Preliminary Report on Human Filariasis in Mozambique, East Africa

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Abstract: Blood examinations for microfilariae of bancroftian filaria and a mosquito survey were made during the evening in April, 1981 and 1982, in a village of Quelimane, Mozambique, East Africa. The microfilariae were found in an African (male). The worms appeared to be *Wuchereria bancrofti*. In the area, *Anopheles coustani*, *Culex pipiens quinquefasciatus*, and *Mansonia uniformis* were usually collected by human baited traps. The mosquitoes were dissected to find the filarial larvae. In *Mansonia uniformis* the larvae of the lst and 3rd stages were found, and while in *Culex pipiens quinquefasciatus*, those were only in the lst stage. The species of these filariae is unknown. In this village, this disease is supposed to be mainly transmitted by the mosquito of *Culex pipiens quinquefasciatus*.

Key words: Filariasis, Vector, Mosquito, East Africa

INTRODUCTION

Bancroftian filariasis is known to be indigenous to Mozambique, East Africa (Fraga de Azevedo, 1964; Sasa, 1976). However, there are scarcely found studies on the vector biology in relation to the transmission dynamics of this disease. In 1981 and 1982, we conducted a mosquito survey and blood examinations for bancroftian filariasis in Quelimane, Mozambique. The results are reported here.

PLACES AND METHODS

The Mosquito survey and blood examinations for microfilariae from Africans were made on April 12-16, 1981 and April 15-20, 1982 in a small village of Quelimane, Mozambique of East Africa (Fig.1). The village is situated in a coastal area and consists of about 30 small dwellings made of grass and mud.

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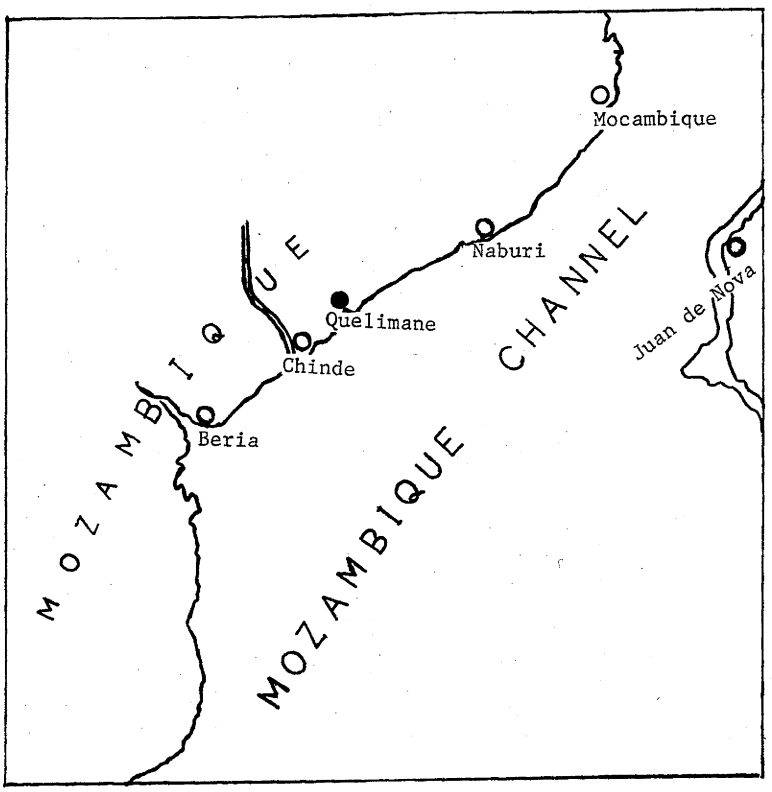

Fig. 1. Map of Mozambique, East Africa. The mosquito survey was conducted in Quelimane.

The population consisted of approximately 300 Africans (aborigines), and villagers showing such typical symptoms as elephantitis, and chylocele of bancroftian filariasis were usually found. Around the houses there were many foul ground pools, which appeared to be the main breeding places of the *Culex pipiens quinquefasciatus* mosquito. Inside and outside the village were the ponds and swamps; ground water was thought to be important breeding places of *Anopheline* and *Mansonia mosquitoes*.

During the observation periods, the temperature (28°C) and humidity (about 75% RH) were high.

