

Experimental Infection of *Culex (Culex) quinquefasciatus* and *Aedes (Stegomyia) aegypti* with *Wuchereria bancrofti*

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A study was conducted to determine the susceptibility of local strains of Culex quinquefasciatus and Aedes aegypti to infection with the strain of Wuchereria bancrofti that occurs in Maceió, State of Alagoas, Brazil. Cx. quinquefasciatus blood fed simultaneously on the same microfilariae carrier ingested more blood and 2-3x more microfilariae than Ae. aegypti. Survival rates of both species of insects living for 21 days after blood feeding on microfilaraemic patients were not significantly different from the survival rates of mosquitoes that blood fed on amicrofilaraemic individuals. W. bancrofti parasites underwent normal development in Cx. quinquefasciatus, with third stage larvae first being recorded on the 11th day post infection, and their numbers increasing thereafter. Development of filariae in Ae. aegypti did not proceed beyond the first larval stage, and there was a progressively increasing number of non-viable larvae with the passage of time. It is concluded that Ae. aegypti is not involved in the transmission of W. bancrofti in Maceió.

Key words: *Culex quinquefasciatus* - *Aedes aegypti* - experimental infection - *Wuchereria bancrofti* - filariasis - microfilariae

Culex quinquefasciatus is the principal vector of Bancroftian filariasis in areas where *Wuchereria bancrofti* has nocturnal periodicity, but, in other places, other species of mosquitoes may serve as insect hosts of the parasite (White 1989, WHO 1992). In Brazil, Causey et al. (1945), Rachou et al. (1955) and Rachou (1956) incriminated species of *Aedes* and *Anopheles* as hosts of secondary importance.

In Maceió, capital of the State of Alagoas, northeast Brazil, Deane et al. (1953) and Fontes et al. (1994) established that *Cx. quinquefasciatus* is the most important insect host of *W. bancrofti*. However, *Ae. aegypti* is frequently found in human habitations in Maceió and it was considered necessary to determine if this species plays a role in the transmission of *W. bancrofti* in the city.

MATERIALS AND METHODS

Routine maintenance of mosquito colonies - Adult females of *Cx. quinquefasciatus* and *Ae. aegypti* were collected inside houses in urban areas of Maceió. After identification, each species was placed in a separate cage containing bowls of dechlorinated water for oviposition.

The mosquitoes were maintained in an insectary at $27 \pm 2^\circ\text{C}$ and of $80 \pm 10\%$ RH, and provided a 10% glucose solution.

After eclosion, larvae were fed with ground food for laboratory mice. The cubes of mouse food had been previously sterilized by autoclaving. Pupae were transferred to water bowls and placed in clean insect cages for eclosion of adults. Emerged adult females of both species were provided a blood meal on pigeons weekly.

The established colony of *Cx. quinquefasciatus* has been maintained since March 1993. For public health safety, related to the possible transmission of Yellow Fever and Dengue, the *Ae. aegypti* colony was maintained only for the duration of the study (January to August 1996).

Selection of volunteers - Four microfilariae carriers were randomly selected as hosts for mosquito blood meals. Each was informed of the nature of the study, and all gave written consent for their participation. The volunteers males, over 18 years of age, and not having been treated for filariasis, were microfilaraemic and with no clinical signs of lymphatic filariasis. Densities of microfilariae in the peripheral blood of the volunteers were determined by the method of Chularerk and Desowitz (1970). At the end of the study, the volunteers were treated with diethylcarbamazine in accordance with the therapeutic scheme recommended by the World Health Organization (WHO 1987).

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Experimental infection of mosquitoes - Nulliparous 2-5 day-old females of both species were used in the experiments. The sugar solution was removed 24 hr before the blood meal to increase blood-feeding. Females of both species were exposed simultaneously to either the upper surface of the hand or on the forearm of the same volunteer between 23 hr and midnight. The time of blood feeding coincided with the peak of microfilaraemia recorded in Maceió (Rocha et al. 1991). To reduce discomfort, blood feeding times were limited to 5-10 min for *Ae. aegypti* and to 10-20 min for *Cx. quinquefasciatus*. Female mosquitoes that did not engorge were discarded.

