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EXPERIMENTAL INFECTION OF *ANOPHELES GAMBIAE* AND *CULEX PIPIENS FATIGANS* WITH *WUCHERERIA BANCROFTI* IN COASTAL EAST AFRICA[1,2,3]

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Abstract: Laboratory strains of *Anopheles gambiae* A and *Culex pipiens fatigans* from Tanzania, East Africa were experimentally infected with *Wuchereria bancrofti* to compare vector potential in the 2 species. Uptake of microfilariae, survival of the vector after an infective blood meal, number of filarial larvae reaching the infective stage and percentage of mosquitoes harboring infective larvae after 14 days were compared in the laboratory. *C. p. fatigans* ingested more than 3 times as many microfilariae when the 2 species were fed simultaneously on the same carrier. Fifty-four percent of the *C. p. fatigans* contained infective larvae after 14 days compared to 9.0% of the *A. gambiae*. Feeding on a carrier with lower microfilaremia produced comparably lower values for each species. The mean number of infective larvae per positive mosquito was nearly twice as high in *C. p. fatigans*. *A. gambiae* showed a lower mortality rate than *C. p. fatigans* after an infective blood meal, but data indicated that the extremely low infection rates in *A. gambiae* nearly equated infected groups with controls. The extreme differences in vector potential between the 2 species, in conjunction with the apparent role of *A. gambiae* as a vector in nature, suggest that strain rather than natural susceptibility may have been a major factor in the results of this study.

Three mosquito species, *Anopheles gambiae* sensu lato, *Anopheles funestus* and *Culex pipiens fatigans* are known to transmit Bancroftian filariasis in coastal East Africa. Studies in coastal Kenya (Nelson et al. 1962) revealed that numerous other mosquito species carried Filaroidea of wild and domestic animals but only the 3 aforementioned mosquito vectors harbored *Wuchereria bancrofti*. The importance of these vectors and the degree to which each participates in actual transmission may vary considerably in different localities. Anopheline species are considered the major vectors in most rural situations; *C. p. fatigans* is most likely the sole vector in urban areas. In foci where the 3 vectors co-exist, the efficiency of each species requires much investigation.

A. gambiae and *C. p. fatigans* are easily colonized and can be subjected to experimental infection in the laboratory. Although both are known to act as vectors, comparable efficiency under controlled conditions is of interest to better assess results obtained from the field. The uptake of microfilariae from a carrier, survival after an infective blood meal, percentage of mosquitoes which successfully develop infective stage larvae and the number of filarial larvae reaching the infective stage are useful criteria for assessing vector potential. A series of experiments designed to compare these aspects of vector-parasite interaction are the subjects of this paper.

MATERIALS AND METHODS

A. gambiae is recognized as a complex which can be identified with certainty only by characteristic banding on the polytene chromosomes (Coluzzi 1966, Coluzzi & Sabatini 1967, 1968). The specimens utilized in these experiments were taken from an inbred colony of *A. gambiae* species A (Kisumu strain), which was maintained at the East African Institute of Malaria and Vector-borne Diseases. *C. p. fatigans* were taken from a newly established colony originally collected at Tanga, Tanzania.

Several hundred 3-day-old *A. gambiae* and *C. p. fatigans* were placed in separate cages and deprived of sugar water for 12 hr. They were then simul-

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taneously fed on 2 carriers of nocturnally periodic Bancroftian filariasis; microfilaremia was determined after taking 100 mm³ of blood immediately prior to feeding the mosquitoes at 20:00 hr and again when feeding was terminated 30 min. later. A similar group of each mosquito species was fed on the author to serve as controls. The procedure was replicated once each week for a total of 10 weeks.

To assess the uptake of microfilariae, all mosquitoes feeding on 1 carrier were immobilized with chloroform and visually sorted according to blood-meal size. Mosquitoes which had not engorged completely were discarded. One hundred fully engorged mosquitoes of each species were randomly selected and individually preserved in 0.5 ml of 3% acetic acid. The acetic acid solution gradually decomposed mosquito tissue but preserved microfilariae. After several days, mosquitoes were little more than a hollow exoskeleton containing microfilariae from the infective blood meal. Mosquitoes were dissected in a Sedgewick-Rafter counting cell in acetic acid solution. Wings and legs were removed from each specimen; head, thorax and abdomen were opened and scraped clean with dissecting needles. Large pieces of exoskeleton were well flushed in the chamber and removed; small debris was left in the counting cell during examination. Each sample was thoroughly scanned with a compound microscope at 35 × and microfilariae were counted and recorded.

