

## MALAYAN FILARIASIS STUDIES IN KENDARI REGENCY, SOUTHEAST SULAWESI, INDONESIA I :

### Parasitological survey

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### ABSTRACT

Observasi penyakit filaria telah dilakukan pada penduduk di desa-desa Teteona, Lalohao, Pondidaha dan Wawolemo, Kecamatan Wawotobi, Kabupaten Kendari, Sulawesi Tenggara antara bulan November 1980 dan Oktober 1982. Sejumlah 3,499 jiwa atau antara 71.2% sampai 83.8% dari penduduk di desa-desa ini telah diperiksa darah jarinya masing-masing sebanyak 20 cumm terhadap adanya parasit filaria. Morphologi dan periodisitas dari embrio parasit yang ditemukan di dalam darah penduduk diperiksa dan begitu pula gejala-gejala klinis yang disebabkan. Nyamuk penular dari parasit di desa-desa ini ditentukan pula. Adanya jenis parasit yang sama pada binatang di sekitar kampung dipelajari dan diteliti lebih lanjut dengan percobaan eksperimental di laboratorium menggunakan hewan percobaan.

Dari hasil observasi ini ditemukan bahwa penduduk desa-desa ini telah diserang parasit filaria, masing-masing dengan derajat infeksi sebesar 9.6%, 15.8%, 9.3% dan 19.7%. Parasit yang ditemukan adalah dari jenis *Brugia malayi* dengan tipe mikrofilaria yang periodik nokturna. Sekitar 57.3% dari mikrofilaria ini melepaskan diri dari selubungnya. Gejala klinis berupa adenolymphangitis, lymphadenopathy, lymphscars, dan lymphedema pada penduduk masing-masing desa adalah 15.8%, 30.8%, 35.0% dan 52.0%. Gejala elephantiasis ditemukan pada tiga desa kecuali pada desa Teteona. Nyamuk dari jenis *Anopheles barbirostris*, *Anopheles nigerrimus*, *Mansonia uniformis* dan *Mansonia indiana* merupakan nyamuk penular alamiah dari parasit ini. Pada pemeriksaan darah kucing di sekitar kampung ini ditemukan pula embrio parasit: mikrofilaria yang menyerupai mikrofilaria malayi pada darah penduduk namun pada penelitian lebih lanjut dengan percobaan eksperimental menggunakan hewan percobaan belum dapat dipastikan jenis mikrofilaria dari kucing ini berasal dari *Brugia malayi*. Penelitian lebih lanjut dari parasit filaria pada binatang seperti kucing dan kera di desa-desa ini masih perlu dilanjutkan.

### INTRODUCTION

Malayan filariasis was surveyed in different parts of Sulawesi except Kendari Regency of Southeast Sulawesi, and the disease was found to be endemic in most of the areas surveyed

(Arbain Joesoef & Cross, 1978, Gandahusada et al., 1980). Preliminary studies of malayan filariasis in six villages at Kendari Regency revealed high prevalence of the disease among the population. Four of these villages which have higher incidences of the disease rates were

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selected as a pilot project for a field control trial.

Pre control surveillance of the entomological and parasitological studies were carried out, the former for a 24 months period, while the latter was done on two occasions in the four villages. The present paper reports the results of the parasitological survey, and confirmation of the vectors.

## MATERIALS AND METHODS

Four villages (Teteona, Lalohao, Pondidaha and Wawolemo) in the district of Wawotobi were selected (Figure 1). These villages are easily accessible by the main trunk road from Kendari city. The topography, ecology, and socio-economic status of these villages are similar (Table 1).