Volume of ingested blood - The amount of ingested blood was determined by weighing 20 unfed females of each species immediately before blood feeding, and weighing 20 engorged females immediately after their blood meals. The volume of blood ingested was estimated by dividing the weight differences by 1,055 (1,055 mg/ml being the approximate density of human blood).

Number of ingested microfilariae - The expected number of microfilariae ingested by the mosquitoes was calculated by multiplying the microfilaraemia of each volunteer by the mean volume of ingested blood. The actual numbers of ingested microfilariae were calculated by dissecting out the contents of the gut of a sample of the blood fed adults. The midgut was opened and a smear was made of the contained blood. The smear was fixed in methanol, stained with Giemsa, and the number of ingested microfilariae determined.

Development of W. bancrofti in Cx. quinquefasciatus and Ae. aegypti - Blood fed mosquitoes were maintained in the insectary for up to 21 days and provided with a 10% glucose solution as previously described (the usual time needed for complete development of *W. bancrofti* in an insect host is less than the period of observations in the present study).

After blood feeding, 2-5 females of both species of mosquitoes were removed daily, stunned by placing them in a freezer at -10°C for 30 sec, and then dissected in a drop of buffered saline on a slide. After removal of the wings and legs, the head, thorax and abdomen were separated. Each of the body parts was transferred to separate drops of buffered saline and macerated to liberate filaria larvae. The numbers of larvae were recorded and their stage of development was noted.

Assessment of mosquito mortality - Mortality was recorded twice daily and dead mosquitoes dissected and examined for *W. bancrofti* larvae, as previously described. Survival rates were calculated by dividing the numbers of mosquitoes surviving for 21 days after the infective blood meal

by the number of mosquitoes that took complete blood meals. Control mosquitoes of both species were allowed to blood feed on uninfected persons. The accumulative numbers of dead mosquitoes were plotted against time, and the correlation coefficients were calculated and compared.

RESULTS

Survival rates of mosquitoes - Observations on mortality and survival are summarized in Table I. Of the 1,084 *Cx. quinquefasciatus* mosquitoes which blood fed on *W. bancrofti* microfilaraemic volunteers, 476 survived for more than 21 days, a survival rate of 0.66. For 926 female *Ae. aegypti* exposed to infection, the survival rate was 0.71. Survival rates for mosquitoes that blood fed on individuals without microfilaraemia were 0.73 for *Cx. quinquefasciatus* and 0.79 for *Ae. aegypti*.

Among species, survival rates were not significantly different in mosquitoes exposed to infection ($\chi^2 = 3.21$, $p > 0.05$ for mosquitoes exposed to infection, and $\chi^2 = 0.49$, $p > 0.05$ for mosquitoes that blood fed on individuals without microfilariae). Likewise, there was no significant differences in the survival of the same species after blood feeding on persons with or without microfilariae (*Cx. quinquefasciatus*: $\chi^2 = 2.17$, $p > 0.05$; *Ae. aegypti*: $\chi^2 = 2.49$, $p > 0.05$).

Fig. 1 shows the accumulative daily mortality of mosquitoes that blood fed on subjects with or without microfilariae. The four curves have the same correlation coefficient ($r = 0.99$).

Volume of ingested blood - The mean volume of blood ingested was 3.37 ± 0.63 ml and 2.37 ± 0.53 ml for *Cx. quinquefasciatus* and *Ae. aegypti*, respectively (Table II).

Number of ingested microfilariae - Sixty-one *Cx. quinquefasciatus* were examined soon after they had taken an infective blood meal. Fifty-nine (97%) harboured 3-102 microfilariae in the midgut (mean 19.8 ± 19.5 microfilariae/female). Similarly, 76 female *Ae. aegypti* were examined after blood feeding. Sixty-four (84%) were infected with 1-22 microfilariae (mean 5.7 ± 5.6 microfilariae/female).

