Modelling Lymphatic Filariasis Transmission and Control

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Photos: *Wuchereria bancrofti* microfilaria, L3 and adult worm

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Modelling Lymphatic Filariasis Transmission and Control

Modellering van transmissie en bestrijding van lymfatische filariasis

Thesis

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by

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born at Melasengalmedu, India

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To my father Shri R. Swaminathan

and my mother Smt. S. Chinnammal

Contents

Preamble

Human lymphatic filariasis (LF) is an infectious mosquito borne disease of tropics. The disease is caused by the lymph-dwelling nematode parasites *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. Several species of mosquitoes are involved in the transmission of disease. LF is prevalent in 73 countries in tropical and sub-tropical regions of the world. About 1.1 billion people are exposed to the risk of infection and 120 million are apparently infected and several million more show blood antigenaemia, which is also an indication of infection prevalence. The disease constrains the social and economic development of the affected communities. More than one-third of 'at risk' and infected population lives in India.

LF control, until recently, has received poor attention because of meagre information on the disease burden and lack of feasible control tools and strategies. Nevertheless, recent research studies led to appreciation of the socio-economic impact of the disease and development of new diagnostic and control tools. Simultaneously, in 1993, an International Task Force for Disease Eradication has identified LF as one of the six diseases with good prospects for eradication. In 1997, recognising the public health importance of the disease, the 50th World Health Assembly passed a resolution (WHA50.29) to eliminate LF from the globe by 2020. The global LF elimination strategy envisages (i) transmission control through annual single dose community wide treatment with anti-filarial drugs and (ii) alleviation and prevention of suffering and disability caused by disease. During the last decade, several countries, including India, initiated pilot scale or full elimination programmes. This marked the beginning of the global campaign to eliminate LF as a public health problem.

Recommending a specific control/elimination program should be based on prospects of its favourable impact on the epidemiology and disease burden. Such judgements are far from easy in macro-parasitic diseases like LF, in which the disease incidence is largely determined by cumulative exposure to parasites over a period of many years. Further, a host of factors viz., efficacy of drugs, efficiency of vectors, parasite epidemiology, immunity of hosts and treatment coverage of communities influence the outcome of the control/elimination programmes. To aid decision-making about control/elimination strategies and to evaluate the long-term effects of control/elimination measures, it is therefore, important to develop epidemiological models, which can describe the full transmission cycle, morbidity, and control options. Such epidemiological models helped the selection, type and duration of control strategy in other diseases such as Onchocerciasis in West Africa. Keeping this in mind, this thesis tries to contribute firstly to knowledge and quantification of the population dynamics of *W. bancrofti*, the most predominant parasite species, and secondly to use this knowledge in the development and application of a model for designing and monitoring control strategies aimed at LF. This model is based on the results of an integrated vector management study in Pondicherry, India, and is intended to be used for prospective evaluation of effectiveness of various chemotherapy based control strategies.

1

General introduction

1.1 Lymphatic Filariasis: burden, parasites, vectors and disease

Human Lymphatic Filariasis (LF) is an infectious vector borne disease caused by three nematode parasites of the order Filariidea [2] namely *Wuchereria bancrofti, Brugia malayi*, and *Brugia timori*. The infection and disease caused by these worms are commonly termed as Bancroftian filariasis, Brugian filariasis, and Timorian filariasis respectively. More than 100 species of mosquitoes are involved in the transmission of LF. Nocturnally periodic *W. bancrofti* [4] transmitted by the tropical house mosquito, *Culex quinquefasciatus* [5] is the most formidable and widely prevalent vector-parasite combination and is the focus of the present study.

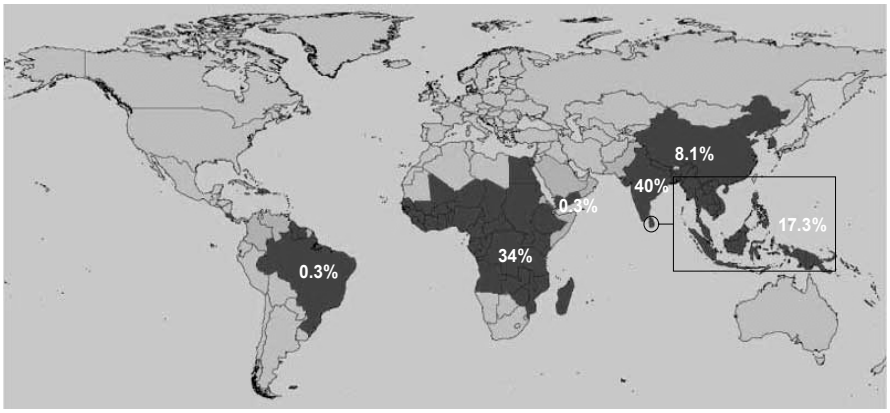

Figure 1 Geographical distribution of lymphatic filariasis. Gray areas are endemic (based on World Health Organization, 2002 [1]). Percentages refer to the worldwide burden of disease due to lymphatic filariasis.

Public health importance

The disfigurement of body parts caused by chronic disease manifestations of LF such as lymphedema and elephantiasis in men and women and hydrocele in men inflict stigma and disability on the affected people. Globally, LF is the second leading cause of permanent and long-term disability [6,7]. LF impairs the mobility, social life, educational, employment, marriage prospects and also the marital relations and sexual life. It restricts the occupational activities leading to lower productivity by as much as 27% [8] and significant economic loss [9-12]. India alone suffers an annual economic loss of nearly 1 billion US $, equivalent to 0.63% of GNP [13]. The burden of the disease is estimated at 5.5 million DALYs, which is 1.5% of the global burden of parasitic and infectious diseases [14,15].

Disease burden

Globally, 1.1 billion people living in 73 countries are exposed to the risk of infection with LF and 119 million people are already infected. The infected population includes 76 million asymptomatic microfilaria (Mf) carriers and 43 million with chronic disease condition. India alone accounts for 40% of the global burden of LF [16]. The sub-Saharan Africa, Southeast Asia and the south Pacific islands and the Americas are the other major endemic regions (Fig. 1). Of the three parasite species, *W. bancrofti* accounts for 90% of the total disease burden and is very widespread. *B. malayi* accounts for 10% of the burden and is prevalent only in a few Asian countries (Fig. 1).

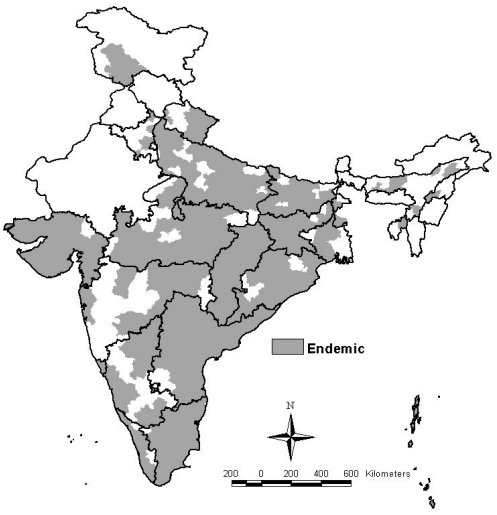

Figure 2 Filariasis endemicity in India (Sabesan *et al.*, 2000 [5]).

Within India, LF is endemic in 21 states or union territories and 45% of its more than 1 billion population is exposed to the risk of infection (Fig. 2) [3,17]. Nearly 48.1 million are infected and another 68 million are estimated to have antigenaemia [18]. Two-third of the 'at risk' and infected population live in rural areas. *W. bancrofti* transmitted by *Culex quinquefasciatus* is the cause of 94.6% of the LF cases.

The parasite

The life cycle of the parasite involves definitive host, man, and intermediate host, mosquito (Fig. 3). The adult filarial worms live in the lymphatic system of man. They are creamy-white thread like worms with a smooth cuticle. The male worms of *W. bancrofti* measure about 40 mm in length and 0.1 mm in diameter and females 80 to 100 mm by 0.24 to 0.3 mm. After mating, the female worms produce large number of embryos (measuring a length of 220-300 µm and diameter of 8 µm) called 'microfilariae (Mf)'. Female mosquitoes ingest Mf while they imbibe blood from an infected person. Within the mosquito body, the Mf penetrate the gut wall and migrate to the thoracic region, where they develop into L1, L2 and finally into L3 or infective stage within 10-12 days under tropical climatic conditions [19,20]. The L1 measures about 200 µm, the L2 300-

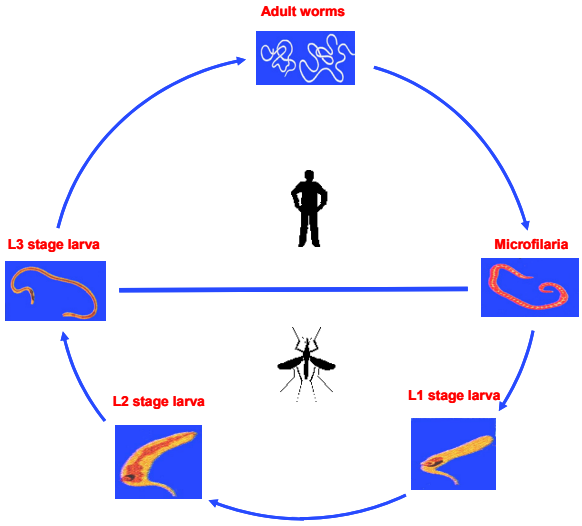

Figure 3 Life cycle of *Wuchereria bancrofti.*

1100 µm and L3 1100 to 1800 µm. Most of the infective stage larvae are found in the head region of the mosquito [21]. They escape through the proboscis of the mosquito and are transmitted to man during the subsequent blood meals of the vector mosquitoes. Within the human body the larvae migrate to the lymphatic system and develop into adult worms in about 8 months to 2.5 years [22,23]. The average life span of the adult worms is about 5 to 10 years [24,25] with a maximum longevity of 40 years [26]. The average number of reproducing female worms per microfilaraemic person is estimated to be about 6.5 [22]. An adult worm can produce millions of Mf during its lifetime.

The vector

LF is transmitted from one person to another by mosquitoes. Though several species of *Culex*, *Anopheles*, *Aedes* and *Mansonia* are vectors, more than 50% of the LF infections all over the world are transmitted by a single vector species, *Culex quinquefasciatus* [27]. In many countries including India, *C. quinquefasciatus* is highly anthropophilic, rests and feeds indoors as well as outdoors. Following a blood meal and ovary development, a female lays eggs (ranging from 100 to 180) on the water surface. Habitats with stagnant and polluted water rich in organic content are the most preferred breeding habitats [28,29]. The eggs hatch and develop through four larval stages into pupae, which emerge into adults. The immature life cycle is completed in 8-10 days under the typical tropical temperature conditions [30]. After about 48 hours of emergence, mating takes place and the females seek a blood meal, which is necessary for the maturation of eggs [31,32]. The daily survival rate of the species ranges from 0.72 to 0.89 and is influenced by temperature and relative humidity [30,33]. The mosquito lives on an average about 10-12 days in nature [20]. *C. quinquefasciatus* is considered as a highly successful species, its reproductive potential is very high and was found in very high density in urban areas. The proliferation and spread of the species from urban to rural areas is facilitated by various unplanned developmental activities.

Infection, morbidity and diagnosis

The population in endemic areas is exposed to and receive infective mosquito bites. The infective stage larvae migrate to lymphatic system, mature, mate and produce the offspring, microfilariae. Several thousands of infective bites were shown to be necessary to establish a patent infection [20], characterized by presence of blood microfilaraemia. During the microfilaraemia stage of the infection, people do not exhibit overt clinical symptoms and hence described as 'asymptomatic' Mf carriers. Mf carriers (patent-infection) are the source of transmission and can remain asymptomatic for many years. Population with no microfilaraemia in endemic areas is generally referred to as 'endemic normals' [34]. However, a considerable proportion of infected population may harbour only adult worms with no presence of Mf [35]. Thus persons in amicrofilaraemic status may be uninfected or infected (harbour worms) but the worms yet to mature and produce Mf (pre-patent infection) or the produced Mf may have been cleared by the immune system or have worm(s) with single sex only [36,37].

All the people with adult worms develop lymphangiectasia, which is caused by the release of toxins by the worms. The host also reacts to the death of or damage to the adult worms leading to acute filarial lymphangitis (AFL) and this marks the beginning of the inflammatory phase of the disease process. The AFL episodes cause acute hydrocele as well as acute lymphedema. In most of the instances, the acute hydrocele and acute lymphedema get resolved and rarely lead to chronic lymphedema. However, the inflammatory host responses can precipitate some chronic disease manifestations such as hydrocele, chylocele and chyluria. Adult worm burden may also be an important factor in

the pathogenesis of hydrocele. Swelling of the skin due to accumulation of interstitial fluid following repeated bacterial infection and associated lymphatic dysfunction causes lymphedema. Hypertrophy and fibrosis of the skin and subcutaneous tissues after recurrent bacterial episodes may lead to elephantiasis, a prominent disfiguring clinical manifestation of LF [35].

For a long time the only way to measure infection in man is through the microscopic examination of Mf in peripheral blood samples. About 20 or 60 µl of finger prick blood sample is collected between 20.00-24.00 hours, thick smeared on a glass slide, dried overnight and dehaemoglobinized and stained on the next day and examined under microscope for the detection of Mf. A more sensitive method is to filter at least 1 ml of venous blood through a 3-5 µm NucleporeTM filter (Nuclepore Corporation, Pleasanton, CA). Other methods, which are less commonly used in detecting Mf in blood, are counting chamber, and Knott's concentration technique [23]. Recently filarial antigen based assays have been developed which can identify circulating antigens of *W. bancrofti* in microfilaraemic and amicrofilaraemic infected persons [38,39]. These assays can be used on finger-prick blood collected at any time of day. These assays can detect not only persons with Mf but also persons with single sex and those with worms as yet not produced Mf. Although these assays have considerably eased the task of diagnosis, they are very expensive and this limits their wider use in large-scale surveys.

1.2 Control of LF

Control of LF involves both preventing the spread of infection (transmission control) and alleviating the suffering caused by the disease (morbidity control). Transmission control can be achieved in two ways: by reducing the vector population (vector control) and by reducing the intensity of blood microfilaraemia (parasite control).

Transmission control

Vector control

Several measures are possible to control the vectors of LF. These include measures against adult mosquitoes and immature stages at community level and personal protection at individual and household level. Short-term vector control may not be useful for LF control and effective control measures for a period of 5-10 years, which is equivalent to the life span of the parasite, are necessary to prevent new infections and totally remove the existing infections. Such long-term vector control through residual spray of insecticides - an important malaria vector control tool - is not preferred because of logistic, cost and insecticide resistance problems. Larval control is the widely practiced method for LF vector control. Almost all types of anti-larval measures viz., environmental, chemical and biological methods play important role because the vector breeding habitats are of wide variety and no single method may be suitable for all situations.

Larval control is the main stay of the National Filaria Control Programme (NFCP) in India. An integrated vector management strategy in Pondicherry, India, that envisaged environmental, chemical and biological methods reduced the *C. quinquefasciatus* density by 80-90% over a period of five years [40,41]. However, subsequent withdrawal of the strategy leads to tremendous resurgence of the vector species. Polysterene beads were successfully used in the breeding habitats in India [42] and Tanzania [43]. Vector control had also been successful in eliminating *W. bancrofti* from Solomon Islands and parts of Papua New Guinea, where *Anopheles* species were the vectors of malaria and filariasis [44-46] and from Australia where *C. quinquefasciatus* was a vector [47]. While most of the vector control programmes still require both evaluation of their long-term impact and assessment of their cost-effectiveness [48,49], extension of larval control to the entire endemic areas in a large country like India may not be possible due to poor infrastructure and resource constraints.

Personal protection measures are gaining momentum in developing countries. Insecticide Treated Bed Nets (ITBNs), coils and repellents are widely used, particularly in urban areas, where *C. quinquefasciatus* density is very high [50,51]. A recent study showed that use of ITBNs reduced the prevalence of microfilaraemia significantly [52,53]. The impact of

ITBNs in reducing malaria morbidity and mortality is encouraging; however, their value against lymphatic filariasis infection and disease is yet to be established.

Parasite control

Parasite control aims at reducing the number of Mf and adult worms in the human population and consequently the uptake of Mf and transmission of infection by mosquitoes. The parasite population can be controlled through selective or mass treatment. Selective treatment is expensive and cumbersome as it involves detection of all Mf carriers and night blood screening of entire population using invasive blood sampling procedures. In mass treatment programmes, all persons in a community, irrespective of their Mf-status, are given treatment. Two anti-filarial drugs are widely used in mass treatment programmes: diethylcarbamazine (DEC) and ivermectin (IVR). However, co-administration of either of these drugs with albendazole is recommended for mass treatment under the LF elimination programme.

Both DEC and IVR are very effective microfilaricidal drugs. Several clinical trials showed that a single dose of DEC (6 mg/kg body weight) or IVR (200-400 μg/kg body weight) can reduce Mf intensity by 80-90% and these reduced levels can be sustained for about one year [54,55]. Most of the community trials with DEC standard 12 days-course [56-59] and repeated spaced (weekly, monthly or yearly) single-dose have demonstrated marked reduction in Mf-prevalence and intensity [60-71]. A recent community level study showed that six rounds of annual mass treatment of DEC or IVR could reduce Mf prevalence by 86% and 72% and the geometric mean intensity of Mf by 91% and 84% respectively [71].

DEC-salt trials in China [72-74], India [75-78] and in Taiwan [79] indicated that when salt is consumed for a minimum period of 6-9 months, it decreases Mf-prevalence by 70-100% [80]. Though short-term evaluations of most of the trials are encouraging, their long-term effectiveness and cost effectiveness needs assessment.

The effectiveness of ivermectin has been tested only in a limited number of community trials[42,69,70,81-84]. These studies indicate that a single dose ivermectin reduces the prevalence and intensity of Mf considerably.

Results from clinical and community trials suggest that albendazole (400 μg/kg) was as effective as DEC (6 mg/kg) or ivermectin (400 μg/kg) when it is given alone or in combination with DEC or ivermectin [85-91]. However, this drug is now advocated for use together with DEC or ivermectin for elimination.

Morbidity control

Studies on the role of bacterial and fungal infections in triggering ADL episodes have shown the need for the management of morbidity due to lymphatic filariasis [35,92-96]. Simple hygienic measures supplemented with antibiotics can have profound effect in preventing debilitating and damaging episodes of ADL [92,97,98] and halt or even to reverse the lymphedema and elephantiasis [99].

Control /elimination of LF

Several research findings during the last one and a half decade, particularly the discovery of the effect of DEC and IVR in single dose [48], availability of newer tools to manage morbidity [100] and new diagnostic tools such as Immunochromatography Test (ICT), lead to development of the global programme to eliminate LF as a public health problem by the year 2020. The strategy envisages (i) annual mass treatment with single dose of DEC or IVR in combination with albendazole and (ii) to alleviate the suffering and prevent or decrease the disability caused by the disease [101]. Several countries have already initiated LF elimination programme, including India.

1.3 LF control in India

A National Filaria Control Programme (NFCP) was initiated in India in the year 1955. The major goals of the programme were to delimit the endemic areas and to undertake control activities. The NFCP operates only in some urban and rural areas, where two-third of the affected population lives, are left with no organized control activities. As of now only 11% of the population is protected by the NFCP activities. The control operations under NFCP include vector control through the application of insecticides in breeding habitats and selective treatment of Mf carriers [17].

Following recent research advancements in control, diagnostic and rapid assessment tools, India has initiated steps to move from control to elimination. As an important step in this direction, in 1996 a pilot project has been initiated to assess the operational feasibility of mass administration of annual single-dose DEC (6 mg / kg body weight) in 13 districts covering a population of 41 million spread over 7 states (Bihar, Uttar Pradesh, West Bengal, Orissa, Andhra Pradesh, Tamil Nadu and Kerala). The programme also includes morbidity control through referral services at selective centres, and IEC (Information, Education, and Communication) components for creating health awareness and cooperation among the public. While the state governments implement the strategy, the national bodies such as National Institute of Communicable Diseases (NICD), Regional offices of Ministry of Health, Indian Council of Medical Research (ICMR) are involved in reviewing, monitoring and evaluating the programme in collaboration with the concerned state health authorities. In February 2001 the mass annual single dose DEC has been augmented with single dose albendazole (400μg) in 3 states (six districts in Tamil Nadu, two in Orissa, and one in Kerala) covering a population of 20.5 million.

Preliminary results from a south Indian state of Tamil Nadu, where an annual mass treatment programme has been initiated in all the endemic districts, indicate that there are several operational problems [102]. As a result, only 70-80% of the population received DEC and 45-55% consumed the DEC tablets. Several steps have been taken to resolve the operational problems. The possibility of extending this strategy throughout the endemic zones in India will depend on the results of this pilot study.

1.4 The Filariasis Control Demonstration Programme in Pondicherry, India

Pondicherry town, located between 11.45°E to 12.15°E north latitude and 79.35°E to 80.00°E east- longitudes (Fig. 4), has been endemic for bancroftian filariasis for many decades [103]. The Mf-prevalence increased from 6.3% in 1957 to 8.4% in 1981 [104,105]. Increase in human population (due to migration of people from villages to towns and cities), inadequate civic amenities, decrease in the level of environmental sanitation are factors contributing to the creation of mosquitogenic condition and thus for the upsurge of vector population, and filariasis prevalence in Pondicherry [106]. Recognising similar problems in many parts of India and the limitations posed by existing control strategies by NFCP, the Vector Control Research Centre (VCRC) of the Indian Council of Medical Research (ICMR) launched the Filariasis Control Demonstration Project (FCDP) in Pondicherry. The project was in operation from January 1981 to December 1985. The major objective of the project was to interrupt transmission of *W. bancrofti* by applying the Integrated Vector Management strategy for controlling the principal vector *C. quinquefasciatus* [40].

