

## On the Inefficiency of Transmission of *Wuchereria bancrofti* from Mosquito to Human Host

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*The high biting rate of Culex pipiens fatigans in Rangoon, combined with a low prevalence of microfilaraemia due to Wuchereria bancrofti, suggested a poor efficiency of transmission. Data obtained by the WHO Filariasis Research Unit in Rangoon were analysed, and the efficiency of the parasite from Stage III larva in the mosquito to the production of microfilariae was estimated as  $6.04-6.71 \times 10^{-5}$ , indicating that an average of around 15 500 bites by "infective" mosquitos is necessary to produce 1 case of microfilaraemia. This surprising result prompted a step-by-step analysis of the success of the parasite through departure from the mosquito, penetration of host tissues, survival to maturity, and encountering a mate in the human host. It was hoped that the second approach would identify some discrepancy in the original analysis, but when all sources of loss were combined, it was estimated that the expected efficiency was  $4.78 \times 10^{-5}$ . The two estimates are within observational error of each other. The degree to which the two approaches agree leads to the conclusion that survival of the parasite is reasonably well known at all stages, and the calculations indicate that a high proportion of the population of Rangoon must be carrying parasites that are either dead or immature. A complete quantitative statement of the epidemiology of W. bancrofti seems to be attainable.*

### INTRODUCTION

It is common practice to refer to mosquitos carrying Stage III larvae of *Wuchereria bancrofti* as infective, and to bites by such mosquitos as representing transmission of the parasite. For some time, evidence has been accumulating that there are many more bites by "infective" mosquitos than are reflected in the prevalence of microfilaraemia at one point in time or in the rate at which blood-negative people become blood-positive. This is further supported by the experience of one of us (B. de M.) while serving as Project Leader, WHO Filariasis Research Unit, in Rangoon, where local testimony and records show that during the last 30 years no European has become blood-positive in spite of frequent exposure during gatherings at night in the open. Similarly, mem-

bers of the Filariasis Research Unit, both European and Burmese, have not so far become blood-positive in spite of repeated exposure in the field over several years.

The apparent paradox of large numbers of infective mosquitos and a low rate of becoming blood-positive will be documented in this paper with data collected by the WHO Filariasis Research Unit and the Burmese Government. The preliminary calculations gave such a surprising result that we made an attempt to identify the source of what seemed to be a major discrepancy in understanding the epidemiology of the parasite. This attempt required a quantitative analysis of the probability of success of the larval parasite and of its survival through the successive steps between development in the body of the mosquito and the production of microfilariae in man. This approach to the problem, instead of identifying the source of any discrepancy, seems to confirm the surprising conclusion of the original estimate that very many bites by "infective" mosquitos are required to produce microfilaraemia.

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We are thus at a loss to explain the apparent inefficiency of the parasite. It is true that in order to carry out the calculations, we have made some assumptions that may not be strictly valid, and we have used some data for *Brugia* where experiments with *Wuchereria* have not been performed or are not possible. One obvious possibility can be eliminated, since *W. bancrofti* is the only filarial parasite present in man and vector in Rangoon.

We therefore present the results as they stand, hoping that they will stimulate others to bring new evidence to bear on the subject.

#### THE OBSERVED EFFICIENCY OF TRANSMISSION

The efficiency of transmission is best regarded as the fraction :

$$\frac{\text{No. of people becoming positive per year}}{\text{Total no. of bites by infective mosquitos per year}}$$

In order to use information which is readily obtainable it is necessary to express the fraction in terms of average values per person. The data needed for the denominator are average biting rates per man-hour, both over 24 hours and by season, and the proportion of biting mosquitos that are carrying stage III larvae. The average number of "infective" bites per person per year can then be calculated.

De Meillon, Grab & Sebastian (1967) give the results of observations on the biting rate by *Culex pipiens fatigans* in Rangoon. The average rate was 18 bites per man-hour for the first half of the night, after taking seasonal differences into account. The first half of the night accounts for approximately 47% of the bites recorded on repeated observations carried out over the 12-hour period 18.00–06.00 hours (de Meillon & Sebastian, 1967). Combining the two sets of data, it can be calculated that over the year there are 227 bites by *C. p. fatigans* per person per day, for an annual total of 82 873. The proportion of mosquitos carrying stage III larvae varied over the year, but followed no pattern because of the virtually constant mortality rate of mosquitos (de Meillon, Grab & Sebastian, 1967). The over-all proportion of 0.0036, when applied to the calculated number of bites per year, yields the estimate that there were 298 "infective" bites per person per year as an average value for the Kemmendine Experimental Area in Rangoon. This figure will be used

as the denominator of the fraction denoting the efficiency of transmission.

