IMPLICATION OF FOREST MOSQUITOES IN THE TRANSMISSION OF *WUCHERERIA BANCROFTI* IN THAILAND

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ABSTRACT. Three species of mosquitoes, *Aedes harinasutai, Ae. desmotes* and *Mansonia dives* were implicated as vectors of *Wuchereria bancrofti* in an endemic area of rural Thailand. This represents a unique situation for Southeast Asia in that forest-dwelling mosquitoes were responsible for transmitting the causative agent.

In 1970 Harinasuta et al. described an endemic focus of bancroftian filariasis in rural villages near the headwaters of the Kwae Noi river in Sangkhlaburi district of Kanchanaburi province, Thailand. Prevalence rates of infection with *Wuchereria bancrofti* of up to 30% were observed in some of these villages, and many cases of filarial hydrocoele were encountered. These investigators reported that microfilaremia in the infected villagers was nocturnally subperiodic, with peaks between 1800 and 2000 hours, but with microfilariae present in significant numbers in the peripheral blood during daylight hours. Infective stages of *W. bancrofti* were found in wild-caught mosquitoes of the *Aedes (Finlaya) niveus* complex. Mosquitoes of this group are among the most common diurnally active species attacking man in the forested regions of Southeast Asia. Females of the 24 presently recognized members of this complex are extremely difficult to differentiate, and the examination of the terminalia of associated male mosquitoes is usually required for positive identification (Colless 1958, 1959; Knight 1978).

This report summarizes the results of studies conducted between November 1973 and June 1975 in an effort to obtain a definitive identification of the vector(s) of *W. bancrofti* in the Sangkhlaburi district.

MATERIALS AND METHODS

Sangkhlaburi district is located in the northern half of Kanchanaburi province approximately 200 km northwest of Bangkok (Fig. 1). The district has an area of 3655 km^2, and the topography is hilly with elevations ranging from 160 m above sea level in the river valleys and up to more than 700 m in the hills. Much of the district is covered by a mixed tropical deciduous and evergreen forest (Suvarnasuddhi 1976).

Five villages in Sangkhlaburi district—Kupadu, Lawa, Nithae, Nong Padong and Wang Kalang—were selected as study sites because of the high prevalence rates of microfilaremia and/or accessibility from our field laboratory at Wang Kabon (Fig. 1). Nithae was the most densely populated village, with two temples and a market area, while the other four were hamlets in which houses were scattered and separated by paddy fields, gardens, orchards, bamboo thickets and forest.

Blood samples were taken by finger puncture from all available adults of each village. Two thick films of approximately 20 cmm each were prepared from each sample, stained with Giemsa and examined with a compound microscope for microfilariae. Whenever possible blood samples were collected in the evenings between 1800 and 2000 hours.

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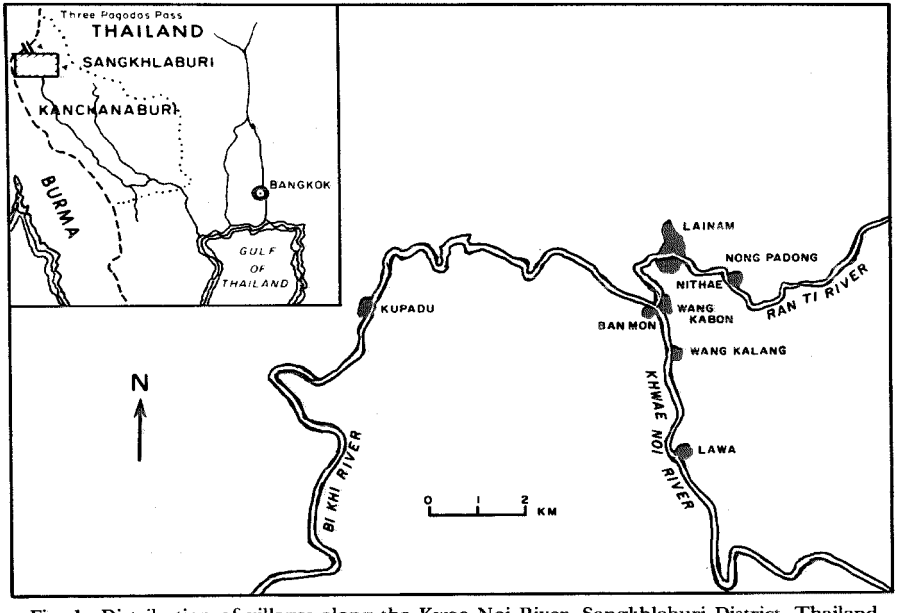

Fig. 1. Distribution of villages along the Kwae Noi River, Sangkhlaburi District, Thailand.

Mosquitoes were collected while biting humans around or inside homes of filariasis cases between 0700 and 2000 hours. Mosquitoes were captured in screened vials, then identified and dissected as soon after collection as possible. Filarial larvae were mounted in glycerine on depression slides.