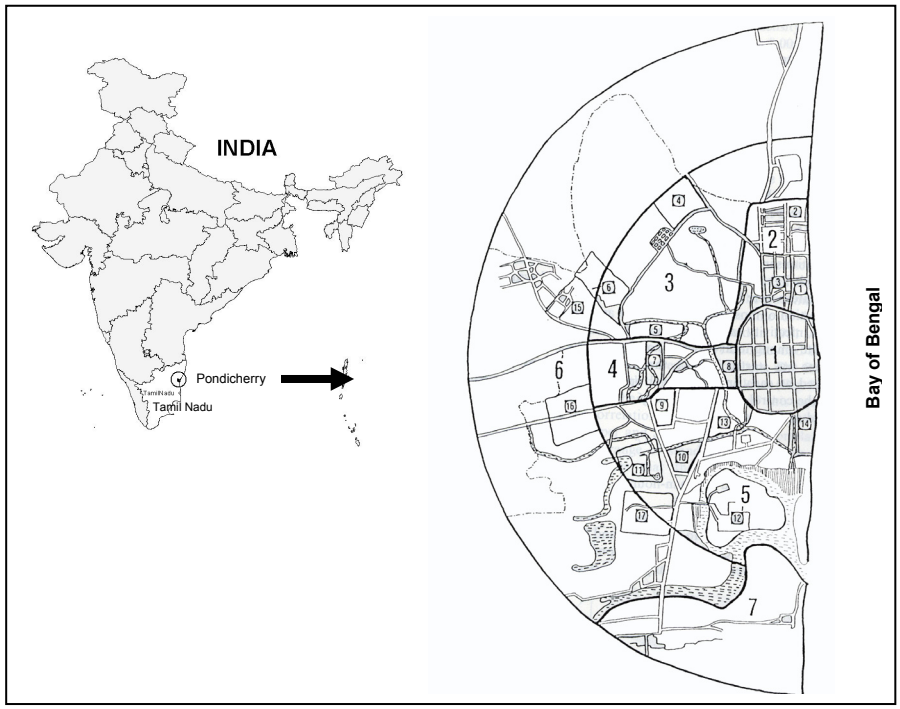

Figure 4 Pondicherry and its location in India along with the zones under integrated vector management (3, 4, 5 & 6) and conventional programmes (1, 2 & 7). Numbers in box are sites where entomological evaluations were carried out.

For operational convenience the entire area of Pondicherry town (59 Km^2; population, 1981 census: 272,000) was divided into 7 zones (Fig. 4) comprising 60 localities (sites). While the VCRC implemented the IVM strategy in one part of the town (zones 3, 4, 5 & 6; area: 48 Km^2; population: 164,000), the state government under the National Filariasis Control Programme (NFCP) applied the conventional strategy (larviciding with malarial oil with a spreading agent, and selective DEC-therapy) in the remainder of Pondicherry (zones 1, 2 & 7; area: 11 Km^2; population: 109,000). The IVM strategy aimed at reducing vector breeding through environmental management and judicial use of insecticides for larval control [40].

Throughout the IVM period VCRC evaluated the programme by monitoring both larval and adult vector density. Larval evaluation was done in all the 60 localities to provide feedback to the control operations. Entomological evaluation was done by weekly collection of man landing mosquitoes from 5 fixed sites and fortnightly collection of mosquitoes resting indoors from 17 fixed catching sites spread all over Pondicherry (Fig. 4 numbers in boxes). Parasitological data were collected during pre- (1981) and post-control periods (1986, 1989 and 1992) from approximately 10% of the population

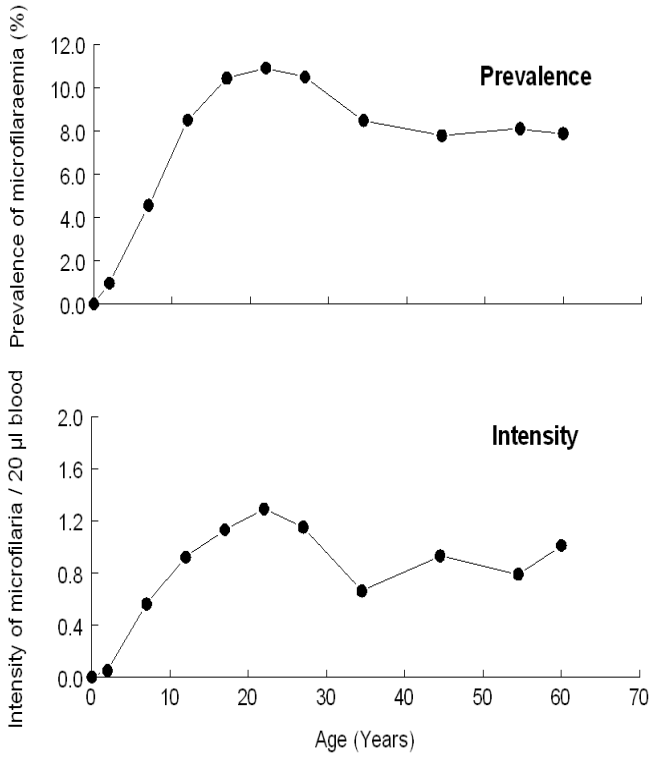

Figure 5 Pre-control epidemiological situation in Pondicherry in 1981.

(272,000; 1981 census) in Pondicherry using a stratified random sampling protocol with household as the sampling unit. Figure 5 depicts the pre-control epidemiological situation in Pondicherry. Both prevalence and intensity of Mf increases monotonically until 20 years of age, following which there was a decline until about 40 years to become relatively stable in older age classes [104].

Entomological and parasitological evaluation of the programme in 1986 demonstrated that the IVM strategy achieved significant reductions in vector density, intensity of transmission and prevalence of Mf in human and that the conventional larviciding did not achieve similar reductions in the NFCP area [107]. Further epidemiological evaluations indicated that there were few new infections in children born (i.e. children of age 1-5 years) during the intervention period in both IVM and NFCP areas. However, the number of new infections was lower than the pre-control period. This would indicate that both the strategies though reduced the transmission they did not completely interrupt. On the other hand, the impact of vector control on epidemiological parameters can only be seen many years after stopping control, as vector control can only prevent new infections but not the existing one. Therefore it was felt necessary to have an assessment of the long-term effectiveness and cost-effectiveness of IVM for making a rational decision about control strategies. Such an assessment, however, would largely depend on the population dynamics of the parasite in human and vector.

1.5 Modelling LF: a review[1]

Reasoning about consequences of interventions on the epidemiology of infectious diseases is very difficult, because of the feedback of the prevalence of infectious cases on the future incidence. This is why mathematical models for infectious diseases are an essential part of research in infectious disease epidemiology, and also why they tend to be more complex by this feedback from prevalence to incidence than models for non communicable diseases. Indeed, mathematical/statistical models provide the necessary quantitative framework for investigating key issues about parasite's population dynamics, making credible predictions of epidemiological trends, and aiding decisions about control strategies. There are several examples of the successful and practical application of mathematical models in disease control, the most recent being the application of a simulation model in the Onchocerciasis Control Programme in the West Africa [108]. In lymphatic filariasis, simple models have been applied to gain valuable insights into the dynamics of *W. bancrofti* infection both in human and in the vector mosquitoes. The models developed and applied for lymphatic filariasis can be broadly classified into two categories: analytical and simulation. Analytical models were used to describe certain fundamental characteristics of the dynamics of transmission, infection and disease. They are usually based on sets of differential equations that keep track of few important variables such as the number susceptible/infected. Simulation models do not provide explicit relationships between key characteristics, but they provide a comprehensive framework for the quantitative description of the dynamics of transmission, infection and disease and of the impact of control.

Modelling dynamics of infection and disease in human

Two analytical approaches for describing the dynamics of lymphatic filariasis infection and disease in human are: prevalence models and intensity models. Prevalence models consider only the changes in infection status of individuals (i.e. microfilaraemic or amicrofilaraemic). Intensity models explicitly consider the number of microfilaria per host. If an assumption about the distribution of the number of Mf per host is made, these models can also be used to describe the prevalence of infection.

Prevalence models

Catalytic models are originally developed for describing chemical reactions between molecules of a substance and a catalytic substance [109]. These models were applied in lymphatic filariasis or other filarial diseases especially onchocerciasis to represent the age-distribution of Mf prevalence in the human population [22,110-112]. Hayashi [113] was the first

[1] This part of the text has been adapted from Chapter 4, Section 4.2: Modelling the epidemiology, transmission and control of lymphatic filariasis

to apply catalytic models to describe the age-specific incidence of infection and disease using cross-sectional data. Hayashi assumed that susceptible individuals can become infected and recover and that, individuals who are or have been infected will always be resistant to further infection. Later, in a 'historic' development in the epidemiology of lymphatic filariasis, Hairston and Jachowski [22] applied a modified version of Muench's catalytical model to gain quantitative insights into the dynamics of filarial infection. Unlike Hayashi, Hairston and Jachowski assumed that both infection and recovery were reversible. This means that individuals could switch from being amicrofilaraemic to microfilaraemic and vice versa. Analyses of cross-sectional data by Hairston and Jachowski [22] provided the first estimates of the duration of the fecundic lifespan of *W. bancrofti* and of the mean number of adult worms in each infected individual. Applying the same reversible catalytic model to a longitudinal data from Pondicherry, India, Vanamail *et al.* [24,114] estimated the age-specific rates of gain and loss of infection. Their results indicated that the rate of loss of infection is independent of age whereas the rate of gain is age-dependent until adulthood and then declines or stabilizes. This pattern suggests that there may be some density-dependent limitation on the prevalence of human infection. The loss rate provided an estimate of the fecundic life span (patent period) of the adult worm (5-10 years). In further analyses of the same dataset from Pondicherry, attempts were made to define the relationship between microfilaraemia and the development of chronic lymphedema in the infected population [115,116]. After demonstrating a significant correlation between cumulative loss rate and age-specific disease prevalence, Srividya *et al.* [115] and Bundy *et al.* [116], inferred their results as that people develop disease after clearing their Mf and that the immune system may play an important role in combating the infection and provoking disease symptoms. However, recent research findings do not support the above preposition that development of disease is always sequential (microfilaraemic, amicrofilaraemic, acute and chronic manifestations). The new tenet is that development of disease depends on the cofactors such as adult worm burden, degree and extent of lymphangiectasia, location of adult worm nests and chronology of adult worm death [35,117].

Intensity models

When differences in number of parasites between persons in a population would only be due to chance, the variability between persons would follow a Poisson distribution. If the variability between persons is greater than Poisson variability ("over-dispersion") we use the term "aggregation". Most of the macro-parasite populations are aggregated in their host populations [118-123]. The degree of aggregation plays an important role in the dynamics of host-parasite system. Two measures are commonly used to characterise the degree of aggregation: variance to mean ratio ('vmr'), and the parameter 'k' (which is an inverse measure of aggregation) of the negative binomial distribution, which is a generalization of the Poisson distribution. For vmr =1 or equivalently 'k' tends to infinity, the distribution is completely random (negative binomial becomes Poisson) and for 'vmr' significantly greater than unity or for finite values of 'k', the distribution is aggregated [124].

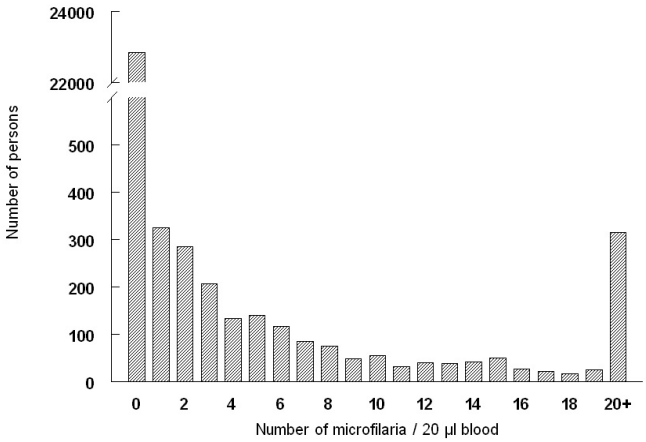

Figure 6 Frequency distribution of microfilaria counts in humans in Pondicherry.

Since the assessment of adult worm burden is difficult in lymphatic filariasis, the frequency distribution of Mf in blood samples was used instead. Figure 6 depicts the frequency distribution of Mf-counts in humans prior to the introduction of FCDP in Pondicherry. The aggregated nature of the distribution is reflected in a small value of 'k' (0.019) and the large 'vmr' (35.9). Figure 6 also indicates that a large proportion of the individuals are Mf-negatives, which is similar to earlier observations in other parts of the world [125-128]. Individuals who are apparently uninfected on the basis of blood diagnosis may either be true Mf-negatives, or only apparently negative ('false negatives') as a result of sampling process, including the volume of blood examined [129,130]. Attempts were made to reasoning the unusually large number of Mf-negatives arising from microfilaria surveys. Poisson [22], lognormal [130] and negative binomial [128] models have been fitted to describe the observed microfilaria distributions in humans. These models helped to quantify both prevalence and intensity of Mf in humans, and the resulting potential transmission rate to the vector population.

Grenfell *et al.* [131] developed a model for the relationship between microfilarial burden and the prevalence of adult (macrofilarial) worms which indicated that most of the observed amicrofilaraemic persons are probably 'true zeros', arising from the absence of macrofilarial infection or presence of adult worms of only one sex, rather than being 'false-negatives' attributable to blood-sampling process. The distribution of Mf should therefore follow a Poisson mixture (negative binomial), arising from the sampling of Mf-positives with an additional proportion of 'true' Mf-zeros. By fitting zero-truncated

negative binomial distributions to the counts of Mf in different age groups, Das *et al.* [132] examined the effects of host age and sex on the distribution of *W. bancrofti* infections in the human host. They have shown a reduction in degree of aggregation ('k' increased) with increasing age. This effective shrinking of the tail of the Mf-distribution with host-age provided indirect evidence for the operation of density-dependent constraints such as acquired immunity on the intensity of Mf. These findings corroborate with the evidence of immunological constraints on parasite establishment both from the cat model [133,134] and from field data [135,136]. Further, the analysis by Das *et al.* [132] also provided estimates of the numbers of microfilaraemics who are identified as amicrofilaraemic because of sampling errors or deficiencies (around 5% of all of those recorded as amicrofilaraemic).

Modelling dynamics of infection in the vector

Quantifying parasite transmission is important for understanding the dynamics of infection in the vector and for assessing the impact of control programmes. Transmission studies in the laboratory [112,127,137-146] and in the field [19,138,141] have been carried out to understand the transmission and dynamics of infection in the vector. In most of these studies, statistical models have been used to describe the relationship between the parasite load in the mosquito and the Mf-density in human blood. Some of these models focused on the infection dynamics in the vector host [140,143] whereas others considered the overall success of transmission through the parasite's lifecycle [139,147,148]. The results of most of the experimental studies in which *C. quinquefasciatus* were fed on human volunteers infected with *W. bancrofti* have shown that the number of Mf in the mosquito blood meal is related linearly to the human Mf-density. However, the relationship between the numbers of L3 developed in the mosquitoes and the Mf-density in human was found to be non-linear and saturating, indicating a density-dependent regulation of parasite numbers within the mosquito [140,143]. The key issue in all of these studies was the mechanism that regulates parasite number in the mosquitoes.

Analysis of the frequency distribution of the counts of parasites per mosquito in wild-caught mosquitoes suggested *W. bancrofti*-induced mortality of *C. quinquefasciatus* [138,141,149]. This was confirmed in experimental studies, when the survival distributions of mosquitoes fed on microfilaraemics were found to differ markedly from those of similar mosquitoes fed on amicrofilaraemics [137,150]. The results of experimental and field studies, involving *W. bancrofti* and *C. quinquefasciatus*, provide evidence for the regulation of *W. bancrofti* larvae in *C. quinquefasciatus*. Knowledge of the functional relationship between the numbers of L3 developed and the Mf-density in human blood is crucial to the development of mathematical models and to the role that they can play in the design and monitoring of interventions. Such knowledge needs to be generated for all the mosquito vectors involved in the transmission of *W. bancrofti*, including the Anophelines [151].

Depending on the number of Mf ingested and the vector-parasite combination three types of relationships have been identified for the yield of L3-larvae (number of L3 per ingested Mf), as a function of the number of ingested Mf: proportionality, a constant

ratio of L3 yield to ingested Mf; facilitation, an increase in this ratio; and limitation, a decrease in this ratio [143]. The epidemiological significance of these relationships have been discussed elsewhere [112,140,143-145,149,152,153]. In case of limitation it may be difficult to interrupt transmission even when control programmes reduce Mf-prevalence and intensities to very low levels; whereas in case of facilitation it is relatively easy to block transmission and eradicate the parasite from the human population. Limitation has been observed in *C. quinquefasciatus* and in certain species of *Aedes* transmitting *W. bancrofti*; facilitation only in Anopheline mosquitoes transmitting *W. bancrofti*; proportionality in *Manosonia bonneae* transmitting *B. malayi* [143]. Although most of the transmission studies provide convincing evidence for the existence of a limitation phenomenon in *W. bancrofti*-*C. quinquefasciatus* complex, its epidemiological significance is not well known [154]. Further, since limitation was found to occur only at very high Mf-densities in a small percentage of the vector population, its implication for parasite regulation in the vector is expected to be minimal [154].

Development of comprehensive models

Application of the models discussed above provides valuable insights into the dynamics of infection both in human and mosquitoes. These models describe a specific process (e.g. dynamics of infection in human, and mosquitoes) in the transmission cycle of the parasite, and are not intended to take account of the other inter-relationships between the parasite and human and vector hosts. Thus from the point of view of the total transmission cycle and implication for dynamics, they can be considered "partial models". Since all components of the transmission cycle are likely to be affected by long-term control measures, partial models are not a complete modelling tool for planning, evaluation and monitoring of control programmes. Furthermore, in order to use available public health resources most efficiently and effectively it becomes increasingly important to assess the costs and effectiveness of various control strategies. All these strategic issues necessitate developing models that include the full transmission cycle of the parasite.

Rochet [155] was the first to develop a simulation model for lymphatic filariasis transmission dynamics. The model is deterministic and relates the fluctuations in mosquito population with the environmental variables such as climate and vector control using a differential equations approach. The model has been applied to describe the observed entomological and epidemiological trends in the IVM area in Pondicherry. The model described the trends in most entomological parameters well but not the infection rate in mosquitoes. The model was detailed enough to describe the dynamics of the vector population but did not consider the mechanism that regulates the parasite population in both humans and vector.

Recognising the lack of a quantitative framework for describing the dynamics of infection and morbidity, a deterministic model called EPIFIL has been developed [156]. In EPIFIL, the infection dynamics is modelled using the immigration-death formulation, incorporating the acquisition of immunity to infective larvae over time. The dynamics of

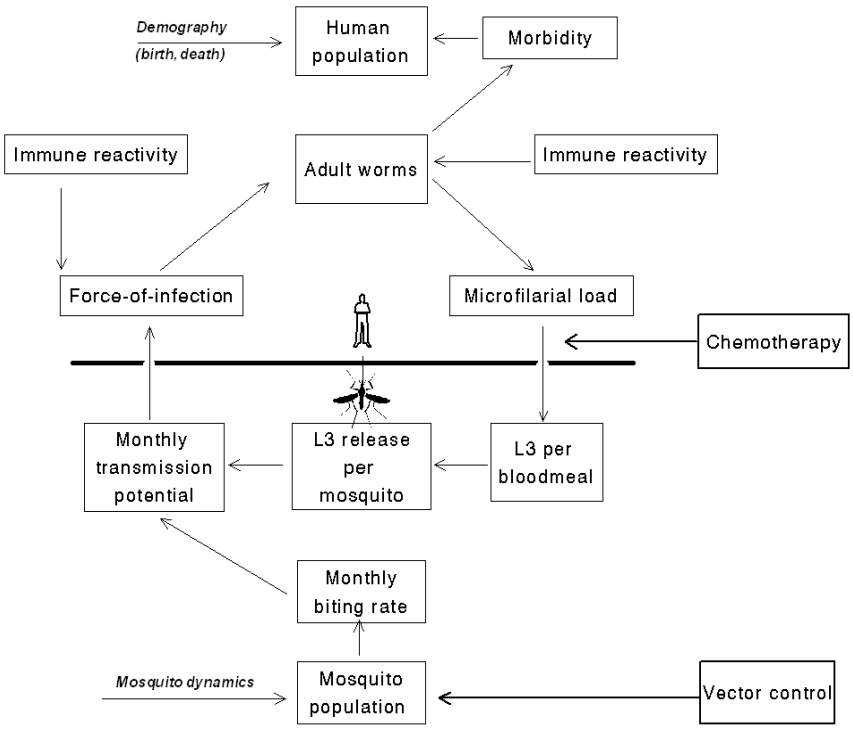

Figure 7 Structure of the LYMFASIM model.

disease is modelled as a function of worm load and the impact of immunological responses. The model parameters are quantified using the pre-control cross-sectional observations from the same IVM area in Pondicherry. The good fit of the model to these data suggests that both hydrocele and lymphedema are irreversible conditions that develop as a consequence of lymphatic damage caused by the worms, with the risk of disease being higher for hydrocele than lymphedema. The fit of the model to the data did not support the hypothesis that disease progression is immune mediated. Application of the model to simulate the effectiveness of various control measures indicated that chemotherapy (DEC or ivermectin) has a larger short-term impact on the intensity of Mf than vector control but the effects of vector control can last longer beyond the 5-year period of intervention [157]. Further, evaluation of the impact of 10-year annual single-dose mass DEC or ivermectin showed that DEC is superior to ivermectin in reducing the Mf-load and that combining DEC and ivermectin has little benefit. Recently EPIFIL was modified to provide a comprehensive framework for investigating the mechanisms linking filarial transmission intensity, infection and disease in endemic communities and the model was applied to examine the role of two anti-filarial immunities, one reflecting

accumulated experience of worms and the other reflecting cumulative exposure to infective larvae [158]. Application of the modified version of EPIFIL to East African data indicated that acquired immunity in filariasis is transmission driven and may be significant only in areas of high transmission. Further, prevalence of both hydrocele and lymphedema might increase disproportionately with transmission intensity without immunopathological involvement but progression of lymphedema might be accelerated with the onset of immunopathology.

Deterministic macro-simulation models, like EPIFIL, assume that there are no individual differences in exposure, immune response, and human demographic characteristics and therefore the outcomes of these models do not suffer from stochastic variability. Stochastic models account for such variability and therefore the outcomes are variable between simulations. The variable outcomes are useful to have an assessment of the risk involved in making decisions about certain control options. But the validation of stochastic models is hampered by the variable output and the difficulty in using the maximum likelihood procedures.

