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Low-density microfilaraemia in subperiodic bancroftian filariasis in Samoa*

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Among microfilaria (mf) carriers of subperiodic bancroftian filariasis in Samoa, the low-density level of microfilaraemia was defined as 1-20 mf/ml, and the occurrence of low-density carriers (90 in the present study) was analysed by age, sex, and village in relation to the microfilarial prevalence rate. The low-density carriers were more numerous among those under 20 years and over 60 years old than in other age groups. The ratio of low-density carriers to the total of mf-positive subjects in a village decreased as the prevalence rate of Wuchereria bancrofti *in the village increased.*

*The epidemiological significance of low-density carriers was assessed in connection with the infectivity of vector mosquitos (*Aedes polynesiensis*) produced by them, the possible change of these carriers to carriers of a higher density, and the production of new low-density carriers by diethylcarbamazine citrate (DEC-C) treatment. The mosquito infectivity produced by the low-density carriers accounted for only 2.16% of the total infectivity produced by all the carriers, suggesting that these carriers are of minor importance in the transmission of filariasis. The change of microfilarial count over time among untreated mf-positive subjects was not remarkable during a 60-252-day observation period. However, the low-density carrier group showed a mean increase of 36%, the younger such carriers (under 30 years old) showing a 132% increase. The production of low-density carriers by DEC-C single-dose treatment (6 mg/kg body weight) was not as great as expected.*

The treatment of human subjects with *Wuchereria bancrofti* microfilaraemia using diethylcarbamazine citrate (DEC-C) has not always been successful in eliminating the microfilariae from the peripheral blood. Often a low-density microfilaraemia persists which may not be easily detected. The importance of this residual microfilaraemia in the epidemiology of filariasis has been discussed by Jordan (*1*), Burnett & Mataika (*2*), Sasa (*3*), and Marshall & Yasukawa (*4*).

In field surveys, membrane filtration techniques using 1 ml of venous blood are highly sensitive in detecting microfilaria (mf) carriers with very low microfilarial densities that would not normally be detected by conventional blood smear methods. Kimura et al. (*5*) recently demonstrated in Samoa that the nuclepore membrane filtration method (with 1 ml blood) detected 21% more microfilaria carriers than did the fingerprick blood smear method (with 60 mm^3 blood). Desowitz & Hitchcock (*6*) had also shown that, in 5-9-year-old children in the Kingdom of Tonga, the millipore filter concentration technique (using 1 ml blood) detected 7.8 times as many infections as did thick films of fingerprick blood (60 mm^3). Based on these observations, questions were raised about the validity of the usual parasitological diagnostic techniques, which use a small amount of fingerprick blood, and about the epidemiological significance of low-density carriers in the transmission of filariasis.

In 1979, a nationwide prevalence study of microfilaraemia was conducted by a joint WHO/Samoa Filariasis Research Project team, employing both the nuclepore method (1 ml blood) and the fingerprick method (60 mm^3). The present studies on low-density microfilaraemia make use of the data obtained dur-

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ing this prevalence study for the following purposes: to define low-density microfilaraemia; to record its prevalence in the Samoan population and analyse it by sex and age; to identify the relationship between the rate of low-density carriers and the microfilarial prevalence rate in each village; and to analyse the epidemiological importance of low-density microfilaraemia in filariasis transmission.

MATERIALS AND METHODS

Blood examinations for microfilaraemia, using both the nuclepore and fingerprick methods, were carried out on 7430 subjects. The methods and other information have been described by Kimura et al. (*5*).

To study the change in microfilarial counts among untreated persons, a total of 158 mf-positive subjects by the nuclepore method, who could not be treated for periods ranging from 60 to 252 days, were re-examined by this method before treatment with DEC-C.

Mosquito transmission experiments on carriers with different levels of microfilaraemia were conducted by Samarawickrema et al. using *Aedes polynesiensis* (*7*). The results have been utilized to analyse the transmission potential (mosquito infectivity) produced by the low-density carriers.

To study the effect of DEC-C treatment in the production of low-density carriers, 112 mf-positive subjects detected by the nuclepore method were treated with a single dose of DEC-C (6 mg/kg body weight) and re-examined by the same method 6 months later.

