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# The maximum density of anopheline mosquitoes that can be permitted in the absence of continuing transmission of filariasis

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## Summary

Using two years of mosquito collections from two fixed stations on Guadalcanal it was possible to compare the average man-biting rate with the prevalence of microfilaraemia. One area was found to be free of infection while the other still had one positive case. The DDT residual spraying had caused a change in biting habits of *A. farauti* No. 1 allowing a greater density of mosquitoes to bite man at times when they had lower microfilaria densities. This difference was compared with the pre-spray density so that the maximum permissible number of mosquitoes biting man without transmission of filariasis taking place, could be calculated. A theoretical value for *m* of 19·84 had been worked out previously; this study gave a corrected observed value of *m* of approximately 20, very close to the calculated theoretical value. The situation studied here probably showed incomplete interruption of transmission, so it was considered preferable to recommend a figure of 0·66 average vector man-biting rate per hour indoors for the critical value.

## Introduction

In Solomon Islands, the prevalence and intensity of infection with the local nocturnally periodic strain of *Wuchereria bancrofti* have declined steadily during the course of a Malaria Eradication Programme (MEP) (WEBBER, 1975a). In this island group, both malaria and filariasis are transmitted by the same mosquito vectors, members of the *Anopheles punctulatus* complex, mainly *A. farauti* No. 1. (BRYAN, 1973a, b and c, 1974).

In following up the original field studies, it was possible to work out the rate of decline of the infection, as well as noting the unexpected finding that the degree of reduction of the vector required to cause a decline did not need to be so great for filariasis as for malaria (WEBBER, 1975b, 1977). By good fortune, the MEP had fixed stations in an area known previously to be highly endemic for filariasis on Guadalcanal, and it was hoped that the density of vector mosquitoes that could be permitted without continuing transmission of filariasis could be determined quantitatively.

## Materials and Methods

*Geographical area of the study*

The location of the two entomological fixed stations was approximately 30 km east of Honiara, the capital, at Gilutae and Tumunagela in a region called Koli point (Map 1). Each fixed station was surrounded by a group of villages, but separated from each other and other collections of people by distances beyond the normal flight range of vector mosquitoes. It is reasonable to presume that the mosquitoes took their blood meals from the people examined and that importation of parasites or mosquitoes, if this occurred, was of no importance as the same conditions of residual spraying were in operation in all areas.

*Parasitological methods*

Surveys for microfilariae (mf) were conducted in the surrounding villages, around Tumunagela on 21 December 1976 and around Gilutae between 4 January 1977 and 11 January 1977. People had been examined here so many times for malaria that slides could be taken from only 76% of the population, the rest refusing. The technique used was the conventional one of three 20 $mm^3$ films from each person on one slide, for reasons given by WEBBER (1976). The slides were taken between 20·00 and 24·00 hours, stained with Giemsa and examined at × 60 magnification.

In the Gilutae area, following the first survey results, a further examination was made using the membrane-filtration technique (CHULARERK & DESOWITZ, 1970) but here the resistance to venepuncture was even greater, and only some 20% of the population agreed to have blood taken. In this method, more fully described by DESOWITZ *et al.* (1970), 1 ml of venous blood was haemolysed and filtered through a 5 μ pore-size Millipore filter, stained in hot Harris's haematoxylin and when dry, cleared with immersion oil and examined at × 60 magnification.

