<table>
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<th>Health</th>
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<td>Variation in susceptibility of Aedes aegypti (L.) strains to Wuchereria bancroft (Cobbold)</td>
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**Abstract**

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VARIATION IN SUSCEPTIBILITY OF AEDES AEGYPTI (L.)

STRAINS TO WUCHERERIA BANCROFTI (COBBOLD)

by

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Consultant's Report to MBU/ICIPE,
Summer, 1972
INTRODUCTION

From a conversation with Professor Craig, I learned that Dr. P. Rodriguez of the Vector Biology Laboratory, University of Notre Dame, has recently demonstrated that Aedes aegypti formosus strains are more susceptible to infection with Brugia pahangi than the type form Aedes aegypti aegypti.

Thinking that the same phenomenon might hold for W. bancrofti, this short project was therefore undertaken to study the susceptibility of Aedes aegypti to W. bancrofti, emphasis being placed on comparisons of the type form strains with the feral strains.

MATERIALS AND METHODS

The strains of Aedes aegypti mosquitoes used are shown in Table I. They were all obtained from colonies maintained by the Mosquito Biology Unit of the International Centre of Insect Physiology and Ecology, at Mombasa, Kenya. The mosquitoes were reared in an insectary maintained at a tropical ambient temperature of about 25°C and about 70% relative humidity.

When approximately five days old, the adult female mosquitoes were deprived of sugar and water for a period of about 14 hours, then they were fed between 21 and 23 hours (local time) on the arm of a man naturally infected with periodic W. bancrofti. The enclosed photograph shows Mr. Jefa Wawara of Garashi Location, Malindi District who kindly supplied the microfilariae. Mr. Waware did not have a previously recorded history of treatment for filariasis. All mosquito feeding proceeded inside a laboratory room, just adjacent to the insectary.

Before the mosquito feeding began, a temporary wet blood film was made by pricking the tip of a finger and examining the blood for microfilariae. At the same time, thick blood films were made for future references. These thick films were later stained with Weigert's Hema-toxylin in the Department of Parasitology and Medical Entomology Laboratory, Faculty of Medicine, University of Dar-es-Salaam. (It was not necessary to stain the thick smears at Mombasa since in this part of the world live W. bancrofti microfilariae are easily recognized on site). No attempt was made at determining the microfilarial densities, this can be estimated from the accompanying stained blood films.

The mosquitoes were allowed to engorge on the donor's arm, which was introduced through the sleeve of a 'gallon' cage. In all the three feedings, the two strains being compared were engorged within thirty minutes, (two attempts at feeding Culex pipiens fatigans which we previously collected as immatures from tyres with residual rain water, completely failed after exposing an arm for at least 30 minutes on each occasion).
Because of unforeseen timing difficulties the infected mosquitoes were held on sugar and water for 18 to 22 days (instead of 9 to 11 days) inside the insectary, after which the surviving ones were dissected, the abdomens examined immediately and the thoraxes and heads fixed in 85% alcohol, then shipped to Dar-es-Salaam. In the Dar-es-Salaam laboratory, the fixed mosquitoes were brought down to water, then stained for 24 hours in Mather's Acid Hemalum, after which they were washed in tapwater to "blue" the stain, and then stored in glycerol for subsequent dissections (Shute and Maryon, 1966).

RESULTS

Three of the enclosed microscopic slides show *W. bancrofti* microfilariae on the three days of mosquito feeding. On these occasions, an average thick drop would contain more than 50 microfilariae.

Two of the enclosed microscopic slides show infective larvae of *W. bancrofti*.

Table II shows the susceptibility of four strains of *Aedes aegypti* to *W. bancrofti*. Two of the strains (Ganga and Tree-Hole) did not show any larvae. The Ganga strain did not show any larvae in two separate infection attempts. However, two other strains (Mkwaia and Mnazi) developed infective larvae. These infective larvae were found mainly in the head, although a few were still in the thoraxes. From abdomen examinations of all these mosquitoes, Dr. Hausermann recorded two infective larvae from the haemocoel of one Mnazi female.

Each infected mosquito had one to four infective larvae, the majority having two.

