Bancroftian filariasis: a 13-year follow-up study of asymptomatic microfilariae carriers and endemic normals in Orissa, India

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SUMMARY

The natural history of human filarial infections leading to development of disease has been a subject of intense debate. The models proposed so far have largely been based on cross-sectional data on microfilariae (Mf) and disease prevalence in filariasis endemic areas. In an attempt to study the parasitological and clinical consequences of filarial infection in Beldal (Orissa, India), an area endemic for Bancroftian filariasis, cohorts of 59 asymptomatic Mf carriers (AS) and 187 asymptomatic and amicrofilaraemic subjects or 'endemic normals' ('EN'), were followed-up and a fraction (73% and 46% respectively) re-examined after 13 years to monitor (a) Mf prevalence, (b) Mf density, (c) circulating filarial antigen (CFA) and (d) chronic disease manifestations. The Mf prevalence and density were also monitored in Mf carriers after 1 and 4 years. Both Mf prevalence and density decreased progressively in the cohort of Mf carriers over a period of 13 years in Beldal. Only 37% of them continued to be microfilaraemic and the Mf density in these subjects was only 10% of the original level. However, loss of circulating Mf in this cohort did not result in loss of CFA and 95% remained CFA positive regardless of Mf status. About 23% of males in the 'EN' cohort developed hydrocoele while only 5·7% of male Mf carriers, who were not treated with DEC, had developed hydrocoele after 13 years. A cohort of Mf carriers in another area, Jatni, was also examined after 10 years to study the parasitological and clinical outcome. In this area, about 59% of the Mf carriers continued to be microfilaraemic after 10 years. These results reveal that in Mf carriers adult filarial worms persist for several years and that loss of circulating Mf with or without chemotherapy with DEC (single 12-day course) does not influence adult worm survival. The findings have been discussed in the context of 'static' and 'dynamic' models describing the relationship between infection and disease in human filariasis.

Key words: *Wuchereria bancrofti*, Bancroftian filariasis, circulating filarial antigen, longitudinal follow-up, infection *vs* disease.

INTRODUCTION

The natural history of human filarial infection and disease has been a subject of intense debate and speculation for several years. Based on clinical presentation, presence of circulating microfilariae (Mf) and circulating filarial antigen (CFA, a metabolic product of lymphatic-dwelling adult worms), subjects living in areas endemic for Bancroftian filariasis can be classified into the following 5 groups: (a) asymptomatic Mf carriers; (b) patients with history of 1 or more episodes of acute filariasis such as adenolymphangitis (ADL); (c) patients with chronic disease such as hydrocoele, elephantiasis, etc; (d) asymptomatic, amicrofilaraemic individuals with cryptic filarial infection as demonstrated by presence of CFA and (e) 'endemic normals' ('EN') who are asymptomatic, amicrofilaraemic and with no demonstrable CFA (Weil *et al*. 1999; Sahoo *et al*. 2000). The last two categories are separable only by detection of CFA using an immunoassay made available only in recent years (More & Copeman, 1990). It is not clear if individuals follow any particular pattern of shifting from one of the above mentioned states to the other.

In the absence of definitive studies on periodical long term follow-up of cohorts of human subjects displaying any of the above filarial condition, analysis of cross-sectional epidemiological data and follow-up of Mf carriers for a few years have been the mainstay to propose models of filariasis population biology (Bundy, Grenfell & Rajagopalan, 1991; Ottesen, 1992; Chan *et al*. 1998; Plaisier *et al*. 1998). It is widely perceived that exposure to infective larvae (L3) results in development of worms to maturity leading to peripheral microfilaraemia and that the subsequent loss or elimination of circulating Mf and death/killing of adult worms, presumably by

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immunological mechanisms, result in acute and chronic disease manifestations (Bundy *et al*. 1991). Very broadly, a 'static immunological viewpoint' on the one hand and a 'dynamic model' on the other have been forwarded to explain the natural history of filariasis. According to the former, it has been proposed that individuals displaying filarial-specific hypo-responsiveness (characterized by decreased antigen-specific T-cell proliferation) harbour mature adult-stage parasites resulting in a microfilaraemic state while those displaying filarial-specific T-cell hyper-responsiveness develop pathology associated with the disease and are generally free of active infection (Ottesen, 1992). Briefly, the model envisaged that filarial infection could lead to one of two consequences in a given individual patient, infection or pathology depending on the quality of immune response generated by the host. In the second model, the primary thrust has been on the premise that lymphatic-dwelling parasites mediate development of pathology (Bundy *et al*. 1991; Chan *et al*. 1998; Plaisier *et al*. 1998). For this hypothesis, cross-sectional epidemiological data collected in endemic areas were used to develop mathematical models to propose that all microfilaraemic individuals would essentially proceed to develop pathology resulting in chronic filarial disease. Empirical proof for the immunological viewpoint of filarial disease comes only from experimental studies in nude mice infected with *Brugia malayi* in which microfilaraemic animals were shown to rapidly develop symptoms of filarial disease on reconstitution of infected animals with filarial-specific immune lymphocytes (Vickery *et al*. 1991). There are no reported studies in human filariasis which provide direct evidence for the immunological viewpoint of disease development. A follow-up of a cohort of asymptomatic amicrofilaraemic subjects, also described as endemic normals (EN), can be expected to test the 'immunological viewpoint' of filarial disease development. Similarly, long-term follow-up of asymptomatic, microfilaraemic individuals can be expected to address the 'parasitological viewpoint' which states that development of disease is an inevitable sequel of patent infection.

A 16-year follow-up study from Tanzania in areas endemic for Bancroftian filariasis showed remarkable stability of the prevalence of Mf carriers as well as of amicrofilaraemic subjects (Meyrowitsch, Simonsen & Makunde, 1995). Only 18% of the Mf carriers became free of circulating Mf and only about 18% of amicrofilaraemic subjects acquired Mf in circulation after 16 years. Furthermore, there was no significant change in the prevalence of clinical filariasis in the overall population after 16 years. In the present study we report a follow-up of cohorts of asymptomatic Mf carriers (AS) and 'EN' over a 13-year period in an area endemic for Bancroftian filariasis in India. The infected individuals were followed up at different time-points for microfilaraemia (unlike the Tanzanian study by Meyrowitsch *et al*. 1995) and were analysed for persistence of infection by detection of CFA and development of chronic clinical symptoms of filariasis after 13 years.

