

# ANOPHELES DONALDI INCRIMINATED AS A VECTOR OF PERIODIC BRUGIA MALAYI IN GRIK, PERAK, MALAYSIA

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**Abstract.** Studies were carried out to observe the species composition of mosquitos and to determine the vectors responsible for the transmission of filariasis in Grik, Perak, Malaysia. A total of 2,155 mosquitos belonging to 7 genera and 30 species were collected. *Anopheles donaldi* comprised 24.1% of the collection. Twelve out of 519 *An. donaldi* were infected with L3 larvae of *Brugia malayi*. The peak biting time was around 23.00-24.00 hours. The infective bites per month ranged from 0 to 6.3.

## INTRODUCTION

Filariasis in Malaysia is caused mainly by *Brugia malayi* and *Mansonia* mosquitos have been incriminated as the vectors (Cheong, 1983). In studies carried out by Wharton *et al* (1963) in the Aboriginal settlements in Malaysia, *Mansonia dives* and *Anopheles donaldi* were found to be vectors of periodic *B. malayi* in Selangor while in Kelantan the vector remained unknown.

In Malaysia diethylcarbamazine (DEC) has been the drug of choice for filariasis. No control measures have been instituted for the vectors. The current drug regimen used is 36 mg/kg over 6 doses. A study was carried out to determine the effectiveness of mass single dose DEC treatment with the standard regimen among inhabitants of Aboriginal settlements in Perak, Malaysia (Lokman Hakim *et al*, 1995). At the same time entomological studies were carried out to determine the mosquitos responsible for the transmission of filariasis in this area. This report deals with species composition, biting habits and filarial infections of *An. donaldi* from Grik, Perak Malaysia.

## MATERIALS AND METHODS

### Study area

This study was carried out in 2 Aboriginal resettlements situated in Perak which is about 400 km north from Kuala Lumpur. It has borders with the State of Kelantan and Thailand. The two localities RPS Air Banun and Dala are about 100 km apart

and the villages are located close to lakes. Both areas are of hilly terrain and the houses are situated in the clearings of jungle. The houses in this area are mainly built of wood and have either zinc or thatched roofs. Wild animals still reside in these areas.

### Mosquito collections

Mosquito collections were carried out simultaneously indoors and outdoors by two collectors each from 19.00 to 07.00 hours. All mosquitos landing on human bait were caught using small tubes which were subsequently plugged with cotton wool. Two houses were selected in each village and two to four nights of collections were carried out depending on circumstances and weather conditions. The study started in November 1992 and ended in May 1994. In some months one of the study site was not accessible.

All mosquitos collected were counted and brought back alive to the field laboratory the next day, identified and dissected to determine their parity and filarial infection. All larvae were mounted using the technique of Wharton (1959) and identified.

This study protocol was approved by the Human Use Ethical Committee, Ministry of Health, Malaysia.

### Statistical analysis

Data were analysed using the primer program. Difference in the means of mature larva rate were analysed using Student's *t* test.

Table 1

Total number of mosquito species caught from November 1992 to May 1994 at RPS Air Banun and Dala.

Species	No. caught	No. dissected	Infected with <i>B. malayi</i> ( )*	<i>B. malayi</i> infected rate ( )*
<i>Anopheles</i>				
<i>donaldi</i>	519(24.1)	516	23 (12)	4.5(2.3)
<i>hyrcanus</i> sp grp	8	8	-	-
<i>introlatus</i>	3	3	-	-
<i>maculatus</i>	31	31	-	-
<i>nigerrimus</i>	485 (22.5)	476	7 (1)	1.5 (0.2)
<i>nitidus</i>	1	1	-	-
<i>peditaeniatus</i>	4	4	-	-
<i>philippinensis</i>	3	3	-	-
<i>sinensis</i>	1	1	-	-
<i>tessellatus</i>	4	4	-	-
<i>Aedes</i>				
<i>albopictus</i>	39	22	-	-
<i>lineatopennis</i>	2	0	-	-
<i>niveus</i> sp grp	5	4	-	-
<i>poicilius</i>	2	2	-	-
<i>vexans</i>	129	17	-	-
<i>Aedeomyia</i>				
<i>catasticta</i>	3	0	-	-
<i>Armigeres</i>				
<i>annulitarsis</i>	4	4	-	-
<i>flavus</i>	5	5	-	-
<i>subalbatus</i>	1	1	-	-
<i>Coquilleltidia</i>				
<i>crassipes</i>	4	4	-	-
<i>Culex</i>				
<i>bitaeniorhynchus</i>	0	6	-	-
<i>fuscocephala</i>	9	3	-	-
<i>gelidus</i>	346	39	-	-
<i>pseudovishnui</i>	81	53	-	-
<i>quinquefasciatus</i>	1	1	-	-
<i>sinensis</i>	3	2	-	-
<i>tritaeniorhynchus</i>	26	10	-	-
<i>Mansonia</i>				
<i>annulifera</i>	1	1	-	-
<i>dives</i>	191	188	2 (2)	1.1 (1.1)
<i>uniformis</i>	234	230	1 (1)	0.4 (0.4)

