto, H.A. (1980). Parasites in the silvered leaf monkey, Presbytis cristatus Eschscholtz 1821, with a note on a Wuchereria-like nematode. J. Parasitol, 66:170-171.
- Palmieri, J.R., Purnomo, Lee, V.H., Dennis, D.T., Marwoto, H.A. (1980). Filariid parasites of South Kalimantan (Borneo) Indonesia. Wuchereria kalimantani Sp. N. (Nematoda: Filaroidea) from the Silvered Leaf Monkey Presbytis cristatus Eschscholtz 1821. J. Parasitol, 66:645-651.
- Masbar, S., Palmieri, J.R., Marwoto, H.S., Purnomo and Darwis, F. (1981). Blood parasites of wild and domestic animals from South Kalimantan (Borneo), Indonesia. South East Asian J. Trop. Med. Pub. Hlth. 12(1):42-46.
- Palmieri, J.R., Masbar, S., Purnomo, Marwoto, H.A., Tirtokusumo, S. and Darwis, F. (1985). The domestic cat as a host for Brugian filariasis in South Kalimantan (Borneo), Indonesia. Journal of Helminthology, 59:277-281.
- Sommerman, K.M., and Simmet, R.P. (1967).
   Versatile mosquito trap. Mosq. News 27:412-417.
- Ramachandran, C.P. (1970). A guide to methods and techniques in filariasis investigations. Bull. Inst. Med. Res. Fed. Malaya, 15:39 p.
- Wharton, R.H. (1959). A simple method for mounting and preserving filarial larvae. Bull. Wld. Hlth. Org. 20:729-730.
- Ramachandran, C.P., Sivanandam, S. (1970). Experimental studies on the transmission of Wuchereria bancrofti to the domestic cat. Southeast Asian J. Trop. Med. Pub. Hlth. 1:150-152.
- Palmieri, J.R., Connor, D.H., Purnomo, Dennis, D.T., Marwoto, H.A. (1982). Experimental Infection of Wuchereria bancrofti in the silvered leaf monkey Presbytis cristatus Eschschlotz 1821. J. Helmintol. 56:243-245.
- Lowrie, R.C. Jr., and Eberhard, M.L. (1980). A new technique for immobilizing filaria larvae using dimethyl sulfoxide (DMSO) J. Parasit. 66:169-170.

- Nelson, G.S. (1960). The identification of filarial larvae in their vectors. Ind. J. Mal. 14:585-592.
- WHO (1987). Control of lymphatic filariasis. A manual for health personnel. World Health Organization, Geneva, 89 p.
- Sivanandam, S and Mak, J.W. (1975). Some problems associated with the processing and staining of blood film for filaria diagnosis. Journal of Medical Health and Laboratory Technology of Malaysia. 2:4-6
- Hawking, F. (1973). The distribution of human filariasis throughout the world. II Asia. WHO mimeograph WHO/FIL/ 73.114.
- Sasa, M. (1976). Human filariasis. A global survey of epidemiology and control. Univ. Tokyo Press, 819 p.
- Lie, K.J. (1970). The distribution of filariasis in Indonesia. A summary of published information. Southeast Asian J. Trop. Med. Pub.Hith. 1:366-376.
- Lim, B.L., Kurniawan, L., Sudomo, M., and Arbain, J. (1985). Status of Brugian filariasis research in Indonesia and future studies. Bul. Penelit. Kesehatan. 13(2):31-55.
- Laing, A.B.C., Edeson, J.F.B., and Wharton, R.H. (1960). Studies on filariasis in Malaya: The vertebrate hosts of Brugia malayi and B. pahangi. Ann. Trop. Med. Parasitol. 54:92-99.
- Barclay, R. (1969). Filariasis in Sabah, East Malaysia.
   Ann. Trop. Med. Parasit. 63:473-488.
- Hii, J.L.K., Kan S., Chin, K.F. and Chan M.R.C. (1984). Anopheles (Cellia) balabacensis Baisas is a vector of Wuchereria bancrofti in Sabah, Malaysia. Trans. Roy. Soc. Trop. Med. Hyg. 78:281-282.

