BANCROFTIAN FILARIASIS IN MACEIO, STATE OF ALAGOAS, BRAZIL: OBSERVATIONS ON CULEX QUINQUEFASCIATUS AFTER BLOOD FEEDING ON INDIVIDUALS WITH DIFFERENT DENSITIES OF MICROFILARIAE IN THE PERIPHERAL BLOOD STREAM

A. C. BRITO, G. FONTES, P. WILLIAMS, AND E. M. M. ROCHA
Departamento de Patologia, Universidade Federal de Alagoas, Maceio, Brazil; Departamento de Parasitologia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Abstract. Females of laboratory-reared Culex quinquefasciatus were allowed to take blood meals on individuals with low (1–100 microfilariae/ml of peripheral blood), moderate (101–500 microfilariae/ml), and high (> 500 microfilariae/ml) microfilaraemia. The mosquitoes ingested 1.39–3.80 μl of blood and infective third-stage (L₃) larvae were first recorded 13–14 days after the infecting blood meal. The number of microfilariae ingested by mosquitoes was proportional to the density of microfilariae in the peripheral blood of the human subjects, but with a concentration factor of up to 6.5 times the expected number. Survival of mosquitoes was not influenced by the density of microfilariae in the peripheral blood of infected individuals. Infectivity indices were proportional to microfilaraemia in human subjects. The number of L₃ larvae/female (intensity of infection) was not influenced by individual microfilaraemia. The highest vector efficiency and the best experimental infection index were recorded in mosquitoes that blood fed on individuals with moderate microfilaraemia. The results are discussed in relation to the transmission on Bancroftian filariasis in the study area.

In his account of the development of Filaria sanguinis hominis in a mosquito host, Manson⁠¹ noted that insects ingested more microfilariae than could be expected from their density in the peripheral blood of human hosts. This phenomenon has been confirmed by many subsequent investigators²⁻⁹ working in countries as widely separated as Malaysia, Fiji, Guyana, Tanzania, and Haiti. There have, however, been contradictory reports. Jordan and Goatly¹⁰ and Gubler and others¹¹ working in Tanzania and India, respectively, found no evidence of concentration of microfilariae during the course of mosquitoes blood meals.

The apparent concentration of microfilariae during the insect blood meal has stimulated research workers⁵, ⁷, ⁸, ¹²⁻¹⁶ to estimate the minimum number of microfilariae that a mosquito needs to ingest to develop sufficient infective larvae to induce an infection in another human host. The results of studies have shown that persons with low microfilaraemia could be of importance in the transmission dynamics of Wuchereria bancrofti.¹⁵

Several investigators³, ⁵, ¹², ¹⁷⁻²⁰ have recorded a higher mortality rate in mosquitoes that blood feed on hosts with high microfilaraemia. In contrast, Rosen did not observe this phenomenon in Culex quinquefasciatus on certain Pacific islands (French Oceania).¹² In the Pacific islands, Cx. quinquefasciatus is considered to be a poor insect host for W. bancrofti, whereas the same species of mosquito seems to be a highly efficient host of the parasite in Africa.⁷

Because of the contrasting results obtained in different parts of the world, a study was undertaken to examine some factors that define the vectorial capacity of Cx. quinquefasciatus in Maceio, the capital of the state of Alagoas, Brazil. Culex quinquefasciatus is widespread in Brazil, but Bancroftian filariasis now has a very limited distribution in this country. Within the city of Maceio, lymphatic filariasis occurs only in three districts.²¹ This paper reports experimental observations on some factors of epidemiologic significance in a well-defined, but small, focus of the disease.

MATERIALS AND METHODS

Hosts for mosquito blood meals. Fontes and others²¹ surveyed the population of Maceio using the filtration method for venous blood described by Chularerk and Desowitz²² to determine microfilaraemia. The filtration method was used only in the screening to detect carriers with different densities of microfilariae. The results of these surveys showed that infected individuals could be grouped into three categories: low microfilaraemia (1–100 microfilariae/ml of peripheral blood); moderate microfilaraemia (101–500 microfilariae/ml of blood); and high microfilaraemia (> 500 microfilariae/ml of blood). Individuals of each category were invited to participate in the study.