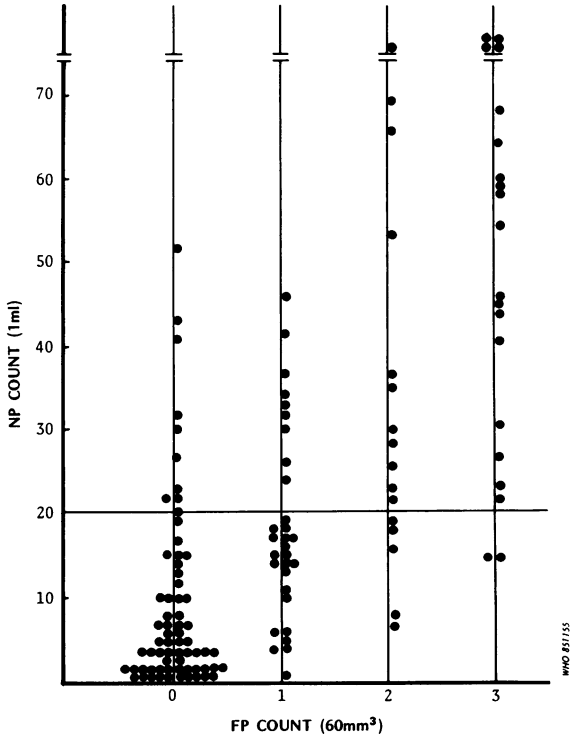

Fig. 1. Distribution of microfilarial counts by the nuclepore filtration (NP) method against 0–3 microfilariae in counts by the fingerprick smear (FP) method.

Definition of low-density microfilaraemia

The term low-density microfilaraemia is usually applied to those densities detectable by the nuclepore method but not by the more usual fingerprick method. However, the theoretical grouping of "low" and "high" counts is not a simple task because of the wide variation of nuclepore counts to a given fingerprick count. The 133 nuclepore-positive subjects who were found to have 0–3 microfilariae by the fingerprick method are plotted in Fig. 1, in which the fingerprick counts from 0–3 are graduated on the horizontal axis and the corresponding nuclepore counts observed are recorded on the vertical axis. On the basis of Fig. 1, an arbitrary decision was made to include microfilarial counts of up to 20 mf/ml within the definition of "low density". By this criterion, the low-density group was found to embrace 87% of subjects with a fingerprick count of zero, 70% with a fingerprick count of 1, 31% with a fingerprick count of 2, and 10% with a fingerprick count of 3.

RESULTS

Prevalence of low-density carriers

Out of 381 mf-positive subjects found by the nuclepore method during the course of the prevalence study in 28 sample villages, 90 (23.6%) were low-density carriers. As the total number of microfilaria carriers estimated for the whole of Samoa was about 7200 in 1979, there were expected to be some 1700 low-density carriers in the whole country.

The ratio of low-density carriers to the total of mf-positive subjects in the population varied from village to village. Fig. 2 shows on the vertical axis the percentage of low-density carriers in relation to the total number of positives in each of 19 villages whose prevalence rate is plotted on the horizontal axis. A tendency can be seen for the proportion of low-density carriers to decrease as the village mf-prevalence rate increases. The regression line was calculated as: $y = -1.833x + 37.57$, where y is the percentage of low-density carriers and x is the village prevalence rate ($r = -0.4897$; $0.02 < P < 0.05$).

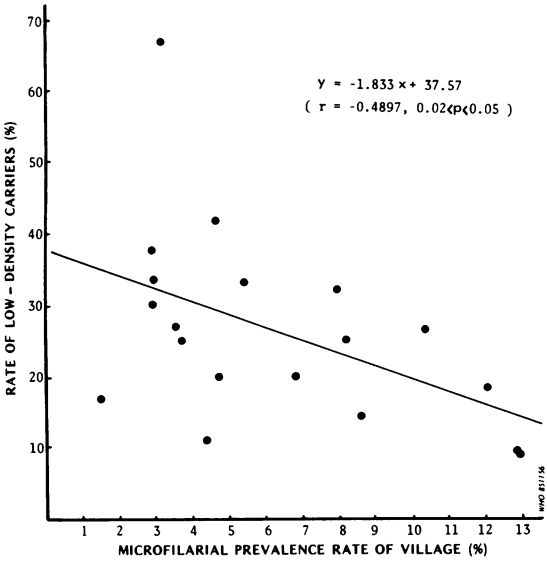

Fig. 2. Relationship between the percentage microfilarial prevalence rate of a village and the percentage rate of low-density microfilaria carriers to the total number of mf-positive subjects in the village (9 villages with less than 5 mf-positive subjects were excluded).

