# Hyperendemic subperiodic Bancroftian filariasis: a search for clinical and immunological correlates of microfilaraemia

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*A study was carried out in the Kingdom of Tonga, an area of hyperendemic Bancroftian filariasis, to determine whether correlations could be made between microfilaraemia, as diagnosed by membrane filter concentration, and immunological (skin test, immunoglobulin levels) or clinical findings. There was no relationship between the presence or degree of microfilaraemia and any clinical manifestation or skin test reaction. The skin test positivity rate for microfilaraemic and amicrofilaraemic individuals was approximately the same for all age groups. Among those aged 0 to 4 years, 48% of microfilaria positives were negative in the skin test. The highest average IgG and IgE levels were found in the groups with the highest microfilarial densities, i.e., in children with a history of fever and in adults with a history of lymphangitis/lymphadenitis. Over a period of a year, the microfilarial density changed significantly in 18 (34%) of 53 adults.*

Blood surveys employing membrane filter concentration (MFC) as the diagnostic method have revealed much higher microfilaraemia rates in populations exposed to endemic Bancroftian and Malayan filariasis than previously estimated from examination of stained blood films (*2, 5, 9, 11*). This has been particularly true for younger age groups when age-stratified surveys have been carried out. The microfilaraemia rate for the 5- to 9-year-old age group, the youngest group studied, was 5% or less by blood film diagnosis but 30% to 70% by MFC (*2, 9*). These new findings suggested that it would be of value to carry out a clinical–immunoepidemiological study in an attempt to correlate these factors with microfilaraemia as determined by MFC. For example, a number of investigators, such as Desowitz et al. (*3*), have observed that skin test positivity rates are much higher than the microfilaraemia rates. Would there be a logical reconciliation in these differences if the low grade MFC positives, hitherto undiagnosed, were accounted for; or would it be possible to identify any particular clinical manifestation or group of manifestations conventionally attributed to filariasis with microfilarial density as estimated by MFC?

A previous survey (*2*) had shown that subperiodic Bancroftian filariasis was hyperendemic in Tonga; that country was therefore considered highly suitable for the study described in this paper. Not only could a well-identified population be examined but there would be further advantage in reexamining some of the individuals from the previous study for any changes in microfilarial density that may have occurred. It would also be possible to extend the investigations to include children under the age of 5 years. Since Tonga had not at that time commenced its planned mass drug administration campaign against filariasis, it was envisaged that the results obtained from the proposed study would provide valuable baseline data for the longitudinal assessment of clinical, immunological, and parasitological changes consequent to the control programme.

## MATERIALS AND METHODS

A total of 297 individuals of all ages from Te'ekiu village, Tongatapu Island and 309 from Pangai Island, the Ha'apai Group of Tonga, were exam-

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ined. A citrated venous blood sample was obtained from each individual and 1 ml examined for microfilariae by the MFC method described by Desowitz & Southgate (*4*). An aliquot of plasma was stored at —20°C in Tonga and subsequently at —70°C in Hawaii for further serological study.

Each individual was skin tested with Sawada's FST 3-1 *Dirofilaria immitis* antigen obtained from the World Health Organization and the test performed according to their recommendations. The reaction was determined 15–20 min after injection by outlining the wheal and flare with a ball-point pen, transferring the ink to a piece of alcohol-moistened filter paper, and measuring the maximum diameter of the reaction.

On a form prepared for this survey, the following data were entered: name, age, sex, occupation, height, weight, information on the clinical history, and the findings of a physical examination. The clinical history (provided by the parents for children) sought information on the occurrence of fever, recurring lymphangitis, lymphadenitis, abcesses, and chyluria. Those giving affirmative answers were further questioned as to age of onset and frequency of attacks. The individuals were asked whether they considered themselves to have " kulakula " (the Tongan term for filariasis, which literally means " hot and red rash ") and if so the reasons for this belief. Each individual was examined for the presence of lymphadenopathy, lymphangitis, lymphoedema, hydrocele (in males), and elephantiasis.