After the development and application of the stochastic epidemiological simulation model ONCHOSIM for onchocerciasis [108,111,159], the Department of Public Health, Erasmus University Rotterdam and VCRC jointly developed a simulation model called 'LYMFASIM' for lymphatic filariasis transmission and control. LYMFASIM is a micro-simulation computer model. The micro-simulation techniques allow for simultaneous simulation of the life histories of humans, parasites, the vector population and the impact of interventions on both parasite and vector (Fig. 7). The initial quantification of model parameters was based on cross-sectional epidemiological data collected in two areas of Greater Recife, Brazil.

A range of model assumptions was found to be compatible with these data. In order to apply LYMFASIM under operational programmes, it was necessary to narrow down the range of model assumptions. In response to this, it was decided to adapt, quantify and validate LYMFASIM on the basis of longitudinal parasitological observations generated by the IVM programme in Pondicherry. The development of LYMFASIM to simulate the impact of IVM on transmission dynamics is the topic of the thesis.

1.6 Aims of the thesis

The thesis aims: (i) to contribute to knowledge through quantification of the population dynamics of *W. bancrofti* both in human and in vector, (ii) to contribute to the development and application of "integrated transmission models" for lymphatic filariasis that could aid in making policy decisions about control programmes, and (iii) to create a situation that the model can be used for studying the dynamics of recrudescence of infection after cessation of control. The thesis tries to accomplish these aims through answering the following research questions:

1. Is the integrated vector management a better method than the conventional vector control programme to control transmission of filariasis?
2. Which of the epidemiological parameters – overall or age-specific prevalence or intensity of Mf in humans – is sensitive to assess the impact of vector control?
3. What would be the impact of recovery of the vector population on the dynamics of *W. bancrofti* infection after cessation of 5 years of IVM?
4. Is there any regulation in the processes of *W. bancrofti* Mf-uptake by, and larval development, within the vector host *C. quinquefasciatus*?
5. Does parasite regulation occur through parasite-induced vector mortality?
6. Is regulation of parasite numbers in humans immune mediated?
7. What would be the impact of human immune mechanisms regulating *W. bancrofti* infections on lymphatic filariasis control programmes?
8. What could be the long-term impact of IVM programme in Pondicherry?
9. What are the prospects for elimination of LF by mass drug administration programmes?

1.7 Structure of the thesis

The epidemiological evaluation of the effect of integrated vector management (IVM) and conventional control programmes in Pondicherry is presented in Chapter 2. Since independent evaluation of the IVM and conventional programmes, based on entomological and epidemiological parameters, indicated that the programmes have achieved their objective of reducing transmission, emphasis was on a comparative assessment of the IVM and conventional programmes. In Section 2.1, the effect of IVM is compared with conventional programme by assessing the relative changes in epidemiological parameters between pre (1981)- and post (1986)-intervention periods. In **Section 2.2,** the impact of recovery of the vector population 3-years after terminating the IVM-programme is evaluated by relating the changes in entomological and epidemiological parameters. Further, the data used in **Section 2.1** is the basis for quantification and validation of model in the later part of the thesis.

The mechanisms that regulate the parasite numbers both in human and vector are the important determinants for the persistence of infection and disease in the human population. Further, as argued earlier, parasite regulation in mosquitoes has important implications for the control of filariasis. Therefore extensive analyses of data from field and experiment have been carried out to understand and quantify the transmission dynamics of the *W. bancrofti* infection in *C. quinquefasciatus* (**Chapter 3**). In **Section 3.1**, through a semi-experimental transmission study, the relationships of human Mf-density with uptake and development of *W. bancrofti* Mf by *C. quinquefasciatus* was quantified and the data was further used to identify the vector-parasite relationship. This quantification has become an important input towards the development and application of LYMFASIM. The conclusion of the experimental study was confirmed using data on indoor resting mosquitoes collected from 17 sites in Pondicherry (**Section 3.2**). Vector life expectancy is an important factor influencing the success of parasite transmission. Mosquitoes have to survive longer than the time taken by the parasite to develop Mf to the infective stage in order to be transmitted to the human host during successive blood meals. In **Section 3.3,** age-distributions of infections in *C. quinquefasciatus* was examined to see if there is any reduction in survival of naturally infected mosquitoes using the same data used in section 3.2 as well as from 5 sites where man-landing collections were carried out. **Section 3.4,** in addition to confirming the findings of Section 3.3, provides quantification of the risk of parasite density related mortality of the vector using data from an experimental transmission study.

In **Chapter 4**, **Section 4.1** deals with the development of LYMFASIM model, its implementation in a computer simulation program and a formal mathematical description of the model. **Section 4.2** reviews the development of the mathematical/statistical models, their application in studies of the epidemiology, transmission and control of *W. bancrofti* and in the optimisation of control strategies and in predicting the outcome of the various interventions. Application of LYMFASIM for quantifying the population

dynamics of the parasite, its usefulness for making long-term predictions of the impact of IVM-programme and for estimating the number of treatment rounds necessary to achieve the goal of elimination by the ongoing mass drug administration (MDA) programmes are presented in **Sections 4.3** and **4.4** respectively. **Chapter 5** discusses the chapters from 2 to 4, and gives conclusions and recommendations.

References

1. World Health Organization. Global Programme to Eliminate Lymphatic Filariasis. Annual Report on Lymphatic Filariasis 2001. Geneva: World Health Organization, 2002.
2. Yamaguti S. The nematodes of vertebrates. NewYork: Interscience Publisher, 1961.
3. Sabesan S, Palaniyandi M, Das PK, Michael E. Mapping of lymphatic filariasis in India. *Annals of Tropical Medicine and Parasitology* 2000;**94**(6)**:**591-606.
4. Cobbold TS. Discovery of the adult representative of a microscopic Filariae. *Lancet* 1877;**2:**70-1.
5. Say T. Descriptions of dipterous insects of the United States. *Journal of Academy of Natural Science Philadelphia* 1823;**3:**9-54.
6. Evans DB, Gelband H, Vlassoff C. Social and economic factors and the control of lymphatic filariasis: a review. *Acta Tropica* 1993;**53:**1-26.
7. World Bank. World Development Report 1993. Investing in health (World development indicators). New York: Oxford University Press, 1993.
8. Ramu K, Ramaiah KD, Guyatt HL, Evans DB. Impact of lymphatic filariasis on the productivity of male weavers in a south Indian village. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1996;**90:**669-670.
9. Sabesan S, Krishnamoorthy K, Pani SP, Panicker KN. Man-days lost due to repeated acute attacks of lymphatic filariasis. *Trends in Life Sciences (India)* 1992;**7:**5-7.
10. Gyapong JO, Gyapong M, Evans DB, Aikins MK, Adjei S. The economic burden of lymphatic filariasis in northern Ghana. *Annals of Tropical Medicine and Parasitology* 1996;**90 (1):**39-48.
11. Babu BV, Nayak AN, Dhal K, Acharya AS, Jangid PK, Mallick G. The economic loss due to treatment costs and work loss to individuals with chronic lymphatic filariasis in rural communities of Orissa, India. *Acta Tropica* 2002;**82**(1)**:**31-8.
12. Ramaiah KD, Radhamani MP, John KR*, et al.* The impact of lymphatic filariasis on labour inputs in southern India: results of a multi-site study. *Annals of Tropical Medicine and Parasitology* 2000;**94**(4)**:**353-64.
13. Ramaiah KD, Das PK, Michael E, Guyatt H. The economic burden of lymphatic filariasis in India. *Parasitol Today* 2000;**16**(6)**:**251-3.
14. World Health Organization. The World Health Report 2000. Health Systems: Improving Performance. Geneva: World Health Organization, 2000.
15. Molyneux DH, Zagaria N. Lymphatic filariasis elimination: progress in global programme development. *Ann Trop Med Parasitol* 2002;**96 Suppl 2:**S15-40.
16. Michael E, Bundy DA, Grenfell BT. Re-assessing the global prevalence and distribution of lymphatic filariasis. *Parasitology* 1996;**112**(Pt 4)**:**409-28 issn: 0031-1820.
17. Sharma RS, Biswas H, Saxena NBL, eds. National Filaria Control Programme India. Operational Mannual. New Delhi: National Malaria Eradication Programme, 1995.
18. Das PK, Ramaiah KD, Augustin DJ, Kumar A. Towards elimination of lymphatic filariasis in India. *Trends in Parasitology* 2001;**17**(10)**:**457-60.
19. Krafsur ES, Garrett Jones C. The survival in nature of *Wuchereia*-infected *Anophels funestus* Giles in North-eastern Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1977;**71:**155-60.
20. Rajagopalan PK, Kazmi SJ, Mani TR. Some aspects of transmission of *Wuchereria bancrofti* and ecology of the vector *Culex pipiens fatigans* in Pondicherry. *Indian Journal of Medical Research* 1977;**66:**200-15.

21. Paily KP, Hoti SL, Manonmani AM, Balaraman K. Longevity and migration of *Wuchereria bancrofti* infective larvae and their distribution pattern in relation to the resting and feeding behaviour of the vector mosquito, *Culex quinquefasciatus*. *Annals of Tropical Medicine and Parasitology* 1995;**89**(1)**:**39-47.
22. Hairston NG, Jachowski LA. Analysis of the *Wuchereria bancrofti* population in the people of American Samoa. *Bulletin of the World Health Organization* 1968;**38:**29-59.
23. World Health Organization. Lymphatic Filariasis: the disease and its control. WHO Technical Report Series, 821. Geneva: WHO, 1992: 1-71.
24. Vanamail P, Subramanian S, Das PK*, et al.* Estimation of age-specific rates of acquisition and loss of *Wuchereria bancrofti* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1989;**83:**689-93.
25. Vanamail P, Subramanian S, Das PK, Pani SP, Rajagopalan PK. 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* 1990;**91:**293-7.
26. Carme B, Laigret J. Longevity of *Wuchereria bancrofti var. pacifica* and mosquito infection acquired from a patient with low level parasitemia. *American Journal of Tropical Medicine & Hygiene* 1979;**28:**53-5.
27. Southgate BA. Recent advances in the epidemiology and control of filarial infections including entomological aspects of transmission. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1984;**78:**19-28.
28. Kaul H, N., Wattal BL, Singha P. Chemical characteristics of *Culex pipiens fatigans* breeding waters in areas around Delhi. *Journal of Communicable Diseases* 1977;**9**(1)**:**8-21.
29. Menon PKB, Rajagopalan PK. Relative importance of different types of breeding habitats in contributing to the population of *Culex pipiens fatigans* in Pondicherry. *Indian Journal of Medical Research* 1980;**71:**725-733.
30. Rajagopalan PK. Population dynamics of *Culex pipiens fatigans*, the filariasis vector, in Pondicherry - Influence of climate and environment. *Procedings of the Indian National Science Academy* 1980;**B46:**745-751.
31. Yasuno M, Harinasuta C. The studies of the beahviour of *Culex pipiens fatigans* with special reference to dispersal, mating and feeding. *Japanese Journal of Experimental Medicine* 1967;**37:**525-534.
32. Subra R. Biology and control of *Culex quinquefasciatus* Say 1823 (Diptera, Culicidae) with special reference to Africa. 1980;**WHO/VBC/80.781**.
33. Nathan MB. Bancroftian filariasis in coastal North Trinidad, West Indies: intensity of transmission by *Culex quinquefasciatus*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1981;**75:**721-30.
34. Ottesen EA. Immunological aspects of lymphatic filariasis and onchocerciasis in man. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1984;**78:**9-18.
35. Dreyer G, Noroes J, Figueredo-Silva J, Piessens WF. Pathogenesis of lymphatic disease in bancroftian filariasis: a clinical perspective. *Parasitol Today* 2000;**16**(12)**:**544-8.
36. Maizels RM, Lawrence RA. Immunological tolerance: the key feature in human filariasis. *Parasitology today* 1991;**7:**271-276.
37. Ottesen EA. The Wellcome Trust Lecture. Infection and disease in lymphatic filariasis: an immunological perspective. *Parasitology* 1992;**104**(9)**:**S71-S79.
38. More SJ, Copeman DB. A highly specific and sensitive monoclonal antibody-based ELISA for the detection of circulating antigen in bancroftian filariasis. *Tropical Medicine and Parasitology* 1990;**41:**403-6.
39. Weil GJ, Lammie PJ, Weiss N. The ICT filariasis test: a rapid-format antigen test for diagnosis of bancroftian filariasis. *Parasitology Today* 1997;**13:**401-404.
40. Rajagopalan PK, Das PK. The Pondicherry Project on Integrated Disease Vector Control. (Filariasis Control Demonstration Project). Pondicherry: Vector Control Research Centre, 1987.

41. Ramaiah KD, Das PK, Arunachalam N, Rajavel AR, Paily KP. Observations on population density of *Culex quinquefasciatus* and transmission indices of Bancroftian filariasis during and after Integrated Vector Management strategy. *Journal of Communicable Diseases* 1992;**24:**173-84.
42. Reuben R, Rajendran R, Sunish IP*, et al.* Annual single-dose diethylcarbamazine plus ivermectin for control of bancroftian filariasis: comparative efficacy with and without vector control. *Annals of Tropical Medicine and Parasitology* 2001;**95**(4)**:**361-378.
43. Maxwell CA, Curtis CF, Haji H, Kisumku S, Thalib AI, Yahya SA. Control of Bancroftian filariasis by integrating therapy with vector control using polystyrene beads in wet pit latrines. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1990;**84:**709-14.
44. Webber RH. The natural decline of *Wuchereria bancrofti* infection in a vector control situation in the Solomon Islands. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1977;**71:**396-400.
45. Webber RH. Eradication of *Wuchereria bancrofti* infection through vector control. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1979;**73:**722-4.
46. Bockarie M. Can lymphatic filariasis be eradicated in Papua New Guinea? [editorial]. *Papua New Guinea Medical Journal* 1994;**37:**61-4.
47. Boreham PFL, Marks EN. Human filariasis in Australia: introduction, investigation and elimination. *Procedings of the Royal Society of Queensland* 1986;**97:**23-52.
48. Ottesen EA, Duke BOL, Karam M, Behbehani K. Strategies and tools for the control/elimination of lymphatic filariasis. *Bulletin of the World Health Organization* 1997;**75**(6)**:**491-503.
49. Burkot T, Ichimori K. The PacELF programme: will mass drug administration be enough? *Trends in Parasitology* 2002;**18**(3)**:**109-15.
50. Mulla MS. New techniques and measures for the suppression of filariasis vectors. *International Congress forTropical Medicine & Malaria 8th Teheran Abstract Review* 1968**:**123-4.
51. Snehalatha KS, Ramaiah KD, Vijay Kumar KN, Das PK. The mosquito problem and type and costs of personal protection measures used in rural and urban communities in Pondicherry region, South India. *Acta Tropica* 2003;**88**(1)**:**3-9.
52. Croft AM, Baker D, von Bertele MJ. An evidence-based vector control strategy for military deployments: the British Army experience. *Med Trop* 2001;**61**(1)**:**91-8.
53. Bockarie MJ, Tavul L, Kastens W, Michael E, Kazura JW. Impact of untreated bednets on prevalence of *Wuchereria bancrofti* transmitted by *Anopheles farauti* in Papua New Guinea. *Medical and Veterinary Entomology* 2002;**16**(1)**:**116-9.
54. Cao WC, Van der Ploeg CPB, Plaisier AP, Ivera van der Sluijs IJ, Habbema JDF. Ivermectin for the chemotherapy of bancroftian filariasis: a meta analysis of the effect of single treatment. *Tropical Medicine and International Health* 1997;**2:**393-403.
55. Subramanyam Reddy G, Venkateswaralu N, Das PK*, et al.* Tolerability and efficacy of single dose diethylcarbamazine (DEC) or ivermectin in the clearance of *Wuchereria bancrofti* microfilaraemia in Pondicherry, south India. *Tropical Medicine and International Health* 2000;**5:**779-785.
56. Wijers DJ, Kaleli N. Bancroftian filariasis in Kenya. V. Mass treatment given by members of the local community. *Annals of Tropical Medicine and Parasitology* 1984;**78:**383-94.
57. Sharma SP, Kosare GJ, Biswas H, Das M, Trivedi GK, Sharma GK. Control of bancroftian filariasis in rural areas through selected treatment with diethylcarbamazine. *Journal of Communicable Diseases* 1986;**18:**283-6.
58. Biswas H, Sharma SP, Das M, Rao VG, Yadava RL, Narasimham MV. Filariasis control in rural areas through detection and treatment with diethylcarbamazine. *Journal of Communicable Diseases* 1989;**21:**272-81.

59. Ramaiah KD, Pani SP, Balakrishnan N*, et al.* Prevalence of bancroftian filariasis & its control by single course of diethyl carbamazine in a rural area in Tamil Nadu. *Indian Journal of Medical Research* 1989;**89:**184-91.
60. Laigret J, Fagneaux G, Tuira E. [Mass chemotherapy with spaced doses of diethylcarbamazine: effects in Tahiti on microfilaraemia due to *Wuchereria bancrofti* var. pacifica]. *Bulletin of the World Health Organization* 1980;**58:**779-83.
61. Ottesen EA. Efficacy of diethylcarbamazine in eradicating infection with lymphatic-dwelling filariae in humans. *Review of Infectious Diseases* 1985;**7:**341-56.
62. Galtier J, Julvez J, Michault A, Isautier H. [Effectiveness of mass chemotherapy campaigns with diethylcarbamazine on blood microfilaria of *Wuchereria bancrofti* in Mayotte]. *Bull Soc Pathol Exot Filiales* 1987;**80:**826-33.
63. Nathan MB, Hamilton PJ, Monteil S, Tikasingh ES. Bancroftian filariasis in coastal north Trinidad: the effects of mass chemotherapy using spaced doses of diethylcarbamazine citrate on human microfilaraemias and vector infection rates. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1987;**81:**663-8.
64. Panicker KN, Krishnamoorthy K, Sabesan S, Prathiba J, Abidha. Comparison of effects of mass annual and biannual single dose therapy with diethylcarbamazine for the control of Malayan filariasis. *Southeast Asian Journal of Tropical Medicine and Public Health* 1991;**22:**402-11.
65. Balakrishnan N, Ramaiah KD, Pani SP. Efficacy of bi-annual administration of DEC in the control of bancroftian filariasis. *Journal of Communicable Diseases* 1992;**24:**87-91.
66. Ottesen EA, Ramachandran CP. Lymphatic filariasis infection and disease: control strategies. *Parasitology today* 1995;**11:**129-131.
67. Meyrowitsch DW, Simonsen PE. Long term effect of mass diethylcarbamazine chemotherapy on bancroftian filariasis: results at four years after start of treatment. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1998;**92:**98-103.
68. Mataika JU, Kimura E, Koroivueta J, Shimada M. Efficacy of five annual single doses of diethylcarbamazine for treatment of lymphatic filariasis in Fiji. *Bulletin of the World Health Organization* 1998;**76**(6)**:**575-9.
69. Bockarie MJ, Alexander ND, Hyun P*, et al.* Randomised community-based trial of annual single-dose diethylcarbamazine with or without ivermectin against *Wuchereria bancrofti* infection in human beings and mosquitoes. *Lancet* 1998;**351**(9097)**:**162-8.
70. Das PK, Ramaiah KD, Vanamail P*, et al.* Placebo-controlled community trial of four cycles of single-dose diethylcarbamazine or ivermectin against *Wuchereria bancrofti* infection and transmission in India. *Transactions of the Royal Society of Tropical Medicine & Hygiene* 2001;**95**(3)**:**336-41.
71. Ramaiah KD, Vanamail P, Pani SP, Yuvaraj J, Das PK. The effect of six rounds of single dose mass treatment with diethylcarbamazine or ivermectin on *Wuchereria bancrofti* infection and its implications for lymphatic filariasis elimination. *Tropical Medicine and International Health* 2002;**7**(9)**:**767-74.
72. Fan PC. Eradication of bancroftian filariasis by diethylcarbamazine-medicated common salt on Little Kinmen (Liehyu district), Kinmen (Quemoy) Islands, Republic of China. *Annals of Tropical Medicine and Parasitology* 1990;**84:**25-33.
73. Fan PC. Filariasis eradication on Kinmen Proper, Kinmen (Quemoy) Islands, Republic of China. *Acta Tropica* 1990;**47:**161-9.
74. Liu J, Chen Z, Huang X, Tu Z. Mass treatment of filariasis using DEC-medicated salt. *Journal of Tropical Medicine and Hygiene* 1992;**95:**132-5.