For studies pertaining to mortality rates and filarial development, fully engorged mosquitoes were divided into groups of 10 and placed in paper cups covered with gauze netting. Mosquitoes were fed daily with a moist sugar-water pad and held for 14 days at 25°C. Dead mosquitoes were counted and removed daily; comparable mortality rates were plotted over the 2-week interval. Surviving mosquitoes were dissected in saline and developing filariae were counted and recorded.

RESULTS AND DISCUSSION

Comparative uptake of microfilariae

The average blood meal of a fully fed *C. p. fatigans* appears to vary from 2.5 to 4.0 mm³ (Wharton 1960, Burton 1964). The fluctuation is most likely due to variations in the size and weight of individual insects (Jordan & Goatly 1962). The microfilaremia of the donor prior to feeding the mosquitoes in this test was 108 per 100 mm³ of blood. After feeding was terminated the microfilaremia had risen to 128 per 100 mm³. On the basis of volume, the average blood meal of *C. p. fatigans* would be expected to contain 3–5 microfilariae. *Anopheles gambiae* ingest a somewhat smaller volume of blood and an intake of 2–4 microfilariae would be anticipated. Other workers (Wharton 1957, Burton 1964) have shown that mosquitoes rarely ingest calculated values; a wide range of microfilarial uptake is the general rule.

TABLE 1. The comparative uptake of microfilariae by *Anopheles gambiae* and *Culex p. fatigans* when feeding on a filariasis carrier.*

No. mf ingested	Number of mosquitoes	
	A. gambiae A	*C. p. fatigans*
0	20	4
1–4	50	24
5–10	23	21
11–20	7	26
21–50	0	21
51–100	0	3
>100	0	1
Total mosq. tested	100	100
Mean no. and range of mf ingested	4.1(0–17)	15.0(0–136)

*Microfilaremia of carrier per 100 mm³ of blood: before feeding: 108, after feeding: 128.

In these studies *A. gambiae* closely approached the expected intake with a mean number of 4.1 microfilariae and a range of 0–17. *C. p. fatigans* ingested approximately 3 times the expected number; the mean was 15.0 and the range was 0–136. TABLE 1 subdivides the numbers of microfilariae ingested into ranges from 0 to 100 ÷ and lists the numbers of mosquitoes in each group.

TABLE 1 shows that 20% of the *A. gambiae* failed to ingest any parasites while only 4% of the *C. p. fatigans* were similarly categorized. The difference becomes extremely important when specimens are infected in the laboratory and compared to a group of controls. Even though the *A. gambiae* in these tests were fed upon a filariasis carrier and therefore assumed infected by laboratory criteria, 20% of the sample was identical to the controls which were fed on a non-carrier.

Further differences in microfilarial uptake are evident in TABLE 1. No *A. gambiae* in these tests ingested more than 20 microfilariae, but 1/4 of the *C. p. fatigans* took in more than 20 parasites with a single specimen containing 136 microfilariae or more than 40 times the expected number. Gorden & Lumsden (1939), working with a frog filarid, found that when mosquito mouthparts were inserted directly into a capillary, microfilariae could be ingested in larger numbers than in cases where a capillary was lacerated. The findings imply that the 2 modes of feeding could account for variations

TABLE 2. Microfilarial uptake in *Anopheles gambiae* and *Culex p. fatigans* based on blood-meal volume.

Mosquito species	Expected no. mf in average blood meal	Percentage of test samples		
		< Expected no.	With expected no.	> Expected no.
A. gambiae A	2 4	32%	35%	33%
C. p. fatigans	3–5	7%	20%	73%

in microfilarial uptake. It is possible that *A. gambiae* and *C. p. fatigans* obtain the blood meal in a different manner, as evidenced by the numbers of microfilariae ingested from a unit volume of peripheral blood. Further evidence to support this hypothesis is presented in TABLE 2, where the test sample is subdivided into the percentage of mosquitoes which imbibed greater and fewer numbers than would be expected in a given volume of blood. With *A. gambiae*, 35% ingested an expected number of microfilariae based on blood-meal volume and the remaining specimens were divided equally between greater and fewer than calculated values. With *C. p. fatigans*, 20% of the test sample ingested the calculated number of microfilariae; however, only 7% took in fewer while 73% took in more. The majority of *C. p. fatigans* in these tests appeared to concentrate microfilariae from the peripheral blood; *A. gambiae* did not have this capacity.