MATERIALS AND METHODS

Study area, blood survey and collection of blood

Beldal. The first part of the study was conducted in the village of Beldal (predominantly agricultural farmers) in the Puri district of Orissa State, India. This coastal district is endemic for Bancroftian filariasis. Mass blood survey was undertaken in this area by collection of 20 µl of nocturnal blood samples from 386 individuals. Thick blood smears were stained with Giemsa and examined for Mf in February 1986 by a team of investigators from The Regional Medical Research Centre (RMRC), Bhubaneswar. The residents of this village strongly favour homeopathic remedies and symptomatic cases were regularly presenting to the filariasis clinic run by the Homeopathic Research Institute for Filariasis at Puri for treatment. None of the asymptomatic Mf carriers thus consumed diethyl carbamazine citrate (DEC) even when offered. Four of the investigators in the present study (B.R., A.K.S., P.K.S. and N.M.) were part of the team that conducted the survey in 1986. In 1999 the individuals in the area were clinically examined for acute and chronic symptoms of filariasis. Detailed clinical history was taken using a proforma and the subjects were classified into 4 categories based on the following criteria. (1) Acute filariasis: patients presenting with a history of 1 or more episodes of adenolymphangitis (ADL). (2) Chronic filariasis: patients presenting with persistent lymphoedema/ elephantiasis or hydrocoele. (3) Mf carriers: individuals with circulating Mf. (4) 'Endemic normals': asymptomatic individuals without circulating Mf. This category has been designated as 'EN' in this work since immunoassays for detection of CFA were not available in 1986. During studies conducted in recent years, we have divided 'EN' into 2 groups i.e. those with cryptic infection and those truly EN based, respectively, on the presence or absence of CFA while both groups are amicrofilaraemic and asymptomatic (Sahoo *et al*. 2000). The above classification of the filariasis spectrum is largely mutually exclusive although it is not uncommon to find a small percentage of patients with acute or chronic disease, particularly hydrocoele displaying microfilaraemia. About 5 ml of blood were collected in 1999 after informed consent from 129 subjects in Beldal (86 'EN' and 43 AS cases). One ml of blood was used for detection of Mf by the filtration method and sera, separated from the rest of the aliquot, was frozen at −20 °C for the CFA assay.

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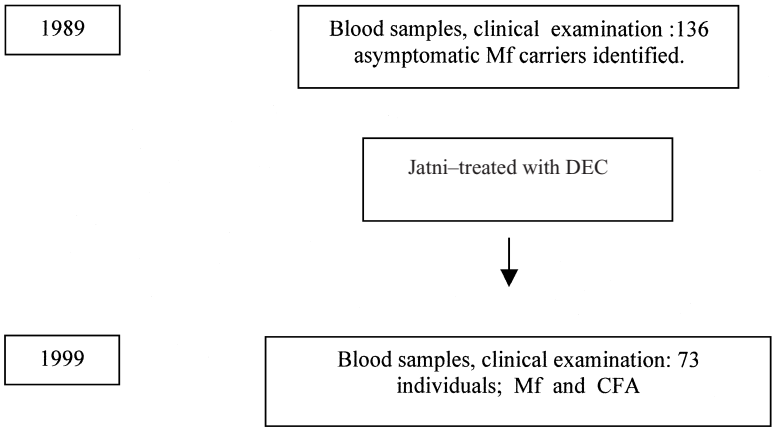

Fig. 1. Flow chart of study design of Mf carriers in Jatni area.

Jatni. In another locality, in the villages of Jatni (Khurda district), also endemic for Bancroftian filariasis, a total of 73 out of 136 Mf carriers identified in February 1989, median age 30 years (range 7–60 years) were re-examined after 10 years in 1999 (Fig. 1). Twenty µl of nocturnal blood samples were used for examination of Mf in Giemsa-stained thick smears and 100 µl of blood were taken in 400 µl of PBS–BSA (0·1%), microcentrifuged and the separated plasma was frozen at −20°C for further use to detect CFA. All the Mf carriers were offered a 12-day course of DEC (6 mg/kg) treatment in 1989.

Follow up of Mf carriers and endemic normals

In February 1986, 62 individuals from Beldal were found to be microfilaraemic by nocturnal blood examination. Those Mf carriers who were asymptomatic were re-examined for Mf in January 1987, September 1990 and August 1999. In the first two instances, 20 µl blood samples were used for Mf detection and in August 1999, 1 ml of blood was used for Mf detection. For the 43 Mf carriers who could be followed for 13 years the median age in 1986 was 35 years (range 3–62 years). Detailed clinical history was taken by clinical examination by using a proforma in August 1999. In the same village 187 'EN' identified in 1986 were re-examined in 1999 for presence of Mf, CFA and clinical symptoms. Only 86 individuals from this cohort were available for follow-up investigation at the end of 13 years. The median age of the cohort of 86 'EN' subjects in 1986 was 39 years (range 6–65 years). The investigations undertaken in these cohorts over 13 years are shown in Fig. 2.

Og4C3 circulating antigen assay

The Trop Bio ELISA kit was used for detecting and quantifying *Wuchereria bancrofti* antigen according to the manufacturer's (Tropical Biotechnology Pty. Ltd, Townsville, Australia) recommendations. The sera samples were boiled with EDTA, centrifuged and the supernatants were used for antigen testing. The results were expressed as arbitrary antigen units per ml using *Onchocerca gibsoni* antigen provided as standard in the kit (cut off = 100 units/ml).

Statistical analysis

The geometric mean microfilarial intensity was calculated for logarithimically transformed microfilaraemia data. Apart from a quantitative description for the presence or absence of CFA and/or Mf in different categories of the filariasis spectrum, the geometric mean index of CFA units (that approximately reflects adult worm burden) was also calculated using non-parametric two-tailed Mann-Whitney U-test. Comparisons of means were conducted on the log-transformed (normalized) microfilaraemia and CFA data. Comparisons of prevalence were performed by the z-test. The 95% confidence intervals for prevalence values were estimated by the normal approximation method with the exception of those values close to 0% or to 100%, when the exact method was applied (Fleiss, 1981).

RESULTS

The study addressed 3 issues, namely: (a) cross-sectional analysis of prevalence of Mf carriers, acute and chronic filariasis and 'EN' in 1986 and 1999, (b) follow-up of a cohort of asymptomatic Mf carriers (not treated with DEC) and 'EN' in 1 village over a period of 13 years to study the clinical and parasitological consequences in these two categories of subjects and (c) follow-up of a cohort of Mf carriers in another village who were treated with DEC to study the clinical and parasitological consequences after a period of 10 years.