75. Rao CK, Panduranga Rao P, Russel S, Hamazakoya KK, Chandrasekharan A, Roychowdhury SP. Control of bancroftian filariasis with common salt medicated with diethylcarbamazine in Lakshadweep. *Indian Journal of Medical Research* 1981;**73:**865-73.
76. Narasimham MV, Sharma SP, Sundaram RM*, et al.* Control of bancroftian filariasis by diethylcarbamazine medicated common salt in Karaikal, Pondicherry, India. *Journal of Communicable Diseases* 1989;**21:**157-70.
77. Kaul SM, Raina VK, Joshi RD, Koya CN, Kumar A, Verghese T. Efficacy of diethylcarbamazine medicated salt in interrupting *Brugia malayi* transmission in hill tribe settlements in Kerala State. *Journal of Communicable Diseases* 1992;**24:**16-9.
78. Subramanyam Reddy G, Venkateswaralu N. Mass administration of DEC-medicated salt for filariasis control in the endemic population of Karaikal, south India: implementation and impact assessment. *Bulletin of the World Health Organization* 1996;**74**(1)**:**85-90.
79. Jingyuan L, Zi C, Xiaohang H, Zhaoping T. Mass treatment of filariasis using DEC-medicated salt. *Journal of Tropical Medicne and Hygiene* 1992;**95:**132-135.
80. Gelband H. Diethylcarbamazine salt in the control of lymphatic filariasis. *American Journal of Tropical Medicine and Hygiene* 1994;**50:**655-62.
81. Cartel JL, Nguyen NL, Moulia Pelat JP, Plichart R, Martin PM, Spiegel A. Mass chemoprophylaxis of lymphatic filariasis with a single dose of ivermectin in a Polynesian community with a high *Wuchereria bancrofti* infection rate. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1992;**86:**537-40.
82. Nguyen NL, Moulia Pelat JP, Glaziou P, Martin PM, Cartel JL. Advantages of ivermectin at a single dose of 400 micrograms/kg compared with 100 micrograms/kg for community treatment of lymphatic filariasis in Polynesia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1994;**88:**461-4.
83. Moulia Pelat JP, Glaziou P, Nguyen LN, Cartel JL. Single doses of ivermectin 400 micrograms/kg-1: the most effective dosage in bancroftian filariasis. *Southeast Asian Journal of Tropical Medicine and Public Health* 1995;**26:**124-7.
84. Nguyen NL, Mouliapelat JP, Cartel JL. Control of bancroftian filariasis in an endemic area of Polynesia by ivermectin 400 mu g/kg. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1996;**90:**689-691.
85. Addiss DG, Beach MJ, Streit TG*, et al.* Randomised placebo-controlled comparison of ivermectin and albendazole alone and in combination for *Wuchereria bancrofti* microfilaraemia in Haitian children [published erratum appears in Lancet 1997 Oct 4;350(9083):1036]. *Lancet* 1997;**350**(9076)**:**480-4.
86. Ismail MM, Jayakody RL, Weil GJ*, et al.* Efficacy of single dose combinations of albendazole, ivermectin and diethylcarbamazine for the treatment of bancroftian filariasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1998;**92**(1)**:**94-7.
87. Beach MJ, Streit TG, Addiss DG, Prospere R, Roberts JM, Lammie PJ. Assessment of combined ivermectin and albendazole for treatment of intestinal helminth and *Wuchereria bancrofti* infections in Haitian schoolchildren. *American Journal of Tropical Medicine & Hygiene* 1999;**60**(3)**:**479-86.
88. Dunyo SK, Nkrumah FK, Simonsen PE. A randomized double-blind placebo-controlled field trial of ivermectin and albendazole alone and in combination for the treatment of lymphatic filariasis in Ghana. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2000;**94**(2)**:**205-11.
89. Ismail MM, Jayakody RL, Weil GJ*, et al.* Long-term efficacy of single-dose combinations of albendazole, ivermectin and diethylcarbamazine for the treatment of bancroftian filariasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2001;**95**(3)**:**332-5.
90. Ottesen EA, Ismail MM, Horton J. The role of albendazole in programmes to eliminate lymphatic filariasis. *Parasitol Today* 1999;**15**(9)**:**382-6.

91. Shenoy RK, John A, Babu BS, Suma TK, Kumaraswami V. Two-year follow-up of the microfilaraemia of asymptomatic brugian filariasis, after treatment with two, annual, single doses of ivermectin, diethylcarbamazine and albendazole, in various combinations. *Annals of Tropical Medicine and Parasitology* 2000;**94**(6)**:**607-14.
92. Addiss DG, Eberhard ML, Lammie PJ. "Filarial" adenolymphangitis without filarial infection [letter]. *Lancet* 1994;**343:**597.
93. Olszewski WL. Bacterial studies of skin, tissue fluid and lymph in filarial lymphoedema. *Lymphology* 1994;**27 (suppl):**345-348.
94. Olszewski WL. Immunohistology of skin in various stages of filarial lymphoedema. *Lymphology* 1994;**27:**512-516.
95. Dreyer G, Addiss D, Roberts J, Noroes J. Progression of lymphatic vessel dilatation in the presence of living adult *Wuchereria bancrofti*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2002;**96**(2)**:**157-61.
96. Suma TK, Shenoy RK, Kumaraswami V. Efficacy and sustainability of a footcare programme in preventing acute attacks of adenolymphangitis in Brugian filariasis. *Tropical Medicine and International Health* 2002;**7**(9)**:**763-6.
97. Pani SP, Srividya A. Clinical manifestations of bancroftian filariasis with special reference to lymphoedema grading. *Indian Journal of Medical Research* 1995;**102:**114-8.
98. Olszewski WL. Episodic dermatolymphangioadenitis (DLA) in patients with lymphoedema of the lower extremities before and after administrattion of benzathine penicillin: a preliminary study. *Lymphology* 1996;**29:**126-131.
99. Partono F. Filariasis in Indonesia: clinical manifestations and basic concepts of treatment and control. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1984;**78:**9-12.
100. Dreyer G, Dreyer P, Noroes J. [Recommendations for the treatment of bancroftian filariasis in symptomless and diseased patients]. *Rev Soc Bras Med Trop* 2002;**35**(1)**:**43-50.
101. Ottesen EA. The global programme to eliminate lymphatic filariasis. *Tropical Medicine and International Health* 2000;**5**(9)**:**591-4.
102. Ramaiah KD, Das PK, Appavoo NC*, et al.* A programme to eliminate lymphatic filariasis in Tamil Nadu state, India: compliance with annual single-dose DEC mass treatment and some related operational aspects. *Tropical Medicine and International Health* 2000;**5**(12)**:**842-7.
103. Nair CP. Filariasis in centrally administered areas. Part I. Filaria surveys of Pondicherry settlement. *Indian Journal of Malariology* 1960;**14:**233-52.
104. Rajagopalan PK, Das PK, Subramanian S, Vanamail P, Ramaiah KD. Bancroftian filariasis in Pondicherry, south India: 1. Pre-control epidemiological observations. *Epidemiology and Infection* 1989;**103:**685-92.
105. Manoharan A, Das PK, Keerthiseelan VB, Ramaiah KD. Trend in *Wuchereria bancrofti* infection in Pondicherry urban agglomeration after withdrawal of a five year vector control programme. *Journal of Communicable Diseases* 1997;**29:**255-261.
106. Vector Control Research Centre. Annual Report. Pondicherry: Vector Control Research Centre, 1981.
107. Rajagopalan PK, Das PK, Pani SP*, et al.* Evaluation of integrated vector control measures on filariasis transmission in Pondicherry. *Indian Journal of Medical Research* 1988;**87:**434-9.
108. Habbema JD, van Oortmarssen GJ, Plaisier AP. The ONCHOSIM model and its use in decision support for river blindness control. Cambridge: Cambridge University Press, 1996.
109. Muench H. Catalytic Models in Epidemiology. Cambridge, Massachusetts: Harvard University Press, 1959.

110. Webber RH. Theoretical considerations in the vector control of filariasis. *Southeast Asian Journal of Tropical Medicine and Public Health* 1975;**6:**544-8.
111. Remme J, Ba O, Dadzie KY, Karam M. A force of infection model for onchocerciasis and its applications in the epidemiological evaluation of Onchocerciasis Control Programme in the Volta River basin area. *Bulletin of the World Health Organization* 1986;**64:**667-681.
112. Wada Y, Tsuda Y, Suenaga O. Transmission dynamics of *Dirofilaria immitis* in a Southwestern part of Japan. *Tropical Biomedicine* 1989;**31:**35-47.
113. Hayashi S. A mathematical analysis on the epidemiology of bancroftian and Malayan filariasis in Japan. *Japanese Journal of Experimental Medicine* 1962;**32:**13-43.
114. Vanamail P, Ramaiah KD, Pani SP, Das PK, Grenfell BT, Bundy DAP. Estimation of the fecundic life span of *Wuchereria bancrofti* in an endemic area. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1996;**90:**119-121.
115. Srividya A, Pani SP, Rajagopalan PK, Bundy DA, Grenfell BT. The dynamics of infection and disease in bancroftian filariasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1991;**85:**255-9.
116. Bundy DA, Grenfell BT, Rajagopalan PK. Immunoepidemiology of lymphatic filariasis: the relationship between infection and disease. *Immunoparasitology Today* 1991;**12:**A71-5.
117. Dissanayake S. In *Wuchereria bancrofti* filariasis, asymptomatic microfilaraemia does not progress to amicrofilaraemic lymphatic disease. *International Journal of Epidemiology* 2001;**30**(2)**:**394-9.
118. Crofton HD. A quantitative approach to parasitism. *Parasitology* 1971;**62:**179-193.
119. Anderson RM, May RM. Regulation and stability of host-parasite interactions. I.Regulatory processes. *Journal of Animal Ecology* 1978;**47:**219-247.
120. Anderson RM, Gordon DM. Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology* 1982;**85:**373-398.
121. Pacala SW, Dobson AP. The relation between the number of parasites/host and host age: population dynamic causes and maximum likelihood estimation. *Parasitology* 1988;**96:**197-210.
122. Guyatt HL, Bundy DAP. Estimating prevalence of community morbidity due to intestinal helminths:prevalence of infection as an indicator of the prevalence of disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* 1991;**85:**778-782.
123. Grenfell BT, Dietz K, Roberts MG. Modelling the Immuno-Epidemiology of Macroparasites in Naturally-Fluctuating Host Populations. In: Dobson AP, ed. Ecology of Infectious Diseases in Natural Populations. Publications of the Newton Institute, Cambridge: Cambridge University Press, 1995: 362-383.
124. Elliot JM. Some methods for the statistical analysis of samples of benthetic invertebrates. Ambleside: Freshwater Biology Association, 1977.
125. Sasa M. Methods for estimating the efficiency of detection of microfilariae in various volumes of blood samples. *Southeast Asian Journal of Tropical Medicine and Public Health* 1974;**5:**197-210.
126. World Health Organization. Expert committee on Filariasis. Third report. Technical Report Series No. 524, 1974: 1-54.
127. Pichon G, Merlin M, Fagneaux G, Riviere F, Laigret J. [Studies of the numerical distribution of microfilariae in foci of lymphatic filariasis (author's transl)]. *Tropenmedizin und Parasitologie* 1980;**31:**165-80.
128. Park CB. Microfilaria density distribution in the human population and its infectivity index for the mosquito population. *Parasitology* 1988;**96:**265-71.

129. Southgate BA. A quantitative approach to parasitological techniques in Bancroftian filariasis and its effect on epidemiological understanding. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1974;**68:**177-86.

130. Sasa M. Human filariasis. A global survey of epidemiology and control. London and Tokyo, 1976.

131. Grenfell BT, Das PK, Rajagopalan PK, Bundy DA. Frequency distribution of lymphatic filariasis microfilariae in human populations: population processes and statistical estimation. *Parasitology* 1990;**3:**417-27.

132. Das PK, Manoharan A, Srividya A, Grenfell BT, Bundy DA, Vanamail P. Frequency distribution of *Wuchereria bancrofti* microfilariae in human populations and its relationships with age and sex. *Parasitology* 1990;**3:**429-34.

133. Denham DA, Ponnudurai T, Nelson GS. Effect of continual reinfection on *Brugia pahangi* infections in cats. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1972;**66:**20.

134. Denham DA, McGreevy PB, Suswillo RR, Rogers R. The resistance to re-infection of cats repeatedly inoculated with infective larvae of *Brugia pahangi*. *Parasitology* 1983;**86:**11-8.

135. Subramanian S, Vanamail P, Ramaiah KD, Pani SP, Das PK, Rajagopalan PK. A simple deterministic model for host-parasite relationship in *Wuchereria bancrofti* infection & its relevance to parasite regulation in human host. *Indian Journal of Medical Research* 1989;**89:**411-7.

136. Day KP, Grenfell B, Spark R, Kazura JW, Alpers MP. Age specific patterns of change in the dynamics of *Wuchereria bancrofti* infection in Papua New Guinea. *American Journal of Tropical Medicine and Hygiene* 1991;**44:**518-27.

137. Jordan P, Goatly KD. Bancroftian filariasis in Tanganyika: a quantitative study of the uptake, fate and development of microfilariae of *Wuchereria bancrofti* in *Culex fatigans*. *Annals of Tropical Medicine and Parasitology* 1962;**56:**173-87.

138. Samarawickrema WA. A study of the age composition of natural populations of *Culex pipiens fatigans* Widemann in Relation to the Transmission of Filariasis due to *Wuchereria bancrofti* (Cobbold) in Ceylon. *Bulletin of the World Health Organization* 1967;**37:**117-137.

139. Hairston NG, DeMeillon B. On the Inefficiency of Transmission of *Wuchereria bancrofti* from Mosquito to Human Host. *Bulletin of the World Health Organization* 1968;**38:**935-941.

140. Pichon G. Relations mathematiques entre le nombre des microfilaires ingereese et le nombre des parasites chez differents vecteurs naturels ou experimentaux de filarioses. *Cahiers ORSTOM serie Entomologie medicale et Parasitologie* 1974;**12:**199-216.

141. Samarawickrema WA, Laurence BR. Loss of filarial larvae in a natural mosquito population. *Annals of Tropical Medicine and Parasitology* 1978;**72:**561-5.

142. Zhang SQ, Zhang QJ, Cheng F, Wang LL, Pen GP. Threshold of transmission of *Brugia malayi* by *Anopheles sinensis*. *Journal of Tropical Medicine and Hygiene* 1991;**94:**245-50.

143. Southgate BA, Bryan JH. Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 4. Facilitation, limitation, proportionality and their epidemiological significance. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1992;**86:**523-30.

144. Dye C. Does Facilitation Imply a Threshold for the Eradication of Lymphatic Filariasis? *Parasitology today* 1992;**8:**109-110.

145. Dye C. Approaches to vector control: new and trusted. 5.The epidemiological context of vector control. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1994;**88:**147-149.

146. Basanez MG, Townson H, Williams JR, Frontado H, Villamizar NJ, Anderson RM. Density-dependent processes in the transmission of human onchocerciasis: relation between microfilarial intake and mortality in the simuliid vector. *Parasitology* 1996;**113:**331-355.

147. Beye HK, Gurian J. The epidemiology and dynamics of transmission of *Wuchereria bancrofti* and *Brugia malayi*. *Indian Journal of Malariology* 1960;**14:**415-40.

148. Gubler DJ, Bhattacharya NC. A quantitative approach to the study of Bancroftian filariasis. *American Journal of Tropical Medicine and Hygiene* 1974;**23:**1027-36.

149. McGreevy PB, Kolstrup N, Tao J, McGreevy MM, Marshall TF. Ingestion and development of *Wuchereria bancrofti* in *Culex quinquefasciatus*, *Anopheles gambiae* and *Aedes aegypti* after feeding on humans with varying densities of microfilariae in Tanzania. *Transactions of the Royal Society of Tropical Medicine & Hygiene* 1982;**76:**288-96.

150. Crans WJ. Experimental infection of *Anopheles gambiae* and *Culex pipiens fatigans* with *Wuchereria bancrofti* in coastal East Africa. *Journal of Medical Entomology* 1973;**10:**189-93.

151. World Health Organization. Defining the Roles of Vector Control and Xenomonitoring in the Global Programme to Eliminate Lymphatic Filariasis. Report of the Informal Consultation Held at WHO/HQ, Geneva. Geneva: World Health Organization, 2002.

152. Bryan JH, Southgate BA. Some observations on filariasis in Western Samoa after mass administration of diethylcarbamazine. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1976;**70:**39-48.

153. Wada Y, Kimura E, Takagi M, Tsuda Y. Facilitation in Anopheles and spontaneous disappearance of filariasis: has the concept been verified with sufficient evidence? *Tropical Medicine and Parasitology* 1995;**46**(1)**:**27-30.

154. Dye C, Williams BG. Non-linearities in the dynamics of indirectly-transmitted infections (or, does having a vector make a difference?). In: Dobson AP, ed. Ecology of Infectious Diseases in Natural Populations. Publications of the Newton Institute, Cambridge: Cambridge University Press, 1995: 260-279.

155. Rochet MJ. A simple deterministic model for bancroftian filariasis transmission dynamics. *Tropical Medicine and Parasitology* 1990;**41:**225-33.

156. Chan MS, Srividya A, Norman RA*, et al.* Epifil: a dynamic model of infection and disease in lymphatic filariasis. *American Journal of Tropical Medicine and Hygiene* 1998;**59**(4)**:**606-14.

157. Norman RA, Chan MS, Srividya A*, et al.* EPIFIL: the development of an age-structured model for describing the transmission dynamics and control of lymphatic filariasis. *Epidemiology and Infection* 2000;**124**(3)**:**529-41.

158. Michael E, Simonsen PE, Malecela M*, et al.* Transmission intensity and the immunoepidemiology of bancroftian filariasis in East Africa. *Parasite Immunology* 2001;**23**(7)**:**373-88.

159. Plaisier AP, van Oortmarssen GJ, Habbema JD, Remme J, Alley ES. ONCHOSIM: a model and computer simulation program for the transmission and control of onchocerciasis. *Computer Methods and Programs in Biomedicine* 1990;**31:**43-56.

2

Epidemiological evaluation of vector control

2.1 Bancroftian filariasis in Pondicherry, South India: 2. epidemiological evaluation of the effect of vector control

Summary

This article examines the evaluation of a bancroftian filariasis control programme undertaken in Pondicherry from 1981-5. Integrated vector management was applied in one half of the town, and routine operations under the national programme (larviciding and chemotherapy) continued in the comparison area. The programme was evaluated by monitoring relative change in the epidemiological statistics of both populations. The results indicate that there was significant reduction in prevalence of microfilaraemia in juveniles in the controlled area. An apparent reduction in intensity of microfilaraemia was also observed but this was a consequence of the reduction in prevalence, since the density of microfilariae remained unchanged. The results suggest that primary constraints on the epidemiological evaluation of the vector control of filariasis are the longevity and the population characteristics of the parasite.

Introduction

Bancroftian filariasis remains a health problem in India despite the efforts of the National Filariasis Control Programme (NFCP) over the last 30 years [1]. This lack of progress is attributable to a number of disease characteristics, which frustrate control [2], but the major problem appears to be the difficulty in achieving sustained and extensive implementation of the current strategy of chemical larviciding and chemotherapy. In an attempt to identify a more appropriate technology, the Vector Control Research Centre (VCRC) of the Indian Council of Medical Research has been evaluating an Integrated Vector Management (IVM) approach with the major emphasis on the use of environmental methods to control vectors [3-5].

Relatively straightforward procedures for evaluating the effect of such programmes on the vector population are well established, but the evaluation of the effects on human infection is compromised by the difficulty in obtaining adequate epidemiological statistics [6-9]. This contrast is illustrated by the attempts to evaluate the VCRC programme, where vector reduction was readily demonstrable [4], while preliminary attempts to detect an effect on human infection achieved equivocal results [10].

In this article we examine epidemiological data collected during the implementation of an integrated vector management programme to control bancrotian filariasis in Pondicherry, South India, with an aim to identify the constraints, which, in practice, limit evaluation.

Material and Methods

Details of the town of Pondicherry and of the control procedures have been described elsewhere [4] as has the pre-control epidemiological situation [11]. During the period 1981-5 control activity was maintained throughout the whole urban area. In the area (approximately half of the town) where the integrated vector management programme was implemented, the major emphasis was on environmental modification to permanently remove or reduce breeding sites of the vector mosquito (*Culex quinquefasciatus*). In the remainder of the town the routine control procedures of the National Filariasis Control Programme, consisting primarily of the application of larvicidal insecticides, were continued, and this area therefore provided a comparison. The VCRC programme did not utilize chemothereapy as a control measure, although individuals found to be infected during blood surveys were referred to the filariasis clinic of the national programme. The NFCP programme specifically includes the detection and treatment of microfilaria carriers. Entomological variables were monitored throughout the 5-year period. Resting density was determined every 15 days at 17 sites, and biting density every week at 5 sites. Details of entomological procedures have been described previously [4].

In order to determine the epidemiological features of the infection, a mass blood survey of the population was carried out during January to March 1986, following the design of the pre-control survey conducted in 1981 [4,11]. As in 1981 the target sample was 10% of the population from each geographical locality, to give a minimum coverage of 5% of the population in any one age-class. A stratified random sampling protocol was adopted. The blood collection teams visited the households between 8 p.m. and 12 p.m. on the day after each inhabitant had been informed of the details of the project by a social worker. A 20 mm^3 peripheral blood smear was collected for subsequent staining and examination in the laboratory. Permission was received from all individuals, or in the case of children their parents or guardians.

As a result of anecdotal reports that treatment of infected individuals had been inadequately followed up by the filarial clinic after the 1981 survey, a sub-sample of referred individuals were interviewed to determine their treatment history.

Results

Blood specimens were collected from 34615 persons (12.2% of the total population of Pondicherry) in 1986. The target minimum of 5% sampled in each age class was achieved for all age classes except 0-5 years where 4.92% was sampled. This sampling distribution is similar to that achieved in the pre-control survey [11] and appropriately reflects the age distribution of the population.

The effect of the control programmes on the resting and biting density of *C. quinquefasciatus* in the two areas is shown in Figure 1. It is apparent that mosquito densities in the VCRC area were markedly lower than those in the comparison area throughout the 5 years of the programme.

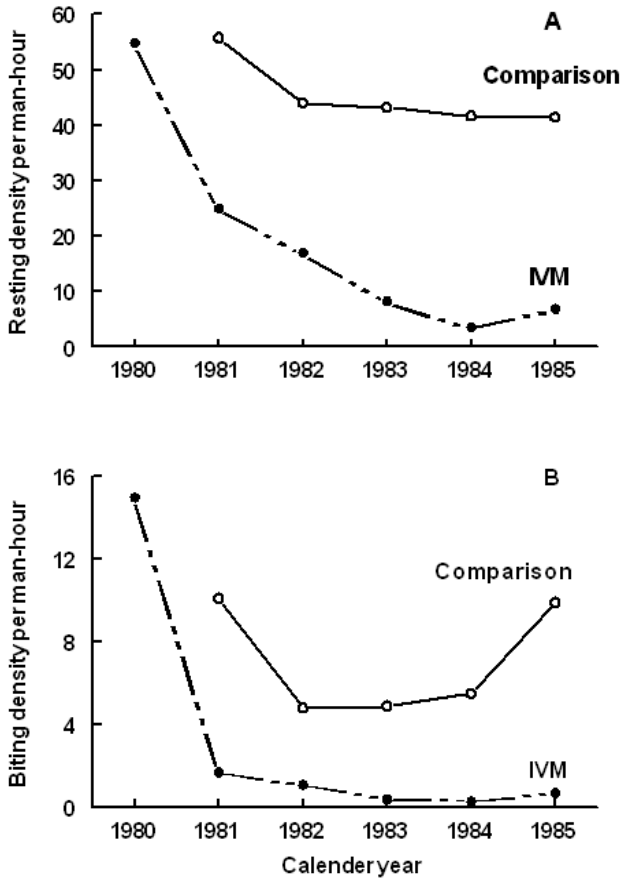

Figure 1 Change in *Culex quinquefasciatus* numbers in the VCRC control (IVM) and comparison areas between 1981 and 1985. (A) Resting density (mean number collected per hour of search), each point represents the mean of 24 fortnightly observations. (B) Biting density (mean number biting per host per hour, each point represents the mean of 52 weekly collections.