Plasma IgG, IgM, IgA, and IgD levels were determined using immunoplates supplied by Meloy Laboratories, Springfield, VA, USA and that of plasma IgE using plates supplied by Hyland Laboratories, Costa Mesa, CA, USA.

## RESULTS

### *Comparison of microfilarial densities in some adult Te'ekiu villagers in 1972 and 1973*

Of the 53 adult Te'ekiu villagers whose blood was examined by MFC in August 1972, 30 were reexamined by this method 1 year later. In 1972, twelve were amicrofilaraemic but in 1973, microfilariae were detected in five of these individuals with densities of 1, 1, 2, 19, and 77 microfilariae/millilitre (mf/ml). The other seven remained negative. Two individuals with 1 and 2 mf/ml in 1972 were negative in 1973. Four persons with moderate microfilaraemia (100–300 mf/ml) and nine with high microfilaraemia had about the same densities in both years. Two persons had much higher counts in 1973 than in 1972 (43–446 mf/ml and 2–226 mf/ml), while the count fell in one individual from 36 to 2 mf/ml. Thus the microfilarial density remained essentially the same in 66% of the group, changed from positive to negative in 17%, changed from negative to positive in 7%, showed an increase in density in 7%, and showed a fall in count in 3.5%.

### *Microfilaraemia*

The findings relating to microfilaraemia are summarized in Table 1. The microfilaraemia rates and densities were similar in the Te'ekiu and Pangai populations and the data for these groups were combined. There were no significant differences in rates or densities between the sexes in any age group. We used the level of <25 mf/ml as categorizing " occult " microfilaraemia, since previous experience had shown that below this density very few infections are detected by examination of the conventional 20-mm³ stained thick blood film.

The high prevalence in the very youngest groups, from under 1 year to 4 years of age, was remarkable. It appears that the prevalence for all age groups up to 20–49 years is similar and is established by the age of 1 year. It is also of interest to note that the geometric mean density did not markedly increase

Table 1. Microfilaraemia rates and densities in Tongans of different ages

| Age group (years) | No. in group | Percentage microfilaria-positive | Percentage of all positives with < 25 microfilariae/ml | Geometric mean density (microfilariae/ml) |
|---|---|---|---|---|
| <1 | 9 | 44 | 100 | 1.00 |
| 1 | 15 | 53 | 88 | 5.19 |
| 2 | 14 | 21 | 100 | 3.17 |
| 3 | 20 | 45 | 100 | 1.86 |
| 4 | 24 | 33 | 88 | 6.23 |
| 5–9 | 117 | 26 | 87 | 3.82 |
| 10–15 | 103 | 31 | 72 | 8.31 |
| 16–19 | 53 | 42 | 45 | 21.84 |
| 20–49 | 188 | 56 | 42 | 40.18 |
| 50 + | 63 | 75 | 47 | 56.80 |
| total | 606 | 45 | 57 | |

Table 2. Relationship of microfilaraemia to frequency of clinical findings

| Finding | Age group (years) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0-4 | | | 5-9 | | | 10-14 | | | 15-19 | | | 20-49 | | | 50 + | | |
| | mf+[a] | mf−[a] | total | mf+ | mf− | total | mf+ | mf− | total | mf+ | mf− | total | mf+ | mf− | total | mf+ | mf− | total |
| Lymphoedema | | | 0 % | | | 0 % | | | 0 % | | | 0 % | | | 0 % | 5 % | 8 % | 7 % |
| Hydrocele | | | 0 % | | | 0 % | | | 0 % | | | 0 % | 16 % | 46 % | 18 % | 54 % | 69 % | 55 % |
| Lymphangitis | | | 0 % | | | 0 % | | | 0 % | | | 0 % | | | 0 % | 5 % | 0 % | 4 % |
| Lymphadenopathy | 24 % | 55 % | 37 % | 57 % | 53 % | 56 % | 53 % | 48 % | 50 % | 100 % | 29 % | 38 % | 14 % | 18 % | 15 % | 18 % | 0 % | 18 % |

[a] mf + = microfilaria-positive; mf− = microfilaria-negative.

until early adulthood. It is therefore difficult if not impossible to estimate an annual incidence from these data. It is also impossible to determine whether the higher average microfilaraemia of the older age groups is due to superinfection or to suppression of an immunological control directed against the microfilariae.

