

# In *Wuchereria bancrofti* filariasis, asymptomatic microfilaraemia does not progress to amicrofilaraemic lymphatic disease

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<b>Background</b>	In lymphatic filariasis due to <i>Wuchereria bancrofti</i> infections, the relationship between the natural course of infection and development of clinical disease remains controversial. The two hypotheses that are widely considered are (1) microfilaraemia represents an early stage of infection which progresses to amicrofilaraemic clinical disease and (2) microfilaraemia and clinical disease are two sequentially unrelated independent entities of the filarial infection and disease.
<b>Aim</b>	To determine whether microfilaraemic individuals develop lymphatic disease.
<b>Methods</b>	The study was conducted in Sri Lanka during the period 1982 to 1998. There were two components, firstly a cross-sectional study and then a longitudinal study. Microfilaraemia was determined by microscopic examination of night blood films. Microfilaraemia associated anti-filarial antibodies were determined by ELISA. Clinical examinations were performed to determine if the test subjects had evidence of acute and chronic lymphoedema.
<b>Results</b>	Two major observations were made. First, the incidence and development of adenolymphangitis and lymphoedema in microfilaraemic individuals were very rare and these subjects maintained asymptomatic microfilaraemic status for very long periods of time. Second, in contrast to microfilaraemic subjects, the incidence and development of lymphangitis and lymphoedema were significantly higher in amicrofilaraemic anti-filarial antibody-positive subjects.
<b>Conclusion</b>	Microfilaraemia does not represent a precondition to development of clinical disease (except male genital involvement).
<b>Keywords</b>	<i>Wuchereria bancrofti</i> , microfilaraemia, lymphangitis, lymphoedema, elephantiasis
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Lymphatic filariasis caused by *Wuchereria bancrofti* is a major public health problem in many Asian, African and some South American countries with an estimated ~70 million people infected and 750 million at risk.<sup>1</sup> The continual detection of new infections in many of these countries reflects the inadequacy of current control programmes. As complete elimination of the parasite seems unrealistic, two of the most desired objectives of most anti-filarial control programmes are the reduction of the microfilarial reservoir in infected individuals and morbidity in the endemic populations. These require a clear definition of the relationship between clinical disease and microfilaraemia. One school of thought is that microfilaraemia precedes or is a precondition to development of clinical disease.<sup>2–6</sup> Others<sup>7–9</sup> support the opposite view that microfilaraemia and clinical disease are independent events.

If microfilaraemia precedes onset of clinical disease, and as the progress of clinical disease is essentially irreversible, it is predicted that at a population level, the microfilaraemia rate should negatively correlate with age whereas prevalence of clinical disease should positively correlate with age. Many epidemiological studies appear to contradict this prediction. Surveys by Knight *et al.*<sup>10</sup> in Papua New Guinea have shown very high levels of microfilaraemia in 1–4 year olds, followed by a drop in 5–14 year olds and a considerable increase in the 15–45 year olds, with highest rates in the 45+ year olds. In north-eastern Tanzania, microfilaraemia prevalence generally increased with age, but often levelled off in older age groups.<sup>9,11</sup> In an earlier study in Sri Lanka,<sup>12</sup> microfilaraemia rates in the 20–70 year age groups remained almost constant (at ~5%) while the incidence of clinical disease (~3–8%) showed a significant decrease with age, especially after 40 years of age. Similar findings were reported 20 years later.<sup>7</sup> In other endemic foci, the age/sex relationship to microfilaraemia is not clear (e.g. Egypt<sup>13</sup> and

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Haiti<sup>14</sup>). In all these studies, microfilaraemia was rarely seen in chronic lymphatic filariasis except when there was genital involvement. In fact, many studies indicate a positive correlation between microfilaraemia and male genital involvement.<sup>6,7,9,11,15-17</sup>

## Hypothesis, Aims and Conclusions

In bancroftian filariasis, asymptomatic microfilaraemia and amicrofilaraemic clinical disease represent two dichotomous courses of infection rather than sequential events. It is also proposed that while some early clinical cases (e.g. lymphadenitis, lymphoedema) may progress towards chronic disease (e.g. elephantiasis), others (tropical pulmonary eosinophilia [TPE], filarial arthritis, orchitis/hydrocoele) represent independent manifestations of filarial infection.

