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PRELIMINARY STUDIES ON THE LABORATORY COLONIZATION OF *ANOPHELES SINENSIS* WIEDEMANN AND ITS SUSCEPTIBILITY TO *BRUGIA MALAYI* (BRUG) (SUBPERIODIC STRAIN) AND *BRUGIA PAHANGI* (BUCKLEY AND EDESON)[1]

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A laboratory colony of *Anopheles sinensis* Wiedemann was started from wild females collected from the coastal village of Lubuk Pusing, Selangor, Malaysia. The species was reared satisfactorily in a room maintained at approximately 80°F and 80% R.H. A sufficient number of adult mosquitoes was obtained by rearing the larvae in hay infusion (deionized water plus dried rice stalks) and feeding with a larval food composed of 1 part casein-Farex-liver powder-dried yeast-Vitamin B complex mixture (75:25:3:3: 1.5), 1 part Quaker oats, 1 part wheat germ and 1 part dried yeast.

After obtaining a sufficient number of mosquitoes (starved, 4-day old adult females), infection studies were done using laboratory animals infected with *Brugia malayi* (Brug) (subperiodic strain) and *Brugia pahangi* (Buckley and Edeson). Extensive chitinization of the larvae of both filaria species in the thoracic muscles of the mosquitoes dissected after 11 days was observed. It was concluded that *Anopheles sinensis* is a poor intermediate host of *Brugia malayi* (sub-periodic strain) and *Brugia pahangi.*

One of the recent suspected vectors of brugian filariasis in Peninsular Malaysia is *Anopheles sinensis* due to the fact that a single adult was collected from the coastal village of Lubuk Pusing infected with three L_1 larvae of *Brugia malayi* (Chiang *et al.*, 1984). Lubuk Pusing is endemic for periodic *B. malayi* and the established anopheline vector along the coast from Selangor to the north in Perlis is *Anopheles campestris* (Poynton and Hodgkin, 1938; Cheong, 1983). This research work was done therefore to investigate further the vectorial susceptibility of *An. sinensis* to 2 species of *Brugia* by (a) colonizing *An. sinensis* in the laboratory and (b) by conducting susceptibility experiments on *B. malayi* (s.p.) and *B. pahangi.*

Anopheles sinensis belongs to the *hyrcanus* group of mosquitoes along with seven other species including *lesteri* and *crawfordi.* Reid (1968) noted that *An. sinensis* is common in Peninsular Malaysia, a zoophilous species which is reluctant to bite man indoors and not a vector of disease. It is said to range from Japan (Hokkaido) and Korea in the north, southward to China, including Taiwan (Formosa), to the Malay Peninsula and Sumatra. However, the mosquito is absent in the Philippines, Java and Borneo. Its immature

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stages could be found in rice fields and in grassy ponds and ditches exposed to the sun.

For a mosquito species to be studied conveniently, it must first be reared to a sufficient number. Thus, in this study, attempts were made to colonize *An. sinensis* in the laboratory, and when sufficient adults were reared out, its susceptibility to *B. malayi* (subperiodic) and *B. pahangi* was tested using laboratory infected animals. These studies were done from April to August, 1984 in the insectary and animal house of the Institute for Medical Research, Kuala Lumpur, Malaysia.

REVIEW OF LITERATURE

Anopheles hyrcanus variety *sinensis* is a reported important vector of both *Wuchereria bancrofti* and *Brugia malayi* in China (Feng, 1931; 1933; 1936; 1938; Feng and Yao, 1935). According to Dr. Feng, *An. hyrcanus* var. *sinensis* is probably the most important vector in China because of its relative abundance in the villages, and because filariasis is more common in the villages than in towns. In Amoy for example, he noted that filariasis due to *W. bancrofti* is of low incidence possibly due to the comparatively small number of *An. hyrcanus* var. *sinensis* in households, despite the abundance of *Culex fatigans* (= *Cx. quinquefasciatus*).

Dr. J.A. Reid, in his book Anopheline Mosquitoes of Malaya and Borneo (1968), states that in China *An. hyrcanus* var. *sinensis* could be actually a group of at least five very similar species, two of which are the true *An. sinensis* described from Canton by Wiedemann in 1928, which is very common and widespread there, and, the less common but more anthropophilic *An. lesteri.* Dr. Reid is of the opinion that many of the past records of *sinensis* as a vector of malaria in China probably refer to *lesteri.* However, he did not mention the studies on *sinensis* as a vector of filaria worms.

Chiang *et al.* (1984) were able to collect 625 adult females *An. sinensis* out of 13,603 specimens caught from Kampung Lubuk Pusing from April 1982 to March 1983. Out of the 625 mosquitoes, one was found to be infected with 3 L_1 larvae of *B. malayi.* By comparison, in the swamp forest in Jalan Tanjong Karang, only 66 females were collected out of 15,917 total catch, and no infection was observed.