The earliest infections detected were in two infants, one 6 and the other 8 months old. The density in both cases was 1 mf/ml. The majority of the microfilaraemias up to the age of 15 years were of an occult nature, although there were individuals in the 0- to 4-year-old groups with densities greater than 2000 mf/ml. While the average density was highest in adults, even in these groups over 40% of the microfilaraemias were at an occult level.

*Clinical findings*

A summary of the clinical findings is given in Table 2. Lymphoedema, hydrocele, and lymphangitis were present only in adults, particularly those over 50 years of age. Hydrocele was the most common concomitant of infection, 55% of males over 50 years of age being affected. No correlation could be made between the presence or absence of microfilaraemia or the degree of microfilaraemia and any of the physical findings. Thus, in men over 50 years of age, 2 out of 3 who were negative for microfilaria had hydroceles as did 6 out of 8 with microfilarial densities over 1000 mf/ml.

Children had the highest frequency of adenopathy. Again, no correlation could be found between adenopathy and microfilarial positivity or density. Of the enlarged lymph glands detected in children, 12% were axillary, 16% epitrochlear, 24% cervical, and 49% inguinal.

Table 3 summarizes the responses elicited by interview regarding the perceived history of filariasis (" kulakula "). Two pictures of " kulakula " emerge from the histories: in children, a syndrome of rash and skin abscesses without fever; in adults, fever and rigors, the onset of which was said to occur usually just before a rainstorm. In addition, some adults reported rash and other signs, e.g., lymphangitis and oedema, which they associated with the condition. A considerable number of adults stated that they believed they had "kulakula" because they developed a fever and rash after eating octopus or, in a few instances, other foods.

Table 3. Percentage of interviewees giving a positive history of filariasis reporting different clinical conditions

| Clinical condition | Age group (years) | | | | | |
|---|---|---|---|---|---|---|
| | 0-4 | | 20-49 | | 50 + | |
| | mf +[a] | mf−[a] | mf + | mf− | mf + | mf− |
| Fever | 0 | 0 | 76 | 77 | 81 | 25 |
| Lymphadenopathy | 0 | 0 | 41 | 62 | 81 | 38 |
| Lymphangitis | 0 | 0 | 44 | 54 | 56 | 31 |
| Lymphoedema | 0 | 0 | 24 | 23 | 37 | 16 |
| Chyluria | 0 | 0 | 7 | 0 | 18 | 0 |
| Skin abscesses | 0 | 83 | 58 | 68 | 79 | 67 |
| Rash | 0 | 33 | 95 | 100 | 69 | 57 |
| No. of interviewees giving a positive history of filariasis | 0/29 | 6/49 (13 %) | 17/78 (22 %) | 13/61 (21 %) | 16/41 (39 %) | 4/13 (31 %) |

[a] mf + = microfilaria-positive; mf− = microfilaria-negative.

Table 4. Percentage positive skin test reactions in microfilaria-positive and negative groups of various ages

| Age group (years) | Positive reactions (%) | |
|---|---|---|
| | microfilaria-positive | microfilaria-negative |
| 0–4 | 52 | 50 |
| 5–9 | 72 | 75 |
| 10–15 | 84 | 86 |
| 16–20 | 86 | 97 |
| 21–50 | 84 | 90 |
| < 50 | 94 | 94 |

*Skin test reactions*

Skin test positivity rates for MFC-diagnosed microfilaraemic and amicrofilaraemic groups of various ages are shown in Table 4; the rates for both groups were essentially the same at all ages. For all age groups, there were a number of skin test-negative individuals who had a demonstrable microfilaraemia. This was particularly true of those in the younger age groups: 48% of those 0–4 years old and 28% of those 5–9 years old.

The wheal size of the positive skin test reactions did not appear to be related to age, to the presence or absence of microfilaraemia, to the degree of microfilaraemia, or to any aspect of clinical status.