Prevalence of filarial disease and infection in cross-sectional surveys

The results of the 1986 and 1999 cross-sectional prevalence surveys in the general population of 4

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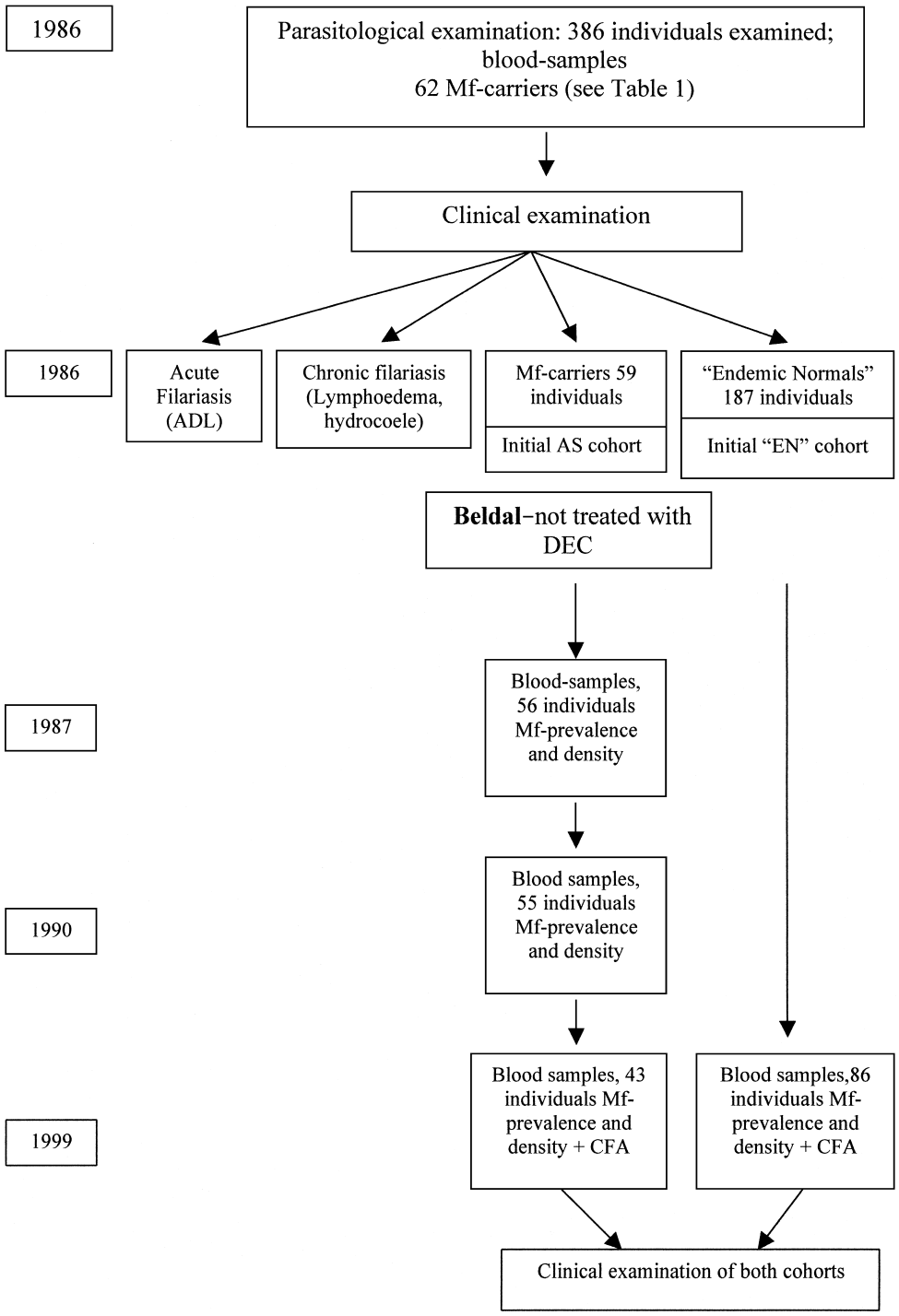

Fig. 2. Flow chart of study design in village Beldal.

categories of the filarial spectrum i.e. Mf carriers, acute and chronic filariasis, and 'EN' for Beldal are shown in Table 1. The proportion of Mf carriers was higher than the proportion of those with chronic disease (lymphoedema and hydrocoele combined) in 1986 ($z = 2{\cdot}50$, $P = 0{\cdot}006$), but similar in 1999 ($z = 1{\cdot}63$, $P = 0{\cdot}052$) and there was no significant change in prevalence of these two categories after 13 years in the general population ($z = 0{\cdot}38$, $P = 0{\cdot}352$ for Mf carriers, and $z = 0{\cdot}39$, $P = 0{\cdot}348$ for chronic disease). However, Mf density was significantly lower in the area in 1999 in comparison to the value in 1986 (Table 1). There was a significant increase in the prevalence of cases with history of acute filarial episodes and a significant decrease in asymptomatic and amicrofilaraemic 'EN'. The reasons for this increase in prevalence of acute filariasis is not clear. Since these cases were recorded based on the feed-back of the examined subjects to the questionnaire applied by the investigators, recall bias could have

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Table 1. Crude prevalence of Bancroftian microfilariae, acute, chronic filariasis and 'Endemic Normals' in 1986 and 1999 in Beldal, Orissa

Year of survey Total (male/female)	Mf carrier (%) 95% CL	GM of Mf/ml‡	Chronic disease (%) 95% CL	Acute disease (%) 95% CL	'Endemic Normals' (%) 95% CL
1986	62 (16·06)	1025·58	39 (10·10)	98 (25·39)	187 (48·45)
386 (218/168)	12·53, 20·12		7·28, 13·56	21·12, 30·04	43·35, 53·55
1999	31 (14·90)	59·59	23 (11·05)	73* (35·09)	81† (38·95)
208 (124/84)	10·34, 20·50		7·13 , 16·15	28·61, 42·01	32·27, 45·94

* z test: $z = 2{\cdot}49$, $P = 0{\cdot}006$.
† z test: $z = 2{\cdot}22$, $P = 0{\cdot}013$.
‡ Mann-Whitney U-test for GM of Mf/ml: $z = 10{\cdot}34$, $P < 0{\cdot}01$.

Table 2. Status of Bancroftian Mf positivity and CFA for those individuals participating in both 1986 and 1999 survey in Beldal, Orissa, India

			Microfilariae		CFA	
Category	Median age (range) years	Mf status 1986/1999	No. (males/females)	Percentage	No. +ve (males/females)	Antigen units (GM)*
AS ($n = 43$)	35	+ve/+ve	16 (14/2)	37·21	15 (13/2)	5707·58
	(3–62)	+ve/−ve	27 (21/6)	62·79	25 (20/5)	1150·63
EN ($n = 86$)	39	−ve/+ve	6 (6/0)	6·91	6 (6/0)	3811·56
	(6–65)	−ve/−ve	80 (46/34)	93·02	17 (9/8)	920·18

* Geometric Mean antigen units of positive cases only; Mann-Whitney U-test for AS cases between Mf +/+ and +/−, $z = 3{\cdot}09$, $P < 0{\cdot}01$.

contributed to this difference. Increased awareness about the disease amongst the people over the last 13 years as a result of the several visits paid by the research team could also have contributed to increased response of individuals giving a history of acute filariasis. The decrease in prevalence of asymptomatic, amicrofilaraemic 'EN' individuals may also be a consequence of increased reporting by the population about acute filarial episodes.