All participants were greater than 16 years of age. Each was informed of the purpose and scope of the study. Written consent was obtained from all participants, and each underwent a clinical examination before being exposed to mosquito bites. At the end of the investigation all participants were treated with diethylcarbamazine at the World Health Organization recommended dose of 6 mg/kg/day for 12 days.²³ This project was reviewed and approved by Joao de Freitas (Professor of Legal Medicine, Universidade Federal de Minas Gerais).

Rearing of mosquitoes. The laboratory colony of Cx. quinquefasciatus originated from mosquitoes collected in Maceio. The female mosquitoes exposed to infection by W. bancrofti were maintained in an insectary with a temperature of 27 ± 1°C and a relative humidity of 80 ± 10%.

Exposure of mosquitoes to infection. In each experiment, batches of 100–300 female mosquitoes 2–10 days after eclosion and without access to a sugar meal for 24 hr were given a 15–30-min opportunity to take blood meals on the forearm of human subjects infected with W. bancrofti. Blood meals were offered from 10:00 PM to midnight to coincide with the peak of microfilaraemia²¹ and with the peak of Cx. quinquefasciatus biting activity.²² After 30 min, unfed mosquitoes were separated from blood-engorged females.
Formulas used to assess the vector potential and vector efficiency of *Culex quinquefasciatus*

Table 1

<table>
<thead>
<tr>
<th>Formula</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Infectivity index (A)} = \frac{\text{Number of mosquitoes with } L_3 \text{ larvae}}{\text{Number of mosquitoes surviving through the incubation period}}$</td>
<td></td>
</tr>
<tr>
<td>$\text{Survival rate (B)} = \frac{\text{Number of mosquitoes surviving through the incubation period}}{\text{Number of blood-fed mosquitoes}}$</td>
<td></td>
</tr>
<tr>
<td>$\text{Intensity of infection (C)} = \frac{\text{Number of } L_3 \text{ larvae}}{\text{Number of mosquitoes with } L_3 \text{ larvae}}$</td>
<td></td>
</tr>
<tr>
<td>$A \times B \times C = \frac{\text{Number of } L_3 \text{ larvae}}{\text{Number of blood-fed mosquitoes}}$</td>
<td></td>
</tr>
<tr>
<td>$\text{Experimental infection index} = \frac{A \times B \times C}{\text{Number of microfilariae/µl of peripheral blood}}$</td>
<td></td>
</tr>
<tr>
<td>$\text{Vector efficiency} = \frac{\text{Number of } L_3 \text{ larvae/mosquito}}{\text{Number of microfilariae ingested/mosquito}} \times 100$</td>
<td></td>
</tr>
</tbody>
</table>

Unfed mosquitoes served as one of the control groups. The experimental and control groups had unlimited access to a 10% aqueous solution of glucose.

**Estimation of blood meal size.** Twenty unfed female mosquitoes, which did not have access to the sugar solution for 24 hr, were weighed on an analytic balance before and after taking a blood meal. The difference between the two weights divided by 20 provided an estimate of the amount of blood ingested per mosquito. The volume of ingested blood was calculated by dividing the weight difference by 1.055 (the approximate density of human blood is 1.055 mg/ml).

**Measurement of the concentration factor.** In each experiment, the expected number of ingested microfilariae was calculated from each participant’s microfilaremia determined by counting the number of microfilariae in a thick blood film of 20 µl of capillary blood obtained when mosquitoes were blood feeding, and the mean volume of blood ingested by the mosquitoes. The actual number of microfilariae ingested was estimated by individually dissecting 10 females in each experiment immediately after blood feeding.

The digestive tract of recently fed females was dissected out of the insect’s body placed in 20 µl of distilled water, and teased apart to liberate the stomach contents. After drying the preparations, they were fixed and stained with Giemsa. The microfilariae in each preparation were counted using a compound microscope at a magnification of 100×.

**Development of *W. bancrofti* and *Cx. quinquefasciatus.* Every day after the infecting blood meals, five female mosquitoes were killed and dissected to determine the numbers of developing larvae, their stage of development, and their positions in the body of the insect host.