The average exposure is higher in Rangoon than in other Asian cities where it has been observed. Rozeboom, Bhattacharya & Gilotra (1968) estimated that in a part of Calcutta with poor sanitation there were on average 50 bites by "infective" *C. fatigans* per person per year; and in Bangkok, where endemic filariasis is not known, the biting rate is much lower than in Rangoon (Col. J. E. Scanlon, personal communication).

The numerator of the fraction could be obtained by direct observation of a randomly chosen group of people who were negative originally. Repeated observations on them would yield the annual rate of becoming positive. The observations were not made, but the rate can be calculated from age-prevalence data, using the appropriate catalytic model of Muench (1959).

The use of Muench's models depends upon several assumptions. The most fundamental is that the epidemiological situation has remained constant for the length of life of the oldest age-group considered. In the case of Rangoon, this assumption is valid for less than 25 years, since endemic filariasis was virtually unknown there before 1941.

The history of the situation was reviewed by Tin Maung Maung and de Meillon in 1963 (unpublished material). Data from various surveys carried out by the Filariasis Research Unit between 1959 and 1967 indicate that over the past 9 years changes in prevalence have not been demonstrated. For our calculations, all age-groups above 20 years have been pooled.

The second assumption is that the rate of acquiring the parasite does not vary among age-groups. The validity of this assumption cannot be demonstrated, but there appears to be no reason to question it. A third assumption that will be made is that people who are blood-positive become negative at a constant rate. The choice among Muench's models depends upon one further assumption—that prior infection does not alter the rate at which negative people become positive. Hairston & Jachowski (1968) have shown that this assumption seems valid for subperiodic *W. bancrofti*, and that good fits to observations can be obtained by the use of the reversible catalytic model of Muench. Age-prevalence data collected in the Kemmendine Experimental Area are shown in Table 1. The population was almost 93%

TABLE 1  
THE RELATIONSHIP BETWEEN AGE AND PREVALENCE OF MICROFILARAEMIA BY ETHNIC GROUPS  
IN THE KEMMENDINE AREA OF RANGOON<sup>a</sup>

Age-group (years)	Burmese		Indian		Chinese		Total	
	No. examined	% positive	No. examined	% positive	No. examined	% positive	No. examined	% positive
0-4	595	0	58	1.7	21	4.8	674	0.3
5-9	1 383	2.0	84	7.1	45	2.2	1 512	2.2
10-14	1 446	4.6	70	11.4	44	2.3	1 560	4.8
15-19	1 170	7.7	48	16.7	35	8.6	1 253	8.1
20-24	1 096	6.2	68	8.8	26	3.8	1 190	6.3
≥25	4 790	4.8	325	11.1	136	3.7	5 251	5.2

<sup>a</sup> Data based on night blood smears and taken from the unpublished WHO Filariasis Research Unit monthly report for January 1965.

Burmese, a fact reflected in the similarity between the data for Burmese and for the total. The annual rate of becoming positive was calculated as 0.0094, using the reversible catalytic model. With the two-stage catalytic model, which assumes no reversions to positive, the same rate was calculated as 0.012. The difference between the two values is probably not significant. If we accept them as the range of values for the numerator of the fraction representing the efficiency of transmission, this efficiency can then be estimated as falling in the range  $3.15 - 4.03 \times 10^{-5}$ , or, put in inverted form, it is estimated that 24 814 - 31 746 "infective" bites are necessary to produce microfilaraemia.

There are several reasons for considering that these numbers are exaggerated. Returning to Table 1, the prevalence of microfilaraemia among the Indian population is significantly higher than that among Burmese. The possibility exists that the observed biting rates are more representative of the exposure of Indians than of Burmese. Use of the models for Indians alone gives calculations of the following rates of becoming positive: 0.012 for the reversible model and 0.0175 for the two-stage model. These would yield efficiencies of transmission of  $4.03 - 5.87 \times 10^{-5}$ , and would imply that 17 036 - 24 814 "infective" bites are necessary to produce microfilaraemia.