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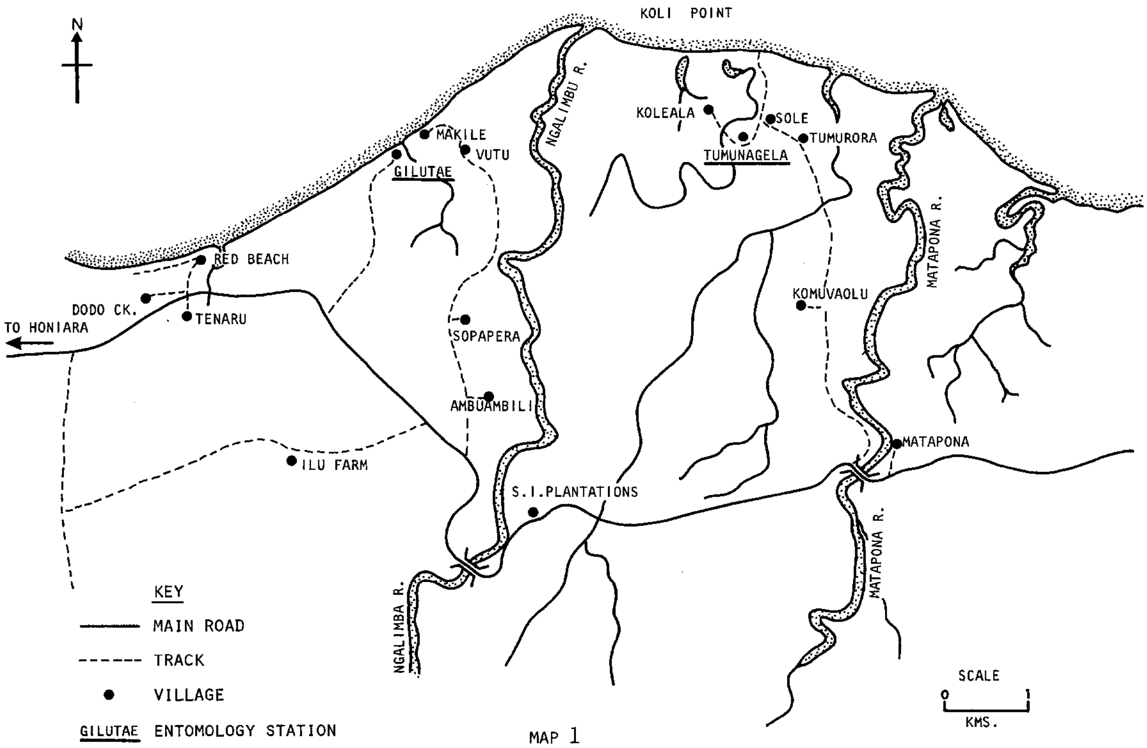


Map 1. The Koli Point area of Guadalcanal, Solomon Islands, showing the fixed entomology stations of Gilutae and Tumunagela.

*Entomological methods*

Once weekly collections were made by entomological technicians from 7 January 1975 to 22 November 1976. Two technicians worked together, one outside and one inside the home (sprayed), collecting all mosquitoes that came to bite them. The technicians changed places every half hour and worked alternately at Gilutae and Tumunagela. Collections were made between 18.30 and 20.30 hours as SLOOF (1964) had found this to be the period of peak biting after a programme of house spraying with DDT. The following morning, all mosquitoes were identified and the numbers of *A. farauti* No. 1 counted.

Since October 1976 there has been a progressive increase in malaria cases, so on 13, 14 and 15 June 1977, in an area close to Tumunagela and Gilutae collections were made throughout the night, both indoors and outdoors, to determine the current pattern of biting activity.

## Results

*Parasitological results*

The results of the human blood surveys in people living close to the two MEP collecting stations are given in Table 1. It is appreciated that the numbers of persons examined were small, but the important consideration was that two years of continuous entomological collections had been made in these places, and then the best possible attempt made to examine the people who were likely to have been fed upon by these mosquitoes. The only requirement in examining the people was to detect the presence and density of circulating mf, and the presence of major clinical signs of filariasis. Only one case of elephantiasis was found and this was in the Gilutae area.

*Entomological results*

The results of the weekly man-biting collections carried out from 18.30 to 20.30 hours between 7/1/75 and 22/11/76 are given in Table II. Continuous collections over such a prolonged period should have removed most of the fluctuations that arise from changing weather conditions, variations in photoperiod and phases of the moon.

The results of the all-night indoor and outdoor biting-catches carried out in June 1977 are given in Table III. Fig. 1 a, b and c, show the hourly percentages of the total man-biting catch recorded indoors, outdoors and for both combined, with the periodicity curve of *W. bancrofti* microfilaraemia for the Solomon Islands superimposed. This curve was derived from the formula $Y = 100 + 1 \cdot 347\ D \cos 15\ (h\text{-}k)^\circ$ where 'D' the periodicity index for the Solomons was calculated as 95·23, and the best estimate of 'k' as 0·6 hr by SASA & TANAKA (1972), SASA (1976), based on data from SCHLOSSER (1945). Each hour (h) was calculated and expressed as a proportion of the peak value (k) in Table IV.

