

A STUDY ON THE MICROFILARIAL PERIODICITY AT BIREUEN, THE TYPE LOCALITY OF BRUGIA MALAYI (BRUG, 1927)*

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Brugia malayi (Brug, 1927) adalah salah satu bibit penyakit filaria yang ditemukan di Indonesia yang dilaporkan pertama kali oleh Brug ditahun 1927 dari daerah endemis Bireuen, Aceh Utara. Walaupun akhir-akhir ini diketahui di Indonesia bahwa *B. malayi* ini mempunyai sifat periodisitas yang periodik nokturna dan juga sub-periodik nokturna, namun dari daerah endemis Bireuen dimana parasit ini pertama kali ditemukan dan dilaporkan belum jelas sifat periodisitasnya. Oleh sebab itulah dilakukan penyelidikan didaerah ini guna menentukan sifat periodisitas dari *B. malayi* didaerah endemis dimana pertama kali bibit penyakit dilaporkan. Dari hasil penyelidikan didaerah endemis Bireuen pada bulan Agustus tahun 1974 ini ternyata bahwa *B. malayi* yang ditemukan mempunyai sifat periodisitas yang periodik nokturna. Disamping itu ditemukan pula bibit penyakit filaria dari jenis *Wuchereria bancrofti* yang juga mempunyai sifat periodisitas yang periodik nokturna.

Brugia malayi (Brug, 1927) is a human parasite described first by Lichtenstein (1927) and Brug (1927) based on the morphology of the microfilariae found in thick blood smears collected from man in Bireuen, northern Sumatra. This parasite is now recognized to be widely distributed in South and East Asia, causing fever attacks, lymphangitis and elephantiasis of legs in a large number of people. It has further been elucidated that there exist two distinct races, or possibly subspecies, within the species complex of *B. malayi*, a nocturnally periodic race and a nocturnally subperiodic race. According to Turner & Edson (1957) and Wilson et al (1958), these two races in West Malaysia differ not only in the pattern of the microfilarial periodicity, but also in a number of other characters, such as the infectivity to certain animal hosts and to various groups of mosquito intermediate hosts.

These two races have been found to occur also in Indonesia (Arbain J, et al. 1973). However, it was not clear which of the two races was the one originally described by Lichtenstein and Brug from Bireuen.

In this connection, a field study was conducted by the present authors in August 1974 in Bireuen, mainly for the purpose to see the pattern of the periodicity of microfilariae of *B. malayi*. In a small scale survey of the people in this area, carriers of both *B. Malayi* and *Wuchereria bancrofti* were discovered, and studied the microfilarial density in blood samples collected at 2 hour intervals over a period of 24 hours, both were shown to be the nocturnally periodic form.

THE AREA SURVEYED AND METHODS EMPLOYED

The people surveyed were from the villages formerly called Bireuen and Peusangan in North Aceh Regency, Aceh Province. The area is situated in the north-western part of Sumatra, at latitude 5°10' - 5°15' N and 96°50' E (Fig). The town of Bireuen could be reached by driving on road for about 240 km eastwards from Banda Aceh, the capital and the westmost city of Aceh. The total popula-