**Determination of vector potential and vector efficiency.** The formulas of Wharton, 4 Ramachandran, 23 and Sabry 26 were used to estimate the vectorial potential in the laboratory of *Cx. quinquefasciatus* mosquitoes fed on individuals with different microfilaremias. Vector efficiency was assessed using the equation of Kartman. 27 The formulas, together with data derived from them, are listed in Table 1. The statistical tests used were the Student’s $t$, chi-square, and correlation tests. The statistical package MINITAB (Minitab Software Release 9.2, 1993; Minitab, Inc., State College, PA) was used.

**RESULTS**

**Size of blood meals.** The mean ± SD weight of unfed female mosquitoes was 1.35 ± 0.30 mg. The mean ± SD weight of ingested blood was 2.49 ± 0.61 mg, and the mean ± SD volume was 2.36 ± 0.58 µl. The mean ± SD volumes ingested by mosquitoes that blood fed on persons with high, medium, or low densities of microfilariae were 2.56 ± 0.61, 2.22 ± 0.65, and 2.28 ± 0.49 µl, respectively. The differences in volumes of blood ingested by the three groups were not statistically significant ($P > 0.05$).

**Ingestion of microfilariae by mosquitoes.** Figure 1 shows the regression line obtained when the percentage of mosquitoes that ingested microfilariae is plotted against the numbers of microfilariae in the peripheral blood of donor hosts. The regression line shows that the proportion of female of *Cx. quinquefasciatus* ingesting microfilariae increases with the density of microfilariae in human peripheral
blood. The correlation test confirms that there is a wide individual variation (r = 0.646). The percentage of mosquitoes that became infected after blood feeding on individuals with high, moderate, or low microfilariaemia was statistically significant (P < 0.05). The numbers of microfilariae ingested by individual mosquitoes (Figure 2) is directly related to the concentration of microfilariae in individual human hosts. In this case, correlation tests (r = 0.836) were significant.

**Concentration factor.** Mosquitoes that blood fed on individuals with high microfilaria densities ingested 3.3 to 6.5 times more microfilariae than the concentration in the peripheral blood of donor hosts. In mosquitoes that blood fed on persons with moderate microfilariaemia, the concentration factor was 0.6 to 5.8 times. Mosquitoes that blood fed on volunteers with low microfilariaemia had a concentration factor of three times. The difference in the concentration factors of the three groups were not statistically significant (P > 0.05).

**Development of W. bancrofti in Cx. quinquefasciatus.** The minimum time for the development of W. bancrofti in the mosquito host was 13 to 14 days. Sausage-shaped, first-stage larvae (L_1_) were first noted four days after the infecting blood meal. The first L_1~L_2_ molts were observed on the ninth day after the blood meal. The L_2~L_3_ transformation was first recorded 13 to 14 days after the infecting blood meal. The L_3_ invasion of the proboscis was first observed 15 days after the infecting blood meal.

The foregoing data refer to the minimum development times. Development of W. bancrofti in Cx. quinquefasciatus of Maceio origin is not uniform. Larvae in different developmental stages were frequently encountered in the same mosquito.

**Survival rates.** Table 2 shows details of survival rates. There were two control groups: mosquitoes that blood fed on persons without microfilariae, and mosquitoes that did not blood feed. The latter control group, which had access to sugar solution, had a lower survival rate than all of the other four groups (P < 0.05, χ² = 1,407.4). There was no statistical difference in the survival rates of the other four groups (P > 0.05).

**Infectivity indices and intensities of infection.** Indices of infection were 0.07, 0.27, and 0.47 in mosquitoes that blood fed on individuals with low, moderate, or high microfilariaemia, respectively. These indices are proportional to the densities of microfilariae in the peripheral blood of the individuals who were hosts for blood meals. There were statistically significant differences (χ² = 312.46, P < 0.05) between the indices for the three experiment groups. The intensities of infection in mosquitoes that blood fed on persons with low, moderate, or high microfilariaemia were 1.22, 1.84, and 2.27, respectively. The differences between the indices for the three experimental groups were not statistically significant (P > 0.05). The number of L_3_ per infected mosquito was not influenced by the density of microfilariae in the blood of the persons who served as sources for blood meals.

**Vector efficiency of Cx. quinquefasciatus.** The highest measure of efficiency was about 35% in mosquitoes that blood fed on persons with moderate microfilariaemia. The next value of efficiency was recorded in mosquitoes that blood fed on volunteers with low microfilariaemia, and the least efficient were the mosquitoes that blood fed on individuals with high microfilariaemia (Table 3). In this group, for every 9.4 microfilariae ingested, only one infective larva developed.