Interviews regarding treatment history were conducted by a social worker with persons referred during the 1981 survey. A definite history was available for 259 persons, of whom only 30.4% had actually received diethyl carbamazine (DEC) therapy. To remedy this situation, all individuals identified as infected in the 1986 survey were given personal letters of referral as well as being directly referred to the filarial clinic, and an additional treatment clinic was established by the VCRC.

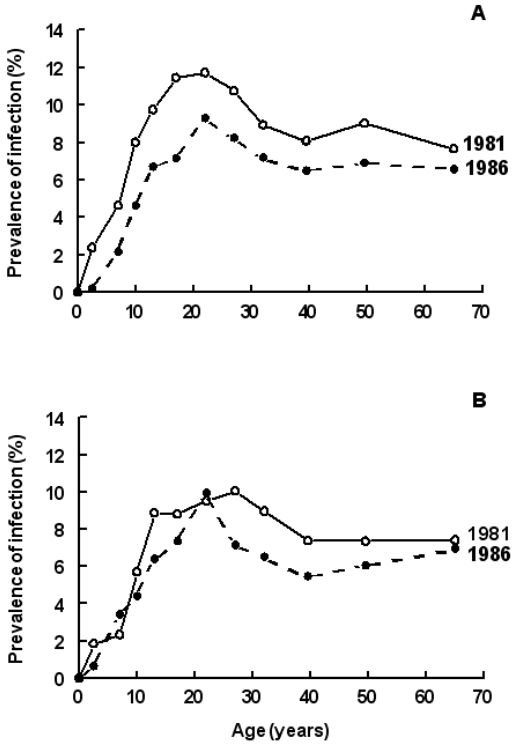

Figure 2 Relationship between microfilaraemia prevalence (proportion with microfilaraemia) and host age during the survey in 1981 and 1986. (A) VCRC control (IVM) area. (B) Comparison area.

The initial (1981) age-prevalence of microfilaraemia and that observed after 5 years of control in both areas are compared in Figure 2. The profiles for both surveys in both areas are qualitatively similar: prevalence rises monotonically over the age range 0-20 years, attains a maximum in the 20 to 25 year age class, and exhibits a declining trend in adults.

In the VCRC area there is an apparent separation of the 1981 and 1986 profiles. This difference is particularly apparent in the younger age classes (Fig. 2A). Statistical analysis indicates a significant difference between the prevalence values for each age class over the age range 1-30 years, but for only one of the adult age classes (Table 1). Similar comparison for the comparison area, however, detected significant differences in only two age classes (Fig. 2B and Table 1).

Statistical analysis (odds ratio interaction test from a log-linear model [12]) of the relative change between the two areas for each age class, however, indicates that the

prevalence in the VCRC area declined significantly (compared with the decline in the comparison area) in only two of the younger age classes (Table 1). A similar analysis of the data summed across juvenile (< 20 years) and adult (≥ 20 years) age groups indicates that, overall, the relative decline in the VCRC area compared to the comparison area is highly statistically significant in the juvenile group (P=0.0009) and not significant in the adults (P = 0.24). Although these results should be interpreted cautiously, they do indicate a significant decline of prevalence in the vector control zone. This is particularly apparent in the 6- to 8-year age class, where microfilaraemia prevalences are increasing relatively rapidly with age (Table 1).

Table 1 Comparison of pre- and post-control age-specific microfilaraemia prevalence in vector control (IVM) and comparison areas

	Sample size in 1986		Prevalence of microfilaraemia		χ^2 probability (1981 vs 1986)		Odds ratio test
Age class (years)	Comparison area	IVM control area	Comparison area	IVM control area	Comparison area	IVM control area	Comparison vs IVM
0-5	618	1405	0.65	0.21	0.067	0.001†	0.114
6-8	728	1531	3.43	2.22	0.134	0.001†	0.002*
9-11	879	1886	4.44	4.67	0.276	0.001†	0.250
12-14	1111	2250	6.39	6.76	0.041*	0.001†	0.696
15-19	1764	3201	7.43	7.12	0.178	0.001†	0.036*
20-24	1384	2586	9.83	9.28	0.858	0.009†	0.080
25-29	1229	2164	7.16	8.32	0.020*	0.011*	0.674
30-34	996	1679	6.63	7.15	0.095	0.100	0.718
35-44	1387	2563	5.48	6.55	0.064	0.070	0.638
45-54	1013	1702	5.92	6.76	0.267	0.027*	0.688
≥55	1046	1493	6.98	6.63	0.811	0.504	0.818
Overall	12155	22460	6.33	6.35	<0.01†	<0.01†	<0.01†

Significant difference: * 0.05 level; †0.01 level.

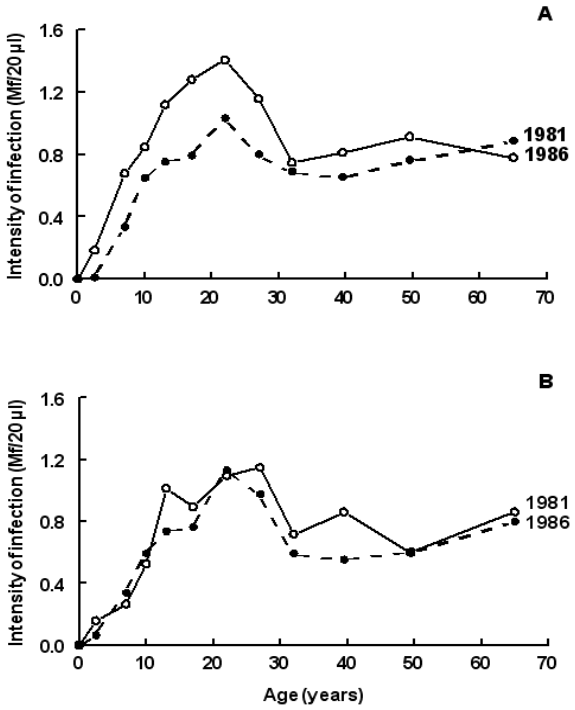

Figure 3 Relationship between microfilaraemia intensity (mean microfilaria density for the total sample in each age class) and host age during the survey in 1981 and 1986 (mean includes amicrofilaraemic individuals). (A) VCRC control (IVM) area. (B) Comparison area.

Figure 3 compares the initial (1981) age intensity of microfilaraemia with that observed after 5 years of control in both areas. The profiles are qualitatively similar: intensity rises monotonically over the 1- to 20-year age range, attains a maximum in the 20- to 25-year age class, declines sharply up to the age of 35, and thereafter remains relatively stable throughout the adulthood. The age-dependent decline in intensity of microfilaraemia in adults is more marked than the decline in prevalence (see Figs. 2 and 3).

Inspection of the comparative age-intensity profiles in the VCRC area gives an initial impression that there was a reduction in microfilaraemia intensity in all age classes less than 60 years, and that the reduction was most marked in the 0- to 25-year age range. In contrast, the 1981 and 1986 age-intensity profiles in the comparison area appear similar to each other, and suggest that the intensity of microfilaraemia was unchanged by that programme. Comparison of the ranked distribution of microfilarial densities in 1981 and 1986 (using non-parametric Mann-Whitney U test) did not however reveal any significant

Table 2 Comparison of proportional frequencies at each count between pre- and post-control for both IVM and comparison areas

	IVM area				Comparison area			
	1981 (n= 15427)	1986 (n= 22460)			1981 (n= 9519)	1986 (n= 12155)		
Mfc	% of n	% of n	Z*	P value	% of n	% of n	Z*	P value
0	91.1	93.6	9.38†	0.0000	92.4	93.7	3.65†	0.0003
1	1.3	1.0	2.86†	0.0042	1.2	1.2	0.17	0.8650
2	1.2	1.0	2.26†	0.0238	1.0	0.9	1.14	0.2543
3	0.9	0.6	3.08†	0.0021	0.7	0.5	1.87	0.0615
4	0.6	0.5	1.82	0.0688	0.4	0.5	0.39	0.6965
5	0.6	0.4	2.96†	0.0031	0.5	0.4	1.72	0.0854
6-10	1.6	1.1	4.28†	0.0000	1.4	1.2	0.99	0.3222
11-50	2.5	1.6	6.23†	0.0000	1.9	1.3	3.82†	0.0001
≥51	0.2	0.3	0.31	0.7566	0.3	0.3	0.11	0.9124

Mfc Microfilaria count
* Two-sample proportion test
† Significant

differences for any age class in either area. This paradoxical result suggests that since the populations were inadequately treated and the longevity of infection is greater than the period of control, the intensity of microfilaraemia in both infected populations did not change over 5 years period of observation. Thus the observed difference in microfilaraemia intensity in the VCRC area between 1981 and 1986 is a consequence of an increased proportion of uninfected individuals, as was indicated by the analysis of prevalence (Fig. 2). The programme may have influenced the rate of acquisition of new infections but not the rate of loss of existing infections.

Comparison of frequency distributions of microfilarial density between 1981 and 1986 (Fig. 4) showed that there is a significant difference between the distributions in both the VCRC area (χ^2 = 71.84; P = 0.0001; D.F., 29) and the comparison area (χ^2 = 65.99; P = 0.0001; D.F., 29). Further analysis was carried out to see whether the observed change in distribution had occurred in low or high microfilaria counts (Table 2). The results indicate that there was a significant increase in proportion of amicrofilaraemic individuals in both areas. It was also observed that, in contrast to the comparison area,

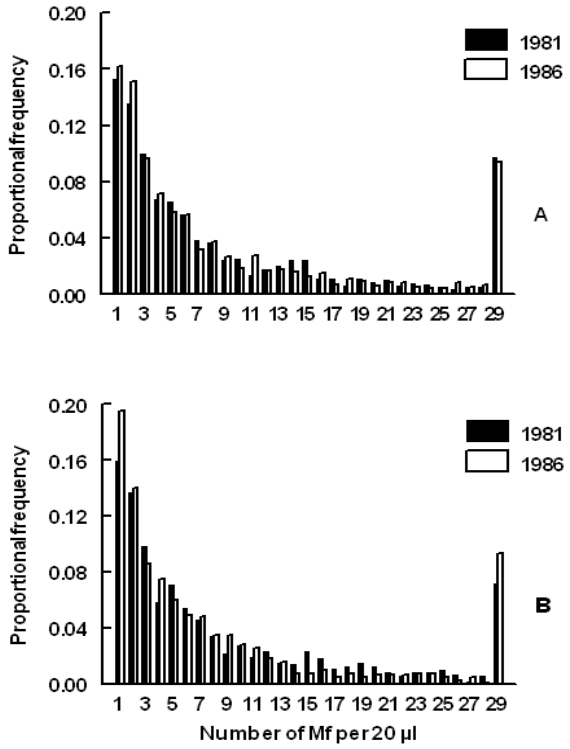

Figure 4 Frequency distribution of microfilarial numbers (expressed as a proportion of total positives) during 1981 and 1986 surveys. (A) VCRC control (IVM) area; (B) Comparison area.

there was a significant reduction in proportion of microfilaraemic individuals with different microfilaria counts in IVM area.

Discussion

The entomological aspects of this study have been discussed elsewhere [4]. The summary data (Fig. 1) clearly demonstrate that the integrated vector management activities achieved a reduction in vector numbers over the course of the programme, and that this reduction was not achieved by conventional larviciding in the comparison area.

The evaluation of the effects of vector reduction upon the filariasis infection status a population is intrinsically difficult [9]. Previous attempts to evaluate the effects of periods of control similar to that of the present study have tended to show little or no measurable impact on epidemiological statistics [13-15], although the effects are apparently

clearer after longer periods of control [6,7]. Adult *Wuchereria bancrofti* have a mean expected lifespan in the range 8-15 years [16]. In the absence of chemotherapy, therefore, it would be expected that most infected individuals would retain their infections over a period as short as 5 years. The present interview data indicate that despite referral of infected individuals by VCRC, and explicit requirement of treatment by the NFCP programme, only 30% of the individuals identified as infected actually received treatment. Since only 10% of the urban population was examined in the pre-control survey, and only 30% of referrals received treatment, the overall treatment coverage of infected individuals in the general population may have been as low as 3.0%. Thus a reduction in the intensity of infected individuals was an unlikely outcome and was not observed in the present study.

The more probable outcome of a reduction in vector biting rate would be a reduction in transmission. This would in turn lead to a reduction in the incidence of new infection, and therefore prevalence. Two factors, however, make such a reduction difficult to identify from epidemiological statistics.

The first is a consequence of the population dynamics of the bancroftian filariasis. The form of the age-prevaence profiles indicates that microfilaraemia prevalence rises at a constant rate over the age range 0-20 years, whereas in adults the prevalence remains relatively constant or actually declines. Since the acquisition of infection in adults is not reflected in an age-dependent change in prevalence and in the relative absence of chemotherapy little reduction in existing adult prevalence is expected, then the adult prevalence would be expected to remain unchanged over a 5-year period even in the presence of perfect vector control. In the age range 0-20 years, transmission is reflected in an age-dependent increase in prevalence, which reflects the rate of acquisition of infection. Thus total interruption of transmission should result in zero increase in prevalence, but not necessarily a reduction; individuals who were already infected would be expected to remain infected 5 years later. Hence, the age-prevalence profile observed for the 0-20 years of age range in 1981 would by 1986, in the presence of perfect vector control, merely be shifted by 5 years along the age axis. The population of 10-year old children in 1986 would be expected to exhibit almost the same prevalence as the population of 5 year olds in 1981. Comparison of Figures 2A and 2B indicates that this approximately describes the situation in the VCRC area but not in the comparison area.

The second constraint on identifying the effect of transmission on human infection is related to the scale of change. Detectable change is only likely to be found in the younger age classes. In the limit case, changes in prevalence should be most apparent in the 0-5 years age class where, with perfect vector control for 5 years, no new cases should occur. These youngest age classes, however, also exhibit the lowest prevalence; even when infection was endemic and uncontrolled in 1981 the prevalence was only 2.39%, or only 23 cases out of nearly a thousand infants examined. In 1986 only 3 cases were detected in the VCRC area out of more than 1400 children examined, a prevalence of 0.21%. Despite the unusually large sample sizes obtained in this study, such data are at the limits of acceptability of significance tests due to the gross asymmetry in the scale of

positive and negative values. Thus the absence of overall significance in comparing relative change between the two control programmes in this age group will be difficult to detect with current statistical techniques and our current level of understanding of the population biology of the filariasis.

This study also illustrates the importance of a comparison group when attempting epidemiological evaluation of vector control programmes. Analysis of the integrated vector management area alone indicates a significant reduction in microfilaraemia prevalence in all the younger age groups. When the analysis include the comparison area, this reduction is only significant for the age class with the highest rate of change of prevalence (although the reduction in the VCRC area is still significant for juveniles overall). Thus without the comparison area the scale of the reduction in the control area would tend to be overestimated.

The analysis of frequency distribution of microfilaria counts indicates that the proportion of amicrofilaraemic individuals increased in both areas in 1986 when compared to 1981. But the significant fall in proportion of individuals with different microfilaria counts in IVM area and not in comparison area may be due to (a) lowered fecundity of adult parasites in people who were already infected at the beginning of the control programmes in course of 5 years (as the rate of acquisition of new infections have been influenced by the IVM programme and not the existing infections) and hence (b) the rise in proportion of amicrofilaraemic individuals.

In conclusion, this study has demonstrated that sustained control of *C. quinquefasciatus* breeding can be achieved by integrated vector management on an extensive scale in a complex urban environment. Reduction in mosquito numbers on this scale has important subsidiary benefits; reducing the general mosquito nuisance and minimizing the negative health impact of other mosquito related diseases [17]. The results indicate, however, that even when vector control is unusually complete, the ability to detect and evaluate the effect of control on epidemiological statistics is compromised by the population biology of the parasite and the statistical distribution of its infections. The major constraint is the unusual longevity of the parasite, which has the important consequence that changes in microfilaraemia prevalence occur on an extended time scale. Some of the problems of analysis and understanding could, in theory, be alleviated by the development of more accurate epidemiological models.

References

1. Sharma SP, Biswas H, Das M, Dwivedi SR. Present status of the filariasis problem in India. *Journal of Communicable Diseases* 1983;15:53-60.
2. Sasa M. Human filariasis. A global survey of epidemiology and control. London and Tokyo, 1976.
3. Rajagopalan PK, Das PK. Integrated vector management for urban filariasis control in Pondicherry. *Indian Council of Medical Research Bulletin* 1985;15:133-140.
4. Rajagopalan PK, Das PK. The Pondicherry Project on Integrated Disease Vector Control. (Filariasis Control Demonstration Project). Pondicherry: Vector Control Research Centre, 1987.
5. Rajagopalan PK, Panicker KN, Das PK. Control of malaria and filariasis vectors in South India. *Parasitology Today* 1987;3:233-241.
6. Webber RH. Theoretical considerations in the vector control of filariasis. *Southeast Asian Journal of Tropical Medicine and Public Health* 1975;6:544-8.
7. Webber RH. Vector control of filariasis in the Solomon Islands. *Southeast Asian Journal of Tropical Medicine and Public Health* 1975;6:430-4.
8. Webber RH, Southgate BA. The maximum density of anopheline mosquitoes that can be permitted in the absence of continuing transmission of filariasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1981;75:499-506.
9. Southgate BA. Recent advances in the epidemiology and control of filarial infections including entomological aspects of transmission. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1984;78:19-28.
10. Sharma GK, Rao CK, Sharma SP*, et al.* Relative impact of integrated vector control strategy vis-a-vis conventional control strategy on bancroftian filariasis in Pondicherry. *Journal of Communicable Diseases* 1986;18:267-75.
11. Rajagopalan PK, Das PK, Subramanian S, Vanamail P, Ramaiah KD. Bancroftian filariasis in Pondicherry, south India: 1. Pre-control epidemiological observations. *Epidemiology and Infection* 1989;103:685-92.
12. Baker D, Nelder JA. Numerical Algorithm Group: GLIM version 3.77: User Manual. Oxford, 1978.
13. Iyengar MOT, de Rook H, Van Dijk WJOM. Interruption of transmission of Anopheles-borne filariasis by indoor residual spraying in Netherlands New Guinea. *Tropical Geographical Medcine Amsterdam* 1959;11:287-290.
14. Ramakrishnan SP, Raghavan NGS, Krishnaswami AK*, et al.* National filaria control programme in India: a review (1955-59). *Indian Journal of Malariology* 1960;14:457-94.
15. Van Dijk WJOM. Control of *Wuchereria bancrofti* filariasis in West New Guinea. *Tropical Geographical Medcine* 1964;16:54-60.
16. Hairston NG, Jachowski LA. Analysis of the *Wuchereria bancrofti* population in the people of American Samoa. *Bulletin of the World Health Organization* 1968;38:29-59.
17. World Health Organization. Lymphatic Filariasis. Fourth report of the WHO Expert Committee on Filariasis. WHO Technical Report Series, No. 702. Geneva: World Health organization, 1984: 112.

2.2 Bancroftian filariasis in Pondicherry, South India - epidemiological impact of recovery of the vector population

Summary

An integrated Vector Management (IVM) strategy was implemented from 1981 to 1985 in one part of Pondicherry, South India, for the control of the bancroftian filariasis vector *Culex quinquefasciatus* (the IVM area). The rest of the town (the comparison area) received the conventional larvicidal input. After 1985 both the areas were managed conventionally. The switch to conventional strategy resulted in an increase of vector density in both areas. The microfilaraemia prevalence in humans showed a general decline ($P < 0.05$) from 1986 to 1989 only in the IVM area whereas its intensity did not change significantly in either area. While the age-specific rate of gain of infection was generally unchanged in the IVM area, an increase in all age classes were observed after 1985 in the comparison area, where the Annual Transmission Index was high during the previous years. In both areas the rate of loss of infection increased during 1986-9 compared to 1981-6. The results suggest that 3 years is too short a period to relate the changes in entomological parameters to those in the microfilaraemia status of the population.

Introduction

An integrated Vector Management (IVM) strategy for the control of *Culex quinquefasciatus*, the vector of *Wuchereria bancrofti*, was implemented in one part of Pondicherry urban area (the IVM area) during 1981-5. In the other part of Pondicherry (the comparison area) the conventional method of control, which is mainly treatment of breeding habitats with the larvicide, malarial, was implemented by the National Filariasis Control Programme [1]. Pre-control observations and the impact of control measures on epidemiological parameters have been reported earlier [2,3]. The control programme was handed over to the state health authorities in 1986 and subsequently the conventional methods of control of were adopted in the whole area of Pondicherry. However, the Vector Control Research Centre (VCRC) continued to monitor the entomological and parasitological parameters in both the IVM and comparison areas. Though the epidemiological impact of vector control programmes has been assessed [3-9], the impact of the recovery of the vector population after withdrawal of programmes has not hitherto been studied. Such a post-control evaluation is an important aspect of the epidemiology of a chronic parasitic disease and could provide information on the process of re-establishment of the infection. In this paper, the epidemiological impact of the recovery of the vector population during the years 1986-89 is assessed.

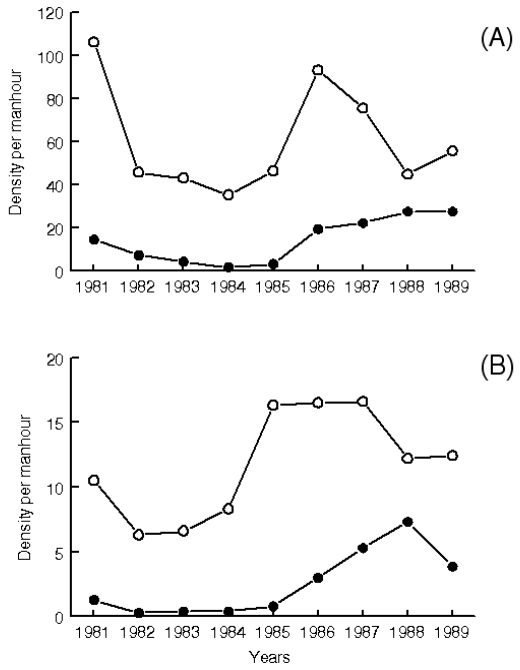

Figure 1 (A) Average resting density (no. of females per manhour) of *C. quinquefasciatus* in the comparison (open circles) and IVM (closed circles) areas. (B) Average biting density (no. of females per manhour) of *C. quinquefasciatus* in the comparison and IVM areas.