In experimental infection studies of *An. sinensis* in Japan with *B. malayi,* Kanda et al. (1975) reported that the mosquito species is not a suitable intermediate host for the parasite. However, in their studies on filariasis in an inland area of Kyungpook, Korea, they concluded that *An. sinensis* appears to be an important vector of *B. malayi* (periodic strain) based on the periodicity of the microfilariae and the mosquito biting behaviour. No infective mosquitoes were collected but several were found to be infected with unidentified L_2 worms. The susceptibility of the mosquito to *B. malayi* was not confirmed.

Several workers had succeeded in colonizing *An. sinensis* in the laboratory. Oguma and Kanda (1974) started their colony with 70 blood-fed females collected from pig pens and cattle sheds. They were able to rear

it to 26 generations through induced copulation and feeding with human blood, and further to another 19 generations by free mating and feeding with mouse blood. The authors reported that the hatchability of eggs obtained through artificial mating was only 20% compared to 92% in naturally inseminated females. The larvae were fed with pulverized food consisting of equal weights of wheat germ, dry yeast and oatmeal.

Pan and Hang (1979) used powdered liver and yeast to feed the larvae of *An. sinensis*. Also, instead of resorting to induced copulation, they kept about 2,000 adults in a 30 x 30 x 30 cm cage and exposed them to blue-light interference illumination for 72 hours to encourage mating. Females were allowed to feed on rabbit blood. The authors report a 52% copulation rate in the tenth generation and the larval and pupal stages lasting 8-10 and 1-2 days respectively.

MATERIALS AND METHODS

Two studies were done from April to August 1984, namely: (a) the laboratory colonization of *An. sinensis* and (b) the susceptibility of *An. sinensis* to *B. malayi* (subperiodic strain) and *B. pahangi*.

A. Laboratory colonization of *Anopheles sinensis*

Wild specimens were collected by bare-leg catch method from Lubuk Pusing, an open swamp ecotype, on the night of April 19, 1984. Mosquitoes attracted to the collectors were actively collected using 50 x 19 mm (length x diameter) vials. Only 18 unfed *An. sinensis* females were caught, fed in the laboratory with human blood and set for egg-laying after 2 days. The colony was started from 5 females which were able to lay eggs.

Larvae were reared in plastic trays measuring 34.5 x 24.5 x 5.5 cm containing 2 cm of deionized water. The composition of the larval food was: 1 part of casein-Farex-liver powder-dried yeast-Vitamin B complex mixture (75:25:3:3:1.5); 1 part Quaker oats; 1 part wheat germ; and 1 part dried yeast. In addition, pieces of dried rice stalks were added to the water to form an infusion.

Pupae were collected from the trays together with a sufficient amount of rearing water and transferred to a Petri dish by a wide-mouth pippette. The Petri dish with the pupae was placed inside a bigger container (5 x 9.5 cm) and covered with a fine netting to confine the emerging adults.

Emerging adults were transferred by a suction tube into a 15 x 15 x 15 cm cage and initially fed with 3% sugar (sucrose) solution. After 4 days, the females were transferred into paper cups, fed with human blood and mated on the same day with males of the same age by induced copulation method (Baker et al., 1962).

Mated and fed females were set for egg-laying after 3 days inside a vial (50 x 22 mm) lined with wet cotton and filter paper. The first instar larvae emerging were collected by a fine-pointed pippette and

transferred to trays containing the rearing medium. As much as 90 larvae were transferred to each tray. The development of the mosquito from egg to adult was observed.

B. Experimental infection of *Anopheles sinensis* with *Brugia malayi* (subperiodic strain) and *Brugia pahangi.*

Starved 4 day-old F_2 adult females were made to feed on infected animals (anaesthesized) on four occasions as follows:

Date	Species of microfilaria	Experimental animal
June 19, 1984	*Brugia malayi* (s.p.)	cat (No. 1037)
July 13, 1984	*Brugia pahangi*	cat (No. 941)
July 16, 1984	*Brugia malayi* (s.p.)	cat (No. 1011)
July 20, 1984	*Brugia malayi* (s.p.)	monkey (No. 4)

Infected mosquitoes were kept in paper cups, provided with 10% glucose-Vitamin B solution and dissected after 11 days. The head, thorax and abdomen of the mosquitoes were examined carefully during dissection under a dissecting microscope. The number, stage and condition of filariae in the mosquitoes were recorded.

The insectary where the colony and infected mosquitoes were kept was maintained at approximately 80^0 F and 80% R.H.