Neither first nor second -stage larvae were observed in any of the mosquitoes.

DISCUSSION

Two of the strains showed no filariae at all. One of these strains (Ganga) was *Aedes aegypti* var. *queenslandensis*, the other (Tree-Hole) was *Aedes aegypti* formosus. Therefore susceptibility like refractoriness is not restricted to one subspecies.

Nelson et al. (1962) site workers in West Africa, the Congo and in the U.S.A. who succeeded in infecting *Aedes aegypti* with periodic *W. bancrofti*. However, Nelson found no natural infections of *W. bancrofti* in *Aedes aegypti* in Kenya; he also failed to infect *Aedes aegypti* with *W. bancrofti* in the laboratory. Those apparently contradictory results are not surprising since different mosquito populations differ in their vectorial capacity (Ramachandran et al. 1960; Kilama, in press). The dichotomous results presented in this report therefore confirm the view that different mosquito populations differ in their vectorial capabilities. As Kilama and Craig (1969) said, "the occurrence of simple monofactorial
factors controlling vectorial capacity should give pause to those medical 
entomologists who, on the basis of a few transmission experiments with 
insects from a single laboratory colony, quickly decide that a given 
species is or is not a potential vector of a given pathogen. It is 
very likely that the observed differences in the susceptibility of Aedes 
aegypti strains to W. bancrofti are not due to environmental factors, 
but are genetic.

The numbers of infective larvae per mosquito was rather low (most 
positive mosquitoes showed only about two infective larvae). As Ramachandran et al. (1960) observed, "the optimum time for dissection was 
about the 9th - 11th days, for after that time there was a loss of larvae 
from mosquitoes the mortality of which also increased."

Moreover, Ramachandran (1966) also observed that "infected 
mosquitoes are maintained after the larvae in them reach maturity a 
loss is likely to occur by the escape of infective larvae from the 
proboscis of the mosquitoes during the act of feeding on substances 
like apple, milk, sugar, water and raisins." Unfortunately in my experi­
ments, the mosquitoes were held for 18 to 22 days. If Ramachandran's 
observations on Brugia malayi in Aedes aegypti also apply here, then 
larvae might have been lost and many mosquitoes may have died. The number of my recorded infective larvae is most probably short of the actual 
numbers, and also the percentage of infective mosquitoes may have decreased. Ramachandran (1966) could not find any infective larvae B. malayi, 18 
days after an infectious blood meal. Because of this time limitation, 
may be it is not proper to compare my results with those of previous 
workers.

MacDonald (1962a and 1962b) observed that 17.1 per cent of parental 
generation Aedes aegypti showed mature larvae of semi-periodic B. malayi. 
In the same experiments he found an average of four infective larvae per 
infected mosquito. My results are therefore in line with MacDonald's.

Jordan and Goatly (1962) studied the infectivity of W. bancrofti to 
Culex fatigans in coastal Tanzania. In their extensive experiments, the 
infection rate of mosquitoes 14½ days after an infectious blood meal 
ranged between 52 and 100 per cent. When their results are compared with 
mine, the obvious conclusion is the C. o fatigans is much more susceptible 
to W. bancrofti than any of the Aedes aegypti strains I tested. However 
the mean number of infective larvae per surviving C.o.fatigans compares 
favourably with that of my infected Aedes aegypti. However because 
of the greater proportion of infected C.o. fatigans and the overwhelming 
(still increasing) numbers of C.o. fatigans in urban East Africa, it is 
most probably that C.o. fatigans is a much more effective vector of urban 
W. bancrofti than is Aedes aegypti. In most rural East Africa, Anopheles 
gambine complex and An. funestus are the major vectors. Since those two 
Anopheles species are also more susceptible to experimental infections 
than I have just shown for Aedes aegypti, then we may regard those Ano­
phales sp. to be more efficient vectors than Aedes aegypti.

Since Aedes aegypti is a diurnal feeder it would not be an efficient 
vector of nocturnal W. bancrofti. However, W. bancrofti periodicity in
in some parts of East Africa is relative, and E. Nnochiri (personal communication) maintains that it is subperiodic in certain areas of Uganda. Under such circumstances diurnal *Aedes aegypti*, that is relatively susceptible, would enhance opportunity of *W. bancrofti* transmission.