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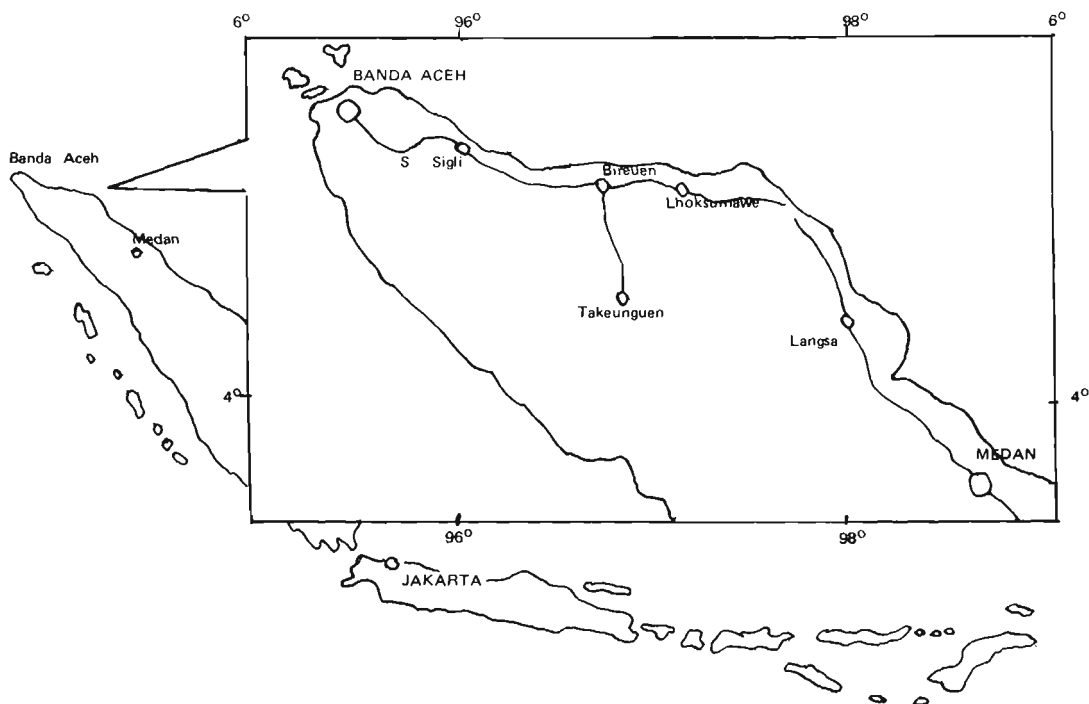


Fig. Map of Aceh province showing the locality of the study area.

tion of Bireuen as of 1974 was 10,950 consisting of 11 subvillages. The population of the subvillages surveyed was 785 in Bireuen Gadung and 676 in Rusip Dayah in Peusangan. The area is a part of the large flat plains on the northern coast of Sumatra, and rice cultivation and coconut growing are the primary occupation.

In each of the villages, people of all ages were requested to visit a village-hall during the hours from 9 p.m. to midnight. Measured blood samples of 20 mm^3 were collected from a finger prick and were smeared on microscope slides. The thick blood smears were examined after dehemoglobinized in tap water and stained with Giemsa solution.

For examination of the periodicity of microfilariae, the carriers were admitted to a hospital, and six blood samples each 10 mm^3 in volume were taken at 2 hour intervals over a period of 24 hours, and the numbers of microfilariae found in each of the 20 mm^3 thick blood smears were recorded after stained with Giemsa.

RESULTS

In the present study, four villages were surveyed during two weeks. At Capa Utara, Bireuen, 75 persons were examined, and one (1.3 percent) was positive for the microfilariae of *W. bancrofti*. At Cot Ketapang, Bireuen, 88 persons were examined, and 3 (3.4 percent) were found carrying the microfilariae of *B. malayi*. At Bireuen Gadung, one *W. bancrofti* microfilaria carrier (0.6 percent) was found out of 158 persons examined. At Rusip Dayah, Peusangan, 3 *B. malayi* microfilaria carriers (3.3 percent) were found out of 91 persons examined. The overall microfilaria rate was 0.49 percent (2 positives of 412) for *W. bancrofti*, 1.46 percent (6 positives of 412) for *B. malayi*, and 1.94 percent for both combined.

Five *B. malayi* carriers and one *W. bancrofti* carriers were selected for the study of periodicity. Six thick blood smears each 10 mm^3 in volume were made from individual carriers at two hour intervals, 12 times star-

Table 1 Observations on the periodicity of the microfilariae of *B. Malayi* (cases I–V) and *W.bancrofti* (case VI) in Bireuen; showing microfilaria counts in six 10–mm³ blood smears collected at 2 hour intervals.