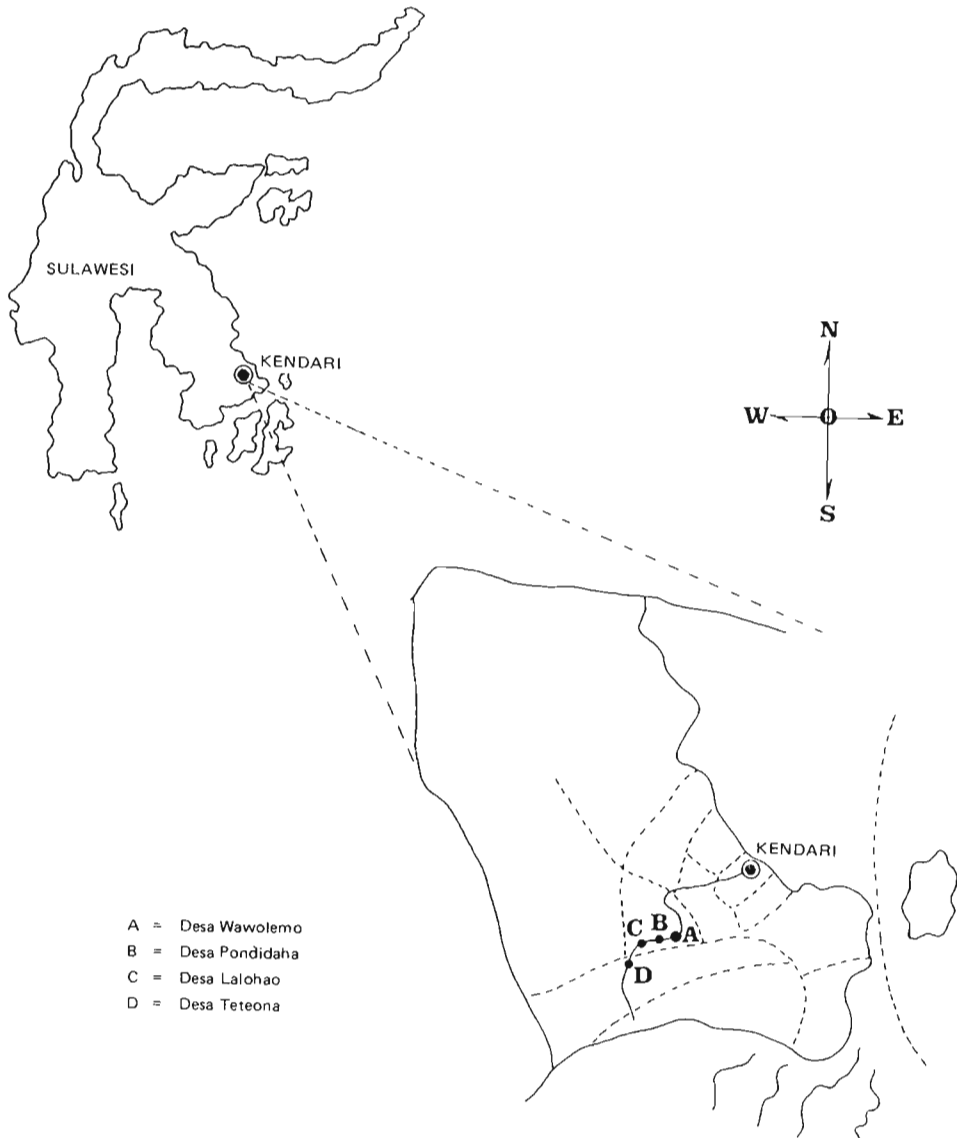


Figure 1. Showing localities of survey areas.

**Tabel 1. Physical structures of the four villages in Kendari Regency, Southeast Sulawesi, Indonesia.**

STATISTIC	District - Wawotobi			
	Teteona	Lalohao	Pondidaha	Wawolemo
Division (RK)	3	5	3	3
Sub-division (RT)	6	10	6	6
Total areas (ha)	3024	4400	4000	4200
% cultivated area (ha)	19.8	18.2	12.8	11.9
% wasteland (ha)	9.9	9.1	22.5	16.7
% swampland (ha)	17.3	20.5	20.0	19.0
% forest (ha)	33.0	25.0	42.1	33.3
% village (ha)	20.0	27.2	2.6	19.1
Total human population	857	1417	1201	1076
Number of household	138	268	188	163
% male	49.0	48.1	50.6	46.3
% female	51.0	51.9	49.4	53.7
% children <15 years	47.6	28.9	38.5	52.5
% adults >15 years	52.4	71.1	61.5	47.5
Farming occupation	80.0%	80.0%	80.0%	80.0%
Domestic animals				
Number of cat per household	1.09	0.7	0.8	1.0
Number of dog per household	0.14	0.4	0.5	0.5
Total number of cattle	49	208	63	50