*Immunoglobulin levels*

Table 5 shows immunoglobulin levels reported by other investigators for normal individuals and for those with endemic filariasis. The average immunoglobulin levels according to parasitological and clinical status in Tongan children are shown in Table 6 and those of adults in Table 7. IgG and IgE levels were higher than values considered normal for populations of the temperate zone. Although these high levels are to be expected where intestinal parasites and infectious diseases are prevalent, filarial infection appears to have some additional influence. For both adults and children, the average IgG and IgE levels were higher in the groups with moderate to high microfilaraemia than in those with occult or low microfilaraemia. There was no apparent relationship between clinical status and immunoglobulin levels, with the possible exception of a very high average IgE level in adults with a history of lymphangitis/lymphadenitis.

## DISCUSSION

There is, at present, no convincing explanation of the underlying mechanisms of pathogenesis in

Table 5. Immunoglobulin levels in normal individuals and in filariasis patients

| | IgG (g/100 ml) | IgM (g/100 ml) | IgA (g/100 ml) | IgD (mg/100 ml) | IgE (IU/ml) |
|---|---|---|---|---|---|
| Normal adult range given by Meloy Laboratories Inc. | 0.77–1.13 | 0.09–0.17 | 0.08–0.2 | 0–36 | |
| Normal adult range given by Hyland Laboratories | | | | | 15–800 |
| American Caucasian children aged 5-17 years (ref. *10*) | 1.01 | 0.092 | 0.120 | | 69 |
| American-born Filipino children aged 5-17 years (ref. *10*) | 1.30 | 0.141 | 0.195 | | 227 |
| Tahitians [a] | level 1.7 times higher in mf+ than in mf− subjects [c] | no difference between mf+ and mf− subjects [c] | no difference between mf+ and mf− subjects [c] | | higher in mf+ than in mf− subjects [c] |
| Onchocerciasis patients of all ages, Republic of Chad [b] | 2.779 | 0.383 | 0.180 | 9.1 | 7823 |

[a] Moreau, J. P. et al. Unpublished document WHO/FIL/72.100; WHO/PDI/72.4.
[b] Buck, A. A. et al. Unpublished document WHO/ONCHO/72.89; WHO/PDI/72.3.
[c] mf+ = microfilaria-positive; mf− = microfilaria-negative.

Table 6. Average immunoglobulin levels in Tongan children aged 1–12 years, according to parasitological and clinical status

| Group | No. in group | IgG (g/100 ml) | IgM (g/100 ml) | IgA (g/100 ml) | IgD (mg/100 ml) | IgE (IU/ml) |
|---|---|---|---|---|---|---|
| All subjects | 36 | 1.93 | 0.20 | 0.18 | 9.88 | 1885 |
| *Parasitological status* | | | | | | |
| Microfilaria-negative | 14 | 2.16 | 0.22 | 0.18 | 4.94 | 1673 |
| Microfilaria-positive | 22 | 1.78 | 0.18 | 0.17 | 13.03 | 2009 |
| occult | 17 | 1.73 | 0.19 | 0.17 | 15.68 | 1832 |
| moderate to heavy | 5 | 1.97 | 0.17 | 0.15 | 4.02 | 2615 |
| *Clinical status* | | | | | | |
| No clinical signs or history | 8 | 1.73 | 0.20 | 0.17 | 7.00 | 2123 |
| History of fever | 4 | 2.45 | 0.20 | 0.17 | 7.05 | 2850 |

lymphatic filariasis. Moreover, there is no general agreement as to what constitute all the manifestations and pathological alterations of filarial disease. There is, however, a consensus that lymphatic disease leading to lymphangitis/lymphadenitis, lymphoedema, hydrocele, and elephantiasis is an insidious process and is rarely present in younger age groups despite, as has been shown, an infection rate in children almost equal to that in adults. The customary measure of filariasis is microfilaraemia and it is with this that attempts have been made to correlate clinical and immunological responses. Other studies have shown there to be little or no association between microfilarial and clinical prevalence. In endemic areas, lymphatic disease has been shown to occur with almost equal frequency in amicrofilaraemic and microfilaraemic adults (*7*, *8*). The present study, in which a sensitive diagnostic method has been used to detect microfilariae, confirms these earlier findings. No correlation could be shown between the presence or absence of microfilaraemia or the degree of microfilaraemia and any one manifestation or group of manifestations attributed to filariasis.