Status of microfilaraemia and CFA in Mf carriers after 13 years

A cohort of 59 Mf carriers was identified in Beldal in 1986 and was re-examined for circulating Mf in 1987, 1990 and 1999. In addition to Mf prevalence and Mf density, CFA was also quantified in 1999. Of the 59 Mf carriers 56, 55 and 43 were available for re-examination after the 1st, 4th and 13th year respectively. This cohort, as mentioned in the Materials and Methods section, was not treated with DEC since all the residents in this village favoured only homeopathic remedies. The results are shown in Table 2 and Fig. 3A and B. The Mf prevalence decreased progressively during the period of observation and by the 13th year, only 16 (37·2%) of the 43 examined subjects continued to harbour Mf in the circulation (Fig. 3A). More interestingly, the Mf density also decreased progressively – a geometric mean Mf density of 1005·54 Mf/ml decreased to a value of 99·92 by the 13th year. The GM of Mf density decreased more sharply than the Mf prevalence (Fig. 3B).

The CFA was quantified in this cohort of 43 subjects in 1999. Unlike Mf prevalence and Mf density which decreased progressively over the period of observation, the prevalence of CFA remained very high (Table 2 and Fig. 4), with about 95% positive for CFA after 13 years. There was no difference in CFA prevalence between the 62·8% of subjects who were amicrofilaraemic and the 37·2% of those who continued to be microfilaraemic after 13 years in Beldal (Fig. 4). However, quantitatively, there was a significant difference in CFA units between these 2 groups ($z = 3{\cdot}09$, $P < 0{\cdot}01$). The microfilaraemic subjects were found to have an intensity of adult worm infection (as revealed by the geometric mean index of antigen units) 5 times as high as those who had cleared peripheral microfilaraemia (Table 2).

Status of Mf/CFA in carriers after 10 years

A second cohort of 73 Mf carriers, identified in 1989, was re-examined in Jatni after a lapse of 10 years in 1999. Unlike the cohort in Beldal, the subjects in this area were not examined during the intervening period and only 1:4 diluted blood for plasma could

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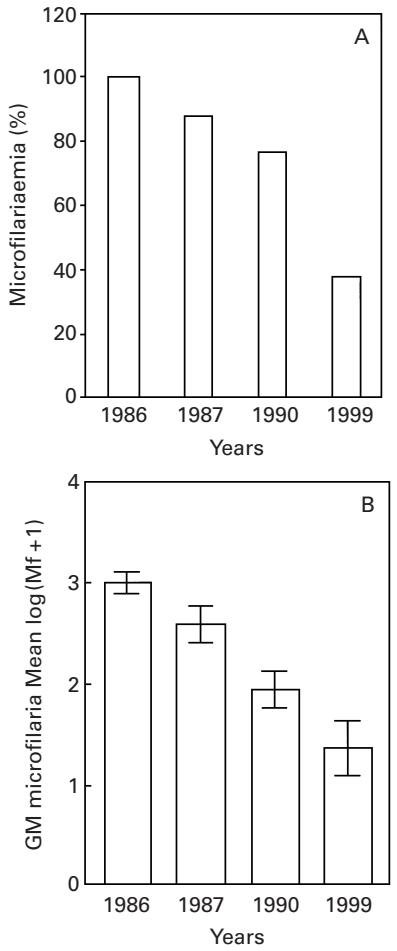

Fig. 3. Follow up of Mf carriers in Beldal. A cohort of asymptomatic Mf carriers ($n = 43$) were re-examined for circulating Mf after 1 year, 4 years and 13 years. (A) Prevalence of microfilaraemia at different time-points. The 95% confidence limits for the years 1986: 83·9129, 107·5135, 1987: 80·429, 101·8976, 1990: 68·5932, 86·4096 and 1999: 19·3503, 53·6994. (B) Geometric mean ± S.E. Mf density/ml log (Mf + 1) of nocturnal blood in 43 individual at different time-points.

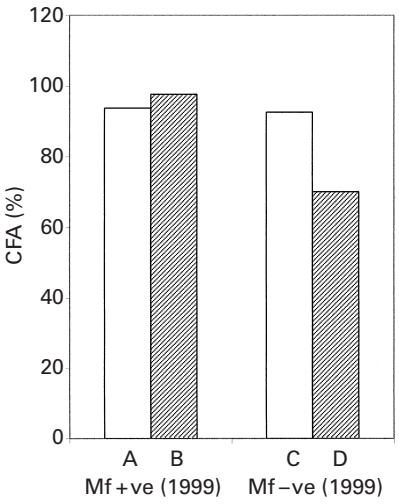

Fig. 4. Prevalence of circulating filarial antigen (CFA) in 2 cohorts of Mf carriers – subjects in Beldal (A and C) were examined after 13 years and those in Jatni (B and D) were examined after 10 years and categorized on the basis of presence of Mf in 1999. The 95% confidence limits for A: 0·5036, 0·9967, B: 0·8544, 0·9993, C: 0·7424, 0·9871 and D: 0·5044, 0·8459.

be collected for performance of CFA. Thus the results of prevalence and units of CFA need to be viewed with caution since the immunoassays were not performed in undiluted sera. Most individuals in this cohort volunteered to be treated with 1 course of DEC distributed through the primary health centre in 1989. The results of follow-up investigations are shown in Table 3. About 59% were found to be still microfilaraemic after 10 years and about 97% of these subjects were found to be positive for CFA. Amongst the 41·1% of those who had cleared Mf in circulation about 64% were found to be positive for CFA (Fig. 4). The low prevalence of CFA positivity in this cohort, as mentioned above, could be due to the use of diluted plasma for detection of CFA – the dilution factor may not have adversely affected the sensitivity in Mf positive cases since quantitatively they contain more CFA units than those who have lost circulating Mf. Although this cohort was from a different area and was largely treated with DEC 10 years ago, the Mf prevalence at the end of l0 years appeared to fit well into the general pattern of Mf loss: the 41·1% loss of microfilaraemia observed after 10 years was in conformity to 23·9% loss by the 4th year and 62·8% loss by the 13th year observed in Beldal. There was a significant difference in CFA units between Mf positive individuals and those who were free of Mf after 10 years (Table 3, $z = 2·13$, $P < 0·01$).