**Experimental infection indices.** Groups of mosquitoes that blood fed on individuals with low, moderate, or high microfilariaemia had indices of 1.97, 2.03, and 0.99, respectively.

**Table 2**

<table>
<thead>
<tr>
<th>Microfilariaemia</th>
<th>Blood fed</th>
<th>Dissected after the blood meal</th>
<th>Retained</th>
<th>Surviving</th>
<th>Survival rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>240</td>
<td>30</td>
<td>210</td>
<td>164</td>
<td>0.78</td>
</tr>
<tr>
<td>1–100</td>
<td>1,063</td>
<td>99</td>
<td>964</td>
<td>755</td>
<td>0.78</td>
</tr>
<tr>
<td>101–500</td>
<td>1,024</td>
<td>99</td>
<td>925</td>
<td>734</td>
<td>0.79</td>
</tr>
<tr>
<td>&gt;500</td>
<td>1,121</td>
<td>138</td>
<td>983</td>
<td>754</td>
<td>0.77</td>
</tr>
<tr>
<td>Control†</td>
<td>2,110</td>
<td>0</td>
<td>2,110</td>
<td>542</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*Microfilaria/ml of peripheral blood.
†Mosquitoes that did not blood feed.

**Table 3**

<table>
<thead>
<tr>
<th>Level of microfilariaemia</th>
<th>Number of mosquitoes</th>
<th>Total L_3_ larvae</th>
<th>L_3_ infection rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>754</td>
<td>805</td>
<td>10.0</td>
</tr>
<tr>
<td>Moderate</td>
<td>734</td>
<td>360</td>
<td>1.4</td>
</tr>
<tr>
<td>Low</td>
<td>755</td>
<td>62</td>
<td>0.47</td>
</tr>
</tbody>
</table>

*Expected L_3_ infection rate (E) = number of microfilariae ingested per mosquito.
†Observed L_3_ infection rate (O) = number of L_3_ larvae per mosquito.
DISCUSSION

The volume of blood ingested by female *Cx. quinquefasciatus* originating in Maceio was within the range (2.8–3.5 μl) recorded by Burton in Guyana and Lowrie and others in Haiti. Jordan and Goatly, who worked in Tanzania, suggested that variations in the size of blood meals is probably related to differences in the overall size and weight of individual mosquitoes.

The concentration of microfilariae during the course of a mosquito’s blood meal has been known for more than 100 years. It has not been observed in some foci of lymphatic filariasis. In the present study, the numbers of microfilariae ingested by mosquitoes belonging to each of the three experimental groups were higher than expected on the bases of microfilaraemia in the individuals who were the sources of blood meals. Some insects ingested as much as 6.5 times the concentration of microfilariae in the peripheral blood of the donor hosts. This is much higher than the concentration factor of 2–3 times recorded by Burton and Crans.

The apparent concentration of microfilariae during the blood meal of a mosquito is probably related to differences in blood feeding behavior of the same species of mosquito. A mosquito is likely to ingest fewer microfilariae when feeding directly from a capillary than when pooled feeding. The number of microfilariae ingested is influenced by the particular vessel from which a mosquito obtains blood. In the present study, it was noted that thick blood films of capillary blood had a significantly higher density of microfilariae than that recorded for filtered venous blood. The chemical composition of mosquito saliva and the effects of injection of saliva into mammalian skin tissue need to be examined in relation to concentration of microfilariae.

The present studies confirm observations made in Japan by Kobayasi and in Fuji by Symes. At temperatures of 23–32°C, larvae of *W. bancrofti* developing in *Cx. quinquefasciatus* reach the infective (L₃) larval stage in a minimum of 12 days after the infecting blood meal. The L₁ larva continued to be observed in different parts of the mosquito body, including the proboscis, for 15–30 days after the infecting blood meal. McGreevy and others suggested that egress of L₃ larvae from the body of the mosquito host is stimulated by a combination of factors: the taking of a blood meal, curving of the labrum, and skin temperature of the human host. Earlier, Pratt and Newton suggested that a decrease in the numbers of L₃ larvae is independent of a new blood feeding.