**Table I—Results of blood surveys for mf of *W. bancrofti* carried out in December 1976 and January 1977.**

| Place | Technique | Number of persons examined | Number of persons positive | % Positive | Number of mf detected |
|---|---|---|---|---|---|
| Tumunagella | 60 $mm^3$ blood film | 50 | 1 | 2 | 2 |
| Gilutae | 60 $mm^3$ blood film | 101 | 0 | 0 | 0 |
| Gilutae | 1 ml blood Millipore filtration | 25 | 0 | 0 | 0 |

**Table II—Results of weekly man-biting collections from 18.30 to 20.30 hours, January 1975 to November 1976**

| Place | Site of collection | Number of *A. farauti* No. 1 collected | Number of night collections made | % of bites | Mean man-biting rate per hour |
|---|---|---|---|---|---|
| Tumunagela | Indoors | 2509 | 116 | 23·7 | 10·8 |
| | Outdoors | 8081 | 116 | 76·3 | 34·8 |
| | Combined | 10590 | 232 | 100·0 | 22·8 |
| Gilutae | Indoors | 2644 | 122 | 35·3 | 10·8 |
| | Outdoors | 4840 | 122 | 64·7 | 19·8 |
| | Combined | 7474 | 244 | 100·0 | 15·3 |
| Total | Indoors | 5153 | 238 | 28·5 | 10·8 |
| | Outdoors | 12921 | 238 | 71·5 | 27·1 |
| | Combined | 18074 | 476 | 100·0 | 19·0 |

**Table III—Results of all-night biting catches in the study area, June 1977**

| Place | Site of collection | Number of *A. farauti* No. 1 collected | % of bites | Mean man-biting rate per hour |
|---|---|---|---|---|
| Station 1 | Indoors | 229 | 33·5 | 19·1 |
| | Outdoors | 455 | 66·5 | 37·9 |
| | Combined | 684 | 100·0 | 28·5 |
| Station 2 | Indoors | 382 | 32·1 | 31·8 |
| | Outdoors | 808 | 67·9 | 67·3 |
| | Combined | 1190 | 100·0 | 49·6 |
| Station 3 | Indoors | 60 | 27·4 | 5·0 |
| | Outdoors | 159 | 72·6 | 13·2 |
| | Combined | 219 | 100·0 | 9·1 |
| Total | Indoors | 671 | 32·1 | 18·6 |
| | Outdoors | 1422 | 67·9 | 39·5 |
| | Combined | 2093 | 100·0 | 29·1 |

## Discussion

LEVINE & HARPER (1947) examined 2,500 people in Guadalcanal and found 22% positive for microfilaraemia. It is likely that this particular area was included in the present survey as this was the part of Guadalcanal occupied by the American forces. MATAIKA (1965) surveyed 502 people on the north coast of Guadalcanal and found 18·8% positive with a mean density of 53·6 mf per 20 $mm^3$ blood and $MfD_{50}$ of 14·0: of these 502 people examined two had elephantiasis. During the present survey one case of elephantisis was found in the Gilutae area. No mass drug administration of diethylcarbamazine has been used in this region, so it is reasonable to presume that the prevalence of filariasis has fallen to the present low level (Table I) as a result of vector control methods (see also WEBBER 1977; 1979).