One further problem requires consideration. It is possible that an unknown proportion of the human population have microfilariae at densities below

the threshold of detectability by the methods used in the surveys. Subsequent to the results reported in Table 1, it was shown that improved techniques and larger volumes of blood gave counts 4 times as high as those found on the survey. In order to estimate the proportion that would be positive if all counts were 4 times as great as observed, it is necessary to have information about the distribution of microfilariae among people. The more uniform the distribution, the greater is the effect on the proportion positive; the more contagious the distribution, the less is the effect of quadrupling all counts. The form of the distribution can be estimated from survey data, if the proportion negative and the mean count of all persons examined are reported (Bliss & Fisher, 1953; Bliss & Calhoun, 1954). This information is available from at least one survey in Rangoon (data from the Filariasis Research Unit's unpublished monthly report for September 1967). With 0.039 positive, the mean count was 0.75 microfilaria per person examined. Assuming a negative binomial distribution, which is common in such data, the degree of contagion can be calculated from:

$$q = \frac{1}{(1 + m/k)^k}$$

where  $q$  is the proportion negative (0.961),  $m$  is the mean count (0.75) and  $k$  is a constant inversely related to the degree of contagion. For the data given,  $k$  is approximately 0.01. If the value of  $k$  remains constant, the same formula can be used to

calculate the effect of quadrupling  $m$ , so long as the initial value of  $q$  is known. In the case of the Indian population from Table 1, the effect would be to raise the prevalence to the following values: for 0-4-year-olds, 2.9%; for 5-9-year-olds, 8.6%; for 10-14-year-olds, 12.6%; and for those older than 15, 12.5%. Thus the distribution is highly contagious and the effect of quadrupling the counts has a small effect on prevalence. Applying the two catalytic models to the revised estimates of prevalence, the rate of becoming positive can be raised to 0.018 for the reversible model or 0.020 for the two-stage model. These values improve the efficiency of transmission to  $6.04 \times 10^{-5}$  and  $6.71 \times 10^{-5}$ , respectively, but the number of "exposures" required to produce microfilariemia remains very high: 16 566 and 14 903, respectively.

Thus, making all reasonable allowances, it is calculated that an average of around 15 500 bites by mosquitos carrying stage III larvae is necessary to produce microfilariemia.

#### THE EXPECTED EFFICIENCY OF TRANSMISSION

Superficially, the calculations in the preceding section indicate an efficiency so small as to be difficult to believe, but there are further considerations which can be shown to have effects that are strong enough to explain the apparent inefficiency.

The first consideration is that both sexes of parasites must be present and mated for the human host to be microfilaria-positive. Thus, it is impossible for a bite by a mosquito carrying a single stage III larva to cause microfilariemia. The same is true for one half of the mosquitos carrying 2 larvae, one fourth of those carrying 3 larvae, etc., on the reasonable assumptions of an equal sex ratio and independent distribution of the two sexes among mosquitos. The effect of this factor can be estimated from the frequency distribution of stage III larvae among mosquitos coming to bite. Table 2 gives data on all such mosquitos reported by the Filariasis Research Unit during 1963 and 1964. From this information, it is estimated that only 53% of bites by mosquitos carrying stage III larvae could possibly result in infection with both sexes of larvae, assuming that all larvae leave the mosquito at one bite, and that all larvae leaving the mosquito reach the tissues of the human host.

TABLE 2  
DISTRIBUTION OF STAGE III LARVAE OF  
*W. BANCROFTI* AMONG FEMALE *CULEX P. FATIGANS*  
COMING TO BITE IN RANGOON<sup>a</sup>

No. of larvae per mosquito	No. of mosquitos
1	32
2	23
3	10
4	6
5	3
6	3
7	4
8	5
9	2
10	4
12	1
14	1
15	3
24	1
28	1
40	1

<sup>a</sup> Total larvae: 454; total mosquitos: 100. Data taken from unpublished WHO Filariasis Research Unit report.

These two assumptions, however, are known to be false. De Meillon, Hayashi & Sebastian (1967) found that a randomly chosen group of 72 mosquitos contained an average of 58.6% as many larvae after a blood meal as did 54 randomly chosen unfed controls. All the mosquitos had been infected at the same time by feeding on the same person. Thus, only 41.4% of the "infective" larvae leave the mosquito at one feeding. The fate of larvae after leaving the proboscis is not known for *W. bancrofti*, but experimental data have recently been published for *Brugia pahangi* (Ewert & Ho, 1967; Ho & Ewert, 1967). These authors found that during a single complete feeding 32% only of the escaping larvae succeeded in penetrating the tissues of experimental hosts. Similar results were obtained when the mosquito had a partial blood meal (31.3%) or made multiple attempts to feed (38.1%). When a single feeding attempt was unsuccessful, 10.2% of the escaping larvae succeeded in penetrating the tissues.