The ability or inability of a mosquito to concentrate microfilariae is of importance in areas where the density of microfilariae in the carrier population is either very high or very low. A mosquito species which does not ingest large numbers of microfilariae may be a better vector in areas where the microfilaria density is high. Those species which take in large numbers of microfilariae might suffer considerable mortality as a result of the worm load. The opposite may be true in areas where the microfilaria density is low. The use of diethylcarbamazine reduces microfilaremia and, when administered to large populations, achieves control by reducing the microfilaria density below the threshold necessary for transmission. If mosquito vectors show differences in microfilarial uptake, filariasis control with diethylcarbamazine could be successful in an area where a vector is unable to obtain microfilariae from low level carriers and unsuccessful in an area where a vector is capable of concentrating the parasites.

It would appear that *A. gambiae* and *C. p. fatigans* show distinct differences in their ability to ingest microfilariae. Although the 2 species do not ingest identical volumes of blood, the variations cannot be attributed to blood-meal volume. There are obviously unknown factors involved in the mode of feeding by these 2 species which require further study.

Comparative mortality after feeding on a filariasis carrier

The mortality rate of the mosquito vector after an infective blood meal is of interest in detecting whether ingestion of microfilariae and subsequent development of the parasites have an effect on mosquito survival. The comparable mortality rates of *A. gambiae* and *C. p. fatigans* were investigated in this study; data from 10 separate trials have been combined.

The mortality rates of 968 *A. gambiae* and 389 controls are plotted in FIG. 1. No detectable differences in mortality between infected and control mosquitoes were evident over the 14-day interval. The mortality rates of 849 infected *C. p. fatigans* and 353 controls are plotted in FIG. 2. The earliest appearance of 1st, 2nd and 3rd stage larvae is marked as a point of reference. Apparently, *W. bancrofti* did have an effect on the survival of this species, as is indicated by the slopes of the mortality curves. Infected *C. p. fatigans* showed a 2-fold higher death rate over the 14-day interval. Although the death rate in infected mosquitoes was double that of the controls, mortality did not occur

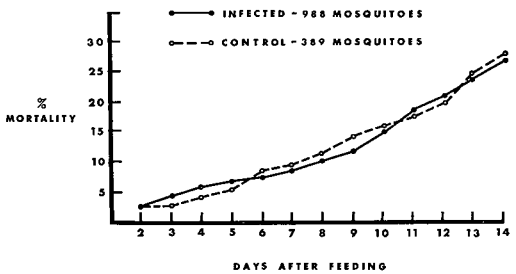

FIG. 1. Mortality in *Anopheles gambiae* A after feeding on a filariasis carrier.

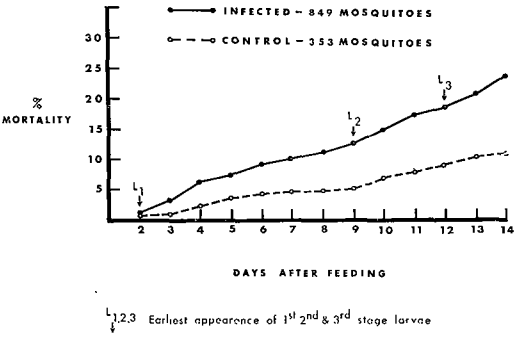

FIG. 2. Mortality in *Culex pipiens fatigans* after feeding on a filariasis carrier.

at specific intervals and cannot be directly attributed to specific stages of the parasite.

Although the data in FIG. 1 and 2 suggest that developing *W. bancrofti* might affect survival of *C. p. fatigans* but not of *A. gambiae*, results of ingestion experiments showed that a significant percentage of *A. gambiae* failed to ingest microfilariae and would have been identical to the control group. Dissection of representative portions of the dead mosquitoes revealed that very low numbers of *A. gambiae* contained developing filarial larvae and the majority of parasites did not pass beyond the microfilarial stage when they were ingested. No attempt was made to ascertain whether the buccopharyngeal armature damaged microfilariae, as suggested by Coluzzi & Trabucchi (1968). Since microfilariae did not develop in the majority of *A. gambiae*, infected and control groups would have been similar by the 2nd day and differences in mortality rates would not have been evident. Conversely, dissection of dead *C. p. fatigans* revealed numerous developing filarial larvae. Since parasites were numerous in *C. p. fatigans* and very rare in *A. gambiae*, comparison of mortality as a result of developing parasites is not possible between the 2 species.