Sex and age distribution

The 90 low-density carriers were analysed by sex and age, and Table 1 summarizes the results. Statistical analyses indicated that there was no significant difference in the occurrence of low-density microfilaraemia by sex ($0.3 < P < 0.4$) but that the age group under 20 years had significantly more low-density carriers than the other age groups ($0.01 < P < 0.025$). Also there was a tendency for low-density carriers to occur more among people aged over 60 years than in the age group 20–59 years, though this was not statistically significant ($0.05 < P < 0.1$).

Epidemiological significance

The epidemiological significance of the low-density carriers was assessed in connection with the following three aspects:

(*a*) the infectivity of vector mosquitos fed on low-density carriers, taking into account the size of the population of such carriers;

(*b*) the possible change from low-density carriers to carriers of a higher density;

(*c*) the production of new low-density carriers by DEC-C treatment.

Mosquito infectivity. The role of low-density carriers in the transmission of filariasis can be measured by calculating the contributions to the total mosquito infectivity of microfilaria carriers of all

Table 1. Low-density carriers classified by sex and age group

Age group (years)	No. with microfilaraemia			No. of low-density carriers			Percentage of low-density carriers		
	M	F	Total	M	F	Total	M	F	Total
0–4	1	0	1	0	0	0	0.0	0.0	0.0
5–9	8	12	20	4	4	8	50.0	33.3	40.0
10–14	10	6	16	3	3	6	30.0	50.0	37.5
15–19	17	10	27	6	3	9	35.3	30.0	33.3
20–24	23	6	29	3	2	5	13.0	33.3	17.2
25–29	22	11	33	6	2	8	27.3	18.2	24.2
30–34	31	12	43	4	1	5	12.9	8.3	11.6
35–39	25	23	48	6	5	11	24.0	21.7	22.9
40–44	24	14	38	3	4	7	12.5	28.6	18.4
45–49	31	6	37	6	2	8	19.4	33.3	21.6
50–54	22	3	25	5	2	7	22.7	66.7	28.0
55–59	15	3	18	2	0	2	13.3	0.0	11.1
60–64	18	5	23	5	2	7	27.8	40.0	30.4
⩾65	21	2	23	7	0	7	33.3	0.0	30.4
Total	268	113	381	60	30	90	22.4	26.5	23.6

densities in the study population. If we assume that all the mf-positive individuals are evenly exposed to mosquito bites, the total mosquito infectivity can be expressed as:

$$\sum_{i=1}^{\text{max.}} \left(\begin{array}{l}\text{Mosquito infection}\\ \text{rate when fed}\\ \text{on a carrier}\\ \text{with } i \text{ mf/unit}\end{array} \right) \times \left(\begin{array}{l}\text{No. of carriers}\\ \text{with } i \text{ mf/unit}\end{array} \right) \quad \ldots\ (A)$$

where max. is the highest microfilarial count obtained and "unit" means 1 ml in the case of the nuclepore method.

For the low-density carriers, the infectivity will be:

$$\sum_{i=1}^{i=20} \left(\begin{array}{l}\text{Mosquito infection}\\ \text{rate when fed}\\ \text{on a carrier}\\ \text{with } i \text{ mf/unit}\end{array} \right) \times \left(\begin{array}{l}\text{No. of carriers}\\ \text{with } i \text{ mf/unit}\end{array} \right) \quad \ldots\ (B)$$

The ratio (B)/(A) will give the portion of the mosquito infectivity due to the low-density carriers.

Samarawickrema et al. conducted transmission experiments in Samoa by using *A. polynesiensis* mosquitos and volunteers with different microfilarial counts, and recorded the mosquito infection rates from carriers with various microfilarial levels (*7*). When their data were analysed on log-log paper, it was revealed that the microfilarial counts (mf/ml) of the blood donors and the corresponding infection rates of the mosquitos (observed percentage + 1) gave a close fit to a linear relationship (Fig. 3). The regression line was calculated as:

$$\log (Y + 1) = 0.5278 \log X + 0.1739$$

where X is the mf count of the blood donor and Y is the percentage infected. This formula was used to calculate the theoretical values for the mosquito infection rates in formulae (A) and (B) (Table 2, column 2).