Numbers in parentheses: total of six smears, adjusted to 60 mm³ when damaged smears are included; numbers in *italic* counts is damaged smears.

Hour	Case I	Case II	Case III	Case IV	Case V	Case VI
18	1, 0, 0, 0, 5, 0; (6)	0, 0, 0; 0, 0, 0; (0)	0, 1, 1; 2, 1, 1; (6)	2, 0, 1; 0, 1, 1; (5)	0, 0, 0; 0, 0, 0; (0)	0, 0, 0; 1, 0, 0; (1)
20	8, 8, 6; 7 12, 3; (44)	6, 2, 2; 6, 5, 2; (29)	2, 2, 2; 4, 6, 4; (22)	1, 1, 0; 2, 2, 2; (8)	0, 0, 0; 0, 0, 0; (0)	15, 6, 3; 3, 7, 4; (42)
22	14, 19, 15, 11, 11, 27; (97)	7, 1, 3; 7, 2, 3; (42)	0, 2, 1; 1, 2, 3; (9)	3, 5, 4; 2, 6, 0; (20)	0, 0, 0; 0 0, 0; (0)	16, 7, 4; 17, 16, 4; (84)
0	21, 22, 11; 10, 15, 10; (89)	3, 4, 0; 13, 19, 10; (84)	1, 1, 2; 2, 3, 8; (17)	4, 3, 5; 1, 4, 2; (19)	0, 0, 1; 0, 1, 1; (3)	9, 17, 11; 11, 2, 4; (72)
2	17, 11, 13; 10, 14, 8; (73)	11, 8, 15; 6, 16, 7; (63)	2, 0, 2; 1, 1, 0; (8)	5, 1, 3; 2, 1, 2; (14)	0, 0, 0; 1, 1, 0; (2)	8, 14, 5; 12, 11, 12; (62)
4	6, 23, 13; 15, 17, 9; (83)	16, 18, 15; 20, 12, 17; (98)	2, 0, 1; 1, 0, 2; (6)	3, 2, 4; 2, 2, 3; (16)	0, 1, 1; 0, 0, 0; (2)	6, 7, 5; 5, 6, 7; (36)
6	10, 0, 0; 15, 12, 13; (75)	6, 9, 7; 12, 11, 12; (57)	not done	not done	not done	5, 0, 1; 7, 7, 1; (20)
8	6, 9, 14; 11, 4, 6; (50)	1, 0, 1; 2, 2, 1; (7)	2, 6, 2; 0, 1, 1; (12)	0, 0, 0; 1, 1, 0; (2)	0, 0, 0; 0, 0, 0; (0)	5, 6, 5; 5, 0, 0; (21)
10	2, 2, 3; 9, 2, 1; (19)	0, 0, 0; 0 0, 0; (0)	not done	not done	not done	2, 0, 0; 0, 1, 1; (4)
12	0, 1, 0, 0, 2, 0; (3)	0, 0, 0; 0, 0, 0; (0)	0, 0, 0; 0, 0, 0; (0)	0, 0, 0; 0, 0, 0; (0)	0, 0, 0; 0, 0, 0; (0)	0, 0, 0; 0, 0, 0; (0)
14	0, 0, 0; 0, 0, 0; (0)	0, 0, 0; 0, 0, 0; (0)	not done	not done	not done	0, 0, 0; 0, 0, 0; (0)
16	0, 0, 1; 0, 0, 0; (1)	0, 0, 0; 0, 0, 0; (0)	0, 0, 0; 0, 0, 0; (0)	0, 2, 0; 0, 0, 0; (2)	0, 0, 0; 0, 0, 0; (0)	0, 0, 0; 0, 0, 0; (0)

ting from 18 hour (6 p.m.). The numbers of microfilariae found in each of these smears are shown in table 1.