( ) \* nature larva

## RESULTS

During the study period a total of 2,155 mosquitos belonging to 7 genera and 30 species were recorded (Table 1). Of this *An. donaldi* comprised 24.1% of the collection followed by *Anopheles nigerrimus* (22.5%). *Mansonia* sp. comprised 19.8% of the total collection. There is an interesting diversity of species in this area. *Culex gelidus* and *Aedes vexans* were also found in great numbers. *Cx. gelidus* was processed for Japanese encephalitis virus but were negative. Filarial worms were present in *An. donaldi*, *An. nigerrimus*, *Ma. dives* and *Ma. uniformis*.

Biting habits of *An. donaldi* expressed as William's mean is shown graphically in Fig 1. *An. donaldi* bites both indoors and outdoors. They come to bite after 19.00 hours and bites continuously throughout the night until dawn. The peak biting time is between 23.00-24.00 hours and there

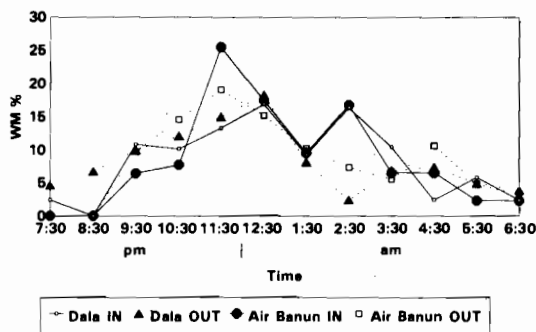


Fig 1—Biting habits of *An. donaldi* indoors and outdoors in RPS Dala and Air Banun.

is another peak between 02.00-03.00 hours.

Table 2 summarizes the filarial infections of *An. donaldi* at the two sites. In RPS Dala a total of 11 *An. donaldi* were positive of which 6 had stage III larvae (L3), while in RPS Air Banun 12 were positive of which 6 had stage III larvae. In both areas the immature larvae were present in greater numbers than mature larvae in the mosquitos.

The parous rates of *An. donaldi* collected during the six surveys are shown in Table 3. In RPS Dala *An. donaldi* was more than 50% parous in five of the surveys. In RPS Air Banun also the parous rate was high.

Table 4 summarizes the effect of control measures on the transmission of *B. malayi* by *An. donaldi*. The mature larva rate was highest in May 1993, ie after treatment was carried out in February 1993. In Air Banun the infective bites per month was highest in September 1993 (5.6) and in Dala it was highest in May 1993 (6.3). There was no significant difference in the mature larva rate in Air Banun and Dala before and after treatment ( $p=0.7$  and  $0.8$  respectively). The infective bites / month also seem to increase after treatment. There was no significant difference in mature larva rate between Air Banun and Dala before and after treatment ( $p=0.6$  and  $0.6$  respectively).

## DISCUSSION

This study, although limited in scope, has provided some useful information on the biting habits of *An. donaldi* and its role as a vector of *B. malayi*.

Table 2

The numbers of *B. malayi* larvae found in naturally infected *An. donaldi* from two different areas.

Area	No. of infections Total (Stg III)	<i>B. malayi</i>			
		Stg III larvae		Immature larvae	
		Mean	Range	Mean	Range
RPS Dala	11 (6)	3	1-6	7.1	2-21
RPS Air Banun	12 (6)	3	1-6	11.3	1-47

Table 3

Age composition of *An. donaldi* from 12 hour landing collections at RPS Air Banun and RPS Dala.

Months	Air Banun			Dala		
	Total dissected	Nulli parous	Parous (%)	Total dissected	Nulli parous	Parous (%)
Nov 92	57	30	27 (47.4)	27	8	19 (70.4)
Feb 93	73	26	47 (64.4)	19	4	15 (78.9)
May 93	15	7	8 (53.3)	49	17	32 (65.3)
Sep 93	130	72	58 (44.6)	22	11	11 (50)
Mar 94		-	-	70	35	35 (50)
May 94		-	-	36	14	12 (33.3)

Table 4

The effect of control measures on the transmission of *B. malayi* by *An. donaldi*.