Adult mosquitoes were collected, using aspirators, inside the dwellings for 30 minutes from 18:20 in 1981. In 1982, the collections were made by using human baited traps from 17:15 to 22:45. Some of the mosquitoes collected were kept as dry specimens and the others were preserved into 70% alcohol. The dry specimens were identified by using a binocular microscope. The mosquitoes preserved in 70 % alcohol were first divided into 3 groups of the genera *Anopheles*, *Culex* and *Mansonia*, and then dissected and stained with dilute Giemuza's solution to detect the bancroftian filarial larvae.

Blood examination for microfilariae was made by placing a thick film of blood (about

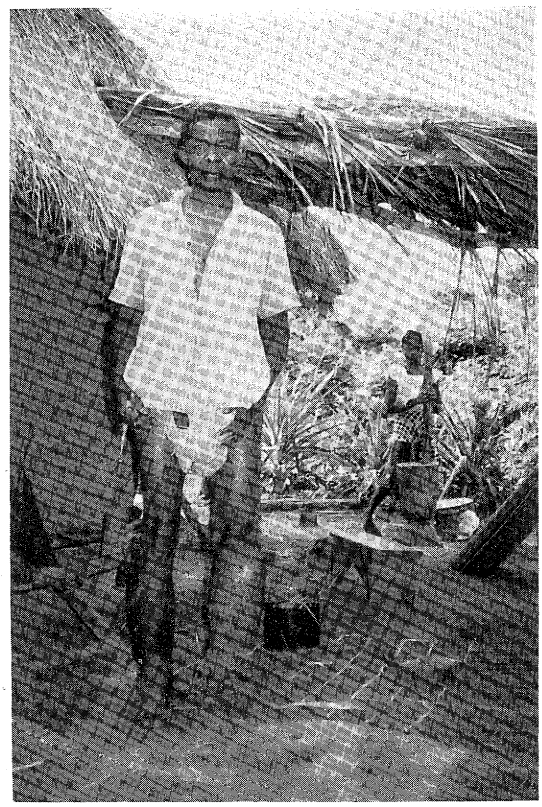

Fig. 2. An African with symptoms of the bancroftian filariasis (Chylocele) and his house in Quelimane.

60 mm^3) obtained from a finger of Africans on a slide. The blood was collected at 21:00 and dried outdoors for one day. The smear was fixed with 70% alcohol and stained as above.

Also, in the laboratory of our department, blood examinations for *Brugia pahangi* microfilariae were made by placing a thick film of blood (about 60 mm^3) obtained from eye vein of Jirds on a slide. The blood smears were kept about one day at 25°C, in a wet-chamber consisting of a Petri-dish (diameter 12 cm and depth 3 cm) that contained cotton moistened with a little water. After fixing and staining as above, the bodylength of the microfilariae was measured under the microscope.

RESULTS

1. *Blood examinations for microfilariae*

Microfilariae were found in one male among the 8 Africans examined. There were 55 in a blood smear; 35 were large from 130 to 160μ and had a sheath (attached

empty sheafh) and 20 were small from 80 to 90μ and without a sheath (Figs. 3 A and B, and Table 1). The variatoin in microfilarial size and existence of a sheath are assumd to have been caused by blood smears in being kept outdoors at a high temperature and humidity for about one day and then fixed with 70 % alcohol.