For taxonomic purposes, a sibling series of offspring were obtained from mosquitoes of the *niveus* complex, by allowing engorged females to oviposit on dampened filter paper. Skins from larvae and pupae reared from these eggs were preserved in 70% ethanol, and the emerged adults were mounted on points. Collections of larval mosquitoes were also made in the five study villages from a variety of habitats including tree holes, bamboo nodes, ground pools and artificial containers located in and around houses.

RESULTS

Overall, 9% (37/410) of the blood films obtained from humans in the five villages contained *W. bancrofti* microfilariae. Microfilaremia prevalence rates ranged from 0.4% of the villagers in Nithae up to 29% of those sampled in Kupadu (Table 1). Only microfilariae of *W. bancrofti* were found in the films.

Table 1. Prevalence rates of *Wuchereria bancrofti* microfilariae in blood of humans, Sangkhlaburi District, Thailand, 1973–74.

Village	Number examined	Number positive	Per cent positive
Kupadu	62	18	29.0
Lawa	50	7	14.0
Nithae	224	1	0.4
Nong Padong	35	8	22.8
Wang Kalang	39	3	7.7
Total	410	37	9

Table 2. Results of dissection of mosquitoes for filariae—Sangkhlaburi District, Thailand, 1974–75.

Mosquito species	Number dissected	Number positive
Aedes (Aedimorphus) vexans (Meigen)	50	0
Aedes (Edwardsaedes) imprimens (Walker)	125	2
Aedes (Finlaya) formosensis Yamada	83	0
Aedes (Finlaya) harinasutai Knight	659	9
Aedes (Finlaya) niveoides Barraud	207	0
Aedes (Ochlerotatus) vigilax (Skuse)	30	0
Aedes (Stegomyia) albopictus (Skuse)	1784	2
Aedes (Stegomyia) annandalei (Theobald)	39	0
Aedes (Stegomyia) desmotes (Giles)	165	7
Aedes (Stegomyia) gardnerii (Ludlow)	126	19
Aedes (Stegomyia) mediopunctatus (Theobald)	103	5
Anopheles (Anopheles) barbirostris Van der Wulp (s.l.)	149	0
Anopheles (Cellia) maculatus Theobald	29	0
Anopheles (Cellia) nivipes (Theobald)	27	0
Armigeres (Armigeres) subalbatus (Coquillett)	215	0
Armigeres (Armigeres) theobaldi Barraud	35	0
Armigeres (Leicesteria) annulitarsis (Leicester)	545	6
Armigeres (Leicesteria) dentatus Barraud	42	0
Armigeres (Leicesteria) digitatus Edwards)	20	0
Armigeres (Leicesteria) dolichocephalus (Leicester)	66	0
Armigeres (Leicesteria) flavus (Leicester)	21	1
Armigeres (Leicesteria) magnus (Theobald)	22	0
Armigeres (Leicesteria) omissus (Edwards)	120	0
Culex (Culex) tritaeniorhynchus Giles	26	0
Culex (Culex) vishnui Theobald (s.l.)	228	0
Culex (Culex) whitmorei (Giles)	22	0
Heizmannia spp.	53	0
Heizmannia (Heizmannia) covelli Barraud	53	0
Heizmannia (Heizmannia) mattinglyi Thurman	165	0
Heizmannia (Heizmannia) reidi Mattingly	36	0
Mansonia (Mansonioides) dives (Schiner)	46	2
Other species*	208	0
Total	5499	53

* Less than 20 specimens per species dissected: *Aedes alboscutellatus* (Theobald), *Ae. caecus* (Theobald), *Ae. albotaeniatus* (Leicester), *Ae. christophersi* Edwards, *Ae. dissimilis* (Leicester), *Ae. litoreus* Colless, *Ae. poecilius* (Theobald), *Ae. prominens* (Barraud), *Ae. lineatopennis* (Ludlow), *Anopheles aitkenii* James s.l., *An. campestris* Reid, *An. balabacensis* complex, *An. kochi* Dönitz, *An. minimus* Theobald, *An. vagus* Dönitz, *Armigeres aureolineatus* (Leicester), *Ar. durhami* Edwards, *Ar. kuchingensis* Edwards, *Ar. malayi* (Theobald), *Ar. maximus* Edwards, *Ar. moultoni* Edwards, *Ar. longipalpis* (Leicester), *Ar. pectinatus* (Edwards), *Ar.* sp (near *subalbatus*), *Culex fuscocephala* Theobald, *Cx. gelidus* Theobald, *Cx. quinquefasciatus* Say, *Cx. sinensis* Theobald, *Cx. whitei* Barraud, *Cx. infantulus* Edwards, *Cx. rubithoracis* (Leicester), *Cx. halifaxii* Theobald, *Heizmannia aureochaeta* (Leicester), *Hz. chengi* Lien, *Hz. communis* (Leicester), *Hz. macdonaldi* Mattingly, *Hz. taiwanensis* Lien, *Hz. achaetae* (Leicester), *Hz. thelmae* Mattingly, *Tripteroides aranoides* (Theobald).