In order to test the above hypothesis, cross-sectional and longitudinal follow-up studies of both microfilaraemic and amicrofilaraemic individuals were carried out in two *W. bancrofti* endemic locations in Sri Lanka. Data reported in this paper support the hypothesis that microfilaraemia does not precede development of clinical disease.

## Materials and Methods

### Study population

This study was conducted in two *W. bancrofti* endemic areas in Sri Lanka (Kurunegala and Gampaha).<sup>7</sup> A total of 3504 subjects were included in the study. Informed verbal consent was obtained in all cases.

### Determination of microfilaraemia levels and anti-microfilaricidal treatment

Microfilaraemia was determined by microscopic examination of Giemsa stained thick blood films in triplicate (~60 µl blood, 3 drops per smear) obtained between 20.00 and 24.00 hours. To be considered amicrofilaraemic, a subject had to be negative on two consecutive examinations over a period of one week. Where possible, a venous blood sample for serological testing was also obtained from volunteer donors. All microfilaraemic individuals were treated with diethylcarbamazine citrate (DEC) at a dosage of 6 mg/kg bodyweight daily, for 12 days.<sup>1</sup>

### Clinical examinations

Clinical examinations were performed at two levels. The first was a preliminary examination conducted in a house-to-house night blood survey. Specific clinical evidence was sought for adenolymphangitis (retrograde lymphangitis associated with fever), lymphoedema (pitting and non-pitting oedema with loss of skin elasticity and fibrosis in limbs) and elephantiasis (chronic lymphoedema in limbs). Investigations on genital involvement were not performed, as such investigations were not practical in a house-to-house survey and under conditions in which this study was conducted. At the second level of clinical examination, those subjects with suggestive clinical evidence of adenolymphangitis and lymphoedema identified during field surveys were asked to attend a regular anti-filariasis clinic held at the respective base hospitals and an independent assessment was made by a medical officer.

### Serum antibody assays

Microfilaraemia diagnostic anti SXP-1 antibody and total anti-filarial IgG4 antibody to adult *Brugia malayi* soluble antigens were carried out as described previously.<sup>18,19</sup> All sera were tested in triplicate at a single dilution of 1:200. For both assays, an ELISA optical density reading >0.1 was considered positive, which represented the mean +2 SD for non-endemic controls from Sri Lanka.<sup>18,19</sup>

### Data management and analysis

Each individual subject enrolled in the study was assigned a unique identification number. Those found to be microfilaraemic were registered with the Anti-Filariasis Control Programme of Sri Lanka. All data were maintained as Microsoft Excel files. Statistical analysis was performed using StatView® software for Macintosh (Abacus Concepts Inc, Berkeley, CA).

## Results

### Microfilaraemic individuals

#### *Cross-sectional study of microfilaraemic subjects detected during 1982-1994*

A total of 1076 microfilaraemic subjects from the field station Kurunegala were investigated for evidence of clinical filariasis in cross-sectional surveys conducted during the period 1982-1994. These subjects were detected during night blood surveys and represented ~5% of the total population screened.<sup>7</sup> For all subjects, triplicate blood smears were collected on two nights within a week. The microfilaraemia count was the estimated average assuming the examined blood volume was ~60 µl (Table 1). Unambiguous clinical evidence of adenolymphangitis, lymphoedema or elephantiasis was not found in any of the 1076 microfilaraemic subjects.

#### *Follow-up investigation of microfilaraemic subjects from Kurunegala (1987-1998)*

The follow-up examination of microfilaraemic subjects in the endemic location Kurunegala began in 1987. All microfilaraemic subjects detected during the period 1982-1987 (n = 758, Table 1) were enlisted in the follow-up programme. However, only 57 subjects could be found and re-examined during 1992-1998 (Table 2). None of the subjects in the 5-year, 7-year or the 8-year follow-up study developed any evidence of clinical filariasis. In total, in the 11-year follow-up group (1987-1998), only one subject developed adenolymphangitis (1/57, ~2%) (Table 2).

