

Ecology of Bancroftian Filariasis

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OBJECTIVE: To investigate the ecology of bancroftian filariasis in rural areas of Sangkhlaburi district, Kanchanaburi Province. Specific objectives include the following:

1. To identify the vector(s) of *Wuchereria bancrofti* by A) the demonstration of filariae in wild-caught mosquitoes and B) by feeding laboratory-reared strains of potential vector species on known microfilaria-carriers.
2. To determine the prevalence of human infections and the periodicity of microfilaremia, applying the techniques of direct chamber counting and membrane filtration for the isolation of microfilariae.
3. To collect information on the distribution, larval habitats and bionomics of suspected vector species and to obtain correlated series of larvae, pupae and adults of these mosquitoes for taxonomic studies.
4. To evaluate the clinical expression of human infections.

DESCRIPTION: In 1970 Harinasuta and associates (1) described an endemic focus of bancroftian filariasis in rural villages located near the headwaters of the Kwai River in the Sangkhlaburi district of Kanchanaburi Province. Prevalence rates of infection with *W. bancrofti* up to 30 per cent were observed in some villages, and many cases of filarial hydrocoele were reported. Bancroftian filariasis is rarer in Thailand than the type caused by *Brugia malayi*; moreover, in this area it differed from the type usually seen in Southeast Asia in that microfilaremia was nocturnally subperiodic, with peaks near 2000 hours, but with microfilariae present in significant numbers in the peripheral blood during daylight hours. Infective stage larvae of *W. bancrofti* were found in wild-caught mosquitoes belonging to the *Aedes (Finlaya) niveus* complex. The females of *A. niveus* and possibly 20 other closely related species are so similar that they cannot be differentiated with certainty at the present time; these mosquitoes are among the most common diurnal man-biting mosquitoes in the forested areas of Southeast Asia, Harinasuta et al also reported finding *Aedes (Finlaya) harveyi*, *Anopheles maculatus*, *Anopheles minimus* and *Anopheles vagus* infected with immature filarial larvae. Subperiodic *W. bancrofti* infections transmitted by *Anopheles minimus flavirostris* and species of the *Aedes niveus* complex have also been reported from the Philippines by Cabrera and Rozeboom (2) and *Anopheles leucosphyrus* was identified as the vector of *W. bancrofti* in Sarawak (3).

The detection of microfilaremia, most often by examination of thick films prepared from 20 to 40 c. mm. of blood obtained from the finger, has been commonly relied upon to determine filariasis prevalence rates. The thick film technique has the advantage of being easy to use in the field; however, in recent years it has been shown that prevalence rates and the apparent age distribution of microfilaremia, based upon this survey method, have been imprecise (4). A relatively new technique, that has been shown to be as sensitive as the thick film and as easy to perform under field conditions, is that of direct counts of microfilariae in specially constructed chambered slides (5). Another new survey procedure, the isolation of microfilariae by filtration of blood through Millipore (6) or Nucleopore filters (7), has yielded higher positivity rates, in areas of nocturnally periodic infections, from daytime bloods than the standard thick blood film taken at night during microfilaremia peaks (8). The value of this technique in identifying

microfilaremia carriers with low density infections is obvious. These newer survey techniques should prove valuable in clinical practice, in the evaluation of control and treatment schemes and in attaining a better understanding of the mechanisms of filarial transmission. During the previous reporting period seven villages located in semiforested areas of Sangkhlaburi district were surveyed for microfilaremia. Five of these villages—Kupadu, Lawa, Nithae, Nong Padong and Wang Kalang—were selected as sites for further studies because their high microfilaremia rates and/or accessibility.

PROGRESS: Between July and December 1974, 6169 mosquitoes were caught in 1832 man—hours of collections from human hosts, made during both daylight and evening hours, in the five study sites. Eight genera of mosquitoes, comprising 86 species, were represented in these collections; however *Aedes albopictus*, *Armigeres annulitarsis* and members of the *Aedes niveus* complex together formed the major portion of those collected. A total of 5141 mosquitoes were dissected, and 45 mosquitoes, belonging to six species (*Aedes niveus* species "A", *Aedes desmotes*, *A. mediopunctatus*, *A. gardneri*, *A. imprimens* and *Armigeres annulitarsis*), were found naturally infected with filarial larvae (Table 1). Identifications of the mature larvae are not yet complete, but some are characteristic of *W. bancrofti* while others appear to be species of *Setaria*, *Diptelonema* and/or *Breinlia*.