**Table IV—*A. farauti* No. 1 biting patterns and the probability of mosquito-mf contact**

| Times (hours) | Mf prevalence as proportion of maximum prevalence | Proportion of combined indoor and outdoor mosquito biting per hour | | | Mf ingestion potential per hour | | |
|---|---|---|---|---|---|---|---|
| | | Before spraying | Recently sprayed | Prolonged spraying | Before spraying | Recently sprayed | Prolonged spraying |
| –19·00 | 0·50 | 0·068 | 0·209 | 0·222 | 0·034 | 0·104 | 0·111 |
| –20·00 | 0·64 | 0·054 | 0·119 | 0·302 | 0·035 | 0·076 | 0·193 |
| –21·00 | 0·77 | 0·104 | 0·093 | 0·126 | 0·080 | 0·072 | 0·097 |
| –22·00 | 0·88 | 0·084 | 0·088 | 0·052 | 0·074 | 0·077 | 0·046 |
| –23·00 | 0·95 | 0·103 | 0·077 | 0·029 | 0·098 | 0·073 | 0·028 |
| –24·00 | 0·99 | 0·094 | 0·077 | 0·022 | 0·093 | 0·076 | 0·022 |
| –01·00 | 1·00 | 0·141 | 0·078 | 0·023 | 0·141 | 0·078 | 0·023 |
| –02·00 | 0·96 | 0·094 | 0·076 | 0·011 | 0·090 | 0·073 | 0·011 |
| –03·00 | 0·89 | 0·103 | 0·080 | 0·003 | 0·092 | 0·071 | 0·003 |
| –04·00 | 0·79 | 0·075 | 0·054 | 0·005 | 0·059 | 0·043 | 0·004 |
| –05·00 | 0·67 | 0·056 | 0·036 | 0·004 | 0·037 | 0·024 | 0·003 |
| –06·00 | 0·53 | 0·024 | 0·014 | 0·022 | 0·013 | 0·007 | 0·012 |
| –07·00 | 0·38 | 0·000 | 0·000 | 0·179 | 0·000 | 0·000 | 0·068 |
| Totals | | 1·000 | 1·001 | 1·000 | 0·846 | 0·774 | 0·621 |
| | | | | | 100% | 91·5% | 73·4% |

**Table V—Results of indoor and outdoor human bait collections, Solomon Islands, 1963-64, *A. farauti* No. 1. (Number of man-hours spent in collecting in brackets). Alves (1965)**

| Time and relation of collections to spraying | Man-biting rate per hour Indoors | Outdoors |
|---|---|---|
| April 1963 (before spraying) | 46·7 (1·5) | 50·6 (1·5) |
| June 1963 (1 month after first spraying) | 1·3 (3·0) | 6·0 (3·5) |
| August 1963 (3 months after first spraying) | 0·0 (3·0) | 19·3 (1·5) |
| January 1964 (1 month after second spraying) | 1·3 (4·5) | 7·8 (4·5) |
| April 1964 (4 months after second spraying) | 7·4 (8·0) | 45·6 (8·0) |
| May 1964 (5 months after second spraying) | 0·7 (3·0) | 83·0 (3·0) |
| November 1964 (3 months after third spraying) | 0·0 (3·0) | 4·7 (3·0) |

Residual spraying commenced in Guadalcanal in May 1963 with DDT 75% wettable powder at 2 g per square metre applied to all structures, particularly to inside walls, roofs, eaves and underneath floors. Spraying was repeated every six months but in the past four years the frequency has been increased to every four months as malaria still persists.

Entomological data collected by ALVES (1965) during the Pilot Project of the MEP are shown in Table V. By comparing the data in this Table with those in Tables II and III, it can be seen that man-biting rates for *A. farauti* No. 1 have declined considerably since the inception of the MEP. Indoor man-biting rates have dropped proportionately more than outdoor rates. However, they were still at appreciably high levels in the Koli point area in 1975-77 after 12 to 14 years of DDT spraying. Despite the prolonged period of insecticidal attack, the vector remains susceptible to DDT. Susceptibility tests with 4% DDT discriminatory dose conducted on specimens collected in 1977 gave 100% kill.

SLOOF (1964) investigated the effects of DDT house spraying in the *A. punctulatus* group in West New Guinea (now Irian Java) and found that following residual spraying the pattern of man-biting of *A. farauti* No. 1 changed from predominantly indoor (80·8%) to outdoor (62·6%) and that peak biting was between 18·30 and 20.30 hours. The biting cycle in the Solomons was considered to be the same but this might not necessarily be so.

Fig. 2a, b and c (adapted from SLOOF, 1964) shows that before spraying *A. farauti* No. 1 had a fairly uniform biting pattern throughout the night, with a peak at 01·00 hours both indoors and indoor-outdoors combined. The peak biting rate outdoors was at 21.00 hours, but a secondary peak occurred at 01.00 hours. The calculated *W. bancrofti* microfilarial periodicity curve of the numbers of circulating microfilariae is superimposed on these diagrams and shows the same pattern as that of vector man biting activity.

