STUDIES ON FILARIASIS IN THE PACIFIC

3. COMPARATIVE EFFICACY OF THE STAINED BLOOD-FILM, COUNTING-CHAMBER AND MEMBRANE-FILTRATION TECHNIQUES FOR THE DIAGNOSIS OF *WUCHERERIA BANCROFTI* MICROFILARAEMIA IN UNTREATED PATIENTS IN AREAS OF LOW ENDEMICITY

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INTRODUCTION

The microfilarial density of infected individuals may range from 0 to several hundred or more per unit volume of blood in a population subject to endemic filariasis; the density distribution curve varying according to the existing conditions of endemicity. Several authors (Kessel, 1957; Sasa, 1967, 1969) have devised mathematical treatments of blood survey data to describe the epidemiology of the infection. These authors have recognized, however, that because "cases with demonstrable microfilaremia are only a fraction of people who are actually infected with the parasite" (Sasa, 1969) their mathematical models have been imperfect epidemiological descriptions.

Calculation of the 2 epidemiologic parameters, microfilarial rate and microfilarial density is almost invariably based on data provided by examination of stained thick blood films, often 20 c.mm spots, although in some instances 60 c.mm has been routinely taken. Undoubtedly this conventional parasitologic method, particularly when performed under field conditions, is not sufficiently sensitive to detect many of the low-grade microfilaraemias present in the population. While concentration methods employing larger amounts of blood might reveal many of the low-density micfofilaraemias, they have rarely been applied to epidemiological surveys due mainly to difficulties in carrying out the procedure under field conditions, and in persuading populations in many parts of the world to undergo venepuncture.

In the preceding papers of this series, it was shown (Southgate, 1973; Desowitz and Southgate, 1973) that a counting-chamber technique (Denham *et al.*, 1971) was an efficient epidemiological tool, and that a modified membrane-filtration technique (Chularerk and Desowitz, 1970; Desowitz, 1971) could be easily carried out under tropical field conditions and was highly sensitive in detecting low-grade microfilaraemias persisting after a therapeutic course of diethylcarbamazine.

The present report describes our experience with these three methods in a survey of some untreated populations on Viti Levu Island, Fiji, in areas where filarial endemicity is low.

MATERIALS AND METHODS

A total of 284 blood samples was obtained from adults living in a number of villages of northern and eastern Viti Levu. These villages had not been subject to mass drug administration with the exception of Bivienua

village (Tailevu province) where diethylcarbamazine had been given in 1953. A 1 ml heparinized or citrated venous-blood sample was taken from all individuals and concentrated by the membrane-filtration method described previously (Desowitz, 1971; Desowitz and Southgate, 1973). Simultaneously, three 20 c.mm spots of finger-puncture blood were made and dried overnight, stained with Giemsa, and examined for microfilariae at × 40 magnification. Concurrent chamber examination (Denham *et al.*, 1971; Southgate, 1973) of 60 c.mm finger-puncture blood was made on 83 of the 284 blood samples. Since the Fijian *Wuchereria bancrofti* is sub-periodic or aperiodic, blood samples were collected during the day, between the hours of 1000 and 1400. Blood for membrane concentration was usually processed within a few hours of collection although in some instances it was stored overnight in a refrigerator at 5°C.

RESULTS

A comparison of results obtained from the stained thick film and membrane concentration is given in Table 1. The data indicate that filariasis is hypoendemic in the population studied, with a microfilaria rate of 1.1 per cent based on 20 c.mm blood samples and 2.5 per cent based on a 60 c.mm stained blood film of finger-puncture blood. The average microfilarial density for the seven positives was also low, 2.7 microfilariae per 20 c.mm. Membrane filtration detected three times as many infections as did the 60 c.mm stained thick blood film. If only a single 20 c.mm blood film had been examined, the expected number of positives would have been 4.7, using the formula of Sasa (1967), $P_{20} = \frac{1}{3}N_1 + \frac{2}{3}N_2 + N_3$. Thus membrane filtration of 1.0 ml venous blood detected about 4.5 times the number of positives found by a 20 c.mm stained blood film.

Table 1

The comparative microfilaraemia rate of 284 Fijians as detected by the stained thick blood-film and membrane-filtration methods.