Material and Methods

The data

The study area and methods used in the control and evaluation aspects of the programme have been described elsewhere [1-3]. A mass blood survey was conducted from January to June 1989 to detect to microfialriae (Mf) in peripheral blood by the finger prick method. The sampling design was similar to that of earlier surveys carried out in 1981 and 1986 [1-3]. The samples (both areas combined) were age stratified and weighted according to the demography of Pondicherry as a whole (since the census did not provide age-structure of the population for the two areas separately) with a minimum target of 5% sample in each age group. Entomological parameters were also monitored continuously after the changeover to conventional control operations. Resting and biting densities were determined every 2 weeks at two sites, one each from the IVM and comparison areas. Details of the entomological procedures have been described elsewhere [1].

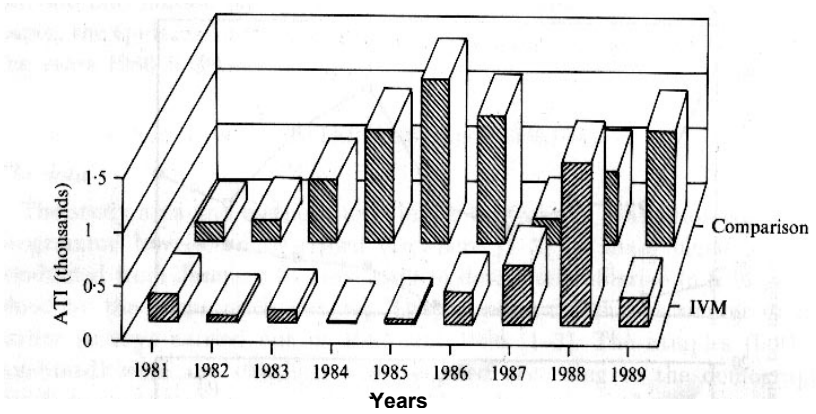

Figure 2 Annual Transmission Index (ATI) in the comparison and IVM areas

Statistical methods

The reduction in Mf prevalence within each age group between the surveys carried out in 1986 and 1989 was compared using Pearson's χ^2 - statistic. The Mantel-Haenszel χ^2 test stratified by age group was used to compare the reductions in the overall prevalence of microfilaraemia between the two surveys. The relative changes in prevalence between the IVM and comparison areas from 1986 to 1989 were compared by the log odds ratio interaction test based on a log-linear model [10]. The changes in Mf density distribution were compared using the non-parameteric Mann-Whitney U test for independent samples. The method of Bekessy and colleagues [11] was used for estimating the loss and gain rates of infection from the resurvey data. Their model is based on the assumption that the phenomenon of patent parasitaemia can be represented by a reversible two-stage catalytic model, which is a Markov process. Application of this model involves estimation of the two parameters h and r (the per capita gain and loss rates of infection respectively) from the data of those individuals whose blood was examined in two consecutive surveys. The individuals were classified as Mf +ve and Mf -ve for each survey. Transition frequencies from positive to negative and reciprocally were calculated as follows:

Let 'a' = the proportion of individuals who were –ve in the first survey and became +ve in the second (N-+ / N-).

'b' = the proportion of individuals who were +ve in the first survey and became -ve in the second (i.e. N+- / N+)

The loss 'r' and gain 'h' rates were then estimated from the proportions a and b by the following formula:

$$h = \frac{a}{t(a+b)} \log\left(\frac{1}{1-(a+b)}\right)$$

Table 1 Age structure of population compared with sample in 1989 (IVM and comparison areas combined)

Age class (Years)	Population size	Percent of total population	Sample size	Percent of total sampled	Percent of age class
0-3	29789	8.85	798	2.59	2.68
4-5	19860	5.90	1068	3.47	5.38
6-10	49649	14.75	3833	12.44	7.72
11-15	42076	12.50	4806	15.60	11.42
16-20	29284	8.70	4234	13.74	14.46
21-25	26592	7.90	3245	10.53	12.20
26-30	24909	7.40	2938	9.53	11.80
31-40	42412	12.60	4280	13.89	10.09
41-50	31641	9.40	2646	8.59	8.36
> 50	40392	12.00	2965	9.62	7.34
Total	336604	100.00	30813	100.00	9.15

$$r = \frac{b}{t(a+b)} \log\left(\frac{1}{1-(a+b)}\right)$$

where, t is the interval in years between the two surveys.

According to Bekessy and colleagues [11] these parameters can be estimated only when (a+b) < 1. If (a+b) ≥ 1, it means that either the process is non-Markovian or the parameters were not constant between successive observations. The loss and gain rates were estimated only for those age classes, which satisfied the above condition.

Table 2 Age specific prevalence (%) and significance of reduction of microfilaraemia in the comparison and IVM areas

Age class (years)	Prevalence of microfilaraemia				χ^2 probability (1986 vs 1989)		Odds ratio Test Comparison vs IVM (*P*)
	Comparison area		IVM area		Comparison area	IVM area	
	1986	1989	1986	1989			
0-3	0.00	0.00	0.00	0.18	-	-	-
4-5	0.94	0.63	0.31	0.66	0.64	0.29	0.3104
6-10	4.09	2.31	2.94	1.80	0.01*	0.01*	0.7667
11-15	5.92	4.14	6.60	4.66	0.02*	< 0.01*	0.9652
16-20	7.72	6.03	8.10	6.90	0.07	0.08	0.6001
21-25	10.06	8.29	8.49	7.46	0.13	0.19	0.6804
26-30	7.10	8.22	8.56	6.27	0.33	0.01*	0.0147*
31-40	5.20	5.16	6.49	5.39	0.95	0.07	0.3284
41-50	6.28	5.57	6.72	5.60	0.50	0.17	0.7782
> 50	6.94	6.33	6.78	7.49	0.54	0.41	0.3184
Overall	6.34	5.32	6.36	5.21	0.48	0.001†	0.7100

* Denotes significant difference at 0.05 level using Pearson χ^2-test.

† Denotes significant difference at 0.05 level using Mantel-Haenszel χ^2 test.

Results

Entomological parameters

The yearly resting and biting density of *C. quinquefasciatus* from 1981 to 1989 is illustrated in Figures 1A and 1B respectively. While in the IVM area the resting density increased steadily after 1985, in the comparison area it declined after an initial increase (1985-6). In the comparison area, the biting density remained high from 1984 to 1987 and then declined, whereas in the IVM area it steadily increased from 1985 to 1988. However, in the IVM area both the resting and biting populations were lower than those of the comparison area throughout the study period.

The biting density per man-hour, infection and infectivity rates varied significantly between the years ($P < 0.05$) and between the IVM and post-IVM periods ($P < 0.05$) in the IVM area. In contrast, in the comparison area, while all these parameters varied significantly between the years ($P < 0.05$), only the biting density per man-hour differed

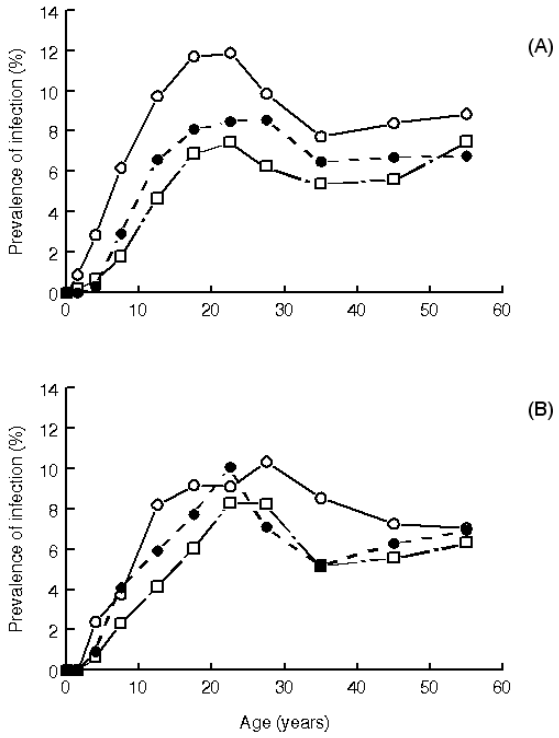

Figure 3 Prevalence of microfilaraemia in the (A) IVM and (B) comparison areas during 1981 (open circles), 1986 (closed circles) and 1989 (open squares)

significantly ($P < 0.05$) between the periods of 1981-5 and 1986-9. Hence the Annual Transmission Index (ATI), which is the product of the number of infective mosquitoes biting a man per year and the average number of infective larvae per infective mosquito [12], was used to show the combined effect of changes in these parameters on the transmission dynamics. The ATI showed an increasing trend in the comparison area from 1981 to 1985 but declined in the IVM area over the same period. In the IVM area it showed an increase from 1986 to 1988 and subsequently declined in 1989. In the comparison area the ATI declined markedly in 1987 but increased thereafter (Fig. 2).

Parasitological parameters

In 1989 blood smears were collected from 30813 individuals from IVM and comparison areas combined. This represented 9.15% of the estimated total population of 336604. The sampling distribution over different age classes was similar to that in the previous two surveys carried out in 1981 and 1986 [2,3]. The target minimum of 5% sampling was achieved in all the age classes except in the 0-3 groups (Table 1). The percentage of males

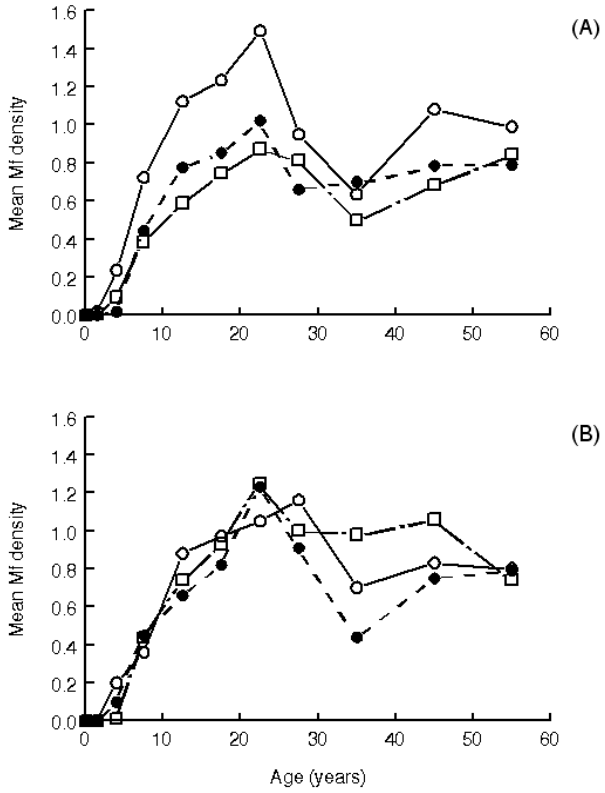

Figure 4 Intensity of microfilaraemia in the (A) IVM and (B) comparison areas during 1981 (open circles), 1986 (closed circles) and 1989 (open squares)

and females in the sample was 47.0 and 53.0 in 1989 and 48.6 and 51.4 in 1986 respectively. The prevalence of microfilaraemia in males and females was 6.7 and 3.9% in 1989 and 7.1 and 5.6% in 1986 respectively and was significantly higher in males than in females in both the surveys ($P < 0.05$). However, the decline in prevalence between the two surveys (1986 and 1989) was significantly higher in females than in males (Mantel-Haenszel test, $\chi^2=132.549$, $P < 0.001$).

Age prevalence and intensity

The age prevalence of microfilaraemia in 1981, 1986 and 1989 for the comparison and IVM areas is shown in Figures 3A and 3B respectively. The age profiles in all surveys in the two areas remained qualitatively similar but differed quantitatively. The separation of age prevalence profiles between the three surveys was well marked in all age classes in the IVM area. In the comparison area the separation was well marked in older age classes

Table 3 Comparison of 1989 microfilaraemia prevalence (%) between freshly surveyed and resurveyed people

Age class (years)	Comparison area surveyed			IVM area surveyed		
	Once	Twice	Thrice	Once	Twice	Thrice
	n=5859	3239	1299	11680	6353	2383
0-3	0.00	0.00	0.00	0.18	0.00	0.00
4-5	0.66	0.00	0.00	0.69	0.00	0.00
6-10	2.47	1.96	0.00	1.96	1.45	0.00
11-15	4.59	3.83	2.81	5.38	3.81	4.11
16-20	7.09	5.42	3.70	9.16	4.58*	4.55*
21-25	9.61	6.65	6.57	7.84	6.14	8.73
26-30	8.01	9.26	6.73	7.10	5.22	4.39
31-40	4.71	6.13	4.35	6.83	4.35*	2.25*
41-50	7.66	4.48	2.84*	7.31	4.73*	3.57*
> 50	7.79	5.76	4.28	8.74	6.95	5.64
Overall	5.58	5.28	4.23	5.75	4.44†	4.57†

* Significant at 0.05 level compared to people of the same age-group surveyed once (Pearson χ^2).
† Significant at 0.01 level compared to people surveyed once (Mantel-Haenszel χ^2)

(above 25) between 1981 and 1986 and in younger age classes (below 25) between 1986 and 1989. The reduction in prevalence from 1986 to 1989 was significant in two age classes in the comparison area and in three age classes in the IVM area (Table 2). While in the IVM area the overall prevalence of microfilaraemia in 1989 was significantly lower than that of 1986, the difference was not statistically significant in the comparison area (Mantel-Haenszel χ^2 test). Statistical analysis based on a log-linear model (odds ratio interaction test) indicated that the relative change in the prevalence of microfilaraemia between the two areas was not significant (Table 2), as also observed between 1981 and 1986 [13].

The relationship of microfilaraemia intensity with age for the comparison and IVM areas is shown in Figure 4. The intensity increased from 0 to 20 years of age and then tended to decline. Comparison of the age intensity profiles in the IVM area showed that the reduction between 1981 and 1986 was marked in almost all age classes, whereas between 1986 and 1989 a marginal decline was observed only in some of the adult age classes. The temporal age intensity profiles in the comparison area appeared generally similar in most adult age classes except for an apparent increase from 1986 to 1989 in those aged 25-45. Comparison of the microfilaria densities between 1986 and 1989

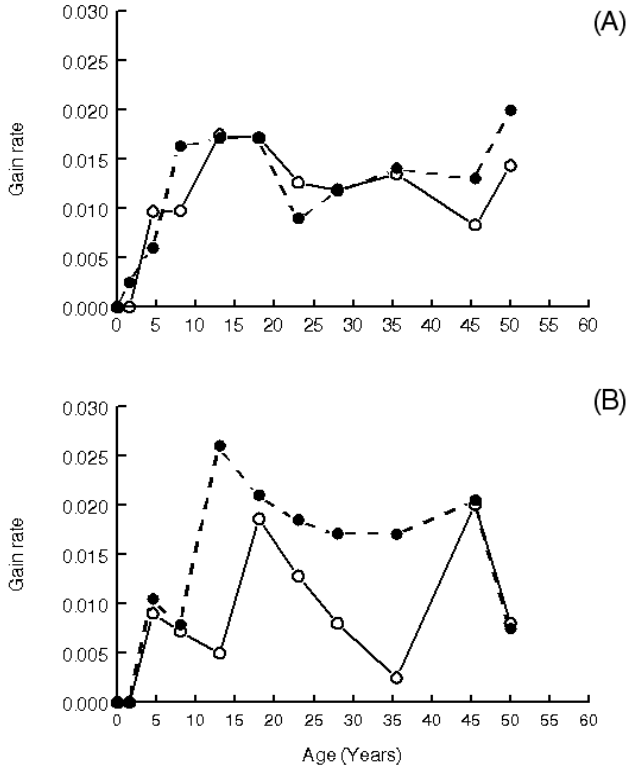

Figure 5 Rate of gain of infection in the (A) IVM and (B) comparison areas during 1981-86 (open circles) and 1986-89 (closed circles)

showed no significant difference for any age class in either area (non-parametric Mann-Whitney U test).

Prevalence and intensity among resurveyed people

The 30813 persons surveyed in 1989 are grouped into (i) 17539 sampled only in 1989, (ii) 9592 persons recruited twice, i.e. in 1981 or 1986 and 1989, (iii) 3682 surveyed three times, i.e. in 1981, 1986 and 1989. Since the administration of DEC to the microfilaria carriers in 1986 in both IVM and comparison areas might have influenced the microfilaria status of the population in 1989, the prevalence of microfilaemia and its intensity in these three groups were analyzed and compared (Table 3). Though the overall prevalence of microfilaraemia was significantly lower in the resurveyed people (groups ii and iii) than in the freshly surveyed (group i) in the IVM area, the difference was not significant in the comparison area (Mantel-Haenszel χ^2 test, Table 3). However, the overall microfilaria intensity was significantly lower (Mann-Whitney U test) in resurveyed people (groups ii and iii) than in freshly surveyed people (group i) in both the areas.

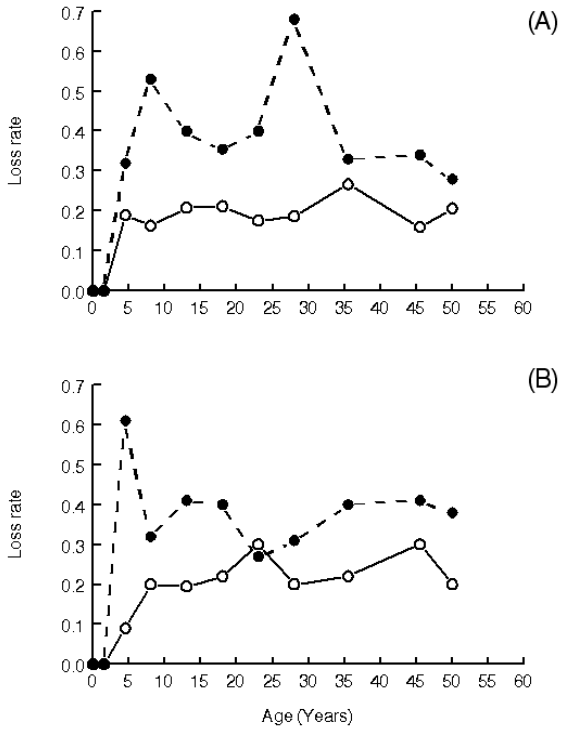

Figure 6 Rate of loss of infection in the (A) IVM and (B) comparison areas during 1981-86 (open circles) and 1986-89 (closed circles)

Loss and gain of infections

The rates of loss and gain of microfilaraemia were estimated for both the areas for 1981-6 and 1986-9. The overall rate of gain increased marginally from 0.0126 per year during 1981-6 to 0.0142 per year during 1986-9 in the IVM area. In the comparison area also the overall rate of gain increased from 0.0094 per year to 0.0158 per year over the same period. While the age-specific rates of gain were generally unchanged in the IVM area (Fig. 5A), an increase was observed in the comparison area (Fig. 5B) in the 11-35 year age class.

The overall loss rate increased from 0.215 to 0.361 per year in the comparison area, and 0.196 to 0.409 per year in the IVM area. The age-specific rates of loss of microfilaraemia in the comparison and IVM areas are shown in Figure 6. In both the areas, the rate of loss was higher than the rate of gain in all age classes both between 1981 and 1986 and in 1986-9. Furthermore, the rate of loss of infection for the period 1986-9 was higher when compared to 1981-6 in both areas.

Discussion

The relative impact of the IVM and conventional strategies on entomological and parasitological parameters has been reported in our earlier publications [1-3]. The present analysis highlights the effects of the withdrawal of IVM, on the pattern of vector recovery and consequent changes in the dynamics of the parasite.

Entomological aspects

The relative impact of the two strategies (IVM and conventional) between 1981 and 1985 was reflected in the changes in resting and biting densities of the vector and other epidemiological parameters such as ATI and the Risk of Infection Index [1]. However, the gains achieved in the IVM areas could not be sustained after switching over to the conventional methods, and a gradual recovery of the vector population and rise in ATI was noticed in this area. The vector densities, though continuously increasing in the IVM area from 1986 onwards, were always lower than those of the comparison area. This could be due to permanent ecological changes brought about in the IVM area by environmental manipulation, which was the main plank of the IVM strategy. However, in the absence of the pre-control data (before 1981), interpretation as to the influence of ecological differences, between the two areas, on the changes in vector population cannot be made with certainty. The decline in the vector densities after 1986/7 in the comparison area could partly be due to ecological changes and localized and need/demand based interventions. Relatively high vector densities in the comparison area throughout the study period (1981-9) and in the IVM area after 1985 suggest that IVM yielded better results than the conventional measures.

Parasitological aspects

While a significant reduction in the prevalence of microfilaraemia was observed in 1986 compared to 1981, in the IVM area, the relative change in the prevalence between the IVM and comparison areas was not significant during the same period [3]. In the IVM area a clear separation of the age prevalence profiles between 1986 and 1989 was observed, but it is difficult to ascertain whether this was due to the prolonged effect of IVM or chemotherapy or combined effect of both. The chemotherapy in 1986 was ethically necessary and imparted to the Mf carriers in both the areas. The significant reduction in microfilaria density and high loss rate of microfilaraemia in the resurveyed people between 1986 and 1989 compared to between 1981 and 1986, in both IVM and comparison areas suggest the beneficial effect of chemotherapy. However, the prevalence declined significantly only in the IVM area possibly due to prolonged effect of IVM. This is further evident from nearly unchanged gain rate in the IVM area during 1981-6 and 1986-9, unlike in the comparison area where it increased considerably during 1986-9.

Relating entomological parameters to parasitological variables

Though the vector density increased steadily in the IVM area after 1985, the rate of acquisition of infection did not change perceptibly, suggesting that either the vector

density did not reach a level which could enhance the rate of acquisition of microflaraemia in humans or the time period of 3 years (1986-9) was too short to reflect the impact of vector recovery on the prevalence of microfilaraemia. The latter possibility is more likely in view of the fact that though the exposure of human hosts to infective larvae was higher during 1986-9 than during the IVM period (1981-5) as is evident from the increased ATI from 1986 onwards (Fig. 2), a corresponding increase in the rate of gain was not observed. In contrast, though the vector density increased (at least in 1986 - 7) in the comparison area, the ATI was markedly lower after 1986 than during 1981-5. The observed increase in the rate of gain of microfilaraemia during 1986-9 in this area could be due to the high ATI during 1981-5.