The lack of uniformity in the development of filarial larvae in arthropod hosts has been previously reported. Williams provided drawings of the disparity of the larval forms of *Loa loa* encountered in experimentally infected *Chrysops silacea*, the prime insect host of human loiasis in West Africa. Rosen attributed the lack of uniformity in larval development to competition of the parasites within their mosquito hosts, with the more developed larvae inhibiting the development of the more slowly developing worms.

The lack of synchronized development of larvae in a mosquito host could be of epidemiologic importance. An infective mosquito could retain L₃ larvae for the rest of its life, and well after the first appearance of such larvae in the head and proboscis. Such a possibility will need to be taken into consideration when it becomes possible to construct a mathematical model of the epidemiology of lymphatic filariasis in Maceio and other foci of the disease in Brazil.

Previous studies on other combinations of filaria infections in arthropod hosts have suggested that infections shorten the lives of infected arthropods and that increased mortality can be correlated with different developmental phases of the parasites. Adverse affects of larval *W. bancrofti* on insect hosts have been recorded in mosquitoes that blood fed on individuals with high microfilaraemias, and this suggests that this situation adversely affects the longevity of mosquitoes in laboratory conditions. In each of the cited papers, mosquitoes blood fed on individuals with much higher microfilaraemia than those encountered in Maceio. In the present observations, no evidence was obtained to suggest that infection with *W. bancrofti* larvae shortened the life of female *Cx. quinquefasciatus*. This is another factor that needs to be considered in developing a mathematical model of the epidemiology of filariasis in Maceio.

These indices are related to the levels of microfilaraemia in the human hosts in which batches of mosquitoes blood fed. The results confirm observations made in widely separated parts of the world. As a generalization, the higher the density of microfilariae in the peripheral blood of human hosts, a higher proportion of blood feeding mosquitoes become infected.

The intensity of infection is a measure of the number of L₃ larvae recorded in mosquitoes exposed to infection. This is not related to the microfilaraemia of the volunteers that served as sources for blood meals. On dissection of mosquitoes, recently ingested microfilariae were found to be deformed in various ways. Microfilariae that managed to migrate to the thorax and/or abdomen of mosquitoes appeared to be dead. The L₃ larvae encountered were motionless and apparently disintegrating in both the abdomen and the thorax. Possible factors involved in the reduction of the numbers of parasites between the ingestion of microfilariae and the egress of L₁ larvae and the immunologic process between the mosquito host and filarial larvae could be an interesting future research project.

Although these indices are not the only feature that needs to be taken into consideration when assessing transmission of filariae, it is a convenient means of studying host/parasite relationships within mosquitoes. In Maceio, the highest index (2.03) was recorded in mosquitoes that had blood fed on volunteers with moderate microfilaraemia, but the index for mosquitoes that fed on persons with low microfilaraemia was only slightly lower (1.97). These results contrast with the low index (0.99) when mosquitoes fed on persons with high microfilaraemia.

In all experiments, there was a discrepancy between the numbers of microfilariae ingested and the number of L₁ larvae recovered 20 days after the infecting blood meal. Several factors are involved in the decrease in *W. bancrofti* larvae in a mosquito host, and adverse effects can begin during the passage of microfilariae through the foregut of the mosquito host. McGreevy and others reported that the cibarial and
pharyngeal armatures of mosquitoes can be lethal to ingested microfilariae.

The experimental evidence obtained in Maceio suggests that human hosts with moderate microfilaremia are an important source of infection for Cx. quinquefasciatus. In the present study, all volunteers in the low microfilaria group had less than four microfilariae/20 μl of peripheral blood. However, the vector efficiency of mosquitoes that fed on these patients was 17%. These results emphasize that patients with very low microfilaria must be taken into account in epidemiologic studies. There is evidence that individuals with very low microfilaria can be important sources of infection for mosquitoes that subsequently develop L3 larvae. The epidemiologic importance of low microfilaria, especially in patients who have not yet developed symptoms of lymphatic filariasis, indicates the need for the development of highly sensitive diagnostic methods for evidence of scanty microfilaria.

Authors' addresses: A. C. Brito, G. Fontes, and E. M. M. Rocha, Departamento de Patologia, Universidade Federal de Alagoas, Maceio, Brazil. P. Williams, Departamento de Parasitologia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

REFERENCES