If *W. bancrofti* has a similar chance of success at this step in the life-cycle, then only 32% of 41.4% of the "infective" larvae in a mosquito succeed in reaching the tissues of the human host at a single complete feeding by the mosquito.

From the data given, it is possible to calculate an expected efficiency of the parasite from stage III larvae in mosquitos to immature worms in humans. For a mosquito carrying  $N_i$  larvae, the average expectation,  $a$ , of transmitting a female worm is

$$\left(\frac{N_i}{2}\right) (0.414) (0.32)$$

With an independent distribution of equally abundant males and females, the probability that a random bite by a mosquito carrying  $N_i$  larvae will transmit a female worm to the tissues is  $(1 - e^{-a})$ , where  $e$  is the base of the natural logarithms. The combined probability that such a mosquito will transmit both sexes of worms to the tissue is  $(1 - e^{-a})^2$ .

The frequency,  $f$ , of ( $N_i$ ) with which "infective" mosquitos carry  $N_i$  larvae is given as a percentage in Table 2, since exactly 100 mosquitos are reported. The probability that a bite by a randomly selected "infective" mosquito will result in both sexes of larvae reaching the tissues of the human is

$$\sum(1 - e^{-a})^2 (f(N_i))$$

for all  $N_i > 1$ , since a single larva cannot be of both sexes.

Applying the calculation to the data given, the expected efficiency of transmission is 0.0325. This figure should not be taken at face value because the method of calculation does not allow for any accumulation of parasites, nor does it take the prepatent period into account. Thus, if a single worm of either sex can wait for a mate to appear, the expected efficiency would be higher. The most important factor in estimating the extent to which accumulation is possible is the rate of survival of immature parasites in the human. Again, data for *W. bancrofti* are not available, but Edeson & Buckley (1959) obtained a survival to maturity of 0.13 for *Brugia malayi* in experimental animals. Assuming a constant death rate during this immature period of 2½ months, the daily mortality can be calculated as

$$0.13 = e^{-75d}$$

when  $d$  is the instantaneous death rate per day and  $e$  is the base of natural logarithms. From the

equation,  $d = 0.027/\text{day}$ , and *W. bancrofti* is known from unpublished WHO information to have a minimal prepatent period of 8 months and 4 days. Thus if we apply the calculated death rate, the proportion of larvae surviving would be 0.00147.

It has been calculated in the preceding section that 16 566 bites by "infective" mosquitos are required to produce microfilaraemia. The data in Table 2 show that these mosquitos carry an average of 4.54 larvae, of which  $0.414 \times 0.32 \times 4.54$  or 0.60 succeeds in penetrating the tissues of the human host. Thus, the host acquires an average of 9923 larvae before becoming positive on blood examination. At a survival of 0.00147 to maturity, only 14.6 of the larvae would survive to reproduction age. While this seems ample, it should be pointed out that at an average intake of 0.60 larva per bite and 298 bites per year, 56 years would be needed to acquire 9923 larvae. The 14.6 mature worms would presumably be spread evenly over this time, and their death rate must also be taken into account. Hairston & Jachowski (1968) have estimated the death rate of mature female subperiodic *W. bancrofti* as 0.02–0.05 per month. If these estimates are used, it can be calculated that for adult worms the mean length of life would be 2–4 years. If the mature worms were spread exactly evenly over the 56 years, one would appear every 3.8 years, so that the probability of accumulation is small.

Thus, a reasonably accurate estimate of the expected efficiency of transmission can be made by combining the probability of receiving both sexes of worms at a single bite ( $3.25 \times 10^{-2}$ ) with the probability of the immature worm surviving to maturity ( $1.47 \times 10^{-3}$ ). The result,  $4.78 \times 10^{-5}$ , falls within the range of values calculated for the observed efficiency.

The correspondence between observed and expected values is so good that we suspect the operation of luck, as the difference is well within observational and experimental error. More important than the exact correspondence of final values is the indication that reasonably accurate information exists for survival at every step in the life-cycle of the parasite, and that it is unnecessary to postulate additional sources of mortality. Moreover, each of the individual values used seems intuitively reasonable. It is only when all are combined into a single number that a surprising result is obtained.

It should be noted that the calculations lead to the conclusion that a large proportion of apparently negative people are infected with larvae which are immature or which will die before maturity. The general conclusions could be tested by demonstrating the proportion positive on immunological tests, or by demonstrating the prevalence of tropical eosinophilia. Skin testing has been carried out by the Filariasis Research Unit, and a large proportion of persons gave significant reactions, although it has not yet been demonstrated conclusively that *W. bancrofti* was responsible. No data are available on the prevalence of tropical eosinophilia.