Surveys for larval mosquitoes were made in the five study sites between July 1974 and March 1975. Larvae of 82 species, belonging to 14 genera of mosquitoes, were collected from a variety of habitats within the vicinity of the five villages. Dense thickets of bamboo are present around Kupadu, Lawa and Nong padong, and mosquitoes which breed in bamboo nodes, such as *Aedes (Stegomyia)*, *Aedes (Finlaya)* and *Armigeres (Leicesteria)*, were especially numerous there. This accounts for the abundance of adults of *A. albopictus*, *Armigeres annulitarsis* and members of the *Aedes niveus* complex caught in biting collections made during this period in those villages. During the rainy season (July—November), larvae of *Anopheles balabacensis*, the principal malaria vector in Thailand, were present in ground pools in all five of the villages. The domestic mosquitoes, *Aedes aegypti* and *Culex quinquefasciatus*, were not present in the more isolated villages of Kupadu, Lawa and Nong Padong, which, incidentally, had the highest microfilaremia rates of the five study sites. On the other hand, larvae of *A. aegypti* and *C. quinquefasciatus* were found breeding on the premises of 72% and 24%, respectively, of the houses in Nithae, which is the most urbanized and had the lowest microfilaremia rate of the five villages.

Colonized strains of *Aedes aegypti*, *A. albopictus*, *A. togoi*, *Armigeres annulitarsis*, and *Culex quinquefasciatus* were fed upon known microfilaria carriers from the Sangkhlaburi district during this period. Of these five species, only *Aedes togoi* and *Culex quinquefasciatus* developed infections with *W. bancrofti* (Table 2).

Between August 1974 and March 1975, a comparison of the relative sensitivity of the 20 c. mm. thick film, 20 c. mm. counting chamber and the 1.0 ml. membrane filtration techniques for detecting microfilaremia was made in the villages of Kupadu and Nong Padong. Blood specimens were obtained from 117 individuals for comparison of the three techniques; the results are shown in Table 3. Microfilaremia rates by age and sex, obtained by the membrane filtration technique, are shown in Tables 4 and 5. A further evaluation of the three techniques was made in a study of the periodicity of microfilaremia in 10 villagers found positive during the earlier survey. Blood was taken at two hour intervals over a 24 hour period, and the microfilaremia densities at each interval, as measured by each of the three techniques, are given in Table 6—8. The mean values of microfilaremia periodicity determined by membrane filtration for the 10 patients are shown in Figure 1. The nocturnally subperiodic character of the Sangkhlaburi strain of *W. bancrofti* is obvious, for at no time was the microfilaremia level less than 20 per cent of peak count. It appears from the above data, that the direct chamber count is at least as sensitive as the standard thick film, with the advantage of providing an immediate result without the necessity of drying and staining slides. The disadvantage is the need for microscopy at the site of specimen collection. Membrane filtration, although venipuncture is required, is still convenient enough for routine field use, and is more sensitive than either the thick film or the counting chamber. The value of this technique in low density infections is illustrated by the case of periodicity patient No. 10 (Table 8).

Seventy—five individuals in the village of Kupadu submitted to full physical examinations during the course of those surveys. Only five of these yielded positive findings as summarized in Table 6.

Table 1. Mosquitoes Found Infected with Filarial Larvae—Sangkhlaburi, 1974

Species	Mupa Du		Nong Pa Dong		Lawa		Niithae		Wong Ka Lang		Totals	
	No. Coll.	No. Pos.	No. Coll.	No. Pos.	No. Coll.	No. Pos.	No. Coll.	No. Pos.	No. Coll.	No. Pos.	No. Coll.	No. Pos.
<i>Aedes (S) gardnerii</i>	19	1	29	1	45	11	10	1	19	3	122	17
<i>Aedes (S) desmotes</i>	55	2	37	0	42	3	8	0	3	0	145	5
<i>Aedes (S) mediapunctatus</i>	45	1	25	3	23	0	3	1	2	0	98	5
<i>Aedes (F) niveus "A"</i>	421	2	83	1	134	3	6	0	21	0	665	6
<i>Aedes (E) imprimens</i>	63	0	51	2	12	0	0	0	1	0	127	2
<i>Armigers (L) annulitarsis</i>	333	1	125	3	122	1	111	1	5	0	696	6
<i>Armigeres (L) flavus</i>	7	0	0	0	9	1	6	0	0	0	22	1
<i>Mansonia (M) dives</i>	11	0	15	0	1	0	4	0	32	3	63	3
Totals	954	7	365	10	388	19	148	3	83	6	1938	45