From July 1974 to June 1975, 90 collections of adult mosquitoes, representing 584 man/hours of collecting were made in the five villages. A total of 5,499 mosquitoes, representing 7 genera and more than 71 species were dissected and examined for filarial larvae (Table 2).

Eggs reared from females in 24 collections of mosquitoes of the *niveus* complex yielded 115 series of correlated immature and adult mosquitoes. Two species of that complex were most frequently encountered, and a study of the sibling series by K. L. Knight revealed that these were *Aedes niveoides* Barraud and a previously undescribed species that he named *Aedes harinasutai* (Knight 1978). Fortunately, the females of these two species were readily differentiated from each other. Filarial larvae were found in 53 mosquitoes belonging to 9 species (Table 2). Third or infective stage larvae of *W. bancrofti* were found in the head and proboscis of 9 of 659 *Aedes harinasutai* and 7 of 165 *Ae. desmotes* and in the thorax of 2 of 46 *Mansonia dives*. Third stage larvae of a *Dirofilaria* species were found in the heads of *Ae. albopictus*. *Ae. gardnerii* and *Ae. mediopunctatus*. First and second stage larvae of uncertain identity were found in the thorax and abdomen of *Ae. imprimens*, *Armigeres annulitarsis* and *Ar. flavus* (Table 3).

Table 3. Species of filarial larvae found in mosquitoes, Sangkhlaburi District, Thailand, 1974–75.

Mosquito species	Site obtained*	Filarial species
Aedes imprimens	T	Unknown
Aedes harinasutai	P, T	*Wuchereria bancrofti*
Aedes albopictus	P, T	*Dirofilaria* sp.
Aedes desmotes	P, T	*W. bancrofti*
Aedes gardnerii	P, T, A	*Dirofilaria* sp.
Aedes mediopunctatus	P, T, A	*Dirofilaria* sp.
Armigeres annulitarsis	T, A	Unknown
Armigeres flavus	T	Unknown
Mansonia dives	T	*W. bancrofti*

* P proboscis, T thorax and A abdomen.

A total of 82 species of mosquito larvae, belonging to 14 genera were collected in the five villages between July 1974 and March 1975. Larvae of *Ae. albopictus*, *Ar. annulitarsis* and members of the *Ae. niveus* complex were collected most frequently from tree holes and bamboo nodes. The domestic mosquitoes, *Ae. aegypti* and *Culex quinquefasciatus*, were not found in Kupadu, Lawa and Nong Padong, or the villages with the highest microfilaremia rates. On the other hand, *Ae. aegypti* and *Cx. quinquefasciatus* larvae were present in artificial containers in and around 72% and 24%, respectively, of the houses in Nithae. This was the largest and most urbanized of the five villages and had the lowest microfilaremia rate of the five villages.

DISCUSSION

Bancroftian filariasis endemic in rural villages of Sangkhlaburi district is distinctive in that the causative agent, *W. bancrofti*, is apparently transmitted by forest-dwelling mosquitoes. Elsewhere in the Orient this disease has usually been found in cities (*e.g.*, Rangoon and Jakarta) where the tropical house mosquito, *Cx. quinquefasciatus*, serves as vector (Edeson and Wilson 1964). A further distinction of the Sangkhlaburi focus was the subperiodic appearance of microfilaremia. Microfilariae of *W. bancrofti* in urban endemic areas are nocturnally periodic. In contrast to the mosquitoes implicated in Sangkhlaburi, *Cx. quinquefasciatus* is a nocturnal feeder. A modified periodicity of microfilaremia, similar to that observed by Harinasuta et al. (1970) in Sangkhlaburi has been reported from Polynesian islands where diurnally feeding mosquitoes such as *Ae. fijiensis* Marks and *Ae. polynesiensis* Marks are vectors of *W. bancrofti* (Edeson and Wilson 1964). Thus, there would seem to be a strong correlation between the periodicity of microfilaremia of *W. bancrofti* and the preferred hours of feeding of its various vectors.

In this study, *Ae. harinasutai* was impli-

cated in the transmission of *W. bancrofti* while other species of the *niveus* complex, *Ae. niveoides*, for example, although abundant in the study villages, were not found infected. At the present time *Ae. harinasutai* is known in nature only from man-biting mosquitoes collected within Sangkhlaburi district. It is not known whether this mosquito feeds on hosts other than man, and the habitats of the immature stages of *Ae. harinasutai* have not been described.

Neither *Ae. desmotes* nor *Ma. dives* have previously been incriminated as vectors of *W. bancrofti* although the latter species is a recorded carrier of *Brugia malayi* elsewhere in Southeast Asia (Edeson and Wilson 1964). Finally, although we dissected more specimens of *Ae. albopictus* than all other species examined, none were found infected with *W. bancrofti*.

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