Status of microfilaraemia and CFA in 'Endemic Normals' after 13 years

In 1986, a total of 187 subjects who were free of circulating Mf and disease symptoms were chosen as 'EN' and 86 of them were re-examined in 1999 for the presence of Mf as well as CFA. The results are shown in Table 2. Only 7% of the 'EN' had acquired Mf and the vast majority of them (93%) continued to be free of circulating Mf when re-examined after 13 years; however, 21% of amicrofilaraemic subjects in this group were positive for CFA (Table 2) in 1999.

Distribution of filarial spectrum in 'EN' and AS groups after 13 years

An attempt was made to understand the consequence and clinical outcome of filarial infection in Mf carriers (AS) and 'EN' by classifying the 2 cohorts into 5 different groups of the filarial spectrum at the end of 13 years based on Mf, CFA status and expression of chronic disease manifestations. For

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Table 3. Status of Bancroftian Mf positivity and CFA for those individuals participating in both 1989 and 1999 survey in Jatni, Orissa, India

Category	Median age (range) years	Mf status 1986/1999	Microfilariae No. (males/females)	Microfilariae Percentage	CFA No. +ve (males/females)	CFA Antigen unit (GM)*
Asymptomatic Carriers ($n = 73$)	30 (7–60)	+ve/+ve	43 (31/12)	58·90	42 (30/12)	824·35
		+ve/−ve	30 (16/14)	41·09	18 (10/8)	280·62

* Geometric Mean antigen units of positive cases only; Mann-Whitney U-test for AS cases between Mf +/+ and +/−, $z = 2{\cdot}13$, $P < 0{\cdot}01$.

Table 4. Summary of studies on long-term follow-up of microfilariae carriers in Bancroftian filariasis

Sl. No.	Area/Country	Follow-up (years)	Mf detection	% loss of Mf	CFA†	Ref
1	American Samoa	4·5	60 µl	N.S.†	No	Hairston & Jachowski (1968)
2	Pondicherry, India	5	20 µl	60	No	Vanamail *et al.* (1989) Srividya *et al.* (1991)
3	Tanzania	16	DEC*/100 µl	18	Yes	Meyrowitsch *et al.* (1995)
4	Orissa, India - Area-1	1	20 µl	12·50	No	Current study
	Orissa, India – Area-1	4	20 µl	25	No	Current study
	Orissa, India – Area-1	13	1000 µl	63	Yes	Current study
	Orissa, India – Area-2	10	20 µl	47	Yes	Current study
5	Egypt	1	1000 µl	13	Yes	Weil *et al.* (1999)

* DEC, day time blood collection after DEC provocation.
† CFA, detection of circulating filarial antigen.
‡ N.S., Not shown.

this classification, subjects giving history of acute disease were not considered since filarial aetiology could not be definitively ascertained. The results are depicted in Fig. 5A and B – only 1 subject in each group developed lymphoedema; the prevalence of hydrocoele was, however, significantly higher amongst 'EN' in comparison to the cohort of Mf carriers ($z = 2{\cdot}17$, $P = 0{\cdot}015$). While 23% of males in 'EN' group developed hydrocoele over 13 years, only 6% of males amongst AS cases developed hydrocoele. Interestingly, 9 out of 12 'EN' cases which developed hydrocoele were free of CFA and none of them had detectable microfilaraemia. About 62% of 'EN' cases were found to be true Endemic Normals (Fig. 5B) while only 2·3% of AS cases were currently in the true 'EN' group (Fig. 5A). About 56% of former AS cases were found to have cryptic infection i.e. they were asymptomatic and amicrofilaraemic but with evidence of adult worm infection after 13 years according to the CFA test. Comparison of the 2 AS cohorts in Beldal after 13 years (DEC untreated) and Jatni after 10 years (DEC treated) revealed a significantly high conversion rate to chronic disease (hydrocoele and elephantiasis together, males only) in Jatni – 21% in comparison to 6% in Beldal ($z = 1{\cdot}98$, $P = 0{\cdot}024$). Interestingly, conversion to true 'EN' was also significantly higher in Jatni, 13·7% in comparison to 2·3% in Beldal ($z = 2{\cdot}02$, $P = 0{\cdot}022$, Fig. 5A and C).

DISCUSSION

Development of filarial larvae into adult worms with consequent production of Mf has been understood to be the natural course of infection from parasitological studies in experimental animals. However, the time-course and progression of infection resulting in filarial disease (both acute and chronic manifestations) in human communities is far from clear. In the absence of comparable clinical features in experimental models, the natural history of human filarial disease has been deduced by analysis of cross-sectional data on prevalence of infection and disease in endemic areas.

The present study was undertaken to contribute to the debate proposed by the 2 models of natural history of human filariasis by observing cohorts of asymptomatic Mf carriers (AS) and asymptomatic amicrofilaraemic subjects 'EN' over a long period of time for loss or gain of circulating Mf and/or filarial antigens and for development of chronic disease manifestations. Although the current study is somewhat similar to a few other investigations reported in the literature summarized in Table 4, it has several other outcome variables: (a) the Mf carriers were monitored at different time-points (1, 4 and 13 years) for Mf prevalence as well as for Mf density, (b) a cohort of 'EN' from the same village was also monitored (13th year only) for acquisition of Mf and