Table 2. Mosquitoes Fed on *Wuchereria bancrofti* Cases—Sangkhlaburi, 1974

Species	No. Fed	No. Dissected	No. Infected	Percent Infected	No. Larvae
<i>Aedes aegypti</i>	271	207	0	0.0	0
<i>Aedes albopictus</i>	62	46	0	0.0	0
<i>Aedes togoi</i>	138	45	23	51.1	80
<i>Armigeres annulitarsis</i>	27	19	0	0.0	0
<i>Culex quinquefasciatus</i>	333	184	9	4.9	12

Table 3: Results of Comparison of Three Techniques for Detecting Microfilaremia—Kupadu and Nong Padong, 1974

Technique	No. Patients	No. Positive	Percent Positive
Thick Film (20 c.mm. blood)	117	20	17
Counting Chamber (20 c.mm. blood)	117	22	19
Membrane filtration (1. ml. blood)	117	31	26

Table 4. Results of Examinations for Microfilaremia by Age, Using Membrane Filtration Technique—Kupadu and Nong Padong, 1974

Age Group (Years)	Number Examined	Number Positive	Percent Positive
0-3	5	2	40
4-6	8	0	—
7-10	21	2	10
11-15	21	4	19
16-20	13	3	23
21-30	22	7	32
31-40	7	2	29
41-50	12	5	42
50+	8	7	88
Total	117	32	27

Table 5. Results of Examinations for Microfilaremia by Sex, Using Membrane Filtration Technique—Kupadu and Nong Padong, 1974

Sex	Number Examined	Number Positive	Percent Positive
Male	73	17	23
Female	44	15	34
Total	117	32	27

Table 6. Twenty-four Hour *W. bancrofti* Microfilariae Counts in 10 Carriers, by Thick Film Technique

Case No.	Microfilariae counts at hours/20 C.mm. blood											
	1200	1400	1600	1800	2000	2200	2400	0200	0400	0600	0800	1000
1	3	3	7	2	7	3	5	11	10	6	1	3
2	8	20	11	11	19	14	11	11	3	6	3	8
3	5	10	15	20	8	24	13	8	11	5	4	9
4	13	12	25	53	59	82	48	65	57	47	1	1
5	1	4	8	7	3	5	11	8	5	6	1	1
6	3	3	2	1	3	0	1	2	0	1	0	2
7	16	34	46	21	26	51	67	31	16	9	6	29
8	23	29	82	40	67	71	31	56	10	14	6	8
9	5	8	18	23	25	16	9	15	21	13	3	7
10	0	0	0	1	0	0	0	0	0	1	0	0

Table 7. Twenty-four Hour *W. bancrofti* Microfilariae Counts in 10 Carriers, Determined by Counting Chamber Technique

Case No.	Microfilariae counts at hours/20 c.mm. blood											
	1200	1400	1600	1800	2000	2200	2400	0200	0400	0600	0800	1000
1	2	1	1	3	4	12	6	4	7	4	3	2
2	10	19	9	20	23	6	9	10	10	3	5	13
3	4	25	23	23	16	23	16	10	8	1	7	3
4	8	15	31	40	48	62	42	48	49	46	15	11
5	3	2	2	5	5	4	2	7	2	2	4	1
6	6	3	4	3	3	1	1	1	0	1	0	1
7	17	27	53	27	29	49	40	12	23	10	14	17
8	23	30	64	41	56	52	38	45	19	23	7	3
9	6	7	14	14	17	8	15	17	15	11	4	8
10	2	1	0	2	5	2	0	1	1	1	0	1

Table 8. Twenty-four Hour *W. bancrofti* Microfilariae Counts in 10 Carriers, Determined by Membrane Filtration Technique

Case No.	Microfilariae counts at hours/1 ml. blood											
	1200	1400	1600	1800	2000	2200	2400	0200	0400	0600	0800	1000
1	109	96	194	245	334	271	433	318	270	200	128	64
2	444	924	560	1049	966	549	521	456	290	231	198	496
3	371	990	1171	1231	601	727	621	399	601	231	198	257
4	409	685	1560	2282	1789	3124	2653	2462	2841	2244	928	871
5	126	44	327	394	316	294	279	283	183	295	99	109
6	132	210	157	120	197	75	107	85	98	57	49	142
7	861	1824	2508	1686	1231	2102	2093	1917	616	769	756	1310
8	925	1672	3239	2358	3681	2891	2306	2950	877	945	273	334
9	439	564	749	1101	1070	1212	675	1036	473	1033	620	294
10	8	10	22	16	23	43	15	22	2	14	13	5

Table 9. Summary of Clinical Findings in Kupadu Villagers Suggestive of Filariasis

Case No.	Age	Sex	Finding	Microfilariae Present
1	52	M	Large hydrocoele	Yes
53	25	M	Large inguinal nodes, no other apparent cause	No
55	25	M	Large inguinal nodes, no other apparent cause	No
58	21	M	Thickened spermatic cord	No
62	52	M	Thickened spermatic cord	Yes

The pathology normally associated with infections of *W. bancrofti* is apparently at a very low level in this particular endemic area and seems to be limited to minor genital abnormalities in males. No cases of elephantiasis have been observed in the study villages.

