THE ROLE OF *CULEX FATIGANS* IN THE TRANSMISSION OF *WUCHERERIA BANCROFTI* IN NEW GUINEA

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INTRODUCTION

As was pointed out by BUXTON[1] *C. fatigans* is not the original vector of the endemic filariasis in Melanesia, since the species has only recently been introduced into the area. Because of lack of suitable breeding places in rural areas the species is even to day still largely absent from the filariasis endemic areas of New Guinea.

In Malaya WHARTON[2] described a rural focus of *W. bancrofti* — in which *A. letifer* is the vector —, where the parasite showed a poor development in *C. fatigans*, which proved at the same time a good host for an urban strain of the parasite from Singapore. In this connection WILSON[3] considers the possibility that *W. bancrofti* originated as a rural infection and only later became adapted to urban conditions and vectors as *C. fatigans*. It is quite likely that the same situation occurred in early days in several parts of the world, for which urbanization has been a more recent experience than endemic filariasis.

It appeared to be of special interest to investigate the possible role of *C. fatigans* in the transmission of filariasis in New Guinea at this very moment. Although in typical rural areas the importance of this mosquito, restricted as it is by the scarcity of breeding facilities, will remain low, the increasing rapid urbanization of originally rural people will bring together increasing numbers of parasite carriers with increasing numbers of *C. fatigans*. The question arises whether this species will be able to assist or to take over the transmission of filariasis in the new urban and semi-urban settlements, where the parasite reservoir will remain essentially unreduced for several years to come. It is to be noted that the Expert Committee on Filariasis[4] recently considered the application of measures to prevent the spread of *C. fatigans* in expanding towns.

In the present paper the available material concerning the possible role of *C. fatigans* in the transmission of filariasis in New Guinea is reviewed.

The question remains whether it would be possible that through a changing host-parasite relationship an originally relatively refractory species might under natural conditions become a susceptible species in the rather short time available, i.e. before the extinction of the parasite reservoir. Since the indoorspraying operations against malaria will substantially affect the original anopheline transmission of filariasis, while the extension of curative facilities in urban and sub-urban areas will greatly reduce the parasite reservoir, the time available for this adaptation will even be shortened.

The following data are available:

1. BRUG AND DE ROOK[5] found in Tanah-Merah, Upper Digul River, among 13 specimens dissected 14 to 15 days after the infective bloodmeal only two infected mosquitoes. No details are given on the microfilaria carrier or on the larval stages.

2. HOPLA[6] found an infection rate of 12 per cent among 50 specimens dissected in the Sagaray Valley, Milne Bay area.

3. BACKHOUSE AND HEYDON[7] reported on the outcome of experiments in Rabaul: Among 86 specimens — nineteen dissected before, and 67 later than 12 days after the infective bloodmeal — Backhouse found an infection rate of 19 per cent, using a donor with 45 to 238 microfilariae per 20 cmm blood. Of the 16 positive specimens eleven showed only immature larvae, in five mosquitoes mature larvae were found. Heydon found among 29 specimens four mosquitoes with mature larvae — dissection after 12 days — without signs of retarded development, being an infection and infective rate of 14 per cent. The donor used had 79 to 136 microfilariae per 20 cmm fingerblood.

4. On the island of Pam, Radja Ampat, West New Guinea, DE ROOK[8] obtained infection and normal development of larvae in the four specimens fed on a microfilaria carrier. In the mosquito dissected 12 days after the infective bloodmeal mature larvae were found.

5. Using a donor who showed on the average 8.7 microfilariae per cmm fingerblood at the time of mosquito biting, DE ROOK[9] obtained in Inanwatan, West New Guinea, an infection rate of 79 per cent, and with dissection later than ten days after the infective bloodmeal an infective rate of 32 per cent.

6. Using a donor with 105 to 138 microfilariae per 20 cmm blood, MACMILLAN[10] obtained an infection rate of 26 per cent in Maprik, Sepik district, and noted retarded development: mature larvae were only seen 17½ days after the infective bloodmeal.

7. Experiments in Hollandia (Kota Baru), 1960.

Wild mosquitoes, as they came to feed on human bait, have been caught in Hollandia (Kota Baru) using microfilaria carriers from the Mandobo area, Upper Digul, as bait. The mosquito showed itself in the early night fairly reluctant to feed and easily disturbed.