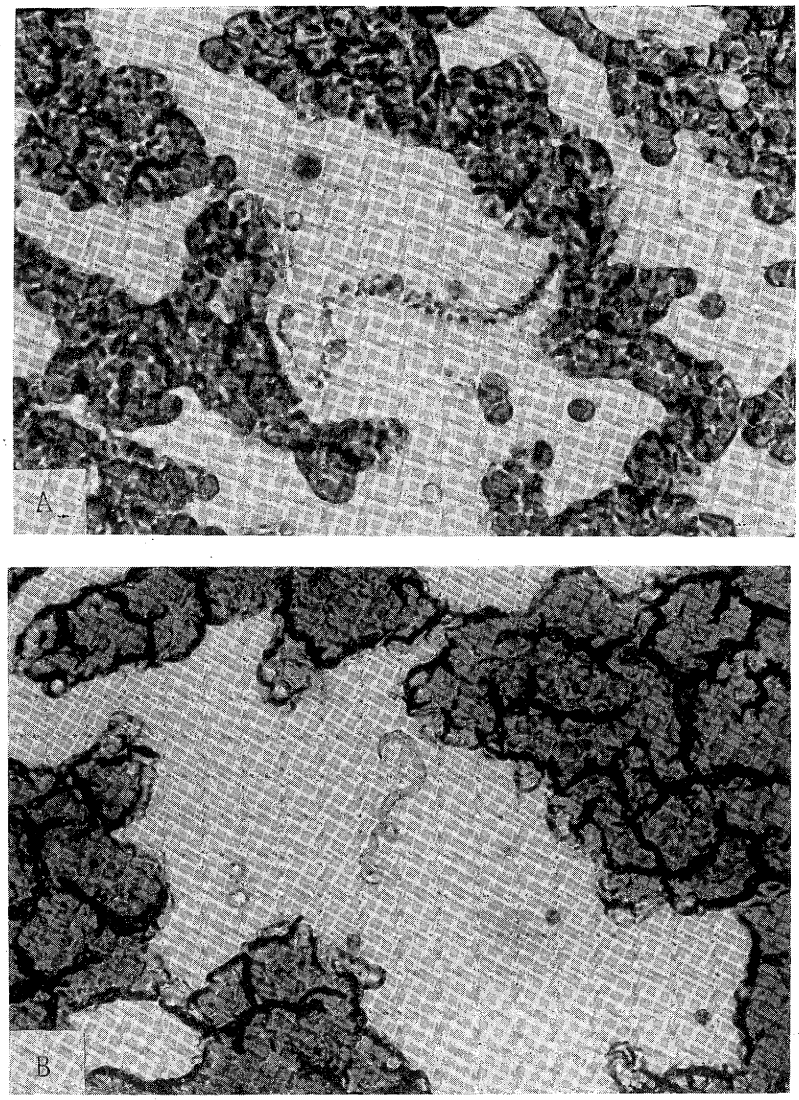

Fig. 3. Microfilariae* taken from peripheral blood of an African in Quelimane at the night.
A: Long microfilariae with sheath (attached empty sheath)
B: Small microfilariae without sheath.
* Microfiariae were kept outdoors for about 24 hours after collection, and then fixed with 70% alcohol and stained with dilute Giemusa's solution.

Table 1. Length of microfilariae* in blood smear collected from the aborigine

Type* of Mf	No, Mf** measured	Length*** of Mf									
		8–	9–	10–	11–	12–	13–	14–	15–	16–	17–
Large-sized Mf	15						1	4	7	3	
Small-sized Mf	10	7	3								

* see Fig. 1.
** Mf : Microfliariae
*** One unit = 10μ

2. *Morphological changes in Brugia pahangi microfilariae in a wet chamber*

To confirm the above assumption, a blood smear (60mm^3) with 225 microfilariae was kept in a wet chamber at a high temperature (27°C) and high humidity for 24 hours. After fixing and staining as above, the body size and existence of a sheath which covers comletely a worm body were examined. The results are given in Table 2.

All of the microfilariae in the dried blood smear (control) had a sheath and they were large (from 220 to 260μ). However, their sizes varied markedly when they were kept in the wet chamber and fixed with 70% alcohol. Most of them were short (140 to 160 μ) and without a sheath. Most of microfilariae with a sheath were also short, just like the exsheathed ones, but some of them were as large as those in dried blood smear (control). These results clearly show that microfilariae become short and exsheath at a low rate when kept about one day and fixed with the alcohol. Thus, the variation in morphology of microfilariae from an African was considered to have been due to the preservation method of blood film.

Table 2. Length of *Brugia pahangi* microfilariae (from Jird) which were kept for about 24 hours in wet chamber at 25°C and then fixed with 70% alcohol

Treatment	Existence of Sheath in Mf	No. Mf* measured	Length** of Mf												
			13–	14–	15–	16–	17–	18–	19–	20–	21–	22–	23–	24–	25–	26–
Control***	With Sheath	15										4	3	3	3	2
Wet chamber	With Sheath	14	1		3	4	2	1						2		1
	Without Sheath	15		7	1	6		1								

* Mf: Microfilariae
** One unit = 10μ
*** Fresh blood smear inclusive Mf were dried one hour at 25°C and thereafter stained with dilute Giemusa's solution.

3. *Relative abundance of female mosquitoes collected*

The total number of female mosquitoes collected are given by species in Table 3, which shows the mosquitoes belonging to 3 genera. The most abundant mosquito was *Mansonia uniformis*. The other species were *Culex pipiens quiquefasciatus*, *Culex* spp., and *Anopheles* spp., and they were about same in number.

