

A STUDY ON THE MICROFILARIAL PERIODICITY AT BIREUEN, THE TYPE LOCALITY OF *BRUGIA MALAYI*

MANABU SASA, RYUKO SHIRASAKA*, ARBAIN JOESOEF**, RAMLI ABDULWAHAS***
and HISASHI YAMAURA*

Institute of Medical Science, University of Tokyo. *Tokyo Women's Medical College.

**Division for Control of Filariasis and Other Helminthic Diseases, Ministry of Health,
Indonesia, and ***Pensangan Health Center, North Aceh, Indonesia.

INTRODUCTION

Brugia malayi 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, Indonesia. This parasite is now recognized to be widely distributed in South and East Asia, causing fever attacks, lymphangitis and elephantiasis of legs in large numbers 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 and Edeson (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 (Joeseof, 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

W. bancrofti were discovered, and in studies of 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 Pensangan in North Aceh Regency, Aceh Province, Indonesia. The area is situated in the northwestern part of Sumatra, at latitude 5° 10' - 5° 15' N and 96° 40' - 96° 50' E. The town of Bireuen could be reached by road for about 240 km eastwards from Banda Aceh, the capital and the westmost city of Aceh. The total population of Bireuen as of 1974 was 10,950, residing in 11 subvillages. The population of the subvillages surveyed was 785 in Bireuen Gadung and 676 in Rusip Dayah in Pensangan. The area is a part of the large flat plain 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 c.mm were collected from a finger prick and were smeared on microscope slides. The thick blood smears were examined after being dehaemoglobinized in tap water and stained with Giemsa.

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For examination of the periodicity of microfilariae, the carriers were admitted to a hospital, and six blood samples each 10 c.mm 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 c.mm thick blood smears were recorded after stained with Giemsa.

RESULTS

Four villages were surveyed during two week period of the present study at Bireuen. At Capa Utara, Bireuen, 75 persons were examined, and one (1.3%) was positive for the microfilariae of *Wuchereria bancrofti*. At Cot Ketapang, Bireuen, 88 persons were examined, and 3 (3.4%) were found carrying the microfilariae of *Brugia malayi*. At Bireuen Gadung,

one *W. bancrofti* microfilaria carrier (0.6%) were found out of 158 persons examined. At Rusip Dayah, Pensangan, 3 *B. malayi* microfilaria carriers (3.3%) were found out of 91 persons examined. The overall microfilaria rate was 0.49% (2 positives of 412) for *W. bancrofti*, 1.46% (6 positives of 412) for *B. malayi*, and 1.94% for both combined.

Five *B. malayi* carriers and one *W. bancrofti* carrier were selected for the study of periodicity. Six thick blood smears each 10 c.mm in volume were made from individual carriers at two hour intervals, 12 times starting from 18 hour (6 pm). The numbers of microfilariae found in each of these smears are shown in Table 2.

In both *B. malayi* (cases Nos. 1-5) and *W. bancrofti* (case No. 6) carriers, the feature of

Table 1

Summary of the differences between *W. malayi* in man in Malaya (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>Mansonia longipalpis</i> .
Natural Vectors	<i>A. barbirostris</i> , <i>hyrcanus</i>	<i>M. longipalpis</i> , <i>annulatus</i> , <i>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.

Table 2

Observations on the periodicity of the microfilariae of *B. malayi* (cases 1-5) and *W. bancrofti* (case 6) in Bireuen; showing microfilaria counts in six 10 c.mm blood smears collected at 2 hour intervals.

Hour	Case No. 1	Case No. 2	Case No. 3	Case No. 4	Case No. 5	Case No. 6
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, <i>1</i> , 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)

Number in parentheses: total of six smears, adjusted to 60 c.mm when damaged smears are included; number in *italics*: counts in damaged smears.

Table 3

Total microfilaria counts in 60 c.mm blood samples of *B. malayi* cases (Nos. 1-5) and *W. bancrofti* case (No. 6) from Bircuen.
(*count estimated by taking the average of the two observed counts)

Hour	<i>Brugia malayi</i> cases						<i>W. bancrofti</i> case						
	No.1	No.2	No.3	No.4	No.5	Total	Ratio to** mean	R-100	(R-100) ²	No. 6	Ratio 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	9312.25
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	88000.51	342	119.99	-0.1	126767.53
Mean count: 1117/12 = 93.08						Mean count: 342/12 = 28.50							
Periodicity Index: D = 88000.51/11 = 89.44						D = 126767.53/11 = 107.35							

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

periodicity of the microfilariae can be estimated from the counts shown in Table 2 as being a nocturnally periodic type. The results were statistically analyzed by the method proposed by Sasa and Tanaka (1972, 1974), as shown in Table 3. 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*.

DISCUSSION

Brugia 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 *Wuchereria 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%) examined at Samalanga, 13 out of 23 (56%) examined at Garoegoe, 10 out of 16 (62.5%) examined at Leuboe, 8 out of 12 (67%) examined at Gloempang Doea, and 26 out of 67 (39%) 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 *Filaria 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, Sir Lanka, 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 *Brugia malayi*, which differ from each other mainly in the pattern of the microfilarial periodicity. Such an evidence was first reported by Turner and Edeson (1957), who observed that the microfilariae of *Brugia 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 1.

According to the nomenclature proposed by a WHO Expert Committee on Filariasis (WHO, 1967) the former is called "the nocturnally periodic form" and the latter to be "the nocturnally subperiodic form". At present, *Brugia malayi* known from India, China, Korea and Japan belongs all to 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 observations have shown that the microfilariae in Kalimantan were represented by the subperiodic form and those in Sulawesi were the periodic form (Joesoef, 1973).

Sasa and 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 indicates, the two forms can be distinctly differentiated by the size of "the periodicity index" calculated from the observed data, and in view of the absence of intermediate forms, they may be regarded as different "sub-species".

SUMMARY

A field study was conducted in August 1974 at Bireuen, Indonesia, at the type locality of *Brugia 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 are 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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