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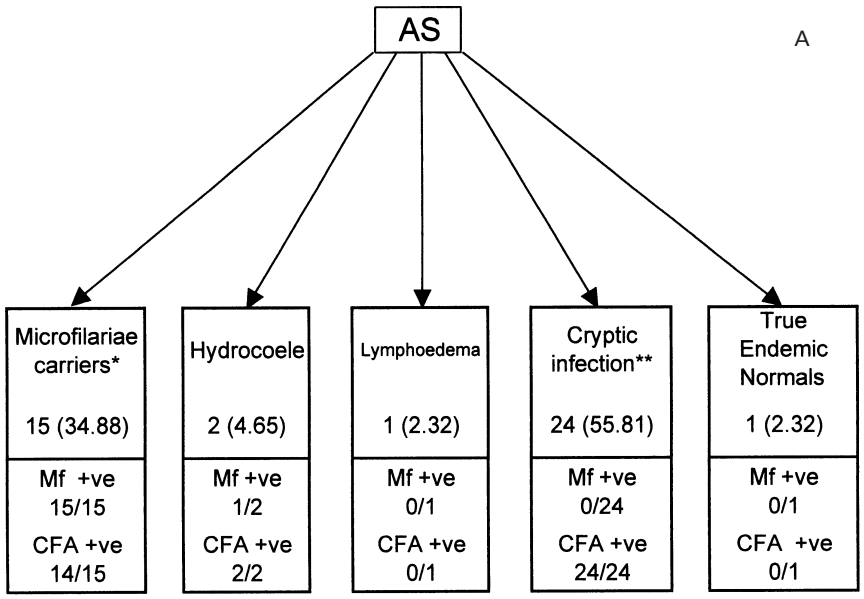
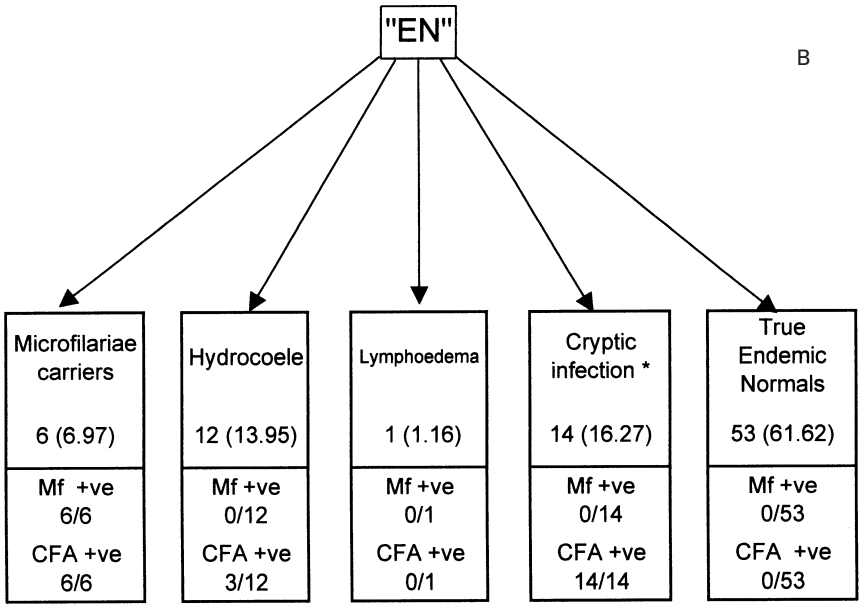


* Of the 16 Mf +ve cases (Table 2) 1 developed hydrocele and the remaining 15 continued to be asymptomatic Mf carriers.

** Of the 25 Mf -ve cases with CFA (Table 2) 1 developed hydrocele and the remaining 24 continued to be asymptomatic.



* Of the 17 Mf -ve cases with CFA (Table 2) 3 developed hydrocele and the rest remained asymptomatic.

Fig. 5. For legend see facing page.

development of chronic filarial disease and compared with the cohort of Mf carriers, and (c) CFA was monitored by a quantitative assay at the end of the observation period in both AS and 'EN'. Inclusion of these criteria in this study has allowed us to analyse and comment about some of the critical aspects of the natural history of filarial infection and disease. This communication attempts to provide a general descriptive study of Mf carriers and 'EN' cohorts over a period of 10–13 years.

The current study has revealed the importance of monitoring Mf density in filarial epidemiology. Most of the investigators analysing the relationship between infection and disease in human filariasis have only used the prevalence of microfilariae in the population (wrongly but often referred to as Mf rate)

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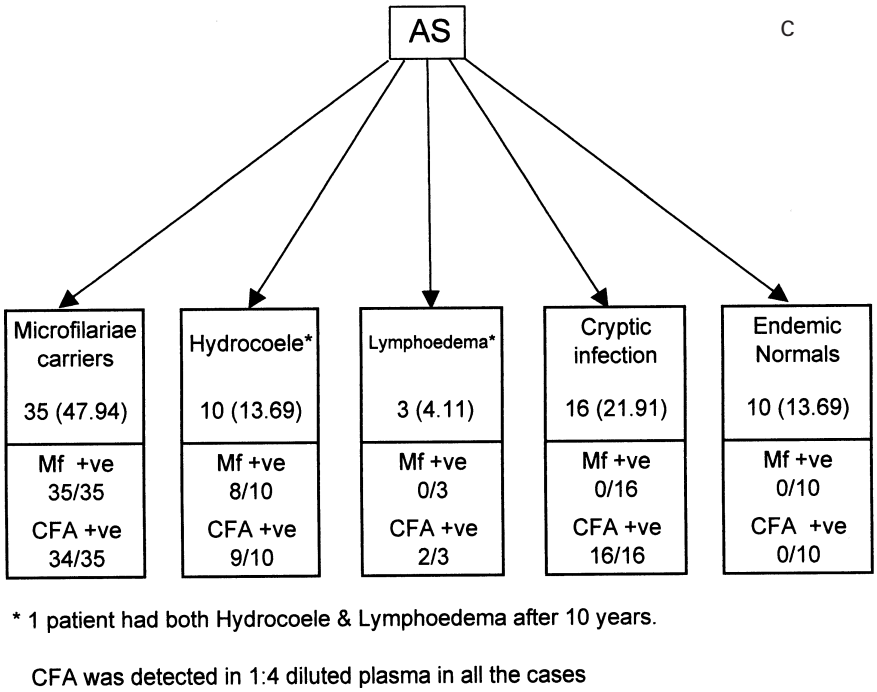

Fig. 5. Current status of asymptomatic, microfilaraemic cases (AS) in Beldal village after 13 years. A total of 43 AS cases (35 males and 8 females) were re-examined for Mf, CFA and development of chronic disease and classified into 5 categories – Number (%) and Mf/CFA positivity in each group are shown. (B) Current status of asymptomatic, amicrofilaraemic, 'EN' in Beldal after 13 years. A total of 86 'EN' subjects (52 males and 34 females) were re-examined for Mf, CFA and development of chronic disease and classified into 5 categories – Number (%) and Mf/CFA positivity in each group are shown. (C) Current status of asymptomatic, microfilaraemic cases (AS) in Jatni area after 10 years. A total of 73 AS cases (47 males and 26 females) were re-examined for Mf, CFA and development of chronic disease and classified into 5 categories – Number (%) and Mf/CFA positivity in each group are shown.

as a parameter of infection (see Michael, Grenfell & Bundy, 1994; Michael, Bundy & Grenfell, 1996). Similarly, the fecund life-span of adult female worms is often calculated by using the duration of Mf persistence in a given population (Vanamail *et al.* 1990, 1996). In the present study, both Mf prevalence and Mf density decreased progressively over the period of 13 years in the 43 Mf carriers who participated throughout the whole study; more significantly cross-sectional analysis of Mf prevalence and density in 1986 and 1999 revealed a significant decrease in Mf density with virtually no change in Mf prevalence. This observation emphasizes the importance of Mf density as a crucial parameter in filarial epidemiology. The observed disparity in duration of Mf persistence in different geographical regions could well be a reflection of variation in Mf densities in these areas. Thus precise monitoring of Mf density and filarial antigenemia over a long period of time could result in more accurate predictions of the life-span of filarial worms, a suggestion also proposed by Vanamail *et al.* (1996).