	Stained thick blood-film				
	3 out of 3 20 c.mm films	2 out of 3 20 c.mm films	1 out of 3 20 c.mm films	Total blood film	Membrane filtration
Number positive	3	1	3	7	21
Percentage positive	1.1%	0.4%	1.1%	2.5%	7.4%

A total of 58 microfilariae were counted in the 7 positive 60 c.mm thick films whereas the total microfilariae on the membranes prepared from the blood of these same 7 infected individuals was 1360, a 23-fold difference. Since the 1 ml sample for membrane filtration is 16.7 times the volume of the 60 c.mm film, it is probable that some microfilariae were lost in dehaemoglobinizing and staining the slides despite them being prepared and processed by experienced filariasis workers.

Concurrent thick-film, counting-chamber and membrane-filtration examination was made on 83 of the 284 blood samples. The results of these examinations are shown in Table 2.

It is obvious that many low-density microfilaraemias would be missed if the 60 c.mm stained thick film were used as the only method of parasitologic diagnosis. The chamber method of examination, using the same amout of blood from the same source as the thick stained film, revealed 3 times as many infections. In the 83 subjects examined by all three techniques, the ratio of micro-

Table 2

The comparative efficacy of the thick stained blood-film, counting-chamber and membrane-filtration techniques for the diagnosis of microfilaraemia in 83 Fijians.

	Technique of Examination		
	Stained blood-film 60 c.mm finger-puncture blood	Counting chamber 60 c.mm finger-puncture blood	Membrane filtration 1.0 ml venous blood
Number positive	4	12	15
Percentage positive	4.8%	14.5%	18.1%
Total microfilariae counted	28	72	604

Table 3

2 × 2 table showing the overlap between 19 Fijians found microfilaria-positive by membrane - filtration and counting - chamber techniques.

		Membrane filtration		Total
		+ ve	− ve	
Counting chamber	+ ve	8	4	12
	− ve	7	64	71
Total		15	68	83

P = 0.2276.

filariae detected per 60 c.mm blood was—blood film : counting chamber : membrane filtration = 1.0 : 2.6 : 1.3.

The membrane-filtration method gave the highest positivity rate in the present study, but as can be seen from Table 3, there is a considerable overlap between subjects detected as positive by the counting-chamber and membrane-filtration techniques. Using the methods of Buck and Gart (1966) and Gart and Buck (1966) the co-positivity of these two tests is calculated as 0.5333 and the co-negativity as 0.9412, when membrane filtration is assumed to be the reference test and the counting-chamber technique the screening test. Membrane filtration was chosen as the reference test because it detected a proportion of 0.1807 of the total population examined as positive, whereas the counting chamber detected 0.1446 of the population as positive. This difference in proportions is not, in fact, statistically significant, the standardized normal deviate with continuity correction calculated by McNemar's test being only 1.2063, giving a two-tail P value of 0.2276. In view of this lack of significance, the reasons for the surprisingly low co-positivity must clearly be examined very critically; the high value of co-negativity is to be expected from the nature of diagnostic tests which depend on seeing microfilariae, a situation in which false negatives are clearly possible, but false positives can quite reasonably be regarded as impossible when there is no chance of transferring microfilariae between individual blood samples or films.

There are four possible explanations for the discrepancy observed in the results of the two techniques:

(a) differing parasite concentrations in venous and capillary blood, the presumptive retardation of microfilariae in the capillary bed leading to higher counts in capillary blood (Yorke and Blacklock, 1917; Hawking and Thurston, 1951a and b; McCarthy, 1956; Burton, 1964);

(b) technical difficulties resulting from the use of an unsuitable anticoagulant during the first day of work in these comparative studies (Desowitz and Southgate, 1973);

(c) the effect of examining 1.0 ml blood in the membrane-filtration technique, but

only 60 c.mm blood in the counting-chamber technique. This difference, in the ratio 16.67:1.0, could obviously outweigh differences due to the capillary: venous ratio discussed under (a) above;

(d) low co-positivity tends to occur with low prevalence.

Further studies are required to determine which explanation or combination of explanations accounts for the discrepancies observed in our study.