- Wharton, R.H. (1960). Studies on filariasis in Malaya, field and laboratory investigations of the vectors of a rural strain of Wuchereria bancrofti. Ann. Trop. Med. Parasit. 54:78-91.
- Wharton, R.H. (1962). The biology of Mansonia mosquitoes in relation to the transmission of filariasis in Malaya, Bulletin No. 11, Institute for Medical Research, Malaysia, 114p.
- Wilson, T. (1961). Filariasis in Malaya a general review. Trans. Royal Soc. Trop. Med. Hyg. 35:107-134.
- Brug, S.L. (1928). Filariasis in Nederlandsch-Indie
   I. Geneesk. Tijdschr. Nederl.-Indie, 68:681-704
- Lie, K.J. and Rees, D.M. (1958). Filariasis in Indonesia: distribution, incidence and vectors. Proc. Int. Congr. Trop. Med. Malaria, Lisbon, 11:360.
- Lim, B.L. (1985). Distribution of Brugia malayi in relation to landscape ecology, reservoir hosts and mosquito vectors in Indonesia. Trop. Biomed. 2:34-39.
- Lim, H.K. (1974). Bibliography of medical parasitology in Indonesia (1855-1968). U.S. Naval Medical Research Unit, Taipei, NAMRU-2-SP-36: 111-125.
- Edeson, J.F.B. and Wilson, T. (1964). The epidemiology of filariasis due to Wuchereria bancrofti and Brugia malayi. Annual Rev. Entomology, 9:245-268.
- Nelson, G.S., Hiesch R.B. and Furlong M. (1962).
   Studies in filariasis in East Africa. II. Filarial infections in man, animals and mosquitoes on the Kenya coast. Trans. R. Soc. Trop. Med. Hyg. 56:202-217.

- Dissanaike, A.S. (1979). Zoonotic aspects of filarial infectious in man. Bull. Wld. Hlth. Org. 5:349-357
- Nelson, G.S. (1965) Filarial infections as zoonoses.
   J.Helminth. 39:229-249.
- Piessens, W.F., McGreevy, P.B., Piessens, P.W., McGreevy, M., Koiman, I. V., Saroso J.S. and Dennis D.T. (1980). Immune Responses in Human Infections with Brugla malayl. J. Clin. Invest. 65:172-179.
- Partono, F., Djakaria, Oemijati, S., Joesoef, A., Clarke, M.D., Cole, W.C., Lien J.C. and Cross, J.H. (1977). Filariasis in West Kalimantan. Southeast Asian J. Trop. Med. Pub. Hlth., 8:549-563.
- Chiang, G.L., Samarawickrema, W.A., Mak, J.W., Cheong, W.H., Sulaiman I. and Yap H.H. (1986). Field and laboratory observations on Coquillettidia crassipes in relation to transmission of Brugia malayi in Peninsular Malaysia. Ann. Trop. Med. & Parasit. 80:117-121.
- 36. Chiang, G.L., Samarawickrema, W.A., Eng, K.L., Cheong, W.H., Sulaiman, I., and Yap, H.H. (1986). Field studies on the surveillance of Coquillettidia crassipes (Van der Wulp) and the isolation of a strain of Cardiofilaria in Peninsular Malaysia. Ann. Trop. Med. & Parasit. 80:235-244.
- Purnomo, Partono F, Dennis, D.T. and Atmosoedjono, S. (1976) Development of the Timor filaria in Aedes togol: Preliminary observations. J. Parasitol; 62:881-885.
- Purnomo, Dennis, D.T., and Partono F. (1977) The microfilaria of Brugla timori (Partono et al, 1977 = Timor microfilaria, David and Edeson, 1964):
   Morphologic description with comparison to Brugla malayi in Indonesia J. Parasitol; 63:1001-1006.