The distribution of the microfilarial counts of the carriers determined by the nuclepore method was then analysed using the negative binomial distribution proposed by Pichon et al. (*8*) as a better and more precise alternative to the log-normal distribution. Based on the results of 358 mf-positive subjects with valid nuclepore counts, this method was used to estimate the theoretical number of carriers having specified microfilarial counts or falling within specified ranges of such counts (Table 2, column 3). These numbers can be used for the numbers of carriers in formulae (A) and (B).

Table 2 shows the calculations. The contribution of the low-density carriers to the total mosquito infectivity was only 251.2/11645.8 or 2.16%.

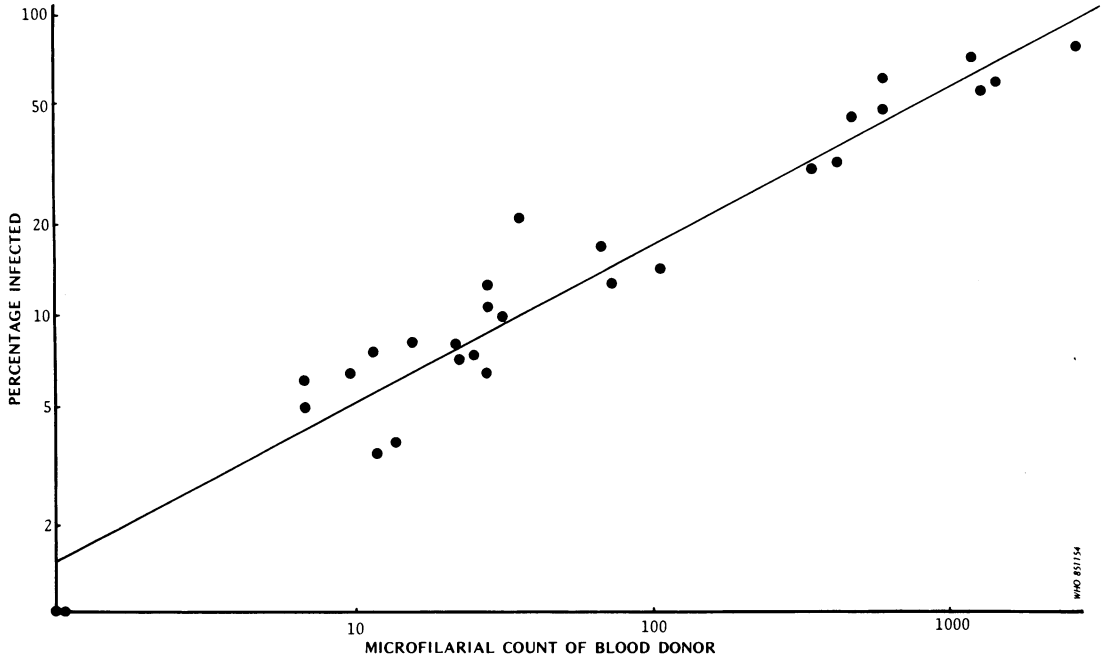

Fig. 3. Infection rate of *Aedes polynesiensis* mosquitos fed on carriers with different microfilarial counts.

Table 2. Mosquito (*Aedes polynesiensis*) infectivity produced by the low-density microfilaria carriers