Blood survey of inhabitants were taken from each of the four villages. From each person, a finger prick sample of 20 mm<sup>3</sup> of peripheral blood was obtained using a glass capillary tube, between 1900 to 2400 hours. Thick smears made in microscopic slides, were dehaemoglobinized the following morning and stained with Giemsa. Smears were examined under compound microscopes. The microfilariae (mf) were identified, counted and recorded.

Clinical examination for evidence of filaria diseases (elephantiasis, lymphoedema, lymphadenitis, and abscess scars along the course of lymphatus and glands) were also made of the sampled population.

Eighteen Mf volunteers from the four villages agreed to take part in the experiments to determine the periodicity of their microfilariae. A fingerprick sample of 20 mm<sup>3</sup> peripheral blood were taken from each volunteer every two hours during a 24 hour period. The pro-

cedure and processing of these blood samples were as above. Morphology of Mf of 5 carriers were studied.

Blood smears taken from the ear-tips of domestic cats (*Felis cattus*) in each of the villages including two capture macaques (*Macaca mauru*), were also examined for filarial infection.

A total of nine laboratory jirds (*Meriones unguiculatus*) were used for laboratory experiments. The jirds were intra-peritoneally inoculated with 3rd stage *Brugia malayi* larvae from laboratory-raised *An. barbirostris* (raised through pupae) infected from human Mf carriers. One was subcutaneously inoculated with 3rd stage *Brugia* larvae from *An. barbirostris* which were infected from a cat carrier. They were intra-peritoneally infected with 3rd stage *B. malayi* larvae from supposedly newly hatched *Ma. uniformis* (based on mosquitoes infested with water mites) infected from human Mf carriers, and two were subcutaneously inoculated with infected *Brugia* larvae recovered from natural infected *An. barbirostris* and *Ma. uniformis*.

Blood smears of infected laboratory jirds were taken at 3 months post infection, and subsequently at monthly intervals. Those animals died during the experimental periods were post-mortemed, and positive jirds were killed to recover adult worms.

A total 29 406 mosquitoes comprised of 17 470 *An. barbirostris*, 615 *An. nigerrimus*, 6895 *Ma. uniformis* and 4426 *Ma. indiana* were dissected for the presence of *Brugia* larvae.

## RESULTS

### Prevalence of Human Filariasis

The results of the brugian filariasis surveys in the four villages showed the prevalence rate was highest in Wawolemo, followed by Lalohao, and about equal in Teteona and Pondidaha (Table 2). The infection rate between male and female was about equal in Teteona, but higher in male than female in the other three villages. All the microfilariae in Mf carriers in these villages were identified as *B. malayi*. The age group of Mf carriers showed the Mf rates between 0-4 years old were low for all the villages

surveyed, but from 5–9, the Mf rates were about 2 times higher in Lalohao and Wawolemo than Teteona and Pondidaha. The Mf rates from 15–49 years old were high in each of the villages sampled. The Mf rates of 50 years old and above were shown to be higher in the few persons sampled. The median Mf density (MfD<sub>50</sub>) of the whole population for Teteona and Lalohao per 20 mm<sup>3</sup> blood was higher than that from Pondidaha and Wawolemo.