Table 3. Species and number of female mosquitoes* collected in a house in Mozambique, in April, 1981

Mosquito species	No. females
Anopheles spp.	11
Culex pipiens quinquefasciatus	9
Culex spp.	8
Mansonia uniformis	70

* Dry specimens

4. *Hourly prevalence in nocturnal feeding activity of mosquitoes*

The results of hourly catches from 17: 15 to 22: 45 are tabulated in Table 4. This table shows that *Anopheles coustani*, *Culex pipiens quinquefasciatus*, and *Mansonia uniformis* were the most common mosquitoes that bit humans in this village. Their nocturnal feeding activity begins from 17: 15. *Anopheles coustani* showed peak activity from 19: 15 to 21: 15, *Culex pipiens quinquefasciatus* was very active from 18: 15 to 19: 15.

Table 4. Hourly prevalence in nocturnal feeding activity of mosquitoes in Mozambique, in April, 1982

Mosquito species	Time										
	17:15 | 17:45	17:45 | 18:15	18:15 | 18:45	18:45 | 19:15	19:15 | 19:45	19:45 | 20:15	20:15 | 20:45	20:45 | 21:15	21:15 | 21:45	21:45 | 22:15	22:15 | 22:45
Aedes spp.	4	8	0	0	0	0	2	0	0	2	0
Anopheles coustani	6	0	0	0	54	52	38	12	14	11	8
Anophles spp.	0	0	0	3	2	10	18	2	0	0	1
Culex pipiens quinquefasciatus	4	0	33	20	8	10	8	15	10	8	16
Culex spp.	8	6	57	40	14	16	10	9	10	8	8
Mansonia africanus	0	0	0	0	5	0	0	0	0	0	0
Mansonia uniformis	18	48	189	120	113	105	61	24	28	22	33
Mansonia spp.	0	0	111	80	4	7	0	20	4	3	0

* Sunset: 18:30

The peak feeding activity of *Mansonia uniformis* also appeared from 18: 15 to 20: 15. In this table, *Aedes* spp., *Culex* spp., and *Anopheles* spp., refer to the mosquitoes that could not be identified because of the lack of morphological features. As for *Culex* spp, and *Mansonia* spp., many of the females are speculated to be *Culex pipiens quinquefasciatus* and *Mansonia uniformis*, based on the results obtained in 1981 (Table 3).

5. *Natural infection of the mosquitoes with filarial larvae.*

Table 5 gives the results of dissection for filarial larvae in the mosquitoes. The larvae were found in both species of *Culex pipiens quinquefasciatus* and *Mansonia uniformis* in low rates. *Mansonia uniformis* had the lst and 3rd stage larvae and *Culex* only the lst stage larvae.

Table 5. No. and percentages of mosquitoes with filarial larvae

Mosquito species	No. dissected (A)	No. and % of mosquitoes with larvae in following stage				
		I	II	III	Total (B)	% (B/A ×100)
Anopheles coustani	35				0	0.0
Culex pipiens quinquefasciatus	46	1			1	2.2
Mansonia uniformis	121	1		4	5	4.1

6. *Morphology of filarial larvae*

Table 6 shows the length of larvae found from *Mansonia uniformis* and *Culex pipiens quinquefasciatus* which are shown in Table 5. The lst stage larvae were short (137μ) while those of *Mansonia uniformis* a little large (248μ). However, the species is not clear. Four larvae in the 3rd stage were defined only by length. There larvae could not be identified or measured, because their bodies were completely coiled and the caudal extremities of the larvae were missing. Only in one larvae could this part be examined (Fig. 4)

As the shape of the caudal extremities in this larvae is quite different from those of the larvae of *Wuchereria bancrofti* and *Brugia malayi*, this larva might be a kind of the filariae living in wild animals.

Table 6. Larval length of filariae found in the mosquitoes shown in Table 5

Mosquito species	Existence of blood meal in midgut	Part of mosquito body	Stage of larvae	No. larvae measured	Larval length (μ)
Culex pipiens quinquefasciatus	Fed	Thorax	I	1	137
Mansonia uniformis	Fed	Thorax	I	3	248 (220-280)
Mansonia uniformis	Fed	Abdomen	III	1	932