Microfilaraemia associated SXP-1 antibody<sup>18,19</sup> levels were available for all 57 subjects. Forty subjects remained SXP-1 antibody-positive confirming exposure and infection in these subjects. Total IgG4 antibody levels were available for all except the 1992 follow-up batch and was positive for 44 out of the available 47 subjects. Taken together, these antibody determinations confirm recent or current exposure and/or infection in these subjects. Therefore, it was concluded that the observed absence of lymphatic disease in these individuals was not due to lack of exposure or infection.

A second re-examination programme was conducted in the endemic location Gampaha<sup>7</sup> in 1992 (Table 2). While the first location Kurunegala lies outside the traditional filariasis endemic belt in Sri Lanka, the station Gampaha is located

**Table 1** Cross-sectional study of microfilaraemic subjects (n = 1076) 1982–1994

Year detected	Mean age (range) (years)	Total no.	Microfilaraemic count (mean + SD)	No. clinical sx <sup>a</sup>
1982	31.6 ± 18.5 (5–90)	109	276 ± 336	none
1983	22.5 ± 15.6 (6–78)	54	411 ± 693	none
1984	25.6 ± 14.7 (4–61)	109	345 ± 394	none
1985	28.2 ± 15.3 (2–71)	167	350 ± 470	none
1986	26.7 ± 17.2 (3–100)	219	381 ± 527	none
1987	26.4 ± 16.6 (2–78)	100	372 ± 450	none
1988	25.4 ± 15.7 (7–63)	47	384 ± 488	none
1989	31.4 ± 17.8 (5–80)	36	252 ± 204	none
1990	25.2 ± 17.3 (5–62)	28	314 ± 255	none
1991	27.4 ± 15.3 (7–68)	81	300 ± 358	none
1992	32.4 ± 20.8 (7–78)	40	477 ± 624	none
1993	32.2 ± 17.3 (8–79)	31	283 ± 457	none
1994	25.7 ± 13.5 (7–62)	55	805 ± 1298	none

<sup>a</sup> Adenolymphangitis, lymphoedema and elephantiasis. Hydrocoele, epididymoorchitis and funiculitis not included.

**Table 2** Development of clinical disease<sup>a</sup> in microfilaraemic subjects

Study period	No. years	No. subjects <sup>b</sup>	Mf +ve/start <sup>c</sup>	Mf +ve/end <sup>c</sup>	Disease <sup>a</sup> /start <sup>c</sup>	Disease <sup>a</sup> /end <sup>c</sup>
1987–1992	6	14	14/14	5/14	0	0
1987–1994	8	10	10/10	0/10	0	0
1987–1995	9	8	8/8	2/8	0	0
1987–1998	12	25	25/25	17/25	0	1
1986–1992 <sup>d</sup>	6	53	53/53	6/53	0	0
Total		110	110/110	30/110	0	1/110

<sup>a</sup> Adenolymphangitis and lymphoedema only. Genital involvement excluded.

<sup>b</sup> Individual subjects in different follow-up periods, same subject not counted twice.

<sup>c</sup> Start and end of the follow-up period.

<sup>d</sup> Field station Gampaha.

~60 km south west of Kurunegala and is almost at the filariasis endemic belt.<sup>7</sup> Of 144 microfilaraemic subjects identified and treated during 1975–1986 at this location, 53 subjects (37%) were re-examined in 1992. The minimum follow-up period for this group was 6 years (1986–1992). In 1992, 6 subjects remained microfilaraemic, 26 subjects were amicrofilaraemic and SXP-1 antibody negative (i.e. likely absence of active infection) and 21 were amicrofilaraemic, but SXP-1 antibody positive (i.e. active or recent infection). None of these subjects had any evidence of lymphangitis, lymphoedema or elephantiasis, thus confirming the Kurunegala data.