**Table 2** Malayan filariasis surveys in four villages at Kendari Regency, Southeast Sulawesi, Indonesia.

Statistics	Teteona	Lalohao	Pondidaha	Wawolemo
Population	856	1417	1202	1076
Population examined	74%	83.8%	71.2%	76.3%
Overall Mf rates	9.6%	15.8%	9.3%	19.7%
Mf rates for males	9.2%	19.5%	12.0%	24.4%
Mf rates for females	10.1%	12.4%	6.5%	15.2%
MfD <sub>50</sub>	7.1	7.2	6.3	6.1

Clinical examination in Teteona, Lalohao, Pondidaha and Wawolemo showed 25.5% (634 person), 47.0% (1187), 35.0% (856), and 52.5% (821) respectively had evidence of the disease. Clinical symptoms of the disease such as, adenolymphangitis, lymphadenopathy, lymphnode scars, and lymphedema observed in these villages were 15.8%, 30.8%, 35% and 52%, while with microfilariemia only were 9.6%, 15.8%, 9.3%, and 19.7% and 8 elephantiasis cases were found in the villages of Lalohao, Pondidaha and Wawolemo.

### Microfilariae Periodicity Studies

Periodicity studies were carried out on 18 Mf carriers from the four villages. The data of each of the Mf carriers from each village were statistically analysed followed that of Aikat and Das's modified method (1976). The F value in each Mf carrier from each village was greater than 4.26, which determined the relationship between the microfilariae count and hour is of the harmonic wave type. Thus, the data of Mf carriers in each village were combined and analysed (Table 3). As observed in

the Table, the peak time (k) of Mf carriers in each village was between 22.54 and 24.11 hours, and the periodicity index (D) was between 88.37 and 117.50 which suggest that the Mf periodicity of these carriers in all the four villages were of the nocturnally periodic type (Figure 2).

**Table 3** Observed and theoretical curves of periodicity of *Brugia malayi* microfilariae in Kendari Regency, Southeast Sulawesi Province, Indonesia.

	Teteona	Lalohao	Pondidaha	Wawolemo
Number of Mf carriers	5	5	4	4
No. of frequencies (n)	12	12	12	12
$\frac{\sum y}{n} = (m)$	140.6	463.4	78.5	211.0
$\sum y^2$	2,335.24	25,263.32	839.86	6,399.63
$\frac{2 \sum y \cos 15 h}{n} = (b)$	9.98	34.09	7.19	20.64
$\frac{2 \sum y \cos 15 h}{n} = (c)$	-2.93	1.50	-0.23	0.99
$(b)^2 + (c)^2 = (a^2)$	10.40	34.13	7.19	20.66
Peak hour = (k)	22.54	24.10	23.53	24.11
$\frac{n}{2} a^2$				
$\frac{1}{n-3} (\sum y^2 - \frac{(\sum y)^2}{n} - \frac{n}{2} a^2)$ = (F <sup>2</sup> ) 9	149.95	165.36	181.04	179.71
Periodicity Index (D)	88.76	88.37	110.04	117.50

### Morphological Study of Microfilariae

The study revealed that 57.3% of the microfilariae in the combined 5 Mf carrier exsheathed only. These characteristics are found to be different in periodic *B. malayi* from Malaysia and Thailand.

### Animal Filariasis

A total of 163 domestic cats, comprising of 30 from Teteona, 70 from Lalohao, 40 from Pondidaha and 23 from Wawolemo were examined for filarial parasites. Of these, 15.7% from Lalohao, 5% from Pondidaha and 4.3% from Wawolemo were found positive with microfilariae of *Dirofilaria* and *Brugia* like parasites. Autopsy of 4 infected cats from La-

lohao, found adults worms namely, *Filarioides* sp., *Anafilaricides* sp., *Vageloides* sp., *Bronchostrongylus* sp., but adult of *Brugia* sp. was not confirmed. Blood examination from two captive macaques were negative with filarial parasite.

### Natural Infection of Mosquitoes

The natural infection of *An. barbirostris* with *Brugia* larvae was 0.21% compared to 0.16% on *An. nigerrimus*. Among the two *Mansonia*, *Ma. uniformis* was 0.4% compared to 0.6% in *Ma. indiana*.