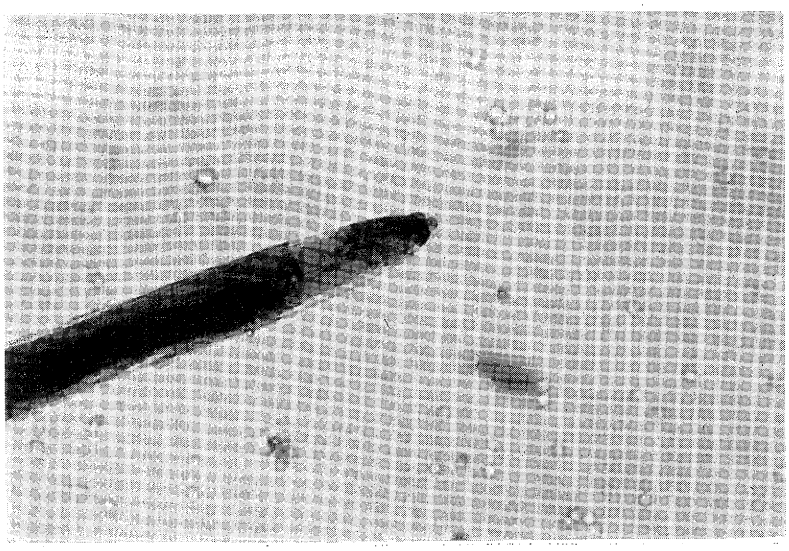

Fig. 4. Caudal extremities of the 3rd stage of larvae indicated in Tables 5 and 6.

DISCUSSION

Fülleborn (1913) pointed out that microfilariae of *Dirofilaria immitis* become shorter and thicker when thick blood smears are more slowly dessicated, or when fixed in hot alcohol. Feng(1933) also stated that if thick smears are slowly dried, or are fixed in hot alcohol, microfilariae of *Brugia malayi* and *Wuchereria bancrofti* shrink. Our findings on *Brugia pahangi* agree well with those observations. Thus, the variation in size of the microfilariae taken from an African is considered to have occurred because they were slowly dried and fixed in 70% alcohol.

The microfilariae of *Wuchereria bancrofti* and *Brugia malayi* are usually exsheathed on thick blood smears, when preserved for about one day at 25°C, but in *Brugia pahangi* the rate of such microfilariae was very low, even when they were kept one day (Aoki, 1968). It is noteworthy that about half of the microfilariae lost their sheaths on thick blood film collected in the present survey.

Blood examinations were made at the night with an African. From the facts mentioned above, microfilariae that were found were considered to be those of *Wuchereria bancrofti*.

In East Africa, *Anopheles coustani* is not thought to be an important vector of *Wuchereria bancrofti*. Infective larvae were not found in *Mansonia uniformis*, even when about 3000 wild females were dissected (Nelson, *et al.*, 1962; Gillett, 1972). Thus, the two species of the mosquitoes mentioned above are not considered td be important vectors of the bancroftian filariasis in Quelimnae, though many females of both species bite

humans. In East Africa, the bancrofitian filariasis is mainly transmitted by the mosquitoes of *Anopheles gamiae*, *Anopheles funestus*, and *Culex pipiens quinquefasciatus* (Nelson, *et al.*, 1962; White, 1971; Wijers and Kiilu, 1977). In the village we surveyed, this disease might be mainly transmitted by *Culex pipiens quinquefasciatus*.

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東アフリカ・モザンビークのフィラリア症について（予報）

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東アフリカ，モザンビークのケリマンの1部落で1981と1982年の4月にバンクロフトフィラリアのミクロフィラリアを検出するため，8人の現地人について夜間採血を実施した．さらに，フィラリア伝搬蚊の調査も行なった．1人の男子現地人からバンクロフトフィラリアと思われるミクロフィラリアがみつかった．採集された蚊の多くは *Anopheles coustani*, *Culex pipiens quinquefasciatus*（ネッタイイエカ）と *Mansonia uniformis*（アシマダラヌマカ）であった．これらの蚊のうちでアシマダラヌマカが最も多かった．これらの蚊の野外での吸血活動の時間的消長をしらべた．*Anopheles coustani* は日没（18:30)後45分から3時間15分の間，すなわち，19時分にかけて最も活発に吸血に来た．ネッタイイエカは18時15分から19時15分頃まで非常に活発に15分から21時45吸血に来た．また，アシマダラヌマカの吸血活動のピークは18時15分から20時15分の間にみられた．ネッタイイエカからⅠ期のフィラリア幼虫が，アシマダラヌマカからはⅠ期とⅢ期幼虫が少数ではあるがみっかった．これらの幼虫の種類はわからなかった．しかし，アシマダラヌマカからとり出された1個体のⅢ期幼虫はその尾端の形態からみて動物に寄生するフィラリアではないかと思われる．この部落でのバンクロフトフィラリアは主としてネッタイイエカによって伝搬されていると想像される．

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