(a) Microfilaria carrier with 30 to 58 microfilariae per 20 cmm fingerblood at the time of mosquito biting, i.e. on the average 2.2 microfilariae per cmm. Twenty-one

TABLE I

RESULTS OF DISSECTIONS OF *C. FATIGANS* FED ON FILARIAL BLOOD (DENSITY 2.2 MICROFILARIAE PER CMM) AND DISSECTED AFTER 12½ DAYS

Serial no.	*Total No. of larvae*	*Sausage stage*	*Second stage*	*Mature larvae in*		
				abdomen	*thorax*	*head*
1–14	0	—	—	—	—	—
15	1	—	1	—	—	—
16–18	1	—	—	—	—	1
19	10	—	3	—	4	3
20	22	—	5	7	3	7
21	22	—	3	8	1	10
Total	58	—	12	15	8	23

afterwards revealed an infection rate of 33 per cent and an infective rate of 29 per cent, while on the average 2.8 larvae per mosquito were found. The details are given in *Table I*. Only one mosquito did not survive for 12½ days: this specimen dissected after 5½ days proved to be negative for filarial larvae.

(b) Microfilaria carrier with 81 to 438 microfilariae per 40 cmm fingerblood at the time of mosquito biting i.e. on the average 6.5 microfilariae per cmm. Nineteen specimens were caught on this donor and kept alive for 12½ days. All specimens survived. Dissection afterwards revealed an infection rate of 63 per cent and an infective rate of 53 per cent while on the average 2.1 larvae per mosquito were found. See *Table II*. It is to be noted that these experiments were carried out simultaneously and under exactly the same conditions.

TABLE II

RESULTS OF DISSECTIONS OF *C. FATIGANS* FED ON FILARIAL BLOOD (DENSITY 6.5 MICROFILARIAE PER 20 CMM) AND DISSECTED AFTER 12½ DAYS

Serial no.	*Total No. of larvae*	*Sausage stage*	*Second stage*	*Mature larvae in*		
				abdomen	*thorax*	*head*
1–7	0	—	—	—	—	—
8	1	1	—	—	—	—
9–11	1	—	—	—	—	1
12–13	2	—	1	—	—	1
14	2	—	—	—	—	2
15	3	2	1	—	—	—
16	3	2	—	—	1	—
17	3	1	—	—	—	2
18	9	—	1	1	3	4
19	11	2	4	4	—	1
Total	39	8	8	5	4	14

THE EXPERIMENTAL INFECTION OF *C. FATIGANS* FROM SORONG, WEST NEW GUINEA, WITH *W. BANCROFTI* FROM THE WEST-INDIES

In Amsterdam, experiments have been undertaken by WILLEMSE[11] who used a microfilaria carrier from the West-Indies and a *C. fatigans* colony originating in Sorong, West New Guinea. With this donor who showed a microfilaria density of only eight microfilariae per 20 cmm blood, he obtained an infection rate of 34 per cent. Retardation in the development of the larvae was common. The temperature at which these experiments were carried out was constant but rather low.

THE EXPERIMENTAL INFECTION OF *C. FATIGANS* WITH *W. BANCROFTI* IN NEIGHBOURING AREAS OF NEW GUINEA

Micronesia: Of 55 mosquitoes examined by PIPKIN[12] in the Carolines 16 days after the infective bloodmeal only four specimens (seven per cent) proved to be infected of which only two with infective larvae.

Philippines: Among 124 and 179 mosquitoes examined ROZEBOOM AND CABRERA[13] obtained an infection rate of 30 per cent in the rural Sorsogon, and of 34 per cent in urban Manila.

Experiments by KARTMAN[33] point to the role played by the rapidity with which the blood clot is formed in the mosquito's gut in the prevention of migration of microfilariae from the gut. From the data supplied by ROSEN[29] from Tahiti, it may be suggested that this preventing mechanism is of such a perfection that higher microfilaria densities are unable to influence the infection rate or the average number of developing larvae per mosquito.

Reviewing the data from New Guinea and leaving out those data which provide insufficiently detailed information, one arrives at a set of rather erratic results. From these, the only line that can be detected is the political division between the western and eastern part of the island of New Guinea, the infection rates in the former part being definitely higher than those in the latter part. See *Table III*.

TABLE III

THE RELATION BETWEEN MICROFILARIA DENSITIES AND INFECTION RATES IN *C. FATIGANS* IN NEW GUINEA

Microfil. density per cmm.	*Infection rate*	*Station*	*Author*
5.4	14%	Rabaul B	G. A. M. HEYDON
7.1	19%	Rabaul A	T. C. BACKHOUSE
6.1	26%	Maprik	B. MACMILLAN
2.2	33%	Holl./Kota Baru	the author
6.5	63%	Holl./Kota Baru	the author
8.7	79%	Inanwatan	H. DE ROOK

It appears that the data from the western part of New Guinea are at least equal to those collected in neighbouring Indonesia and the Philippines, whereas those from the eastern part of the island come closer to the findings in Micronesia. It remains speculation whether this phenomenon has any relation with the fact that *C. fatigans* in the western part originates in Indonesia, and in the eastern part possibly in Micronesia.

There is some evidence for the assumption that the infection rates are related to the microfilaria density of the donor's blood in West New Guinea. Such evidence is lacking in the data from the eastern part of the island.