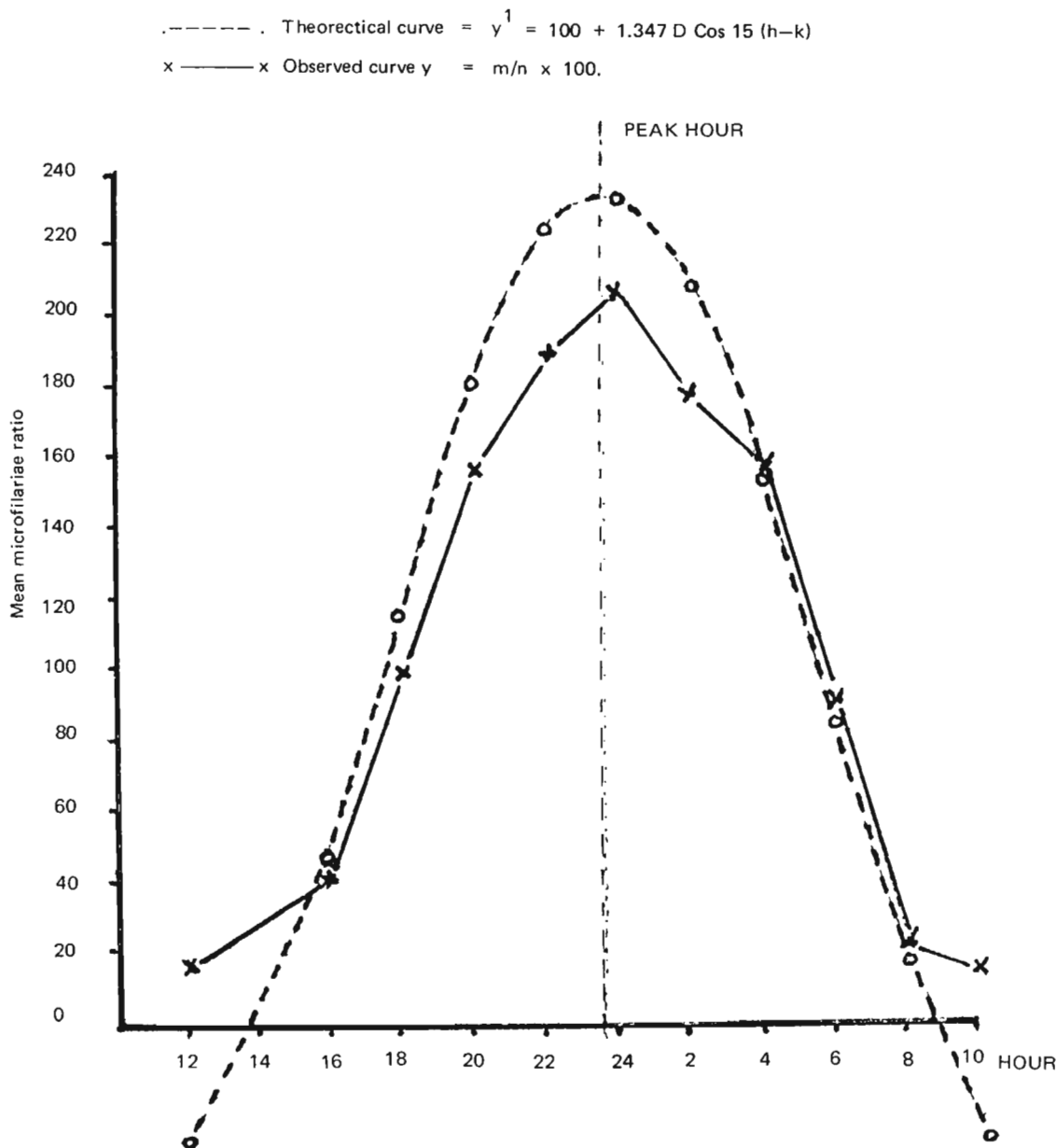


Figure 2. Observed and theoretical curves of periodicity of *Brugia malayi* microfilariae in Kendari Regency, Southeast Sulawesi Province, Indonesia

## Experimental Studies

Two of the three laboratory jirds exposed to infective *Brugia* larvae from experimental infected *An. barbirostris* were positive with Mf 3 month post-infections. Among the two positives, one was killed at the 4th month post-infections, and 5 male and 2 female adult *Brugia* worms were recovered from the testes which were confirmed as *B. malayi*. The second positive which died at the 5th month post-infection, no adult worms were recovered.