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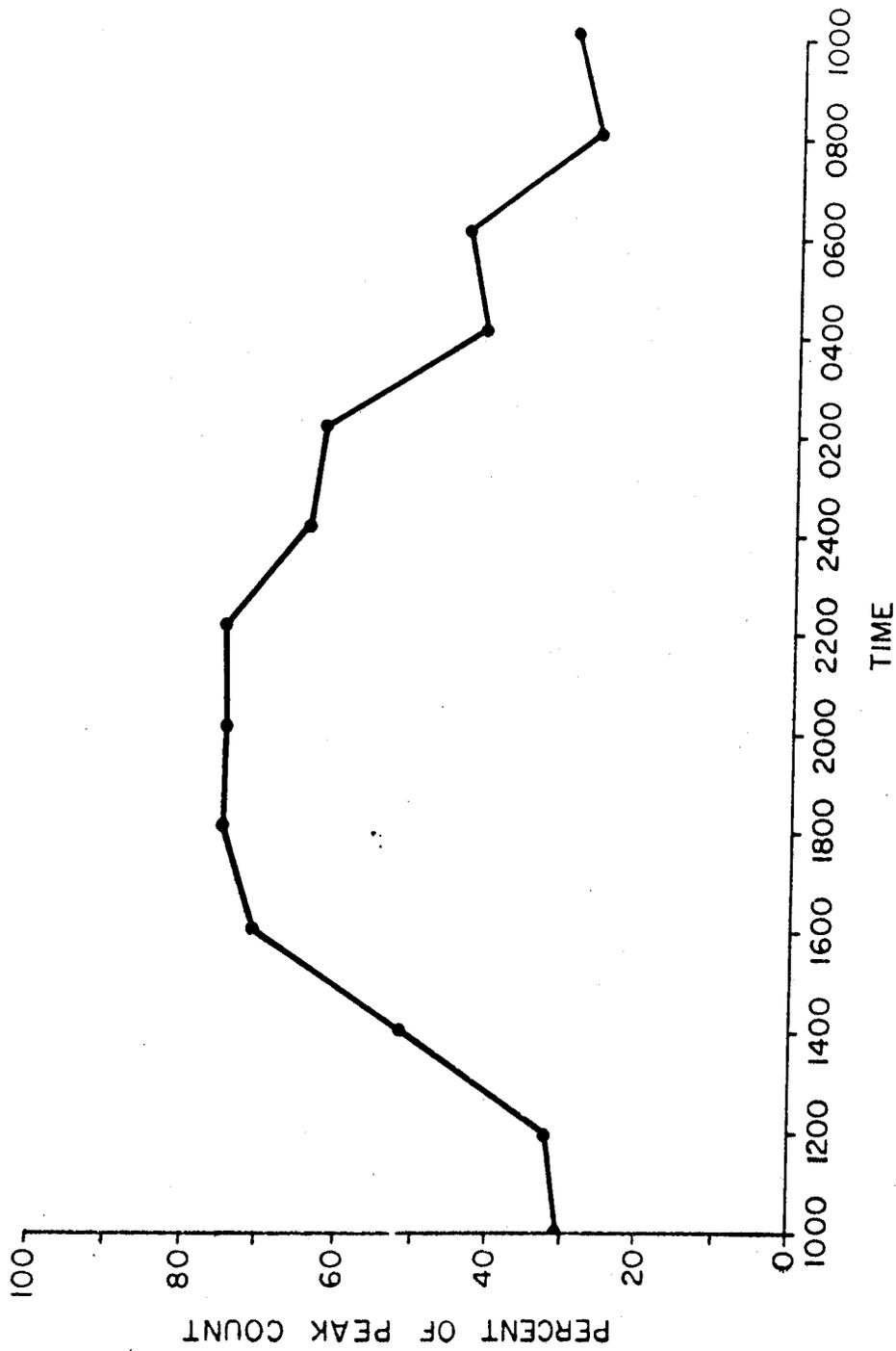


FIGURE 1. MEAN VALUES OF MICROFILARIA DENSITY FOR 10 PATIENTS, DETERMINED AT HOURLY INTERVALS BY 1 ML. MEMBRANE FILTRATION