Fig. 3a, b and c (also adapted from SLOOF, 1964) shows the biting pattern of *A. farauti* No. 1 after residual DDT spraying of houses. Peak biting activity occurred before 21.00 hours, when the mosquito can expect to take a blood meal without having to enter a DDT sprayed structure. This has very worrying implications for mosquito control and reduction of malaria transmission, but also means

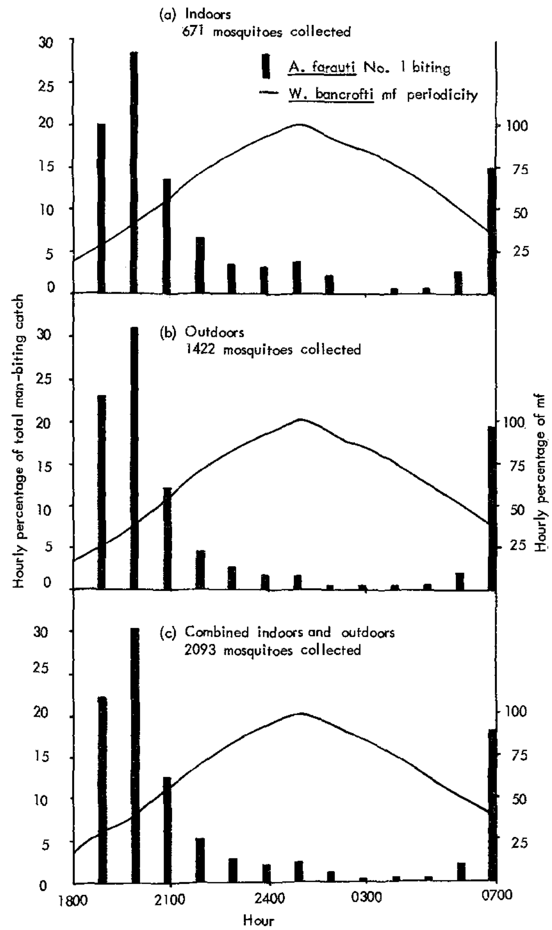


Fig. 1. The distribution of man-biting by *A. farauti* No. 1 throughout the night; periodicity of *W. bancrofti* microfilaraemia superimposed. (Koli point area, Solomon Islands). After prolonged DDT residual spraying.

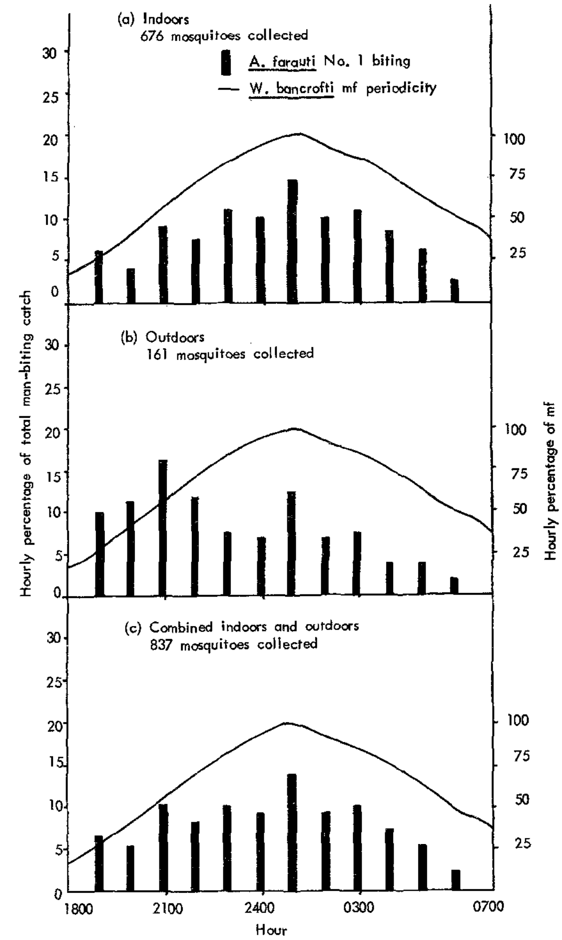


Fig. 2. The distribution of man-biting by *A. farauti* No. 1 throughout the night; periodicity of *W. bancrofti* microfilaraemia superimposed. (Irian Jaya. Modified from SLOOF (1964)). Before DDT residual spraying.

that relatively few mosquitoes are biting indoors at the peak period of prevalence and density of circulating mf.