Comparative success in developing 3rd stage filarial larvae

A. gambiae and *C. p. fatigans* were experimentally infected with *W. bancrofti* to determine comparative infectivity rates in the 2 mosquito species as well as the number and range of infective larvae capable of developing in each. These experiments were conducted using as donors 2 carriers with differing microfilaremias. TABLE 3 presents data obtained from 10 feeding trials; the mean microfilaremia of each carrier before and after mosquito feeding give an indication of relative microfilaria densities in the peripheral circulation at the time of mosquito exposure.

A. gambiae was a potentially poor vector of *W. bancrofti* in these studies. Only 8.9% of the 439 mosquitoes fed on carrier No. 1 developed infective stage filarial larvae. There was a mean number of 1.7 infective larvae in these positive mosquitoes with a range of 1–10. The *A. gambiae* fed on the carrier with the lower microfilaremia showed an even lower infectivity rate. Only 12 or 3.0% of the nearly 400 mosquitoes dissected contained 3rd

TABLE 3. Infection rates of *Anopheles gambiae* and *Culex p. fatigans* fed on 2 different filariasis carriers.

	Mean no. mf/100 mm³ blood			No. mosquitoes		% mosquitoes positive	Mean no. & range infective larvae per positive mosquito
	Before feeding	After feeding	Mosquito species	Dissected	Positive		
Carrier no. 1	92.9	117.6	*A. gambiae* A	439	39	8.9	1.7(1–10)
			C. p. fatigans	431	322	54.1	3.2(1–28)
Carrier no. 2	45.8	54.0	*A. gambiae* A	398	12	3.0	1.6(1–7)
			C. p. fatigans	277	89	32.1	2.6(1–11)

stage larvae. There was a mean of 1.6 larvae per positive mosquito in this sample and a range of 1–7.

By comparison, *C. p. fatigans* was considerably more efficient in laboratory studies. More than 50% of the 431 mosquitoes which fed on carrier No. 1 became infective. There was a mean of 3.2 infective larvae per positive mosquito in this sample and a range of 1–28. Of the *C. p. fatigans* which fed on carrier No. 2, 32.1% became infective with a mean of 2.6 infective larvae per positive mosquito and a range of 1–11.

The results of these experiments suggest that *C. p. fatigans* is more efficient as an intermediate host for *W. bancrofti* than is *A. gambiae* under the laboratory conditions provided. The low numbers of *A. gambiae* which became infective cannot be attributed to the results found with studies of microfilarial uptake. Although 20% of the *A. gambiae* which were experimentally exposed to infection in these tests most likely never ingested microfilariae, the infection rate would not be appreciably altered if this portion were removed from the sample. If 20% of the 439 *A. gambiae* fed on carrier No. 1 were discounted, the 39 positive mosquitoes would still comprise an infection rate of only 11%. With carrier No. 2 the infection rate would rise by less than 1%. *A. gambiae* did not successfully develop *W. bancrofti* in these experiments even though microfilariae were ingested.

The results of these experiments suggest that under laboratory conditions, *C. p. fatigans* is a far more efficient intermediate host of *W. bancrofti* than *A. gambiae*. The *C. p. fatigans* ingested greater numbers of microfilariae, showed a considerably higher infection rate and developed proportionally greater numbers of infective stage larvae. Although these results are very striking, it is quite possible that the differences are attributable to the laboratory strain of the mosquitoes rather than to natural susceptibilities of the species. *A. gambiae* has been shown to be a major vector of *W. bancrofti* in nature by many workers, and studies in rural areas of Tanzania confirm that most natural transmission is attributable to this species (Crans, unpubl. data). All laboratory colonies are highly inbred and natural vector susceptibility can be lost after several generations. Laboratory experiments are conducted in a highly artificial atmosphere and may introduce an important variable unparalleled in nature. *C. p. fatigans* appears to be more susceptible to infection with *W. bancrofti* in the laboratory but it would be unwise to suggest that the same is true under natural conditions until wild strains of each species are tested.

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