The major objective of the study was to evaluate the effect of vector recovery on microfilaraemia in the population. The marked changes in the vector parameters have not been reflected in the parasitological parameters suggesting that a period of 3 years is too short to make a conclusive assessment of changes in the dynamics of the parasite. In the absence of an appropriate method to determine the prevalence of pre-patent infection in the human population, evaluation of the impact of recovery on the dynamics of the parasite is difficult. Therefore, it is of utmost importance to establish a quantitative relationship between transmission potential of vectors and incidence of infection in humans.

References

1. Rajagopalan PK, Das PK. The Pondicherry Project on Integrated Disease Vector Control. (Filariasis Control Demonstration Project). Pondicherry: Vector Control Research Centre, 1987.
2. Rajagopalan PK, Das PK, Subramanian S, Vanamail P, Ramaiah KD. Bancroftian filariasis in Pondicherry, south India: 1. Pre-control epidemiological observations. *Epidemiology and Infection* 1989;103:685-92.
3. Subramanian S, Pani SP, Das PK, Rajagopalan PK. Bancroftian filariasis in Pondicherry, south India: 2. Epidemiological evaluation of the effect of vector control. *Epidemiology and Infection* 1989;103(3):693-702.
4. Hairston NG, Jachowski LA. Analysis of the *Wuchereria bancrofti* population in the people of American Samoa. *Bulletin of the World Health Organization* 1968;38:29-59.
5. Webber RH. Vector control of filariasis in the Solomon Islands. *Southeast Asian Journal of Tropical Medicine and Public Health* 1975;6:430-4.
6. Webber RH. The natural decline of *Wuchereria bancrofti* infection in a vector control situation in the Solomon Islands. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1977;71:396-400.
7. Webber RH. Eradication of *Wuchereria bancrofti* infection through vector control. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1979;73:722-4.
8. Chelbowsky HO, Zielke ZE. Studies on bancroftian filariasis in Liberia. West Africa. III. Efficacy of repeated treatment with Diethyl carbamazine and vector control on the microfilaria reservoir in a rural population. *Tropical Medicine Parasitology* 1980;31:181-193.
9. Dissanaike AS, ed. Filarial infections. Edinburgh: Churchill Livingstone, 1984.
10. Baker D, Nelder JA. Numerical Algorithm Group: GLIM version 3.77: User Manual. Oxford, 1978.
11. Bekessey A, Molineaux L, Storey J. Estimation of malaria incidence and recovery rates of *Plasmodium falciparum* parasitaemia from longitudinal data. *Bulletin of the World Health Organization* 1976;54:685-693.
12. Beye HK, Gurian J. The epidemiology and dynamics of transmission of *Wuchereria bancrofti* and *Brugia malayi*. *Indian Journal of Malariology* 1960;14:415-40.
13. Rajagopalan PK, Das PK, Panicker KN, eds. Environmental and water management for mosquito control. Florida: Boca Raton, 1990.

3

Dynamics of infection in the vector

3.1 The relationship between microfilarial load in the human host and uptake and development of *Wuchereria bancrofti* microfilariae by *Culex quinquefasciatus*: a study under natural conditions

S. Subramanian, K. Krishnamoorthy, K.D. Ramaiah, J.D.F. Habbema, P.K. Das and A.P. Plaisier. *Parasitology* 1998, 116: 243-255

3.2 Frequency distribution of *Wuchereria bancrofti* infection in the vector host in relation to human host: evidence for density dependence

P.K. Das, S. Subramanian, A. Manoharan, K.D. Ramaiah, P. Vanamail, B.T. Grenfell, D.A.P. Bundy, E. Michael, *Acta Tropica* 1995, 60: 159-165

3.3 Rates of acquisition and loss of *Wuchereria bancrofti* infection in *Culex quinquefasciatus*

S. Subramanian, A. Manoharan, K.D. Ramaiah and P.K. Das. *American Journal of Tropical Medicine* & *Hygiene* 1994, 51: 244-249

3.4 Vector survival and parasite infection: the effect of *Wuchereria bancrofti* on its vector *Culex quinquefasciatus*

K. Krishnamoorthy, S. Subramanian, G.J. van Oortmarssen, J.D.F. Habbema and P.K. Das. *ParasitologyI* 2004, 129: 43-50

3.1 The relationship between microfilarial load in the human host and uptake and development of *Wuchereria bancrofti* microfilariae by *Culex quinquefasciatus*: a study under natural conditions

Summary

The uptake of *Wuchereria bancrofti* microfilariae (Mf) by *Culex quinquefasciatus* and their development in relation to human Mf density were quantified by allowing a total of 1096 wild mosquitoes to feed on 13 volunteers sleeping under partially open bed-nets. For each volunteer, each hour between 18.00 and 06.00 h the Mf density in finger-prick blood was determined and engorged mosquitoes collected. Each hourly collection of mosquitoes was kept separately. Half of them was dissected within 18 h post-feeding for the presence of ingested Mf, the other half was reared for 12 days to allow for the development of L3 larvae. About 20% of the latter mosquitoes died during these 12 days and these harboured significantly more larvae than the surviving ones, which could be an indication of excess-mortality among heavily infected mosquitoes. Assuming that variability in Mf uptake and in the number of developed L3 larvae can be described by a negative binomial distribution, a maximum-likelihood procedure was applied to estimate the relationship between human Mf density and both the arithmetic mean Mf uptake and L3 development. Both were adequately described by a saturating hyperbolic function that significantly differed from linearity. The saturation level for Mf was estimated at 29 (CI: 20-54) and for L3 larvae at 6.6 (CI: 4.3-17.0). Next, the L3 yield was related to Mf uptake indicating that the *W. bancrofti-C. quinquefasciatus* complex shows 'limitation', i.e. a decreasing yield for an increasing uptake. Both the number of Mf ingested and the number of L3 larvae developing per mosquito were found to be highly aggregated, with the level of aggregation decreasing in a non-linear way with human Mf density.

Introduction

The capability of vector mosquitoes to ingest microfilariae (Mf) of filarial parasites and to support their development after ingestion are important determinants of filariasis transmission [1]. Three processes, namely (1) uptake of Mf from the human host, (2) development of Mf to the infective stage larvae (L3) and (3) transmission of L3 to human, determine the overall vector competence. Laboratory studies have demonstrated that the uptake of Mf by mosquitoes depends on the density and distribution of Mf in the human host [2,3]. For the number of L3 larvae developing from a particular number of Mf ingested, three possible relationships have been described [4,5]: proportionality, i.e. a constant ratio (≤1) of L3 to ingested Mf, facilitation, i.e. an increase in this ratio, and

limitation, the converse of facilitation. Proportionality has been observed for the *Brugia malayi* (filarial parasite)-*Aedes togoi* (vector) combination in experimental cats and for *B. malayi* - *Mansonia bonneae*. Facilitation was found for *W. bancrofti* - *Anopheles gambiae*, *W. bancrofti* - *An.arabiensis* and *W. bancrofti* - *An. merus* and limitation for *W. bancrofti* – *C. quinquefasciatus* [5]. Although the epidemiological significance of such vector/parasite relationships has been widely discussed [4,6-9], very little theoretical work has been done to demonstrate their effect under control programmes [10]. The argument is that in case of limitation it would be difficult to totally interrupt transmission even when control programmes reduce Mf prevalence and intensity to very low levels; whereas in the case of facilitation it would be relatively easier to block transmission and eradicate the parasite from the human population. However, as argued by Dye [11] and Dye & Williams [12] in speculating about the epidemiological impact of the different parasite-vector relationships it is also crucial to take into account the (often considerable) variation in larval uptake and development and not only consider mean values.

Unlike the *Anopheles* - *W. bancrofti* combination, quantification of the vector parasite relationship in *Culex*-*W. bancrofti* has received little attention [5]. For a periodic strain of *W. bancrofti* it has been observed that the number of Mf ingested by *C. fatigans* (*C. quinquefasciatus*) is non-linearly related to the human Mf density [13]. There are also reports indicating linearity in this vector parasite combination [14-17]. It is further known that a large proportion of the ingested Mf is lost during the development to L3 both in the laboratory [14] and in nature [18].

All earlier reports are based on laboratory studies. Apart from the unnatural conditions, a drawback of such studies is that they often use a single cohort of vector mosquitoes which are allowed to feed on a restricted part of the body of a human Mf carrier. It has been suggested that, while studying the uptake and development of larvae in relation to human Mf density for anticipating the effects of proposed control programmes, it is essential to perform these studies under natural conditions, using local strains of mosquito and parasite [5]. Therefore, such a study was carried out in Pondicherry, India, endemic for periodic *W. bancrofti* transmitted by *C. quinquefasciatus*. Wild mosquitoes, representing overlapping generations, were allowed to engorge on infected human volunteers under natural conditions and were collected throughout the night. Hourly collections were analysed in relation to the human parasite density. Using these data, attempts are made to quantify the relationship between human Mf density, with its periodicity in the host blood, and the uptake of Mf and output of L3 by mosquitoes. The Mf uptake and development of the parasites are considered as distinct processes in order to examine the evidence for density dependence in both processes separately. In analysing the data from the experiments, assumptions will be tested about the heterogeneity of uptake and development.

Materials and Methods

Study design

From the Mf carriers, who were detected during a night blood survey in and around Pondicherry, a total of 13 carriers with varying Mf counts (1 to 394 Mf/20 µl of finger prick blood) and covering an age range of 10-50 years was selected for the present study. Only male patients were chosen in view of poor co-operation of female patients due to social and cultural factors. Informed oral consent of each patient was obtained before starting the collections. Each patient slept under a mosquito net (1 × 2.5 × 2 m) in his own house, with one side of the net partly open for the entry of wild mosquitoes.

Fully fed mosquitoes which were resting inside the net were collected at hourly intervals from 18.00 to 06.00 h. Following collection, mosquitoes were released into one cubic foot mosquito cages and transported to the laboratory. Hourly collections from each patient were kept separately. As the vector mosquitoes have the habit of resting on a nearby object after feeding, the chance of missing fully fed mosquitoes is negligible.

Half of each hourly collection was dissected on the following morning to assess the uptake of Mf. Each mosquito was teased into pieces in a few drops of saline to examine for the presence of Mf and other developmental stages of the parasite. The results of these immediate dissections, carried out within 18 h of the time of collection, were considered to represent the number of Mf ingested at the blood meal. This is justified because there exist no evidence of loss of Mf through dejecta in this mosquito species [14,19]. The remaining mosquitoes were reared for 12 days at 26-28 °C and 70-80% relative humidity. They were maintained on raisin, and ovi-traps were provided for oviposition. Every day raisins were changed, ovi-traps were replaced and all dead mosquitoes were dissected for determining parity status and counting the stage-specific number of filarial larvae. Parity of the mosquitoes was determined by counting the number of dilatations following the method of Polovodova [20]. On the 13th day after collection, all surviving mosquitoes were dissected for infection and parity status. Since no subsequent blood meal was provided, mosquitoes laid eggs only once during the period of observation. Hence, parity of mosquitoes on the day of capturing was determined by subtracting one from the number of dilatations observed on the 13th day.

From 18.30 until 05.30 h, hourly samples comprising two or three smears of 20 µl were prepared from finger prick blood. These moments coincide with the mid-point of each hour of mosquito collection. Paired observations of blood Mf-counts (the arithmetic mean of the smears) and mosquito dissections will be henceforth referred to as 'patient hours' (theoretically 12 hours times 13 patients = 156 patient hours). All volunteers were treated with a standard course of DEC after the experiment.

Statistical methods

The hourly sampling of blood combined with the hourly catches of mosquitoes (patient hours) were used to quantify the relationship between the human Mf density (20 µl of

blood) and Mf uptake by the mosquitoes or the number of L3 larvae developing in mosquitoes.

Since visual inspection of the (arithmetic) mean number of parasites W in the mosquitoes (Mf or L3) in relation to the human Mf density m suggested a saturation at high values for m, the following hyperbolic function was used to describe this relationship:

$$W(m) = a + \frac{bm}{1 + cm} \qquad (1)$$

The interpretation of equation (1) is as follows: The parameter a (intercept), suggesting the possibility of infection of mosquitoes even when $m = 0$, is included to account for false-negative human Mf-counts. At high human Mf-counts, the relationship approaches a saturation level c', with $c' = a+(b/c)$. The initial steepness of the increase of W with m is given by parameter b. Equation (1) was used to explore three possible relationships for W with m: (i) constant (b is indistinguishable from zero); (ii) linear (c is indistinguishable from zero) and (iii) non-linear (hyperbolic: all parameters > 0).

In estimating the parameters of this function, it is assumed that for a given human Mf density m, the variation in the number of parasites/mosquito (either Mf or L3) can be described by a negative binomial distribution with mean W and some unknown overdispersion parameter k (clumping factor). We will explicitly test whether and how (constant, linear or non-linear) this k depends on the human Mf-count m using the following power function:

$$k(m) = k_0 + \alpha\, m^{\beta} \qquad (2)$$

The parameters of equations (1) and (2) are estimated using the maximum likelihood procedure outlined in the Appendix. An important feature of this procedure is that it is based on the larval counts in individual mosquitoes rather than on the mean count or fraction positives/patient hour. The likelihood ratio statistic (which is approximately Chi-square distributed with D.F. equal to the difference between the number of parameters in the models being tested [21] was used to test different assumptions pertaining to W and k.

In order to assess whether the hour of the night is a confounding variable for the uptake of Mf, we carried out a logistic regression analysis (using SPSS) relating the success to engorge at least 1 Mf to the human Mf density and the hour of the night. Hour of the night was expressed as 1 for 18.00 to 19.00 h, 2 for 19.00 to 20.00 h, etc. In the regression equation we included hour itself, its logarithmic and quadratic transformation, and an interaction-term hour × m as independent variables. Non-significant variables were removed through backward elimination based on a likelihood-ratio test [21].

The yield of L3 larvae, defined as ratio L3 output : Mf uptake (see [4,5]), was determined for 9 classes of human Mf density (average of 2 or 3 smears, determined each hour of the night): 0-1, 1.3-4, 4.3-8, 8.3-25, 25.3-50, 50.3-100, 100.3-130, 130.3-200, and

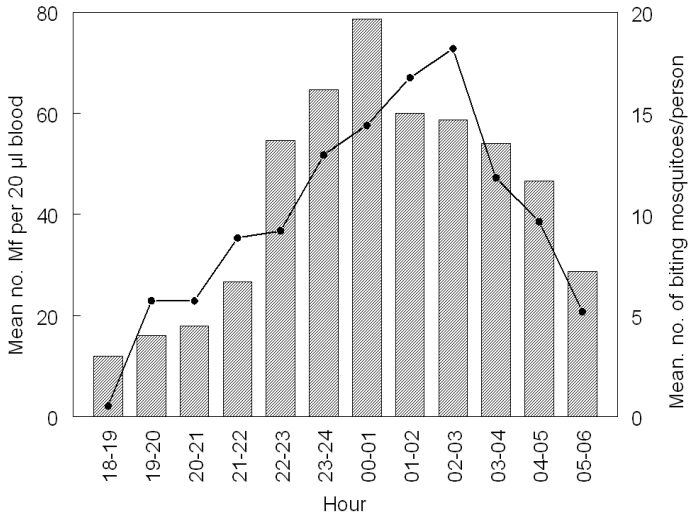

Figure 1 Comparison of periodicity in vector biting (solid line) and microfilaria appearance in the peripheral blood of human (bars) in relation to hour of the night.

more than 200. These classes were chosen such that for each class, the numbers of mosquitoes immediately dissected and dissected after 12 days were at least 30. The observed yields will be compared with the expected yields based on a combination of the estimated relationships (equation (1)) for Mf uptake and L3 output. For a regular series of human Mf densities (0-300, steps of 1), both the expected Mf uptake (x) and the expected L3 output (y) were calculated, and for all these points the yield was expressed as y/x. This combination of the two estimated functions allows for all three Mf-L3 relationships mentioned in the Introduction Section. If we disregard the intercept a, proportionality occurs if $c_{Mf}=c_{L3}$, limitation if $c_{Mf}<c_{L3}$, and facilitation if $c_{Mf}>c_{L3}$.

Throughout the manuscript mean values refer to (either or not weighted) arithmetic means.

Results

Blood smear counts and mosquito collections

Paired observations of human blood Mf density and number of fully engorged *C. quinquefasciatus* are available for 119 (immediate dissection) and 62 (dissected after 12 days) patient hours. Both numbers are considerably smaller than the theoretically expected 156. There were 28 patient-hours, particularly during dusk and dawn, without

Table 1 Summary of mosquito dissection results

	Immediate dissection	Died before 12 days of follow-up	Dissected after 12 days
Number of patient hours	119	29	62
Number of mosquitoes dissected			
All	592	104*	400
Nulliparous	314 (53%)	53 (54%)	114 (29%)
1-parous	207 (35%)	41 (41%)	210 (53%)
2- or 3-parous	71 (12%)	5 (5%)	76 (19%)
Number (and %) of infected mosquitoes			
All	267 (45%)	66 (63%)	166 (42%)
Nulliparous	140 (45%)	28 (53%)	42 (37%)
1-parous	93 (45%)	33 (80%)	84 (40%)
2- or 3-parous	34 (48%)	2 (40%)	40 (53%)
Number with Mf	267 (45%)	15 (14%)	-
Number with L1	-†	15 (14%)	-
Number with L2/L3	-	42 (40%)	166 (42%)
Number with L3	-	11 (11%)	149 (37%)
Mean‡ number of parasites (S.D.)/mosquito			
Mf	9.3 (26.2)	10.5 (45.7)	-
L1	-	1.6 (8.3)	-
L2/L3	-	6.4 (13.5)	4.2 (9.8)
L3	-	1.2 (5.8)	2.4 (5.7)
Mean‡ number of parasites (S.D.)/positive mosquito			
Mf	20.0 (35.6)	73.1 (99.4)	-
L1	-	11.3 (19.3)	-
L2/L3	-	15.9 (17.4)	10.0 (16.4)
L3	-	11.1 (14.5)	6.4 (7.8)

* Parity of 5 mosquitoes could not be determined since they dried up.
† Not applicable / none found.
‡ Arithmetic means.

biting mosquitoes and 9 patient-hours in which volunteers were reluctant for repeated finger pricking. For another 28 patient-hours only a few mosquitoes (2 or 3) were collected and all of them were dissected immediately for assessing the Mf uptake, leaving no mosquito for further observation on L3 development. Finally, in 29 patient-hours, all the mosquitoes died and were dissected before reaching 12 days post feeding. A plot of human Mf load and the number of mosquitoes biting per volunteer against the hourly interval indicates that these variables coincide and both peak between 22.00 and 05.00 h (Fig. 1).

Dissection results

A summary of the dissection results is given in Table 1. None of the mosquitoes for examining Mf uptake died within the 18 h interval needed for dissection. Other developmental stages of the parasite together with Mf were observed in only 3.3% of the immediately dissected mosquitoes, suggesting a previous infective blood meal. As a consequence, it is fairly unlikely that L3 larvae found after 12 days do not originate from the volunteers. Since shortly after feeding, Mf-positive mosquitoes harbour a considerable number of Mf (20 on average), the loss of infection after 12 days is much more apparent from the decline in larval load - from 9.3 to 4.2, i.e. about 55% reduction - than the reduction of the percentage infected mosquitoes (which only declines from 45 to 42%). Of the 504 mosquitoes kept for further observation on parasite development, 20% died before reaching 12 days post-feeding. As many as 64% of these dead mosquitoes harboured a parasite of any stage. This proportion is significantly higher than that observed in mosquitoes dissected after 12 days (42%; $P < 0.05$). Also the mean number of developing larvae (L2 or L3) per infected dead mosquito (15.9 ± 17.4) was significantly higher ($P < 0.001$) than in those dissected after 12 days (10.0 ± 16.4). This could be an indication of parasite-induced mortality among heavily infected mosquitoes.

Mosquito parity

Table 1 also provides details of the parity status of the three groups of mosquitoes. The fraction (1-, 2-, and 3-) parous mosquitoes among those dissected immediately and those dying before 12 days follow-up is about equal (~0.46) and this makes it not very likely that mortality of the latter group is a consequence of relatively older ages. Somewhat counter-intuitive (and difficult to explain) is the large proportion of parous mosquitoes among those dissected after 12 days (0.71; statistically significantly different from the other two groups, $P < 0.05$). The opposite is to be expected should age be an important determinant for survival within the considered interval. None of the dissection results for the 3 groups pointed to statistically significant differences between nulliparous and parous mosquitoes. This applies to both the fraction infected ($P > 0.05$ in all comparisons; see Table 1) and the mean number of parasites (Mann-Whitney *U* test for independent samples, $P > 0.05$; data not shown).

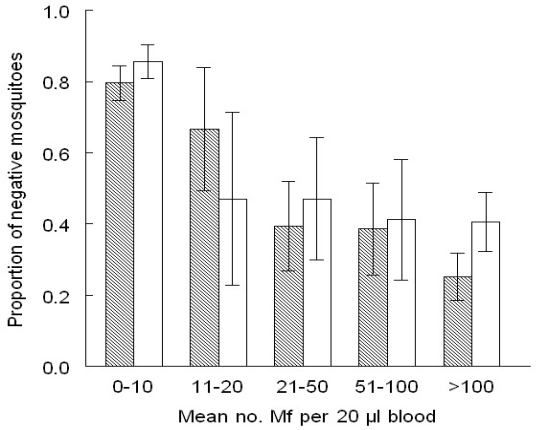

Figure 2 Relationship between observed human microfilarial density and the number of mosquitoes failing to ingest Mf (hatched bars) and to develop infective L3 larvae (empty bars). Error bars are 95% confidence intervals.

Uptake and development of Mf in relation to human Mf density

As stated earlier, both Mf uptake and the number of developing larvae varied considerably among mosquitoes. From Figure 2, showing the proportion of mosquitoes failing to engorge Mf or develop larvae, we can learn that this can only partially be explained from the differences in blood Mf density between patients and patient hours (see Discussion section of this paper). Although the failure rate declines with blood Mf density, even at high densities of more than 100 Mf/20 μl a considerable fraction of mosquitoes remains uninfected. The variability is also clearly shown in Figure 3 where for all dissected mosquitoes the larval load (Mf or L3) is plotted (dots) against the human Mf density in a particular patient hour.