Area		Before control		After control			
		Nov 92	Feb 93	May 93	Sept 93	Mar 94	May 94
Air Banun	No. caught	57	74	15	130	14	na
	Mosq per night	4.8	3.4	1.2	8.1	3.5	
	No. dissected	56	73	15	130	14	na
	Infective	1	0	2	3	0	-
	Infected	3	2	2	5	0	-
	Mature larva rate	1.7	0	13.3	2.3	0	-
	Larvae per mosq						
	Mean	11	4.5	2	12.4	0	-
	Range	1-15	1-8	1-2	1-47	0	-
	Infective bites per month	2.4	0	4.6	5.6	0	-
Dala	No. caught	27	19	50	22	70	36
	Mosq per night	2.3	1	5.1	2.2	3.5	2.3
	No. dissected	27	19	49	22	70	36
	Infective	1	0	2	0	2	1
	Infected	2	1	3	0	4	1
	Mature Larv rate	3.7	0	4.1	0	2.9	2.7
	Larva per mosq						
	Mean	6	4	7.7	0	9.5	2
	Range	6	4	1-21	0	2-15	2
	Infective bites per month	2.6	0	6.3	0	3.0	1.8

During the early 1960s work carried out by Wharton and co-workers found only 2 infected mosquitos with mature filaria larvae out of 146 mosquitos dissected in Aboriginal villages in Malaysia (Wharton *et al*, 1963). This study demonstrates that *An. donaldi* is an efficient vector of periodic form of *B. malayi* as the infective rate found naturally occurring in *An. donaldi* to be high. A total of 12 mosquitos were positive with stage three larvae.

*An. donaldi* bites both indoors and outdoors and also rests outdoors on walls of houses and on the underside of leaves before entering houses to bite. The peak biting time is around midnight when most people are asleep. This agrees with earlier work carried out by Moorhouse and Wharton (1965).

The prevalence of microfilaria in both areas decreased significantly with treatment (Lokman Hakim *et al*, 1995) but suprisingly the mature larva rate in mosquitos were not significantly different before and after treatment.

One possible reason could be that not all people received treatment due to inaccessibility of terrain. Wharton's studies also indicated that there was no significant difference in the mature larva rate before and after treatment (Wharton, 1962).

A large proportion of the mosquitos caught in this area were parous. This means that the mosquitos are very efficient vectors and die only due to natural factors. Due to the nature of the terrain and for security reasons we were unable to carry out larval surveys. This would have given us some indication of the distance between the breeding site and our collection site.

In an earlier study Reid and co-workers incriminated *An. campestris* as the vector of periodic *B. malayi* in North West Malaysia (Reid *et al*, 1962). However Mak *et al* (1976) did not find the vector of periodic *B. malayi* during a study in Perlis, Northern Peninsular Malaysia.

Besides *An. donaldi*, *An. nigerrimus* was also obtained in sufficiently large numbers in this area. Seven were found with *B. malayi* immature stages of which only one stage III larva was found. Its role as a vector is still doubtful. Susceptibility studies carried out in the laboratory showed that *An. nigerrimus* was susceptible to subperiodic *B. malayi* (Reid, 1968). Further studies have to be carried out before *An. nigerrimus* can be incriminated as a vector of *B. malayi*.

Although *Ma. uniformis* and *Ma. dives* were found to be positive in this area, the infection rate was low and was found only on one occassion. This further demonstrates that *An. donaldi* is the main vector in this area. The fact that *An. donaldi* is attracted to man, enters house to feed and has stage III larvae strongly indicates that it is the vector of *B. malayi* in this area.

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#### REFERENCES

- Cheong HW. Vectors of filariasis in Malaysia. In: Filariasis Bulletin Institute for Medical Research, Kuala Lumpur, Malaysia. 1983; 19:37-44.
- Lokman Hakim S, Vythilingam I, Marzuki MI, Mak JW. Single dose diethylcarbamazine in the control of periodic brugian filariasis in Peninsular Malaysia. *Trans R Soc Trop Med Hyg* 1995; 89: 686-9.
- Mak JW, Cheong WH, Abu Hassan Omar, *et al*. Filariasis in Perlis, Peninsular Malaysia. *Med J Mal* 1965; 31:198-203.
- Reid JA. Anopheline mosquitos of Malaya and Borneo. Studies from the Institute for Medical Research, Malaysia, 1986; 31: 520pp.
- Reid JA, Wilson T, Ganapathipillai A. Studies of filariasis in Malaya: The mosquito vectors of periodic *Brugia malayi* in north-west Malaya. *Ann Trop Med Parasitol* 1962; 56: 323-36.
- Wharton RH. A simple method of mounting and preserving filarial larvae. *Bull WHO* 1959; 20: 729-30.
- Wharton RH. The biology of *Mansonia* mosquitos in relation to the transmission of filariasis in Malaya. Bulletin Institute for Medical Research, Malaysia. 1962;11:114 pp.
- Wharton RH, Liang ABG, Cheong WH. Studies on the distribution and transmission of malaria and filariasis among aborigines in Malaya. *Ann Trop Med Parasitol* 1963; 57 235-54.