In both *B. malayi* (cases Nos. I – V) and *W. bancrofti* (case No. VI) carriers, the feature of periodicity of the microfilariae could be estimated from the counts shown in table 1 as being a nocturnally periodic type. The results were statistically analyzed by the me-

thod proposed by Sasa & Tanaka (1972, 1974), as shown in table 2. The periodicity index came out to be 89.44 in *B. malayi* and 107.35 for *W. bancrofti*.

The value of the best estimate of peak hour as calculated with a computer programme was 1.8 hour (48 minutes past one o'clock) in *B. malayi* and 0.4 hour (24 minutes past midnight) in *W. bancrofti*.

Table 2 Total microfilaria counts in 60 mm³ blood samples of *B. malayi* cases (No. I-V and *W. bancrofti* case (No.VI) from Bireuen.

<i>B. malayi</i> cases						<i>W. bancrofti</i> case ratio							
Hour No.	I	No. II	No. III	No. IV	No. V	Total	Ratio @	R-100	(R-100) ²	No. VI	to mean	R-100	(R-100) ²
18	6	0	6	5	0	17	18.3	-81.7	6674.89	1	3.5	-96.5	9.312.25
20	44	29	22	8	0	103	110.7	10.7	114.49	42	147.4	47.4	2246.76
22	97	42	9	20	0	168	180.5	80.5	6480.25	84	294.7	194.7	37908.09
0	89	84	17	19	3	212	227.8	127.8	16332.84	72	252.6	152.6	23286.76
2	73	63	8	14	2	160	171.9	71.9	5169.61	62	217.5	117.5	13806.25
4	83	98	6	16	2	205	220.2	120.2	14448.08	36	126.3	26.3	691.69
6	75	57	*9	*9	*1	151	162.2	62.2	3868.84	20	70.2	-29.8	884.04
8	50	7	12	.2	0	71	76.3	-23.7	561.89	21	73.7	-26.3	691.69
10	19	0	*6	*1	*0	26	27.9	-72.1	5198.41	4	14.0	-86.0	7396.00
12	3	0	0	0	0	3	3.2	-96.8	9370.24	0	0	-100.0	10000.00
14	0	0	0	*0	*0	*0	0	-100.0	10000.00	0	0	-100.0	10000.00
16	1	0	0	0	0	1	1.1	-98.9	9781.21	0	0	-100.0	10000.00
Total	540	380	95	94	8	1117	1200.1	0.1	880000.51	342	11999	-0.1	126767.53

Mean count : 1117/12 = 93.08

Periodicity index : D = 88000.51/11 = 89.44

Mean count : 342/12 = 28.50

D = 126767.53/11 = 107.35

@ Ratio to mean : percentage obtained by dividing the hourly count with the mean count.

DISCUSSION

B. malayi is a human filarial parasite reported as a new species by Lichtenstein (1927) and by Brug (1927) by the name of *Filaria malayi*. The materials used by these authors for differentiating this species from other previously known human filarial parasites were the microfilariae discovered from blood of man in Bireuen. While conducting studies on filariasis in Aceh, Lichtenstein (1927) observed that both elephantiasis and microfilaria carriers were very common among inhabitants in the Bireuen area, and further that the microfilariae failed to complete their development in *Culex fatigans*, in contrast to the already well-known fact that this mosquito species serves as an excellent intermediate host of *W. bancrofti*. Lichtenstein (1927) states that in blood surveys conducted in the Bireuen area, microfilariae (species not mentioned) were found in the blood of 13 persons out of 57 (23 percent) examined at Samalanga, 13 out of 23 (56 percent) examined at Garoegoe, 10 out of 16 (62.5 percent) examined at Leuboe, 8 out of 12 (67 percent)

examined at Gloempang Doea, and 26 out of 67 (39 percent) at Bireuen.