Females of both species of mosquito ingested more microfilariae than the density in the peripheral blood of the volunteers. The proportion of female *Cx. quinquefasciatus* containing microfilariae was significantly higher than that recorded for female *Ae. aegypti* ($\chi^2 = 4.5$, $p < 0.05$). Also, the number of microfilariae ingested by female *Cx. quinquefasciatus* was significantly higher than the number ingested by female *Ae. aegypti* ($p < 0.05$).

The estimated microfilarial "concentration factor" varied from 2.6x to 7.6x for female *Cx. quinquefasciatus*, and between 1.4x and 2.8x for

TABLE I
Summary of *Culex quinquefasciatus* and *Aedes aegypti* survival after blood feeding on *Wuchereria bancrofti* microfilaraemic volunteers

Species of mosquito	Numbers of mosquitoes					Survival rate ^b
	No. taking blood meal	No. dissected immediately after blood meal	No. killed daily	No. found dead ^a	No. maintained in the insectary	
<i>Cx. quinquefasciatus</i>	1.084	61	300	247	723	0.66
<i>Ae. aegypti</i>	926	76	300	161	550	0.71

a: numbers dying before the 21st day after the blood meal;

No. of mosquitoes surviving beyond the 21st day after the blood meal

b: No. of blood fed mosquitoes maintained in the insectary

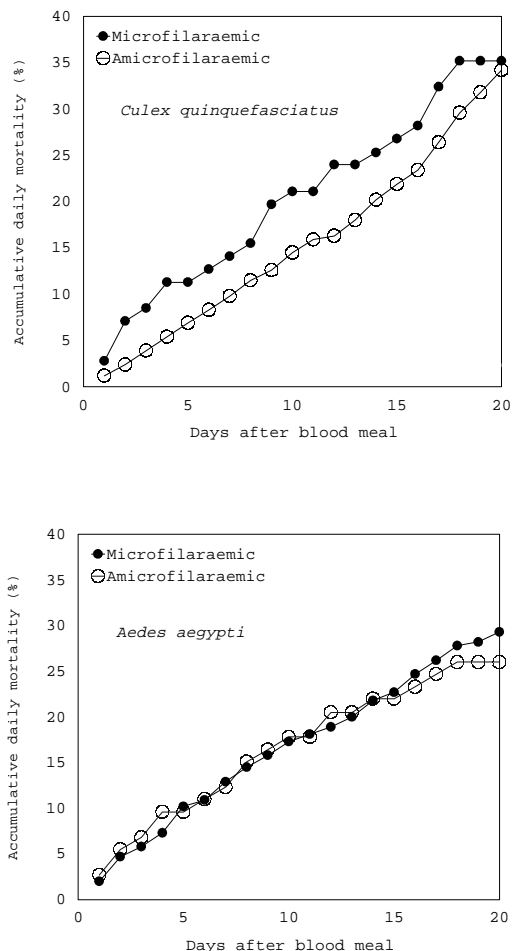


Fig. 1: accumulative daily mortality of female *Culex quinquefasciatus* and *Aedes aegypti* after blood feeding on individuals with or without microfilariae of *Wuchereria bancrofti* in the peripheral blood stream.

female *Ae. aegypti* (Table III). For both species of mosquito, the highest “concentration factor” was recorded in specimens that blood fed on the individual with 1,168 microfilariae/ml in the peripheral blood.