Comparison of Fig. 3 with Fig. 1 shows that after 12 to 14 years of residual DDT spraying on Guadalcanal, *A. farauti* No. 1 has now exaggerated its evening biting peak even more than after a short period of spraying, and that an early morning biting peak now occurs between 05·00 and 07·00 hours (just as people are leaving their houses). Mosquito-DDT contact and mosquito-mf contact are now at very low levels. This explains why one can still have a relative abundance of vector mosquitoes, yet no filariasis.

In attempting to evaluate the absolute number of mosquitoes that can be allowed to come to bite man, still without transmission occurring, the periodicity of the mf must be used. If it is assumed that the unsprayed situation (Fig. 2), where peak mosquito biting and peak mf prevalence and density coincide, is the pattern of maximal opportunity for transmission, alterations from this pattern can then be calculated. There are variations in the ability of mosquitoes to concentrate mf (BRYAN & SOUTHGATE, 1976), in the effects of the pharyngeal armature in destroying mf in different anopheline species (BRYAN *et al.*, 1974), and in the phenomena of facilitation and parasite yield in various *Anopheles* spp.-*W. bancrofti* host-parasite relationships (PICHON 1974; PICHON *et al.*, 1974). For simplicity we have assumed that these effects will not be materially affected by changes in mosquito numbers or in their biting pattern and a simple mathematical relationship is used between vector density and mf prevalence as determined by 60 mm$^3$ blood film examinations. Thus the transmission potential of *W. bancrofti* by *A. farauti* No. 1 is assessed by multiplying the proportion of mosquito bites occurring in each hour by the relative prevalence of the mf in the blood in the same hour (this is calculated from the periodicity curve for each hour where 01·00 hours is the time of maximum prevalence). The products of biting proportion and mf prevalence for three different situations, before spraying, recently sprayed

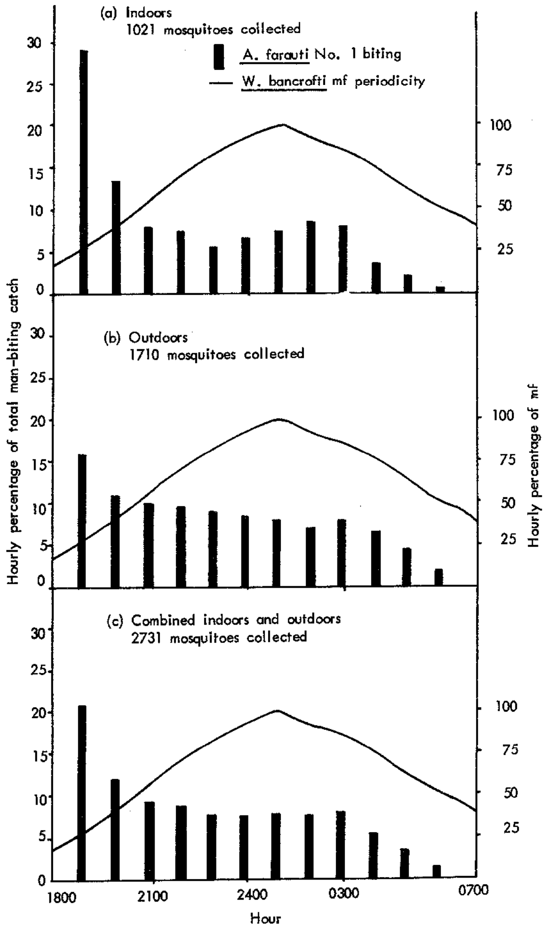


Fig. 3. The distribution of man-biting by *A. farauti* No. 1 throughout the night; periodicity of *W. bancrofti* microfilaraemia superimposed. (Irian Jaya. Modified from SLOOF (1964)). After initial DDT residual spraying.

and prolonged spraying, are set out in Table IV. Shortly after spraying, the transmission potential for *W. bancrofti* is reduced to 91·5% of the pre-control potential, and after prolonged spraying it is reduced to 73·4% of the pre-control potential. These estimates do not depend upon the prevalence of microfilaraemia in the human population, or the reduction in the mosquito population brought about by insecticide spraying, but merely reflect the diminished chances of a mosquito ingesting mf as a result of the behavioural changes in biting pattern brought about by short or prolonged periods of insecticide application.