The blood specimens collected by Lichtenstein from microfilaria carriers in Bireuen were sent to Brug in Batavia for identification. Brug (1927) made detailed studies with these materials, and discovered several morphological characters in these microfilariae with which they could be differentiated as a distinct species from those of *W. bancrofti*, and proposed a scientific name *F. malayi* for this new species. The same parasite was later reported to be widely distributed throughout the Indonesian region by Brug (1928, 1930), and also from a number of countries in South and East Asia (India, Ceylon, Burma, Thailand, Malaysia, Philippines, China, North Vietnam, Korea and Japan). The generic name *Brugia* was created by Buckley (1960) to include several related filarial species, with *malayi* as the type species.

Through recent investigations conducted by a number of workers in South and East Asia, it has been demonstrated that there exist two distinct forms of *B. malayi*, which differ from each other mainly in the pattern of the micro-

filial periodicity. Such an evidence was first reported by Turner & Edeson (1957), who observed that the microfilariae of *B. malayi* in human carriers from Penang (West Malaysia) showed a marked nocturnal periodicity and were rarely found in the peripheral blood during the daytime, while those in human carriers from East Pahang (West Malaysia) showed some nocturnal rise but could be readily found at all times. Wilson et al. (1958) summarized the differences between the two forms as in table 3.

According to the nomenclature proposed by a WHO Expert Committee on Filariasis (Second Report, 1967) the former is called "the nocturnally periodic form" and the latter to be "the nocturnally subperiodic form." At present, *B. malayi* known from India, China, Korea and Japan belongs to all the periodic form, and that from the Philippines has been mostly subperiodic. West Malaysia has the two forms as stated above, though they are geographically isolated. In Indonesia, the two forms are also co-existent, and previous obser-

Table 3 Summary of the differences between *W. malayi* in man in Malaya. (After Wilson et al. 1958)

Feature	Periodic form.	Semi-periodic form
Microfilarial periodicity	Markedly nocturnal : microfilariae rarely found during day.	Some nocturnal rise, but microfilariae readily found at all times.
Microfilarial appearances in Giemsa stained blood films.	Empty sheaths common ! few microfilariae still enclosed in sheath.	Empty sheaths very rare : many microfilariae still enclosed in sheath.
Formalin-fixed microfilariae.	Greater mean length.	Shorter mean length.
Experimental mosquito infections.	Highly infective to <i>Anopheles barbirostris</i> : does not develop readily in <i>Mansonia longipalpis</i> .	Does not develop readily in <i>A. barbirostris</i> : highly infective to <i>M. longipalpis</i> .
Natural vectors	<i>A. barbirostris</i> , <i>A. hyrcanus</i>	<i>M. longipalpis</i> , <i>M. annulatus</i> , <i>M. uniformis</i> .
Experimental infections in cats.	Does not develop well, and microfilaria count remains low.	Infections readily established with high microfilaria counts.
Natural infections in cats	Rare	Common
Terrain	Coastal ricefields and open swamp.	Fresh water swamp forest.

vations have shown that the microfilariae in Kalimantan were represented by the subperiodic form and those in Sulawesi were the periodic form (Arbain J, et al. 1973).

Sasa & Tanaka (1972, 1974) devised a mathematical method with which the pattern of the microfilarial periodicity could be expressed quantitatively, and made statistical analysis of data collected by a number of workers referring to the periodicity of various filarial parasites. These authors have concluded that so far as the present information concerns, the two form could be distinctly differentiated by the size of "the periodicity index" calcula-

ted from the observed data, and in view of the absence of intermediate forms, they might be regarded as different "subspecies".

SUMMARY

A field study was conducted in August 1974 at Bireuen, Aceh Province of the type locality of *B. malayi* from where the parasite was first described by Lichtenstein (1927) and Brug (1927), in order to determine the pattern of the microfilarial periodicity. From the results, it has been demonstrated that both the microfilariae of *B. malayi* as well as those

of *W. bancrofti* in man from Bireuen area were the nocturnally periodic form. The microfilaria rates observed in the present survey were much lower than those recorded by Lichtenstein (1927) some 50 years ago in the same areas.

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