Microfilarial count (mf/ml)	(I) Theoretical percentage of infected fed mosquitos[a]	(II) Theoretical number of mf-positive persons in each mf-density group[b]	(I) x (II)
1	0.492	13.214	6.5
2	1.152	8.584	9.9
-4	2.102	11.999	25.2
-6	2.842	8.774	24.9
-8	3.473	7.074	24.6
-10	4.032	6.000	24.2
-20	6.254	21.729	135.9
		Total:	251.2-----(B)[c]
-30	7.985	15.076	120.4
-40	9.458	11.854	112.1
-50	10.766	9.892	106.5
-60	11.954	8.550	102.2
-70	13.052	7.565	98.7
-80	14.078	6.805	95.8
-90	15.045	6.198	93.2
-100	15.963	5.700	91.0
-200	23.456	41.077	963.5
-300	29.292	26.579	778.6
-400	34.259	19.642	672.9
-500	38.666	15.443	597.1
-600	42.673	12.589	537.2
-700	46.374	10.511	487.4
-800	49.834	8.925	444.8
-900	53.094	7.675	407.5
-1000	56.187	6.666	374.5
-2000	81.449	36.479	2971.2
-3000	100 (101.123)	13.242	1324.2
-4000	100 (117.869)	5.524	552.4
⩾4001	100 (-)	4.634	463.4
		Total:	11394.6
		358.000	11645.8-----(A)[c]

[a] Estimated from log (Y + 1) = 0.5278 log X + 0.1739 where Y = % infected, X = mf count.
[b] Estimation made by Kimura et al. (*5*) by applying the negative binomial distribution.
[c] (B)/(A) = 0.0216 (2.16%).

Change of microfilarial counts over time among untreated low-density carriers. In 158 persons shown to be mf-positive by the nuclepore method, there was a delay of from 60 to 252 days between the initial blood examination and a second blood examination made immediately before starting DEC-C treatment. In Table 3, these persons were classified according to their initial microfilarial count, and the time interval before the second examination and the change in the count are given. Among the low-density carriers, a mean increase of 35.5% in the microfilarial count was obtained and, while this is not statistically significant ($0.1 < P < 0.2$), it is very different from the amount of change in the other categories, where the greatest change was 3.4%.

To allow for the varying intervals before treatment, regression analysis of change in the microfilarial count, which is expressed as log (initial mf count + 1)-log (final mf count + 1), over time (days from initial count to final count) was performed. These results are also shown in Table 3. Again, only in the low-density carriers was there a nearly significant

Table 3. Change in microfilarial count over time in untreated persons in relation to the initial microfilarial count determined by the nuclepore method

Initial microfilarial count	No. of subjects	Mean time interval (days)	Change in mean microfilarial counts			
			Initial count[a]	Final count[b]	Percentage increase	Level of significance
1–20	39	149.5 (57.1)[c]	7.26	9.84	35.5	$0.1 < P < 0.2$
		[$y = 0.317 - 0.00301x$; $r = -0.299$; $0.05 < P < 0.10$][d]				
21–100	38	144.0 (56.1)[c]	48.3	48.9	1.2	$P > 0.5$
		[$y = 0.128 - 0.000926x$; $r = -0.093$; $P > 0.5$][d]				
101–500	49	150.4 (55.5)[c]	231.4	239.2	3.4	$P > 0.5$
		[$y = 0.104 - 0.000785x$; $r = -0.103$; $P > 0.5$][d]				
501–1000	17	156.5 (53.6)[c]	659.7	671.6	1.8	$P > 0.5$
		[$y = 0.122 - 0.000827x$; $r = -0.267$; $0.2 < P < 0.5$][d]				
⩾1001	15	169.9 (44.2)[c]	1353.3	1355.3	0.1	$P > 0.5$
		[$y = -0.0370 + 0.000214x$; $r = 0.032$; $P > 0.5$][d]				
Total	158	151.2 (54.7)[c]	89.4	97.8	9.4	$0.2 < P < 0.5$
		[$y = 0.160 - 0.00132x$; $r = -0.153$; $0.05 < P < 0.10$][d]				

[a] Geometric mean of (initial mf count + 1).
[b] Geometric mean of (final mf count + 1).
[c] These figures in parentheses indicate the standard deviation.
[d] Results of regression analysis of change in mf count over time;
x = time interval (days from initial count to final count);
y = measure of mf change expressed as log (initial count + 1) − log (final count + 1).

degree of regression of change over time ($0.05 < P < 0.1$), indicating that the increase in the microfilarial count was progressive.

A more intensive examination of this category of mf-positive subjects is set out in Table 4, where the low-density carriers have been divided according to age into those under 30 years old and those of 30 years and above. It appears that among the younger people the 132% increase was only just significant ($P = 0.05$), but there is no suggestion that this was progressive with time. In contrast, among older people, the average count was much more stable but it was found to vary with time.