We found that the period 18.30 to 20.30 hours accounted for 60·7% of outdoor man-biting, 56·1% of indoor man-biting and 59·3% of all man-biting during the whole night period (see Fig. 1). Average man-biting per hour is found by multiplying the 22 month collection results (Table II) by 100/59·3 and average effective man-biting per hour (the reduced transmission potential produced by residual DDT spraying) can then be calculated by multiplying by 73·4%. Calculated results are given in Table VI.

It is possible that minimum transmission is still continuing in Tumunagela, but it has ceased in Gilutae (Table I). It would thus appear that in the Solomon Islands, with the marked change in vector biting pattern produced by DDT spraying, transmission of filariasis has ceased when the average vector man-biting rate per hour has been kept below 3·0 for a prolonged period. In other areas of *Anopheles* transmitted filariasis, where patterns of biting activity may be changed in different ways, it would appear that transmission would cease if effective man-biting rates per hour are kept below about 2.0.

It is instructive to compare this figure with the value of $m$, "the critical density of vector mosquitoes below which filariasis transmission will die out", calculated by WEBBER (1975 b) as 19·84. The observed results above give a value of $ma$ of $2·0 \times 12$. The value of $a$ (the average number of men bitten by one mosquito in one day) for the Solomon Islands was found to be 0·4 (WEBBER, 1975b) so $m$ equals 60, over three times the theoretical value.

This value for $m$ depends upon using the man biting rate as opposed to the alternative method of measuring the indoor-resting blood-fed females per inhabitant. GARRETT-JONES & SHIDRAWI (1969) investigated these alternative approaches and found that the man-biting rate was more than three times greater than the fed-indoor resting female mosquito density per person. They suggested correcting the man-biting rate by dividing by three, which now makes the theoretical and observed values of $m$ extremely close, at about 20. It seems reasonable

**Table VI—Average man-biting per hour and average effective man-biting per hour of *A. farauti* No. 1 in a vector control situation in the Solomon Islands**

| Place | Average nightly biting on two collectors, 18·30 —20·30 hours Indoor + outdoor (a) | Total bites expected for whole night on two collectors Indoor + outdoor (a) × 100/59·3 (b) | Average man-biting per hour (b) ÷ 12 ÷ 2 (c) | Average effective man-biting per hour (c) × 73·4% (d) |
|---|---|---|---|---|
| Tumunagela | 45·6 | 76·9 | 3·20 | 2·35 |
| Gilutae | 30·6 | 51·6 | 2·15 | 1·58 |

therefore to suggest that the figure of 19.84 × 0·4/12 = 0·66 for the average corrected man-biting rate per hour indoors would be an appropriate critical level to use.

This means that if any method of vector control is used almost 8 (12 × 0·66) mosquito bites may be allowed per person indoors during the night time period without transmission of filariasis taking place. This low transmission potential would explain why people who take particular precautions against being bitten by mosquitoes by using mosquito nets, repellents and protective clothing can live in endemic areas for long periods without becoming infected.

A further observation from this study has been the behavioural effect DDT has had on this particular vector. It has reduced the indoor man-biting rate from 80·8% (676/837) of the total before spraying to 32·1% (671/2093) after prolonged spraying; the post-spraying percentage is thus 25·9% of the pre-spraying percentage. As was also shown, the microfilarial ingestion potential was reduced to 73·4% of the pre-spraying level. (Table V). This is a cumulative effect of 19·0% (25·9% × 73·4%) of indoor-biting mosquitoes capable of ingesting microfilariae. This is a separate effect from the lethal action of DDT in reducing the expectation of life of the vector. DDT now acts like a huge mosquito net over the house not so much killing the vector, but preventing it from coming into contact with man at the most suitable place and time for it to become infected. These two additional effects of DDT repellency and altering the biting habit are important and it is suggested that more use could possibly be made of these effects in other situations.

Perhaps the task of reducing the amount of *Wuchereria bancrofti* infection transmitted by anopheline mosquitoes could be more easily achieved by applying methods of vector control, or prevention of biting, with the far larger margin of allowable vector densities indicated here.

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