Therefore, the total number of microfilaraemic subjects who were followed up from both stations was 110. The minimum time period was 6 years and the maximum was 11 years. The total number that developed clinical lymphatic disease was 1/110 (<1%). At the end of the study period, 30/110 remained microfilaraemic and 61/110 were SXP-1 antibody positive (evidence of infection).

### Amicrofilaraemic subjects

#### *Cross-sectional study of amicrofilaraemic household contacts of microfilaraemic patients*

Cross-sectional investigations of amicrofilaraemic household contacts of microfilaraemic individuals (n = 244) from Kurunegala

were performed in 1988, 1992 and 1994. This examination was limited to household contacts of microfilaraemic subjects to ensure a comparable level of exposure in the amicrofilaraemic population to that of the microfilaraemic subjects. For all subjects, determination of duplicate night blood microfilaraemia on two nights within a week, SXP-1 antibody levels and a standard clinical examination for adenolymphangitis, lymphoedema and elephantiasis were performed. Of 132 antibody-positive subjects, 4 had evidence of lymphatic disease (3%). Lymphatic disease was not observed in the 112 antibody-negative household contacts (Table 3).

#### *Follow-up investigation of amicrofilaraemic antibody positive subjects (1988–1998)*

During 1988–1998, a total of 80 amicrofilaraemic anti-filarial antibody-positive individuals were followed up. A total of 14 subjects developed lymphatic disease during the study period. Development of clinical disease in this group was 17.5% (14/80). Most importantly, four amicrofilaraemic subjects who became microfilaraemic during the observation period were free of clinical disease. The incidence of clinical disease in the amicrofilaraemic antibody positive subjects (17.5%, 14/80) followed up was significantly higher ( $P < 0.001$ ,  $\chi^2$  test) than that of the similarly investigated microfilaraemic group (1/110, <1%) (Table 4).

**Table 3** Cross-sectional study of amicrofilaraemic subjects; comparison of antibody-negative with antibody-positive subjects

Year of detection	SXP-1 antibody <sup>a</sup>	No.	mean age + SD (years)	No. with clinical symptoms
1988	<0.1	28	17.2 ± 9.6	none
1988	0.4 ± 0.1	09	17.9 ± 3.4	1 lymphoedema
1992	<0.1	57	25.8 ± 14.7	none
1992	0.336 ± 0.187	114	20.7 ± 12.1	2 adenolymphangitis, 1 lymphoedema
1994	<0.1	27	20.9 ± 12.1	none
1994	0.311 ± 0.136	09	22.8 ± 11.2	none

<sup>a</sup> ELISA OD 450 mean ± SD values. Means for <0.1 not given. An OD of 0.1 was the cut-off point for negatives (see text).

**Table 4** Development of clinical disease<sup>a</sup> in amicrofilaraemic antibody-positive subjects

Study period	No. years	No. subjects <sup>b</sup>	Mf +ve/start <sup>c</sup>	Mf +ve/end <sup>c</sup>	Disease <sup>a</sup> /start <sup>c</sup>	Disease <sup>a</sup> /end <sup>c</sup>
1988–1994	6	39	0	0	0	2
1988–1998	10	4	0	0	0	2
1992–1995	3	18	0	0	0	0
1992–1998	6	15	0	0	0	7
1994–1998	4	4	0	0	0	03
Total		80	0/80	4/80	0/80	14/80

<sup>a</sup> Adenolymphangitis and lymphoedema only. Genital involvement excluded.

<sup>b</sup> Individual subjects in different follow-up periods, same subject not counted twice.

<sup>c</sup> Start and end of the follow-up period.

## Clinical filariasis patients

### Cross-sectional survey of clinical filariasis patients

Cross-sectional clinical filariasis surveys of endemic populations in Kurunegala and Gampaha were carried out in 1987 and 1992. A total of 2040 subjects (different from those described earlier) were examined in a house-to-house survey. In all, 69 clinical filariasis patients (excluding male genital involvement) were identified. These 69 patients included 59 cases of lymphoedema, nine cases of lymphangitis and one early case of untreated elephantiasis of the leg. Except for two subjects with lymphoedema, all were amicrofilaraemic by thick blood smear. Thus, the incidence of clinical disease in the endemic population was 3.4%.