Two of the 3 jirds inoculated with infective larvae recovered from experimental infected *Ma. uniformis* were positive with Mf on the 3rd month of post-infections. Both were dissected at the 4th month, and one was found with 1 female adult worm, the other with 2 male and 2 female adult worms. These worms were confirmed as *B. malayi*.

The other two jirds which were inoculated with 32 infective *Brugia* larvae from wild caught *An. barbirostris*, and 38 infective larvae from wild *Ma. uniformis* were found positive with Mf 3 months post-infection. Both the jirds were killed on the 5th month, with 2 female and 1 male adult worms recovered in the first jird, and 3 female and 2 male in the second one. The adult worms were also confirmed as *B. malayi*.

The 4th laboratory jird which was inoculated with infective stage larvae of experimental mosquitoes from a cat Mf carrier was also positive with Mf 3 month post-infection. The jird was sacrificed at the 4th month, and 1 male and 1 female adult worms were recovered from the testes. The worms were identified as probably a new *Brugia* sp.

## DISCUSSION

The prevalence of malayan filariasis in each of the four indigenous villages was very high. Clinical examination showed the disease rates was about 2–3 times higher than the Mf rates in carriers. Periodicity studies of 18 Mf carriers, showed 8 of them with Mf throughout the 24 hours period, and yet each of these demonstrates nocturnally periodicity pattern. Morphological

studies showed 42.7% of the microfilariae sheathed. Mashat (1960) studied the morphology of microfilariae in 13 patients with nocturnal periodic *B. malayi* in Bengkulu, Sumatera and found 52–100% of the microfilariae sheathed. Sudomo et al. (1982) in their studies of 12 nocturnally periodic *B. malayi* patients in north Bengkulu found 44–100% of the microfilariae to be sheathed also.

Based on these three studies, it was apparent that the parasite in Southeast Sulawesi resembles those of Bengkulu in Sumatera, but behaves differently to that of the periodic *B. Malayi* strain in Malaysia and Thailand (Guptavani and Harinasuta, 1971; Sivanandam and Dondero, 1972). The adult worms recovered from experimental jirds inoculated with infective larvae from experimental mosquitoes fed to human carriers confirmed that they were *B. malayi*.

Although 8.9% of 163 cats from the four villages which were naturally infected with *Brugia*-like microfilariae, but adult *Brugia* worms that recovered during autopsy of four cats with heavy Mf densities was not confirmed. Further experimental study of the Mf of a naturally infected cat through mosquitoes and inoculated into jirds, is strongly recommended.

*Ma. uniformis*, *Ma. indiana*, *An. barbirostris* and *An. nigerrimus* were found to be natural vector of *B. malayi*. Experimental studies confirmed that *An. barbirostris* and *Ma. uniformis* are important vectors of periodic *B. malayi* in the study areas. Although no experimental study on *Ma. indiana* was carried out, the fact that 0.6% natural infection was observed, indicates that this species is equally as important vector as that of the other two species. *An. barbirostris* was confirmed as the vector of periodic *B. malayi* in Central Sulawesi (Soeroto Atmosoedjono, 1976).

The present malayan filariasis survey shows that the characteristics of the parasite although behaves like the sub-periodic strain, but exhibits a periodic pattern. The recovery of adult worms of *B. malayi* from human Mf carrier through mosquitoes via laboratory animal models, and based on statistical analysis of 18 Mf carriers

followed Aikat and Das's modified method (1976), confirmed that the brugian filariasis among the infected people in the study is of the nocturnally periodic *B. malayi* type.

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