RESULTS AND DISCUSSION

Anopheles sinensis was reared to a sufficient number needed in the susceptibility studies without much difficulty. However, due to lack of time, it was only reared and observed up to the third generation. The susceptibility tests on the mosquito against *B. malayi* (s.p.) and *B. pahangi* were carried out successfully in cooperation with Division of Filariasis.

A. Laboratory colonization of *Anopheles sinensis*

Tables 1 and 2 present the fecundity of *An. sinensis* in the laboratory and the duration of the various stages in its development. Adequate data was not gathered during the first generation. The average number of eggs was comparatively higher in F_3 than in F_2. Hatchability also increased but the viability of the larvae from first to fourth instar remained almost the same. Compared to the results obtained by Oguma and Kanda (1976), the mosquitoes laid fewer eggs but showed increased hatchability. The viability of the larvae was slightly lower. Oguma and Kanda, in Japan, found *An. sinensis* to lay an average of 235 to 329 eggs with a low hatchability of 18 to 31%, and a larval viability of 68-78%. They used equal parts of wheat germ, dried yeast and oatmeal as larval food but did not mention placing rice stalks into the rearing medium.

The eggs were observed to hatch after 2 days but the duration of hatching varies from 1 to 14 days. The duration was shorter in the third generation (Table 2). Because the eggs do not hatch at the same time, the appearance of pupae was also not uniform. In F_3 for example, approximately 12 days were needed for the larvae to pupate completely. Based on the first appearance of pupae and the duration of pupation, the larval stage was estimated to last for 20 days.

Table 1. **The fecundity of *An. sinensis* in the laboratory.**

Generation	No. of females observed	Total No. of eggs oviposited	Mean No. of eggs per mosquito	Hatchability (%)	Viability from 1st to 4th instar (%)
F_2	7	596	85.1	56.7	63.6
F_3	3	435	145.0	75.6	58.5

Table 2. **The duration (in days) of the various stages of development of *An. sinensis*.**

Generation	Duration of Hatching Mean (Range)	Appearance of pupae after first day of hatching Mean (Range)	Duration of Pupation Mean (Range)	Duration of larval stage
F_2	7.0 (3—14)	12.4 (10—14)	8.4 (6—14)	20.8
F_3	2.7 (1—4)	8.7 (8 — 9)	11.7 (7—15)	20.4

Big and strong adults were obtained from the larvae reared in the rearing medium and larval food used. Bloodfed, 4-day old females mated readily with males of the same age through induced copulation. The males were observed not to feed readily on 10% glucose-Vitamin B solution. However, they fed on 1 to 3% ordinary sugar (sucrose) solution without difficulty. The females, in contrast, can feed on the more concentrated solution. Effort was made to dilute the sugar solution until feeding of the males was observed because unfed ones were totally incapable of mating.

The females were capable of laying up to 3 batches of eggs, laying the most on the second batch. The female mosquitoes could live up to

more than 15 days if provided with sugar, long enough to allow *B. malayi* development to its infective stage.

B. Susceptibility of *Anopheles sinensis* to *Brugia malayi* (subperiodic strain) and *Brugia pahangi* using experimental animals.

Tables 3, 4, 5 and 6 summarize the results of the experimental infection studies. The larvae, chitinized or normal were found in the thoracic muscles of the mosquitoes. No larvae were found in the head and abdomen after careful dissection under a dissecting microscope which could magnify the specimens 7 times.

For the infection study with *B. malayi*, 2 infected cats and 1 monkey were used. The monkey provided comparison on the possible effect of microfilarial density. While the density in the cat was over 200 mf per 20 mm^3 of blood, that of the monkey was only 13 mf per 20 mm^3.

Out of the 39 mosquitoes experimentally fed with blood from cats and monkey containing *B. malayi* microfilariae, and surviving after 11 days, only 3 were observed to contain normal larvae in their thoraces. From Tables 3, 5 and 6, it can be seen that out of these 3 mosquitoes, only 1 was able to allow development of an L_3 or infective larva. In

Table 3. **Summary results of the experimental infection study on *An. sinensis*.**

Expt. No.	Species (Animal	Mf/20 mm^3 blood before feeding	Mf/20 mm^3 blood after feeding	No. of mosquitoes dissected after 11 days	Total No. of larvae mf c	mf n	L_1 c	L_1 n	L_2 c	L_2 n	L_3 c	L_3 n
1.	*Brugia malayi* (cat 1037)	243	242	9		41	0	98	0	0	0	0
2.	*Brugia malayi* (cat 1011)	381	—	18		74	0	371	12	0	8	0
3.	*Brugia malayi* (monkey 4)	13	4	12		2	0	15	0	0	0	0
4.	*Brugia pahangi* (cat 941)	155	—	24		211	0	84	0	0	0	0

c — chitinized
n — normal
mf — microfilaria
L — larval stage

Table 4. **The number of chitinized microfilariae and L_1 larvae per mosquito for each experiment.**