## Discussion

Based on data reported in this paper, two conclusions were made. First, in *W. bancrofti* infections, microfilaraemic individuals do not develop clinical lymphatic disease. Two lines of evidence support this conclusion. A cross-sectional study of 1076 microfilaraemic subjects showed absence of adenolymphangitis, lymphoedema or elephantiasis in all of them (0% lymphatic disease). In a follow-up investigation of 110 microfilaraemic subjects up to 11 years, only one subject developed adenolymphangitis (<1%). Twenty seven per cent (30/110) of these subjects remained microfilaraemic during the study period while 55% (61/110) remained microfilaraemia associated SXP-1 antibody-positive confirming infection and/or exposure. The second conclusion was that amicrofilaraemic anti-filarial antibody-positive subjects were more likely to develop clinical lymphatic disease. Evidence supporting the second conclusion was as follows. A cross-sectional study of 132 anti-filarial antibody-positive amicrofilaraemic subjects showed adenolymphangitis

and lymphoedema in 4 subjects (3%) while 112 antibody-negative subjects did not show any signs of adenolymphangitis or lymphoedema (0%). Follow-up investigation of 80 filarial antibody-positive amicrofilaraemic subjects showed development of adenolymphangitis or lymphoedema in 14 (17.5%) during the examination period of up to 10 years. Cross-sectional survey of 2040 individuals in the endemic area in 1987 and 1992 identified a total of 69 lymphangitis and lymphoedema patients who were amicrofilaraemic (except 2) and had no history of microfilaraemia during the preceding 5 years. This represented a prevalence of 3.4% clinical filariasis in amicrofilaraemic subjects.

Taken together, these findings show that development of adenolymphangitis and lymphoedema is strongly associated with amicrofilaraemic infection. In contrast, microfilaraemic individuals are more likely to remain microfilaraemic without developing clinical lymphatic disease. It is concluded that asymptomatic microfilaraemia and amicrofilaraemic clinical disease are independent outcomes of *W. bancrofti* infection and are not sequential events of progressive infection.

Three arguments which may appear to weaken the conclusions made in this study are (1) that the number of microfilaraemic subjects that were followed up was too small, (2) that optical microscopy using night blood is not the most sensitive technique for microfilaraemia detection and (3) that clinical examinations conducted during house-to-house surveys were inadequate. Considering the long follow-up period (up to 11 years), the number of microfilaraemic subjects (Kurunegala, n = 57; Gampaha, n = 53, total n = 110) that were followed up was sufficient to justify the conclusions in this paper. Further, these conclusions were supported by data from the cross-sectional studies. Although night blood microscopy is less sensitive than the Nuclepore<sup>TM</sup> filtration for microfilaraemia, it is widely used and is sufficiently sensitive to detect the majority of

microfilaraemics. Further, in a recent paper,<sup>20</sup> we showed that even polymerase chain reaction (PCR) based techniques are not much more sensitive than thick blood smear for identification of microfilaraemic subjects. Thus, the main conclusion that lymphatic disease develops in amicrofilaraemic individuals and that microfilaraemics do not progress to amicrofilaraemic clinical disease is most unlikely to be affected by missing a very small number of ultra-low-microfilaraemics by the thick smear technique used in this study. Whether there is a link between ultra-low microfilaraemia and development of clinical disease, is a different question altogether. However, the low sensitivity of the thick blood smear technique for identification of the amicrofilaraemic individuals could theoretically affect the follow-up investigations of the latter group. However, the consistent amicrofilaraemic status of these subjects (data not shown) argues in favour of the conclusions made. A limited number of these subjects were investigated by PCR-based methods described previously,<sup>20</sup> but the majority were PCR negative and as expected, did not show any correlation with serological status (data not shown). Although not performed, antigen detection assays may be useful in resolving this issue. Regarding the third issue that clinical investigations were inadequate, our opinion was the opposite. In the house surveys, all subjects who had suggestive evidence of lymphatic disease were documented and re-examined later at a special anti-filariasis clinic at the base hospital by a medical officer. At the second examination, many subjects who had been misdiagnosed as filarial lymphoedema in the house surveys were excluded based on proper clinical and serological examinations (data not shown). As such, there was no underestimation of the incidence of clinical disease in the study subjects.