The experiments by WILLEMSE would have been of still more value had a better donor been available, under more comparable conditions of temperature. Any retardation of the development of the larvae can be easily attributed now to the low temperature during the experiments while the use of a donor with a microfilaria density of only 0.4 per cmm, and the obtained infection rate of 34 per cent can be explained in different ways. Personally, I feel this rate high considering the really poor donor, much higher than would have been expected on the basis of the findings in Hollandia (Kota Baru). This would mean that *C. fatigans* from Sorong is more susceptible to the West-Indian *W. bancrofti* than the Hollandia strain to the Upper-Digul *W. bancrofti*.

Comparing the infection rates obtained in *C. fatigans* in West New Guinea with those in *A. koliensis*[31], one notices that especially with a donor with a relatively low microfilaria density, the infection in *C. fatigans* is poor. The species might be there-

In the earlier investigations, *C. fatigans* proved to be naturally infected with bancroftian larvae more or less according to expectations. In the later investigations, however, especially in Sabron Samon and Hollandia (Kota Baru) larvae of an advanced stage of development were encountered relatively rarily in wild caught mosquitoes, in spite of the fair infection and infective rates obtained in experiments.

For this reason it was decided to apply the method of DETINOVA on the *C. fatigans* population in Hollandia (Kota Baru). The results are shown in *Table IV*.

These results indicate it is with due reservation that *C. fatigans*, at least in Hollandia (Kota Baru) has a very low natural survival rate. It is felt that more evidence about the applicability of the DETINOVA method to *C. fatigans* as well as about the situation in other centres and sub-urbs are needed for definite conclusions.

SUMMARY

The hospitability of *C. fatigans* to *W. bancrofti* in New Guinea was reviewed. Evidence was gathered for the assumption that *C. fatigans* in the eastern part of the island is less susceptible to *W. bancrofti* than in the western part. Although of some importance as secondary vector, it is doubtful whether the species alone will be able to maintain an endemic filariasis even in the western part of New Guinea. Its possible role in the transmission of filariasis appeared to be further reduced by a very low natural survival rate.

RESUMEN

El papel de Culex fatigans en la transmisión de Wuchereria bancrofti en la Nueva Guinea. Se trató de probar la suposición que *Culex fatigans* es más susceptivo para *W. bancrofti* en la parte occidental que en la parte oriental de la Nueva Guinea. Es dudoso, aunque de algún importancia como vector secundario, que la especie sola sea capaz de mantener filariasis endémico, hasta en la parte occidental.

Parece que el posible papel en la transmision de filariasis se ve reducido por notas muy bajas de supervivencia natural.

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THE FLUORESCENT ANTIBODY TEST FOR SCHISTOSOMIASIS IN EXPERIMENTALLY INFECTED MICE

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The fluorescent antibody test (F.A.) for the diagnosis of schistosomiasis was described by SADUN *et al.*[1] in 1960. The test proved to be reliable and permitted the use of very small amounts of serum. Moreover, the utilization of extracts from a small number of drops of blood collected and dried on filterpaper gave rather good results, as was proved by SADUN *et al.*[2] in 1961. However, further evaluation of the practical merits of the F.A. in schistosomiasis, and especially the filter paper method, is of great importance. In the present experiment we tried to ascertain (a) the earliest date of a positive F.A. test; (b) the titers in the different stages of the infection, and (c) the specificity.

MATERIALS AND METHODS

To obtain optimal results we used freshly emerged cercariae for each reaction. The cercariae were killed by 20 per cent formalin which was equal to the volume of the cercarial suspension. The serum specimens of the mice were obtained by heart puncture. The sera were used either immediately after punction or stored at −20° C. The sera were diluted with P.B.S. (pH 7,2) so as to give final concentrations of 1:2, 1:4, 1:8, 1:16, etc. up to the highest dilution which gave a positive reaction viz. 1:8192. The small amounts of mice sera were inactivated at 56° C for 30 minutes.

The carcariae are incubated in 0,15 ml of serum for 30 minutes at room temperature. After washing twice with P.B.S. (pH 7,2), 0,1 ml of fluorescein conjugated anti-mouse globuline* is added and the cercariae are incubated for 15 minutes at room temperature. Thereafter the carcariae are washed again twice, mounted in buffered glycerol, and the reaction is read. We arbitrarely designated the intensity of the fluorescence as −, ±, +, ++ and +++. As the specimen's antibody titre we regarded the highest dilution at which an intensity of + was obtained. The mice were intraperitoneally infected with between 110 and 160 cercariae each.

RESULTS

I. *The intensity of the F.A. test in the different stages of the infection*

The earliest positive tests were obtained with undiluted sera three weeks after the infection. The fluorescence however, is then weak and according to our designation, the intensity lies between ± and +. Up to the thirtieth day of infection

* Progressive Laboratories Inc.