Table 7. Average immunoglobulin levels in Tongan adults aged over 20 years, according to parasitological and clinical status

| Group | No. in group | IgG (g/100 ml) | IgM (g/100 ml) | IgA (g/100 ml) | IgD (mg/100 ml) | IgE (IU/ml) |
|---|---|---|---|---|---|---|
| All subjects | 70 | 1.90 | 0.13 | 0.17 | 5.19 | 1472 |
| *Parasitological status* | | | | | | |
| Microfilaria-negative | 23 | 1.86 | 0.16 | 0.20 | 5.36 | 1671 |
| Microfilaria-positive | 47 | 2.01 | 0.12 | 0.18 | 5.06 | 1328 |
| occult to low | 22 | 1.79 | 0.12 | 0.14 | 4.82 | 1222 |
| moderate to high | 25 | 2.21 | 0.12 | 0.22 | 5.58 | 1805 |
| *Clinical status* | | | | | | |
| No clinical signs or history | 22 | 2.02 | 0.16 | 0.18 | 5.90 | 1480 |
| Hydrocele | 12 | 2.02 | 0.07 | 0.19 | 3.85 | 1200 |
| History of lymphangitis/ lymphadenitis | 6 | 2.02 | 0.14 | 0.20 | 8.38 | 2366 |
| History of fever | 13 | 1.82 | 0.10 | 0.18 | 5.54 | 1500 |

The immunological techniques employed in this study were the skin test and quantitation of serum immunoglobulin levels. No relationship could be identified between skin test reactivity and the presence or absence of microfilaraemia, the degree of microfilaraemia, or clinical status. For each age group there were a number of individuals with microfilaraemia who were negative in the skin test, the highest percentage of " false negatives " being among children 0–4 years of age. This is consistent with the findings of other workers such as Desowitz et al. (*3*). IgE levels were not lower in these " anergic " individuals than in microfilaraemic subjects giving a positive skin test. An important role of the skin test would be to detect any continuing transmission following an anti-filariasis campaign. However, since a large proportion of the children—the sentinel group for transmission—remained non-reactive, the usefulness of the test was greatly reduced.

No distinct relationship could be discerned between any immunoglobulin level and either clinical or parasitological status. A possible exception was the observation that IgE levels were highest in the groups of adults and children with high microfilaraemia. That some serological correlates to microfilaraemia exist is indicated by the results of a test for precipitating antibody by counter-immunoelectrophoresis with parasite antigens derived from various developmental stages (*6*). It was observed that Tongan adults with no or occult microfilaraemia and dogs similarly infected with *D. immitis* had antibody to microfilarial antigen only, whereas those humans and canines with high microfilaraemia had antibody against antigens derived from the adult filariae. A similar stage-specific immunological pattern has been found to occur in cats with sustained and with resolved *Brugia pahangi* microfilaraemia (*1*).

It is of interest that a considerable number of adults stated that they believed they had filariasis because they developed " heat and rash " after eating octopus or, in a few instances, other foods. This was probably a confusion of a common set of symptoms induced by different agents. Nevertheless, the possibility that filariasis may potentiate homocytotropic antibody production and lead to heterologous hypersensitivity should not be dismissed.

Our study also shows that one cannot elucidate the mechanisms of filarial pathogenicity by " point prevalence " determination of parasitological, immunological, and clinical factors. It is believed that only long-term longitudinal studies beginning in infancy will contribute to solving these problems. Filariasis is not a static condition; we have shown that even microfilarial density may alter greatly over a period of a year in many individuals. The reason that some people tolerate infection while others develop disease can probably only be determined by following the life histories of the host in association with the parasite.