Expt. No.	Species	Mf/20 cmm. before (after) feeding	No. mosquito dissected after 11 days	Chitinized mf + L_1 per mosquito Mean (Range)
1	*Brugia malayi*	243 (242)	9	15.4 (2—56)
2	*Brugia malayi*	381 (—)	18	24.7 (2—65)
3	*Brugia malayi*	13 (4)	12	1.4 (0— 4)
4	*Brugia pahangi*	155 (—)	24	12.3 (1—37)

Table 5. **Details of experiment No. 2 showing the 3 mosquitoes with normal filarial larvae out of 18 dissected specimens.**

Mosquito	Number of Larvae mf		L_1		L_2		L_3	
	c	n	c	n	c	n	c	n
8	0	0	0	2	0	0	0	0
9	0	0	20	9	0	8	0	1
10	0	0	2	1	0	0	0	0

Table 6. **The number of infected and infective mosquitoes.**

Expt. No.	Species	No. mosquito dissected after 11 days	No. of infected mosquito	No. of infective mosquito
1	*Brugia malayi*	9	0	0
2	*Brugia malayi*	18	3	1
3	*Brugia malayi*	12	0	0
4	*Brugia pahangi*	24	0	0

this lone mosquito were 9 normal L_1, 8 L_2 and 1 L_3. However, together with the normal larvae were chitinized ones. This means that the mosquito does not allow the larvae to develop freely in its body.

Based on the results it could be said that *An. sinensis* is almost totally refractory to the development of *B. malayi* in its body. Also, the presence of L_1 and L_2 in some mosquitoes, after 11 days, is abnormal. *Brugia malayi*, in a good host, will develop to L_3 after 11 days. It is interesting to note that no chitinized L_2 was recovered from the specimens. This could mean that all of the normal L_2 larvae, if given several more days, could develop to infective larvae. However, it must be noted that all of the second stage larvae were found in a single mosquito.

Tables 3 and 4 present the stages and number of chitinized larvae isolated from the mosquitoes. Almost all of the mosquitoes dissected contained chitinized microfilariae and L_1 except those fed with monkey blood wherein some negative adults were dissected. With regard to the effect of microfilarial density, it could be said that even at a lower density, *An. sinensis* tends to resist the development of the worms. In Experiment No. 4 for example, all of the microfilariae failed to develop beyond L_1 even though the worms lodging in the thoracic muscle were only 1.4 per mosquito (Table 4).

Brugia pahangi was not able to develop beyond L_1. Normal larvae were never found and fewer larvae reached L_1 compared to *B. malayi*. Out of 24 mosquitoes dissected, only 84 L_1 were isolated compared to 211 chitinized microfilariae. Most of the larvae were chitinized before reaching L_1.

SUMMARY AND CONCLUSION

A laboratory colony of *An. sinensis* was started from wild females from Lubuk Pusing. It was observed that *An. sinensis* could be reared satisfactorily in a room maintained approximately at 80°F and 80% R.H. A sufficient number of mosquitoes was obtained by rearing the larvae in hay infusion (deionized water with dried rice stalks) and fed with a larval food composed of 1 part casein-Farex-liver powder-dried yeast-Vitamin B complex mixture (75:25:3:3:1.5), 1 part Quaker oats, 1 part wheat germ, and 1 part dried yeast. The adults were mated through the usual induced copulation method after feeding with human blood. The average number of eggs, hatchability and viability of the larvae from first to fourth instar were 85.1, 56.7% and 63.6% respectively in F_2, and 145.0, 75.4% and 58.5% in F_3.

After obtaining a sufficient number of mosquitoes, experimental infection studies were done using animals infected with *B. malayi* (subperiodic strain) and *B. pahangi*. It was found out that *An. sinesis* does not allow both *B. malayi* and *B. pahangi* to develop normally in its thoracic muscle. *Anopheles sinensis* therefore is a poor host of both filariae. The mosquito was almost totally refractory to *B. malayi* such that out of 39 mosquitoes dissected after 11 days, only 1 was found to be infective. Extensive chitinization was observed among the other mosquitoes. No normal *B. pahangi*

larvae was ever isolated from the dissections.

Although *An. sinensis* was shown not to allow normal development of both *B. pahangi* and subperiodic *B. malayi*, there is still the possibility that it could be a suitable host of periodic *B. malayi*. Hence, the next logical experiment is to test the susceptibility of *An. sinensis* to periodic *B. malayi*. The fact that the periodic strain of *B. malayi* does not have a known animal reservoir host must be taken into consideration.

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