The findings in this study are in agreement with other reports that show lack of correlation between microfilaraemia and clinical disease. In Kenya,<sup>15,17</sup> it was reported that microfilaraemia rates and clinical disease rates were approximately equal at ~30% and ~28%, with the microfilarial density increasing up to the age of 50 years, an observation that argues against microfilaraemia as a precondition to developing clinical disease. In Ghana, no associations between microfilaraemia and acute adenolymphangitis, lymphoedema or elephantiasis, except hydrocoele were reported.<sup>8</sup> In a 16-year follow-up study in Tanzania, Meyrowitsch *et al.*<sup>9,11</sup> observed that more than 80% of the subjects who were microfilaraemic in 1975 were also microfilaraemic in 1991 without developing clinical disease. Further, these authors did not observe any association between leg elephantiasis and microfilaraemia status. Conclusions reached in this study are also in agreement with the pattern of clinical disease in non-immune subjects. Two well-documented examples are US servicemen deployed in filariasis-endemic Pacific Islands during World War II (reviewed<sup>21</sup>) and that of transmigrants in Indonesia.<sup>3</sup> An intervening microfilaraemic phase was not seen in the migrants who developed clinical disease.<sup>3</sup>

The emphasis of this study was on filarial disease manifestations that are public health concerns. Clinical disease in lymphatic filariasis may be grouped in to three main categories. The first category includes sub-clinical disease diagnosable only by specialized techniques such as lymphosyntigraphy or ultrasonography.<sup>22-24</sup> However, such abnormalities are not considered public health problems as the affected subjects are asymptomatic and appear to remain so throughout life. Also,

the filarial aetiology of leg abnormalities detected by lymphoscintigraphy has been questioned.<sup>25</sup> The second category includes atypical or occult manifestations such as tropical pulmonary eosinophilia (TPE), filarial monoarthritis, nodules etc., which are also not considered major public health problems. In the third category are the clinical manifestations which cause morbidity, and hence are of public health importance. These are retrograde adenolymphangitis with or without fever, lymphoedema, elephantiasis and male genital involvement. Studies on genital involvement were excluded from this study for two reasons. First, it was impractical under conditions of this study and second, the strong positive association of microfilaraemia with male genital filariasis is already well established.<sup>6,7,9,11,15,16</sup>

Since the microfilaraemic individuals detected in this study were treated, the possibility that treatment with DEC may have altered the natural progression of infection to disease development was considered. The ideal experiment to resolve this issue would have been the long term follow-up of treated and untreated microfilaraemic subjects. However, such investigations are unethical and early literature prior to the use of DEC as an anti-filarial drug was searched for evidence. Indeed, several reports prior to DEC era that support the data presented in this report were found.<sup>26</sup> Therefore, it is concluded that the findings in this study were not skewed by effects of DEC treatment and that in general, DEC treatment does not alter the natural course of infection and disease in bancroftian filariasis.

The implications of this study in filariasis control are twofold. First, it provides strong evidence that microfilaraemia and clinical disease (except male genital disease) develop in different segments of the population, but not in the same group of people at different time points of infection. Therefore, if interruption of transmission is the main objective, microfilaraemic subjects identified either by night blood examination or by day-time blood serology<sup>18,19</sup> should be targeted for follow-up investigations. Second, findings in this study clearly demonstrate that the incidence of clinical disease does not reflect the level of microfilaraemia or transmission index in a given population. Therefore, if reduction of morbidity in the population is the main objective of the anti-filariasis control programme, different criteria and strategies need to be evaluated for easy identification and follow-up treatment of those who are at higher risk. The observation that development of clinical disease occurs mainly in amicrofilaraemic, filarial antibody-positive subjects show that these subjects are more susceptible to developing clinical disease than others. The exception is the male with genital involvement, an easily diagnosable condition.

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