The progressive decrease of Mf prevalence with persistent antigenemia in the cohort of Mf carriers observed during the 13 years of follow-up appears to indicate that loss of microfilaraemia was due to progressive loss of fecundity of adult female worms rather than to host immune responses. We had earlier observed that the subjects who had cleared Mf during the 13 years of observation had not elicited antibodies to Mf sheath – this further confirms our contention that Mf elimination in human filariasis is not essentially mediated by antibody to Mf sheath (Satapathy *et al.* 2001). A similar observation of non-appearance of sheath reactive antibodies in Mf carriers after clearance of circulating Mf has been reported by Simonsen & Meyrowitsch (1998). We and others had demonstrated several years ago a significant inverse association between absence of microfilaraemia and presence of antibodies to Mf sheath in Brugian and Bancroftian filariasis (McGreevy *et al.* 1981; Ravindran *et al.* 1990). These associations were interpreted to indicate a protective role for anti-sheath antibodies in elimination of Mf in human filariasis. Our conclusion from the current study that in human filariasis loss of Mf is essentially due to loss of adult worm fecundity is in conformity with our recent findings on the role of anti-sheath antibodies in human filariasis. We had demonstrated recently a significant inverse association between antibodies to Mf sheath and CFA and proposed that absence of Mf in subjects with antibodies to sheath was due to non-maturation of filarial worms and not due to

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elimination of Mf by production of antibodies to sheath in microfilaraemic subjects (Ravindran *et al.* 2000).

About 95% of microfilaraemic subjects as well as those who were free of Mf after 13 years were found to harbour adult worms as shown by the presence of CFA. The finding that loss of Mf in 63% of the subjects is not associated with loss of CFA indicates persistence of adult worms after the Mf carriers become amicrofilaraemic. Similar results on persistence of CFA after loss of Mf have been reported from studies conducted in Tanzania (Simonsen & Meyrowitsch, 1998). More interestingly, in the current study, those subjects who had lost Mf were found to possess 5 times less CFA units than those who continued to be microfilaraemic. We interpret all these results to mean that, Mf carriers become amicrofilaraemic when the fecund adult worms stop producing Mf possibly due to senility, and that the total life-span of adult filarial worms could be several years longer than the fecund life-span of the worms. The long life-span of adult filarial worms is indicated by several immuno-epidemiological studies reporting on prevalence of CFA in age-stratified populations in endemic areas – unlike intestinal worms which follow a convex prevalence curve (Anderson, 1986), filarial antigenemia increases in the younger age groups (< 20 years) and remains as a plateau in older age groups (Ravindran *et al.* 2000; Simonsen *et al.* 1996; Day *et al.* 1991; Weil *et al.* 1999). Based on the above findings we attribute to a long life-span of adult worms rather than to re-infection the persistent CFA positivity observed after 13 years in the cohort of Mf carriers. It is also crucial to note that, unlike schistosomiasis and intestinal helminths, re-infection is yet to be documented in human filariasis.

While categorization of the filariasis spectrum based on Mf, CFA and disease manifestations is broadly recognized, the progression of subjects from one stage to the other is still largely speculative. The 13-year follow-up of cohorts of Mf carriers and 'EN' has revealed interesting features on the progression of disease in subjects with and without demonstrable infection. The prevalence of hydrocoele (males only) was significantly higher in 'EN' than in Mf carriers at the end of 13 years of the follow-up study. None of the 'EN' cases who developed hydrocoele had demonstrable microfilaraemia and only 25% of them had demonstrable infection as shown by presence of CFA in 1999. It may be possible that these subjects were harbouring CFA 13 years ago when they were recruited as 'EN' for the study (they were not tested for CFA since appropriate immunoassays were not available in 1986). As expected, the Mf carriers who developed hydrocoele after 13 years had detectable CFA. Taken together, these results tend to indicate a lack of association between active filarial infection in the host and development of hydrocoele. However, to address the issue of Mf status and DEC treatment as a risk factor for development of hydrocoele, it would be essential to subject these data to appropriate statistical analysis such as survival analysis taking into account censored (lost to follow-up) observations, and adjusting for age, sex, exposure, occupation, and possible selection and monitoring biases in AS and EN cohorts. Such analysis will be presented elsewhere. The relationship between infection and disease in lymphatic filariasis has been a contentious issue – while some of the reports have demonstrated a direct relationship using cross-sectional data there are others who have failed to confirm it (Srividya *et al.* 1991; Meyrowitsch & Simonsen 1995; Michael *et al.* 1994; Gyapong 1998; Dissanayake, 2001). Further follow-up of the two cohorts of subjects in this study during the next 3–5 or more years may indicate the association if any, between filarial infection and development of hydrocoele/lymphoedema, although losses to follow-up will be a major limiting factor.

The current study was not originally conceived to be a long term follow-up investigation and hence there are various problems in the study design. The increased awareness of the studied population in relation to acute filarial manifestations as the study proceeded, indicates, for instance the operation of monitoring biases. However, the observations made in the present investigation are significant to the on-going debate on the relationship between active filarial infection and development of disease. Although the findings do not unambiguously offer proof for either the 'static immunological theory' or the 'dynamic parasitological theory' of disease development in human filariasis, they appear to favour the 'immunological view-point'.

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REFERENCES

ANDERSON, R. M. (1986). The population dynamics and epidemiology of intestinal nematode infections. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **80**, 686–696.

BUNDY, D. A. P., GRENFELL, B. T. & RAJAGOPALAN, P. K. (1991). Immunoepidemiology of lymphatic filariasis: the relationship between infection and disease. *Immuno-Parasitology Today* , A71–A75.

https://doi.org/10.1017/S0031182001001007 Published online by Cambridge University Press

CHAN, M. S., SRIVIDYA, A., NORMAN, R. A., PANI, S. P., RAMAIAH, K. D., VANAMAIL, P., MICHAEL, E., DAS, P. K. & BUNDY, D. A. P. (1998). Epifil: A dynamic model of infection and disease in lymphatic filariasis. *American Journal of Tropical Medicine and Hygiene* **59**, 606–614.

DAY, K. P., GRENFELL, B., SPARK, R., KAZURA, J. W. & ALPERS, M. P. (1991). Age specific patterns of change in the dynamics of *Wuchereria bancrofti* infection in Papua New Guinea. *American Journal of Tropical Medicine and Hygiene* **44**, 518–527.