Epidemiologic investigations on *W. bancrofti* in East Africa (e.g. Nelson et al. 1962, Menu and Kilama 1972) have consistently shown higher infection rates and higher microfilarial densities in men than in women. There is not yet a fully plausible explanations for this observation; among the suggestions made is that hormones may influence the level of filaremia. However, if feral mosquitoes are proved to be efficient vectors, then this might account for the differences in exposure to infection among men and women. While men work in the 'forest', women stay at home; thus in addition to home acquired nocturnal infections, men would also acquire infections from diurnal biting exophagic *Aedes aegypti*.

ACKNOWLEDGEMENTS

I am very thankful to Dr. and Mrs. Walter Hausermann for their help throughout this project, Miss Rosabela for mosquito rearing and maintenance, Mr. Nyiwade of the Division of Insect-Borne Diseases for his administrative assistance and Mr. Joel Save of Malindi District Hospital for locating an infected individual. Last but not least, I am most thankful to Mr. Jefa Wawara, without his participation this project would not have been possible.

SUMMARY

The susceptibility of four strains of *Aedes aegypti* to *W. bancrofti* was studied.

Preliminary results indicate that susceptibility /refractoriness is/are not restricted to any one subspecies of *Aedes aegypti*.
REFERENCES


### Table I. The Strains of A. Aegypti Used for Infection with W. Bancrofti

<table>
<thead>
<tr>
<th>Name</th>
<th>Subspecies, Variety, or Type</th>
<th>Strain History and Collection Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNAZI</td>
<td>FORMOSUS</td>
<td>Eggs collected with dirt samples from steps in coconut palms (Mnazi) in Rabai Location, August - November, 1971.</td>
</tr>
<tr>
<td>TREE-HOLE</td>
<td>FORMOSUS</td>
<td>Eggs collected with dirt samples from tree hole in mango tree at Mazeras, Rabai Location (3° 56' S, 39° 34'E), August 1971.</td>
</tr>
<tr>
<td>GANGA</td>
<td>QUEENSLANDENSIS</td>
<td>Adults caught in an indoor landing biting catch in various houses at Ganga, Rabai Location (3° 57' 10&quot; S, 39° 33' 40&quot; E), October 1971.</td>
</tr>
<tr>
<td>MKWAJA</td>
<td>TYPE FORM</td>
<td>Larvae collected from rain water drums at Mkwaia Village (Tanzania Coast) in backyards of several houses (5° 47' 30&quot; S, 38° 51' 05&quot; E) April 1971.</td>
</tr>
</tbody>
</table>
### Table 2. The Susceptibility of Four Strains of *Ae. Aegypti* to *W. Bancrofti*

<table>
<thead>
<tr>
<th>Time (Hrs) of Expt.</th>
<th>Days Before Dissection</th>
<th>Mosquito Strain</th>
<th>No. ♀♀ Dissected</th>
<th>No. ♀♀ with Infective Larvae</th>
<th>No. ♀♀ Uninfected</th>
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</thead>
<tbody>
<tr>
<td>21:30 - 21:40</td>
<td>22</td>
<td>Ganga</td>
<td>92</td>
<td>0</td>
<td>92</td>
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<tr>
<td>21:20 - 21:30</td>
<td>18</td>
<td>Tree Hole*</td>
<td>85</td>
<td>0</td>
<td>85</td>
</tr>
<tr>
<td>21:30 - 21:40</td>
<td>22</td>
<td>Mkwaja*</td>
<td>117</td>
<td>15</td>
<td>102</td>
</tr>
<tr>
<td>22:00 - 21:10</td>
<td>19</td>
<td>Mnazi**</td>
<td>41</td>
<td>4</td>
<td>37</td>
</tr>
<tr>
<td>22:10 - 22:15</td>
<td>19</td>
<td>Ganga**</td>
<td>127</td>
<td>0</td>
<td>127</td>
</tr>
</tbody>
</table>

* FED JUNE 22RD, 1972
** FED JUNE 23RD, 1972