Development of W. bancrofti in Cx. quinquefasciatus - One day after the infecting blood meal, “young” first stage larvae (=exsheathed microfilariae) were seen. Most of them (78.7%) were observed in the abdomen while 21.3% had reached the thorax. By the 4th day post infection, 94.5% of the parasites were observed in the thorax, but were still in the “young” L₁ stage. Transformation into the sausage-shaped L₁ larvae occurred between the 5th and 7th day post infection. After the 8th day of infection, larvae were observed undergoing the L₁/L₂ moult. The L₂/L₃ moult was first recorded on the 11th day post-infection and,

TABLE II

Estimated weight and volume of blood ingested by females *Culex quinquefasciatus* and *Aedes aegypti*

Species of mosquito	Mean weight of mosquitoes (mg)		Mean weight of blood ingested (mg)	Mean volume of blood ingested (ml)
	Unfed	Engorged		
<i>Cx. quinquefasciatus</i>	1.34 ± 0.25 ^a	5.09 ± 0.52	3.75 ± 0.66	3.57 ± 0.63 ^a
<i>Ae. aegypti</i>	0.95 ± 0.16 ^a	3.45 ± 0.53	2.50 ± 0.56	2.37 ± 0.53 ^a

a: confidence limits 95%; significant differences (p<0.05).

TABLE III

Differences between the expected and observed numbers of microfilariae ingested by females of *Culex quinquefasciatus* and *Aedes aegypti*

Microfilaraemia of volunteers (mf/ml)	Mean number of microfilariae/mosquito				Difference (No. observed/No. expected)	
	Observed		Expected		Culex	Aedes
	Culex	Aedes	Culex	Aedes		
906	8.8	3.5	3.4	2.0	2.6	1.8
1,168	24.4	5.5	3.2	2.0	7.6	2.8
1,215	13.8	6.8	4.3	3.4	3.2	2.0
1,830	26.2	7.2	7.8	5.2	3.4	1.4

thereafter, the numbers of L₃ (infective larvae) increased. Twenty-one days after exposure to infection 216 out of 476 dissected *Cx. quinquefasciatus* mosquitoes yielded 860 infective larvae.

Development of W. bancrofti in Ae. aegypti - During the first four days post-infection, the appearance of *W. bancrofti* in *Ae. aegypti* was similar to their appearance in *Cx. quinquefasciatus*. After the 5th post-infection day, development of *W. bancrofti* showed little progress. A small number of exsheathed microfilariae developed into sausage shaped L₁ larvae, but many of these had a shrunken, wrinkled appearance and were mostly immobile and were therefore considered dead. Development of the Maceió strain of *W. bancrofti* in the Maceió strain of *Ae. aegypti* is shown in Fig. 2. None of the *W. bancrofti* larvae progressed beyond the L₁ stage, dead larvae were recorded as early as 5-7 days post-infection, and, with a single exception, only dead larvae were recorded after the 13th post-infection day.

DISCUSSION

Because the recommendations of Asahina (1964) and Consoli and Lourenço-de-Oliveira (1994) were followed, it is reasonable to assume that the colonies of *Cx. quinquefasciatus* and *Ae. aegypti* were adapted to laboratory conditions before the presently reported experiments were undertaken.

Neither species of mosquito showed adverse effects due to the presence of *W. bancrofti*. This contrasts with studies of Wharton (1960) and Crans (1973) in Malaysia and East Africa, respectively. However, in these earlier studies, patients had much higher microfilaraemia than did the volunteers in Maceió. It is possible that migration of high numbers of developing filarial larvae within the mosquito body have an effect on vector survival.

Previous authors (Manson 1884, O'Connor & Beatty 1936, Burton 1964, Crans 1973, McGreevy et al. 1982), working in different and widely separated parts of world, have recorded the disparity between the density of microfilariae in the peripheral blood of human hosts and the numbers of microfilariae ingested by arthropod hosts of the parasites. Various factors have been suggested to explain the apparent ability of mosquitoes to "concentrate" microfilariae (Hairston & Jachowski 1968, Eberhard et al. 1988, Dickerson et al. 1989, Southgate & Bryan 1992). The simplest explanation is the manner in which mosquitoes acquire blood meals. The classical paper of Gordon and Lumsden (1939), confirmed by Griffiths and Gordon (1952), shows that *Ae. aegypti* either obtains blood directly from a vessel or by lacerating vessels and, thus, forming a subcutaneous haemorrhage (pool feeding). Pool feeders take longer to engorge than do vessel feeders. When a mosquito pool feeds, there is more time for microfilariae to seep from the lacerated vessel into