DISSANAYAKE, S. (2001). In *Wuchereria bancrofti* filariasis, asymptomatic microfilaraemia does not progress to amicrofilaraemic lymphatic disease. *International Journal of Epidemiology* **30**, 394–399.

FLEISS, J. L. (1981). *Statistical Methods for Rates and Proportions*, 2nd Edn. John Wiley & Sons, New York.

GYAPONG, J. O. (1998). The relationship between infection and disease in *Wuchereria bancrofti* infection in Ghana. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **92**, 390–392.

HAIRSTON, N. G. & JACHOWSKI, I. A. (1968). Analysis of the *Wuchereria bancrofti* population in the people of American Samoa. *Bulletin of the World Health Organization* **38**, 29–59.

MCGREEVY, P. B., RATIWAYANTO, T. S., MCGREEVY, N. M. & DENNIS, D. T. (1981). *Brugia malayi*: relationship between anti-sheath antibodies and amicrofilaraemia in natives living in an endemic area of south Kalimantan, Borneo. *American Journal of Tropical Medicine and Hygiene* **29**, 508–513.

MEYROWITSCH, D. W. & SIMONSEN, P. E. (1995). Bancroftian filariasis: analysis of infection and disease in five endemic communities of north-eastern Tanzania. *Annals of Tropical Medicine and Parasitology* **89**, 653–663.

MEYROWITSCH, D. W., SIMONSEN, P. E. & MAKUNDE, W. H. (1995). A 16-year follow-up study on Bancroftian filariasis in three communities of north-eastern Tanzania. *Annals of Tropical Medicine and Parasitology* **89**, 665–675.

MICHAEL, E., BUNDY, D. A. P. & GRENFELL, B. T. (1996). Re-assessing the global prevalence and distribution of lymphatic filariasis. *Parasitology* **112**, 409–428.

MICHAEL, E., GRENFELL, B. T. & BUNDY, D. A. P. (1994). The association between microfilaraemia and disease in lymphatic filariasis. *Proceedings of the Royal Society of London, B* **256**, 33–40.

MORE, S. J. & COPEMAN, B. D. (1990). A highly specific and sensitive monoclonal antibody-based ELISA for the detection of circulating antigen in bancroftian filariasis. *Tropical Medicine and Parasitology* **41**, 403–406.

OTTESEN, E. A. (1992). Infection and disease in lymphatic filariasis: an immunological perspective. *Parasitology* **104**, S71–S79.

PLAISIER, A. P., SUBRAMANIAN, S., DAS, P. K., SOUZA, W., LAPA, T., FURTADO, A. F., VAN DER PLOEG, C. P. B., HABBEMA, J. D. F. & OORTMARSSEN, G. J. V. (1998). The LYMFASIM Simulation Program for Modeling Lymphatic Filariasis and its Control. *Methods of Information in Medicine* **37**, 97–108.

RAVINDRAN, B., SATAPATHY, A. K., SAHOO, P. K. & BABU GEDDAM, J. J. (2000). Protective immunity in human Bancroftian filariasis: Inverse relationship between antibodies to microfilarial sheath and circulating filarial antigens. *Parasite Immunology* **22**, 633–637.

RAVINDRAN, B., SATAPATHY, A. K., DAS, M. K., PATTNAIK, N. M. & SUBRAMANYAM, V. R. (1990). Antibodies to microfilarial sheath in Brancroftian filariasis – prevalence and characterization. *Annals of Tropical Medicine and Parasitology* **84**, 607–613.

SAHOO, P. K., GEDDAM, J. J. B., SATAPATHY, A. K., MOHANTY, M. C. & RAVINDRAN, B. (2000). Bancroftian filariasis: prevalence of antigenaemia and endemic normals in Orissa, India. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **94**, 515–517.

SATAPATHY, A. K., SAHOO, P. K., GEDDAM, J. J. B., MOHANTY, M. C. & RAVINDRAN, B. (2001). Human bancroftian filariasis: Loss of patent microfilaraemia is not associated with production of antibodies to microfilarial sheath. *Parasite Immunology* **23**, 163–167.

SIMONSEN, P. E. & MEYROWITSCH, D. W. (1998). Bancroftian filariasis in Tanzania: Specific antibody responses in relation to long-term observations on microfilaremia. *American Journal of Tropical Medicine and Hygiene* **59**, 667–672.

SIMONSEN, P. E., LEMNGE, M. M., MSANGENI, H. A., JAKOBSEN, P. H. & BYGBJERG, I. B. C. (1996). Bancroftian filariasis: The patterns of filarial-specific immunoglobulin G1 (IgG1), IgG4, and circulating antigens in an endemic community of Northeastern Tanzania. *American Journal of Tropical Medicine and Hygiene* **55**, 69–75.

SRIVIDYA, A., PANI, S. P., RAJAGOPALAN, P. K., BUNDY, D. A. P. & GRENFELL, B. T. (1991). The dynamics of infection and disease in Bancroftian filariasis. *Transactions of Royal Society of Tropical Medicine and Hygiene* **85**, 255–259.

VANAMAIL, P., RAMAIAH, K. D., PANI, S. P., DAS, P. K., GRENFELL, B. T. & BUNDY, D. A. P. (1996). Estimation of the fecund life span of *Wuchereria bancrofti* in an endemic area. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **90**, 119–121.

VANAMAIL, P., SUBRAMANIAN, S., DAS, P. K. & RAJAGOPALAN, P. K. (1990). Estimation of fecundic life span of *Wuchereria bancrofti* from longitudinal study of human infection in an endemic area of Pondicherry (South India). *Indian Journal of Medical Research* **91**, 293–297.

VANAMAIL, P., SUBRAMANIAN, S., DAS, P. K., PANI, S. P., RAJAGOPALAN, P. K., BUNDY, D. A. P. & GRENFELL, B. T. (1989). Estimation of age-specific rates of acquisition and loss of *Wuchereria bancrofti* infection. *Transactions of the royal society of Tropical Medicine and Hygiene* **83**, 689–693.

VICKERY, A. C., ALBERTINE, K. H., NAYAR, J. K. & KWA, B. H. (1991). Histopathology of *Brugia malayi* infected nude mice after immune-reconstitution. *Acta Tropica* **49**, 45–55.

WEIL, G. J., RAMZY, R. M. R., SETOUHY, M. E., KANDIL, A. M., AHMED, E. S. & FARIS, R. (1999). A longitudinal study of Bancroftian filariasis in the Nile delta of Egypt: Baseline data and one-year follow-up. *American Journal of Tropical Medicine and Hygiene* **61**, 53–58.

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