#### DISCUSSION

In spite of the satisfying outcome of the analysis, it should be pointed out that events in nature are not exactly like those postulated for purposes of calculation. Mosquito bites are not randomly distributed among the population, and the resistance of individuals to infection probably varies widely. Quite obviously, it does not always require 56 years to receive enough infective larvae to become blood-positive, since some young children are found with microfilariae. It is possible that what is really required is for a particularly susceptible individual to receive several heavily infective bites, closely spaced in time. The probability of occurrence of such an event would be so low as to

make the average situation appear to be as calculated above. While the distinction might not be important arithmetically, it could well have much significance for a complete understanding of the epidemiology, and for the execution and evaluation of control projects. The assumptions that all mosquitos carrying stage III larvae are "infective" and that their existence in an area implies that "transmission" is taking place are true only as far as very general principles are concerned. In practice, much more precise quantitative statements are needed before an epidemiological situation can be assessed from mosquito data alone, and before the success of control efforts can be judged on the basis of the presence or absence of "infective" mosquitos. It can be shown in principle that there are critical densities of host and vector below which the parasite population cannot maintain itself and that these critical densities are in principle most important for parasites in which the sexes are separate (Hairston, 1962, 1965; Macdonald, 1965). The most realistic and appropriate method for obtaining quantitative estimates of the critical densities appears to be that of Beye & Gurian (1960) and Hairston (1962), in which balanced ecological life-tables would be constructed for each of several situations representing different rates of transmission. The time may not be far distant when the necessary data are available to accomplish this goal for filariasis.

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#### RÉSUMÉ

Les données rassemblées par le Service de Recherches sur la Filariose, Rangoon, Birmanie, ont été soumises à une analyse dont l'objectif était d'évaluer le pouvoir infectant de *Wuchereria bancrofti*, depuis le troisième stade larvaire chez *Culex pipiens fatigans* jusqu'à l'apparition des microfilaires chez l'hôte humain.

De multiples observations ont montré que le taux moyen d'agressivité du vecteur était de 227 piqûres par personne et par jour; 0,36% des moustiques étant porteurs de larves au troisième stade, cela représente une moyenne de 298 piqûres infectantes par personne et par an. Les informations concernant le rapport entre la pré-

valence de la microfilariémie et l'âge de l'hôte ont été analysées au moyen de modèles catalytiques, et l'on a estimé à 0,018-0,020 par an, au maximum, la proportion immédiate des sujets chez lesquels l'examen de sang devient positif. Ainsi, la capacité de transmission du parasite pendant cette période de sa vie peut être évaluée à  $6,04-6,71 \times 10^{-5}$  ou, inversement, on peut dire qu'il faut environ 15 500 piqûres de moustiques infectants pour produire un cas de microfilariémie. Ces résultats surprenants ont été contrôlés en évaluant, étape par étape, les possibilités de réussite offertes au parasite lors de son passage du moustique à l'hôte. De récentes recherches ont montré que 41,4% des larves quittent le moustique au moment de la piqûre et que 32% d'entre elles parviennent à pénétrer dans les tissus de l'hôte. Si l'on tient compte de la fréquence de l'infection chez les moustiques et de la nécessité que les deux sexes soient présents, on peut évaluer à 0,0325 la probabilité que des larves des deux sexes soient transmises à l'homme en une seule piqûre. Les calculs indiquent également que le taux de survie des larves chez l'hôte jusqu'à la matu-

rité est de 0,00147, étant donné que le taux immédiat de mortalité est d'environ 0,027 par jour et qu'il faut plus de huit mois aux larves pour se développer. La capacité probable de transmission correspond donc au produit de 0,0325 par 0,00147, soit  $4,78 \times 10^{-5}$ .

Cette estimation coïncide parfaitement avec les résultats obtenus par le calcul direct et l'on paraît en droit de formuler deux conclusions essentielles. La première est que l'on connaît très bien le taux de survie du parasite pendant une grande partie de son cycle, sinon les deux estimations ne correspondraient pas aussi exactement. La seconde est qu'un nombre important d'habitants de Rangoon doivent être porteurs de *W. bancrofti*, mais que la plupart des parasites qu'ils hébergent sont morts ou immatures. Les résultats des épreuves cutanées pratiquées par le Service de Recherches sur la Filariose ont montré une proportion élevée de réactions nettes, sans que l'on puisse faire la preuve absolue qu'elles sont dues à *W. bancrofti*. On peut espérer rassembler tous les éléments d'une description quantitative de l'épidémiologie de cette affection.

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