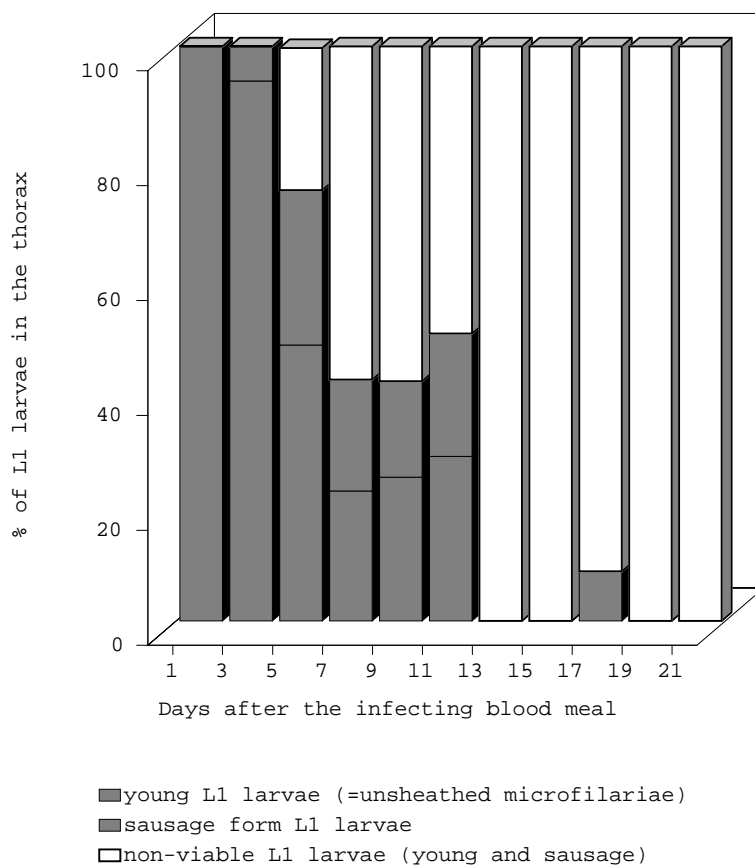


Fig. 2: developing stages of *Wuchereria bancrofti* found in the thorax of experimentally infected female *Aedes aegypti*.

the subcutaneous haemorrhage. However, it is recognized that further studies are necessary to understand the “concentration effect” of microfilariae in mosquitoes (Lowrie et al. 1989).

Complete development of *W. bancrofti* in *Cx. quinquefasciatus* was expected because females of this species have been found naturally infected in Maceió (Fontes et al. 1994).

Experimental infection of *Ae. aegypti* with *W. bancrofti* has given variable results. In some cases (Ponnudurai et al. 1971, McGreevy et al. 1982), the parasites have developed to the L₃ stage, whereas other researchers (Hunt & Gunders 1990, Anosike & Onwuliri 1992) obtained negative results. The present results are most similar to those of MacDonald and Ramachandran (1965) and Anosike and Onwuliri (1992), with larval development only progressing to the sausage-shaped phase.

The available literature provides evidence that susceptibility of *Ae. aegypti* to infection with filarial parasites is controlled by a sex-linked recessive gene (MacDonald & Ramachandran 1965,

Ponnudurai et al. 1971, McGreevy et al. 1974). Although it has not yet been possible to make genetic studies on *Ae. aegypti* in Maceió, it is reasonable to suspect that the Maceió strain of *Ae. aegypti* is genetically incapable of supporting the development of *W. bancrofti*. For this reason, it is concluded that *Ae. aegypti* is not involved in the transmission of Bancroftian filariasis in Maceió.

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