Production of low-density carriers by DEC-C treatment. A total of 112 subjects positive by the nuclepore

Table 4. Change in microfilarial count over time in untreated low-density carriers according to the age of the subjects

Age group (years)	No. of subjects	Mean time interval (days)	Change in mean microfilarial counts			
			Initial count[a]	Final count[b]	Percentage increase	Level of significance
Under 30	10	157.0 (65.7)[c]	6.25	14.5	132	$P = 0.05$
		($y = -0.379 + 0.0000907x$; $r = 0.110$; $P > 0.5$)[d]				
Over 30	29	146.9 (54.9)[c]	7.64	8.61	12.7	$P > 0.5$
		($y = 0.562 - 0.00418x$; $r = -0.397$; $0.02 < P < 0.05$)[d]				

[a] Geometric mean of (initial mf count + 1).
[b] Geometric mean of (final mf count + 1).
[c] Figures in parentheses indicate the standard deviation.
[d] Results of regression analysis of change in mf count over time;
x = time interval (days from initial count to final count);
y = measure of mf change expressed as log(initial count + 1) − log(final count + 1).

method were treated with DEC-C in a single dose (6 mg/kg body weight) and assessed by the same method 6 months later. As a result of this treatment, 17 out of 83 subjects with a microfilarial count of 21 or more per ml (20.5%) had become low-density carriers, and 9 out of 29 previously low-density carriers (31.0%) remained in the same category. In the whole series, the situation had changed from 29 low-density carriers among 112 mf-positive subjects (25.9%) before treatment to 26 low-density carriers among 76 mf-positive subjects (34.2%) after the treatment (Table 5).

Table 5. Production of low-density microfilaria carriers after treatment with DEC-C in a single dose (6 mg/kg body weight) in 112 patients

	Microfilarial count (mf/ml)		
	1–20	Over 20	Total
No. of patients before treatment	29	83	112
No. of patients 6 months after treatment:			
Negative	17	19	36
1–20 mf/ml	9	17	26
Over 20 mf/ml	3	47	50

DISCUSSION

There have been several previous attempts to define "low density" of microfilaraemia. Desowitz & Southgate (*9*), Oemijati et al. (*10*), and Sajidiman et al. (*11*) used the term "low-density" for counts detectable by the 1-ml millipore membrane filter concentration technique but not by the conventional blood smear (20 or 60 mm^3) method. Bryan & Southgate (*12*) used the term "ultra-low-density" carriers for those who had 2–8 mf/ml, while Shibuya[a] defined it as 10 mf/ml or under. Desowitz et al. (*13*) used 25 mf/ml and under as categorizing "occult microfilaraemia" for the purpose of clinical immunological studies. The present study defines the top level of low-density as 20 mf/ml and it is suggested that this may be a suitable level to adopt as a standard in future.

The prevalence study revealed the fact that only 23.6% of the total positives were low-density carriers and that the proportion of these carriers tended to decrease as the prevalence rate in the village increased. This may correspond to our observation that microfilarial density increased in direct proportion to the prevalence rate (*5*). Our study also revealed that more low-density carriers occurred among younger people (under 20 years) and older people (60 years and upward) than in those in between.

Taking account of the vectorial capability of the Samoan vector *A. polynesiensis* and the population size of the human reservoir, the proportion of mosquito infectivity produced by the low-density carriers was calculated by applying the basic concept of the infectivity index (*14*). The results showed that the contribution of low-density carriers to the total mosquito infectivity was only 251.2/11645.8 or 2.16%, which suggests that these carriers are of minor (if not negligible) importance in the transmission of filariasis. More studies are necessary to determine whether this conclusion is true in areas where the pattern of endemicity, mosquito species, and vector-human contact differ from those in Samoa.

The change of microfilarial counts over time is another essential factor in assessing the importance of the low-density carrier group. The speed and degree of the change will undoubtedly depend on the intensity of infection. In Samoa, where the endemicity was considered to be low, the change of counts was not so remarkable within the limited observation period of up to 252 days. The high-count carriers remained with largely the same counts at the two separate determinations. On the other hand, the low-density carriers showed a fairly clear increase (36%) in the microfilarial count during the observation period, although this was not actually significant ($0.1 < P < 0.2$). The younger low-density carriers (under 30 years of age) showed a clear 132% increase in counts ($P = 0.05$), suggesting the potential danger of these people as future sources of transmission.