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## RÉSUMÉ

### LA FILARIOSE HYPERENDÉMIQUE SUBPÉRIODIQUE À *WUCHERERIA BANCROFTI*: RECHERCHE DE CORRÉLATION ENTRE LA MICROFILARÉMIE ET LES SIGNES CLINIQUES ET IMMUNOLOGIQUES

Une étude a été effectuée au Royaume de Tonga, zone d'hyperendémie de la filariose à *Wuchereria bancrofti*, pour déterminer s'il existait des corrélations entre la microfilarémie, telle que peut la diagnostiquer la concentration sur membrane filtrante, et les observations immunologiques (épreuve cutanée, taux d'immunoglobuline) ou cliniques. On n'a constaté aucune relation entre la présence ou le degré de la microfilarémie et une quelconque manifestation clinique ou réaction à l'épreuve cutanée. Le taux de positivité à l'épreuve cutanée pour les individus microfilarémiques et amicrofilarémiques était à peu près le même pour tous les groupes d'âge. Chez les individus âgés de 0 à 4 ans, 48% des sujets positifs à l'égard des microfilaires étaient négatifs à l'épreuve cutanée. Les taux moyens les plus élevés d'IgG et d'IgE ont été observés chez les groupes présentant les densités microfilariennes les plus fortes, c'est-à-dire chez les enfants ayant eu de la fièvre et chez les adultes ayant été atteints de lymphangite lymphadénite. Sur une période d'un an, la densité microfilarienne a changé de façon significative chez 18 adultes (34%) sur 53.

## REFERENCES


1. DESOWITZ, R. S. ET AL. Studies with *Brugia pahangi*. 17. Precipitation antibody in infected cats detected by counterimmunoelectrophoresis. *Journal of helminthology* (in press).
2. DESOWITZ, R. S. & HITCHCOCK, J. C. Hyperendemic bancroftian filariasis in the Kingdom of Tonga: The application of the membrane filter concentration technique to an age-stratified blood survey. *American journal of tropical medicine and hygiene*, **23**: 877-879 (1974).
3. DESOWITZ, R. S. ET AL. Studies on the immunoepidemiology of parasitic infections in New Guinea. 1. Population studies on the relationship of a skin test to microfilaraemia. *Annals of tropical medicine and parasitology*, **60**: 257-264 (1966).
4. DESOWITZ, R. S. & SOUTHGATE, B. A. Studies on filariasis in the Pacific. 2. The persistence of microfilaraemia in diethylcarbamazine-treated populations of Fiji and Western Samoa: Diagnostic application of the membrane-filtration technique. *Southeast Asian journal of tropical medicine and public health*, **4**: 179-183 (1973).
5. DESOWITZ, R. S. ET AL. 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* in untreated patients in areas of low endemicity. *Southeast Asian journal of tropical medicine and public health*, **4**: 329-335 (1973).
6. DESOWITZ, R. S. & UNA, S. R. The detection of antibodies in human and animal filariases by counterimmunoelectrophoresis with *Dirofilaria immitis* antigens. *Journal of helminthology*, **50**: 53-57 (1976).
7. FAN, P. C. ET AL. Filariasis on Kinmen (Quemoy) Islands, Republic of China. II. Clinical investigations. *Southeast Asian journal of tropical medicine and public health*, **5**: 398-407 (1974).
8. KATAMINE, D. On the clinical and pathological problems of filariasis. In: *Progress of medical parasitology in Japan, Vol. III*. Tokyo, Meguro Parasitological Museum, 1966.
9. OEMIJATI, S. ET AL. Studies on filariasis in the Pacific. 4. The application of the membrane filter concentration technique to a survey of *Wuchereria bancrofti* filariasis in Kepu District, Jakarta, Indonesia. *Southeast Asian journal of tropical medicine and public health*, **6**: 186-189 (1975).
10. OGEL, H. A. ET AL. Serum IgG, IgA, IgM and IgE levels and allergy in Filipino children in the United States. *Journal of allergy and clinical immunology*, **53**: 213-222 (1974).
11. SAJIDIMAN, H. ET AL. Studies on filariasis in the Pacific. 5. *Brugia malayi* filariasis in treated and untreated populations of South Borneo. *Southeast Asian journal of tropical medicine and public health*, **6**: 190-194 (1975).