DISCUSSION

The results of this study indicate that under hypoendemic conditions a relatively high proportion of individuals have a low-grade microfilaraemia undetectable by conventional stained thick blood-film examination. The clinical and epidemiological significance of these low-density "carriers" is not known. The clinical manifestations of filariasis may by so protean (Wijetunge, 1967) that a filarial aetiology would not be readily recognizable. That many patients with low-density microfilaraemia or who are amicrofilaraemic have pathophysiological disturbances has recently been stressed by Beaver (1970). It is obvious that accurate parasitological diagnosis of these patients by a sensitive technique such as membrane filtration must precede clinical evaluation.

It is not known with any degree of certainity whether or not low-density microfilaraemias may act as a source of infection to the mosquito vector. Investigations had indicated that a count of at least 10 to 20 *Wuchereria bancrofti* microfilariae per 20 c.mm blood was the minimum density necessary to infect a suitable vector (Rosen, 1955). However the work of Rosen (1955) clearly showed that average densities as low as 0.4 mf per 20 c.mm were infectious for Tahitian *Aedes polynesiensis*. Further investigations are required to determine whether low-level microfilaraemias truly constitute a reservoir of infection. It can be argued that of the mosquitoes feeding on these carriers few would become infected and those that did would harbour only small numbers of infective larvae. In view of the estimate by Hairston and de Meillon (1968) that it requires an average of approximately 15,500 bites by infective mosquitoes to induce one case of microfilaraemia it would seem that the small pool of mosquitoes infected from the "carrier" population would play little or no transmissive role. However, these authors admit that the mathematical model they have constructed may have been imperfect in that: "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." We have now demonstrated that in the hypoendemic situation investigated, 4.5 times more infections were found present by membrane concentration of 1 ml of blood than by the conventional 20 c.mm blood film. Moreover, our study has shown that due to loss of parasites in processing films, thick-film examination may often give a falsely low microfilarial count. Revision of mathematical models of the transmission of filaiarsis may be necessary as a result of these observations.

The application of immunodiagnosis to the epidemiology of filariasis is still another area in which faulty parasitological diagnosis has led to confusion. For example the low regard currently held for the skin test stems mainly from the many "false positive" reactions that have been observed in immuno-epidemiological surveys (Franks *et al.*, 1947; Ciferri *et al.*, 1965; Desowitz *et al.*, 1966; Gidel *et al.*, 1969). Several explanations, such as the presence of microfilaraemia below the threshold of microscopic detection, and antigenic experience with a filaria of non-human origin, have been offered to

account for the large number of reactors without apparent microfilaraemia. Our present study would indicate that many hitherto undiagnosed low-grade microfilaraemias could account for reactions which have been classed as being "false positives". It is strongly recommended that epidemiological studies be carried out in which immunological reactions are correlated with results yielded by the sensitive membrane-filtration and counting-chamber parasitological diagnostic methods.

We have shown that the 20 c.mm stained thick film is the least sensitive epidemiological technique, particularly when carried out under tropical field conditions, for detecting and measuring microfilaraemia. We believe that both the membrane-filtration and counting-chamber techniques should supplant the thick film for epidemiological surveys of populations before and after mass diethylcarbamazine administration. We have found that both methods can easily and rapidly be carried out under field conditions. In addition, a team of trained technicians can prepare and examine as many chamber and membrane samples each day as stained thick films. Recommendation as to the selection of method cannot, as yet, be definitely given. Membrane filtration has produced the highest microfilaraemia rate. However, some individuals with low density microfilaraemia were found to be positive by the chamber method and not by membrane filtration while for other individuals the reverse was true. As stated previously it is not known whether this reflects a difference of distribution of microfilariae in peripheral and venous blood between individuals or was due to a technical difficulty encountered during one phase of the survey. Undoubtedly, for post-therapeutic surveys the membrane filtration technique is by far the most sensitive diagnostic method. A point of interest is that the distribution of microfilariae in the vascular system may be different after diethylcarbamazine treatment than before. Further longitudinal investigations employing the membrane-filtration and counting-chamber methods under conditions of varying endemicity, before and after mass therapy, are planned in order to provide the answers to the problems of filarial epidemiology that this present study has exposed.

SUMMARY

In untreated hypoendemic areas of Viti Levu Island, Fiji, with low microfilarial rates and densities, the membrane-filtration and counting-chamber techniques for the detection and measurement of microfilaraemia were found to be decidedly superior to stained 60 c.mm blood films.

An unexpectedly low co-positivity between membrane-filtration and counting-chamber technique results was observed; possible reasons for this observation are discussed and some recommendations for further research are made.

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