The increase of microfilarial count in the low-density carriers under 30 years old was found not to be progressive with time, but presumably there was an increment in the count from a low density to a high density. In contrast, among older people, the average count was much more stable but it varied with time. An explanation for this is not known.

Another useful result obtained in this study is that 6 out of 39 low-density carriers (15.4%), who had been left untreated for 60–252, days were found to be negative at the second examination. When the microfilarial count was 21–100/ml, only one out of 38 (2.6%) became negative. This does not necessarily mean that the microfilariae had been eliminated from these people without treatment but rather suggests that the detection of low-density microfilaraemia is not completely reliable even when the nuclepore method is used. Kimura et al. reported that this method could give a false negative result in about 10% of the number of cases detected using it (*5*).

[a] Shibuya, T. *WHO assignment report (Samoa)*, 1978 (unpublished).

Our study suggests that the DEC-C single-dose treatment at 6 mg/kg body weight did not give rise to many low-density carriers when the examination was made 6 months after the treatment. This may be because nearly 60% of the original low-density carriers (17 out of 29) became negative and because the high-count carriers tended to remain in the high-count range even after the treatment.

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RÉSUMÉ

MICROFILARÉMIE DE FAIBLE DENSITÉ DANS LA FILARIOSE SUBPÉRIODIQUE À *W. BANCROFTI* AU SAMOA

Les techniques de filtration sur membrane utilisant 1 ml de sang veineux sont d'une très bonne sensibilité pour détecter les porteurs de microfilaires (mf) ayant de faibles densités microfilariennes, ce qui serait impossible avec les frottis de sang habituels. Les très bons résultats de ces techniques de filtration dans les enquêtes sur le terrain, aboutissant à la détection de nombreux porteurs de faibles densités de microfilaires, soulèvent inévitablement la question du rôle épidémiologique de ces porteurs dans la transmission de la filariose.

Au Samoa, où la filariose due à *W. bancrofti* est endémique, une étude de prévalence microfilarienne a été effectuée en 1979, portant sur 7430 sujets, dans 28 villages, en utilisant à la fois la filtration sur membrane Nuclepore et la méthode des frottis après piqûre au doigt. Dans cet article sur la microfilarémie de faible densité, on a utilisé les données provenant de cette étude de prévalence, et les résultats du traitement des sujets reconnus mf-positifs par le citrate de diéthylcarbamazine (DEC-C).

La microfilarémie a été considérée comme étant de faible densité lorsqu'il y avait un taux de 1-20 mf/ml et les porteurs de faibles densités ont été étudiés en tenant compte de l'âge, du sexe, et du village, en fonction du taux de prévalence microfilarienne. Il y avait plus de porteurs de faibles densités chez les moins de 20 ans et chez les plus de 60 ans que dans les autres groupes d'âge. La proportion, dans un village, de porteurs de faibles densités de microfilaires par rapport au total des sujets mf-positifs, diminuait quand le taux de prévalence de *Wuchereria bancrofti* augmentait.

Le rôle épidémiologique des porteurs de faibles densités a été évalué en fonction de l'infectiosité des moustiques vecteurs (*Aedes polynesiensis*) qui leur était due, du passage possible de ces porteurs de faibles densités à porteurs de fortes densités, et du nombre de nouveaux porteurs de faibles densités apparus après traitement par le DEC-C. L'infectiosité des moustiques due à des porteurs de faibles densités ne représentait que 2,16% du total de l'infectiosité due à tous les porteurs, ce qui laisse à penser que ces porteurs ne jouent qu'un rôle mineur dans la transmission de la filariose. Il n'y a pas eu de changement notable de la numération des microfilaires avec le temps chez les sujets mf-positifs non traités, pendant une période d'observation de 60 à 252 jours. Cependant, on a observé une augmentation moyenne de 36% dans le groupe de porteurs de faibles densités, et une augmentation de 132% chez les plus jeunes d'entre eux (au-dessous de 30 ans). L'apparition de porteurs de faibles densités après traitement par une dose unique de DEC-C (6 mg/kg) n'a pas été aussi importante qu'on pouvait l'attendre.

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