Results of fitting relationships

Figure 3 also shows the estimated hyperbolic relationships for the Mf uptake and L3 output as a function of the human Mf density (equation (1)). The dashed line in these graphs is based on the estimated trend in the mean W and clumping factor k (equation (2)) and represents the 95% upper limit of the corresponding negative binomial distributions. Parameter estimates, including 95% confidence interval (CI), for the relationships are provided in Tables 2 (for Mf uptake) and 3 (for L3 output). In these Tables, estimates and log-likelihoods for the 'full models' (i.e. comprising all parameters) are compared with those for simpler hypotheses about how W and k vary with the

human Mf density m. Both for Mf uptake and L3 output the full model results in a significantly better fit to the data than the simpler alternatives (likelihood ratio test, $P < 0.05$ for all comparisons). We have also tested a more complicated relationship, in which the factor bm (see equation (1)) was replaced by bm^d (resulting in a sigmoid function if $d>1$), but this did not improve the fit (d indistinguishable from 1), neither for Mf nor for L3. The intercept a of the hyperbolic functions for Mf and L3 is small when compared to the theoretical saturation level c'. Furthermore, the CIs almost comprise zero, indicating that false negative blood Mf-counts do not constitute an important bias of the experiment. For L3 both the initial slope b (i.e., efficiency of developing larvae at low human Mf-counts) and the theoretical saturation level c' are about a factor of 4 times lower than those for Mf (no overlapping CIs).

If we express the results of the estimated relationships for $k(m)$ in terms of k values at three human Mf densities of 1, 10, and 100 Mf/smear, then we found for the

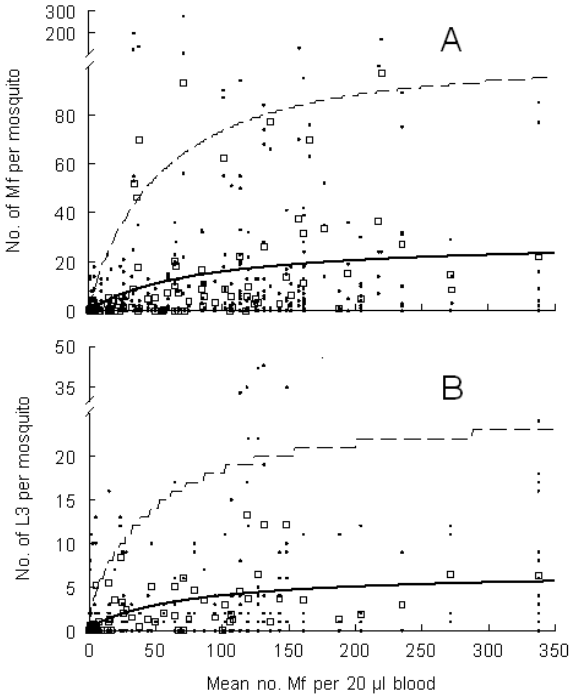

Figure 3 Comparison of observed (dots) and predicted (solid line) number of microfilariae (A) and L3 (B) per mosquito in relation to human Mf density. The dashed line is the estimated 95th percentile of the negative binomial distribution of Mf or L3 in mosquitoes as a function of human Mf density. The empty squares represent the arithmetic mean Mf uptake or L3 output for a particular human Mf density in a particular patient hour.

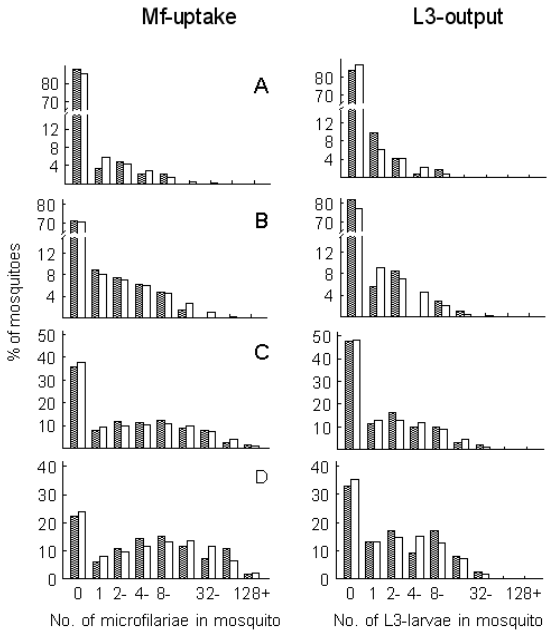

Figure 4 Observed (hatched bars) and expected (empty bars) frequency distributions of larval counts (left series: Mf uptake; right series: L3 output) in relation to the human Mf density: (A) 0-2 Mf/ smear, (B) 2.33-16, (C) 16.33-128, (D) more than 128 Mf/ smear. Larval count classes represent a geometric series with doubling class width's (classes 16-31 and 64-127 are not printed).

Mf uptake k = 0.08, 0.15, and 0.29, respectively and for the L3 output k = 0.09, 0.17, and 0.32, respectively. These values indicate that larval counts are always highly over-dispersed, but that the degree of aggregation decreases with increasing human Mf-counts. Furthermore, the values for Mf and L3 are strikingly similar.

A detailed representation of the estimated relationships is provided in Figure 4. Observed and expected frequency distributions of Mf uptake and L3 output are given for four ranges of human Mf densities: 0-2, 2-16, 16-128, and > 128 Mf/blood smear. The procedure for obtaining the expected distributions is provided in the Appendix. The agreement with the observations is satisfactory: for Mf, $\chi^2_{D.F.=16}$= 11.7, P = 0.75; for L3, $\chi^2_{D.F.=9}$ = 12.4, P = 0.20 (in both cases subtracting the number of estimated parameters - i.e. 6 - from D.F.). The distributions again underline the large variability in Mf- uptake and L3 output.

Table 2 Parameter estimates and associated log-likelihoods (LL) for the relations describing the Mf uptake, $W(m)$ (equation (1)), and the clumping factor of the negative binomial distribution, $k(m)$ (equation (2)), as a function of the human Mf density. (Values in brackets are 95% confidence intervals for the full model)

Hypothesis	Parameter estimates equation (1)				Parameters estimates equation (2)			
	a	b	c	$c'=a+b/c$	k_0	a	β	LL
Constant W	14.4	-*	-*	-	0.00786	0.0258	0.515	-1325.8
Linear $W(m)$	0.411	0.164	-*	-	0.0325	0.0521	0.334	-1300.6
Constant k	0.216	0.294	0.00865	34.2	0.231	-*	-†	-1310.3
Linear $k(m)$	0.206	0.317	0.0108	29.6	0.121	0.00143	-†	-1296.1
Full model	0.168 (0.028-0.56)	0.342 (0.22-0.55)	0.0119 (0.0045-0.026)	28.9 (20-54)	0.0304 (0.004-0.11)	0.0514 (0.008-0.105)	0.355 (0.20-0.68)	-1292.2

* Not estimated, fixed to zero.

† Not estimated, fixed to 1.

Table 3 Parameter estimates and associated log-likelihoods (LL) for the relations describing the L3 output, $W(m)$ (equation (1)), and the clumping factor of the negative binomial distribution, $k(m)$ (equation (2)), as a function of the human Mf density. (Values in brackets are 95% confidence intervals for the full model**)**

Hypothesis	Parameter estimates equation (1)				Parameter estimates equation (2)			
	a	b	c	$c'=a+b/c$	k_0	a	β	LL
Constant W	3.62	-*	-*	-	<0.0001	0.0401	0.435	-674.4
Linear $W(m)$	0.535	0.0288	-*	-	<0.0001	0.0862	0.276	-658.6
Constant k	0.216	0.106	0.0178	6.17	0.260	-*	-†	-665.9
Linear $k(m)$	0.219	0.104	0.0170	6.34	0.135	0.00142	-†	-658.3
Full model	0.276	0.0904	0.0143	6.60	<0.0001	0.0887	0.281	-654.4
	(0.010-0.65)	(0.041-0.24)	(0.0025-0.051)	(4.3-17.0)	(~0-0.091)	(0.051-0.150)	(0.15-0.41)	

* Not estimated, fixed to zero.

† Not estimated, fixed to 1.

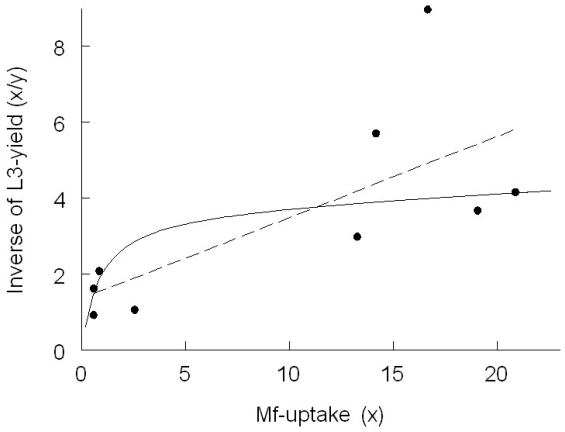

Figure 5 Relationship between the arithmetic mean number of microfilaria ingested (*x*) and the inverse of L3 yield (*x / y*; see text for details). The observations (dots) are obtained by determining the mean Mf uptake and mean L3 output for 9 categories of human Mf density (see Materials and Methods section). The dashed line is the result of a simple linear regression on the observations. The curve (solid line) is based on estimated relationships between human Mf density and the number of Mf ingested and the number of infective L3 larvae developed/ mosquito.

L3-yield

The yield of L3 larvae (ratio L3 output (y) : Mf uptake (x)), was calculated both from the observations (defining 9 classes of human Mf-counts; see Materials and Methods section) and on the basis of the estimated relationships. Following the procedure of Southgate & Brian (1992), the inverse of L3-yield (so: x/y) was plotted against the Mf uptake (x); see Figure 5. Linear regression was performed on the observed points and this showed a significantly better fit than a constant relationship (which signifies 'proportionality'; $P < 0.05$). The positive slope (0.21; 95% CI: 0.023-0.40) suggests 'limitation', i.e. an L3-yield which declines with an increasing Mf uptake. As a result of the considerable variability of larval counts, one of the observations is below the theoretical lower limit of 1.0 (parasites do not multiply in the vector). The solid line of Figure 5 shows the results of combining the estimated relationships for Mf uptake and L3 output. The initial sharp increase suggests that the reduction in L3 yield is most prominent over the lower range of Mf uptake values.

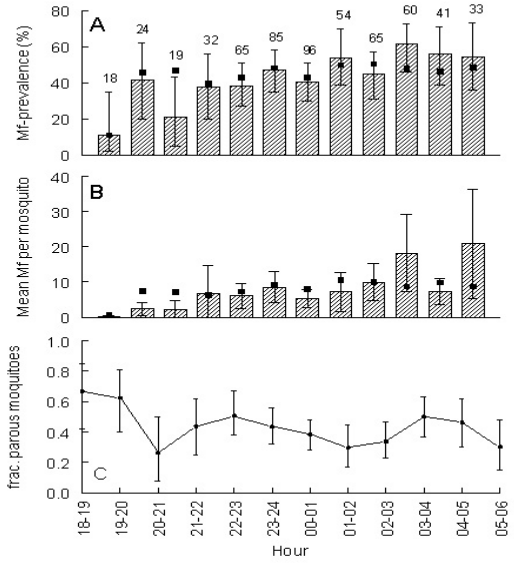

Figure 6 Comparison of observed (bars) and predicted (filled squares) prevalence (A) and intensity (B; arithmetic means) of microfilariae in mosquitoes throughout different hours of the night. Error bars are 95% confidence intervals for the observed prevalence and intensity of microfilariae. The number of dissected mosquitoes is given above the prevalence bars (A). (C) The fraction of parous mosquitoes (with 95% confidence intervals).

Role of periodicity in Mf uptake

The bars in Figure 6 (with 95% CI) show the percentage of mosquitoes engorging Mf (Mf-prevalence; Fig. 6A) and the mean Mf uptake/mosquito (Fig. 6B) for the different hours of the night. Also the expected values based on the fitted relationships are shown (see Appendix for their calculation). It can be seen that most of the expected values are within the CIs of the observed Mf prevalence and Mf uptake, suggesting satisfactory fit of the model to the data. However, the overestimation is rather systematic. A possible reason is that the model (equations (1) and (2)) overestimates the fraction of mosquitoes with high Mf uptake at intermediate (2-16 Mf/smear) human Mf densities (see the upper tail of the predicted distribution for Mf in Fig. 4B).

Figure 6A shows that the Mf-prevalence increases during the night and then stabilizes around 50% in spite of the decline in the human Mf density towards the end of the night (Fig. 1). However, the mean human Mf density during 05-06 h is still twice as high as during 18-19 h and since Mf uptake relates in a non-linear way to blood Mf density one expects an Mf uptake of more than twice the level during the early evening. This is still no explanation for the apparent increase of the observed mean Mf uptake (Fig. 6B), which suggests that the efficiency of the *Culex* vector to engorge Mf increases

during the night. However, in a logistic regression analysis of the success of engorging Mf as a function of the human Mf density and the hour of the night, this latter variable appeared to be non-significant (likelihood-ratio test, $P >> 0.05$). This is also suggested by the wide CIs, which are mainly the result of a few very high counts. In the interval 05-06 h, the exclusion of 2 excessively high uptakes (170 and 196 Mf) would bring the mean uptake down to 10.4 Mf. A plot of the fraction parous mosquitoes (Fig. 6C) demonstrates that a possible increase in uptake efficiency cannot be explained from a trend in mosquito age during the night.

Discussion

This paper reports the analysis of *W. bancrofti* larval counts in wild *C. quinquefasciatus* mosquitoes fed under natural conditions on volunteers sleeping under a partially opened mosquito net. The results thus obtained are of great value for gaining quantitative insight into the transmission of the parasite. Other distinguishing features of the present study are that the Mf density and intake were studied throughout the night, accounting for the periodicity of Mf in the peripheral blood, and that Mf uptake and larval development were studied in parallel permitting the study of possible density regulation during both of these processes.

Uptake of Mf

The results of fitting relationships through a maximum likelihood procedure show that the mean uptake of Mf depends on the human Mf density in a non-linear saturating way (Fig. 3A). The assumption of a straight line had to be rejected in favour of a hyperbolic relationship. It is important to note that a proportional relationship would not have been rejected if our analysis had been based on a simple least-square regression of mean Mf uptake to mean human Mf density, mainly because these mean uptakes are extremely scattered (in some patient hours, only a few mosquitoes could be collected) and sometimes largely dominated by extremely high uptakes (up to 200-300 Mf). Our finding corroborates several publications for different vectors and *W. bancrofti* combinations (*C. quinquefasciatus, A. aegeypti, An. Gambiae*[13]; *An. gambiae, An. arabiensis, An. melas, An. funestus*[2,22]; *A. polynesiensis*[23]), in which a non-linear saturating relationship for the uptake of Mf of periodic *W. bancrofti* was found. Though the experimental settings will not always be comparable, the results disagree with the linear relationship concluded in various other studies [14-17].

Loss of larvae

Irrespective of the human Mf density or Mf uptake, the overall loss of larvae during 12 days, is estimated at an average of 80%. This estimate includes the 104 mosquitoes that died during development and, hence, do not carry larvae at all. If these latter mosquitoes are disregarded, the loss is estimated at 74%. These figures are well in agreement with observations by Jordan & Goatly [14] who found losses of 55-99%. Higher losses were

found by McGreevy *et al.* [13]: 87-96%, and lower by Jayasekara *et al.* [17] who observed a loss of 24-67% of Mf during their development to the L3 stage. The loss of larvae in the *Mansonia dives - B. malayi* complex was reported to be minimal [24]. While the loss of larvae is high, the percentage of mosquitoes losing all larvae is estimated much lower: 17% (when only considering the survivors after 12 days) or 37% (when treating dead mosquitoes as if they lost their infection). The latter percentage is well in agreement with observations on wild mosquitoes caught in Pondicherry where the reduction in the number of infected mosquitoes during development from Mf to L3 was estimated at 25%-33% [18].

Regulation of larval density

More interesting than an over-all percentage loss is to know whether and how this depends on the number of Mf engorged: is there any evidence of density regulation during larval development in addition to the density regulation we concluded for the Mf uptake? The data presented in Figure 5 suggests such a regulation by showing a statistically significant decrease in the L3 yield (or an increase in the inverse) for increasing Mf uptakes. Furthermore, given the sharp initial increase in the curve based on the estimated relationships, this 'limitation' is probably not a phenomenon that only occurs at (extremely) high Mf intakes. This latter phenomenon should however be considered carefully, because it also arises from the fact that both estimated relationships of Figure 3 have a positive intercept *a* (to take account of the few, false-negative blood-smears). Since *a* for L3 output is even slightly higher than for Mf uptake (see Tables 2 and 3), at 'zero' human Mf densities the L3-yield is even higher than the theoretical value of 1. Hence, the sharp rise of the curve in Figure 5 could in part be due to the (lack of) sensitivity of the blood smear for detecting Mf. Another comment to be made is that Figure 5 only applies to mean Mf uptakes while Figure 3A and Figure 4 clearly show that, for a given human Mf density, there is a large individual variation in the uptake of Mf. An important, but as yet unsolvable, question is what happens with the (extremely) high Mf uptakes (the upper tail of the distribution). To answer this question one should be able to examine mosquitoes at a moment that the engorged Mf can still be counted while it is already clear which of these Mf will develop to the L3-stage. For *Simulium damnosum*, this can be done by distinguishing between the Mf encapsulated in the peritrophic membrane and those outside the membrane and / or entering the thoracic muscles Basáñez et al, [25]. In this approach, one should be aware that excess mortality of mosquitoes at later stages of larval development, a potential mechanism of density regulation, is not taken into account.

The density regulation of both Mf uptake and L3 yield can be due to increased mortality of the larvae at high densities or to parasite induced mortality of the vector [10,18,26]. The experimental design of our study does not permit far-reaching conclusions about mosquito-survival because during the 12 days period no record was kept of the day on which a mosquito died and, more important, because no parallel dissections of live

mosquitoes were carried out during this period. However, the results as presented in Table 1 suggest that the excess mortality of highly infected mosquitoes may play a role. Both the percentage infected and the intensity of infection in the positives among the 104 mosquitoes which died before 12 days were significantly higher than those for the survivors (64% vs. 42% and 16 vs. 10 L2/L3 larvae, respectively). Parasite-induced vector mortality was also reported by Crans [27], who observed a mortality rate twice as high as in *C. quinquefasciatus* females harbouring *W. bancrofti* larvae when compared to mosquitoes without infection. Failloux *et al.* [23] observed a mortality rate of 20 to 60% in *A. polynesiensis* populations infected by *W. bancrofti* and concluded that the level of mortality was associated with parasite load.

Heterogeneity in Mf uptake and larval yield

Both the number of Mf ingested and the number of L3 larvae/mosquito show marked variability. Figure 2 shows that, even for high human Mf densities of more than 100 Mf/20 μl blood still 25% of the mosquitoes fail to ingest Mf and 40% do not carry L3 larvae. This variation can in part be explained from variation in the human Mf density, both in time and across different sites of the body. While mosquitoes were collected continuously throughout a (patient) hour, the Mf density was only determined at the mid-point and only from finger prick samples. Though a clear periodicity was observed for all carriers together, considerable hour-to-hour variations were observed for each individual, and this will also imply variation within an hour. Further, while human Mf density is determined in the capillary blood of a finger, mosquitoes bite all over the body. This spatial variability of Mf in the host as one explanation of variation in Mf uptake was suggested earlier by Jordan & Goatly [14] and Ramachandran & Zaini [28]. However, variation in human Mf density can neither be the only nor the most important reason for the large differences in Mf uptake by and larval development within the vector. In our experiment, this is suggested by the virtual absence of 'false positives', i.e. mosquitoes which engorge Mf or develop larvae from patient-hours with a zero Mf density. This number would be larger if the blood-smears were not representative of the blood engorged by the mosquitoes. Most likely, factors related to the feeding itself are responsible for the heterogeneity, such as the presumed ability of mosquitoes to concentrate Mf in the blood close to the feeding site [2], differences in pool and capillary feeding habits of the mosquitoes [29,30], or perhaps the non-homogeneous (clustered) distribution of Mf in the blood [31], which becomes more important as the amount of blood declines.

In our analysis, the variability of the Mf uptake and the L3 output is represented by a negative binomial distribution. Both the mean and the clumping factor k of this distribution were found to vary with the human Mf density m. Among the tested relationships, the assumption of a power-function for describing k as a function of the human Mf density resulted in the best fit to the observations (maximum-likelihood). The low values of this k (ranging from close to zero to 0.3) indicate a high level of aggregation

of the numbers of Mf or L3/mosquito [25,26,32-34]. The increase in k, and hence a decrease in the degree of overdispersion with human Mf density, could imply that the levelling-off of the mean uptake or larval output is partially due to the absence of excessively high Mf uptakes: the upper tail of the distribution is lopped off, reducing the estimates for W and increasing the estimates for k (see also [35,36]).

Methodological issues

Though the relationships in Figure 3 are based on many data-points (592 for Mf and 400 for L3), the number of patients involved in the study is only 13. From these 13 patients we derived 119 (Mf) and 62 (L3) 'patient-hours' by treating the hourly collection of blood together with the hourly catches of mosquitoes as independent samples. Independent, of course, not in the sense that for a patient the successive bloodsmears are not correlated (depends on the worm load of a person), but in the sense that it is exclusively the Mf density in the blood which determines the Mf uptake and not the hour of the night or any (unknown) patient factor. By means of logistic regression analysis we have excluded the hour of the night as a confounder. However, both the considerable within-patient (hour-to-hour) and the between-patient variation in blood smear counts, together with the highly variable Mf uptake make it very difficult to resolve the problem of systematic differences between volunteers in their ability to infect mosquitoes. But even if such patient factors should exist, the implications for our findings are likely to be limited, mainly because the ranges of Mf densities shown during the night by each of the volunteers are considerably overlapping. This implies that it is, for example, not just one volunteer who delivers the data points at the higher end of human Mf densities or just one with the zero and low counts. In order to obtain a second data set for verification of our results, another experiment, similar to the one here presented but with more volunteers, is now being carried out at VCRC.

In contrast to many other studies [5,13-15,25,34], we have based our conclusions on the analysis of larval counts in individual mosquitoes and not on the mean uptake of a batch of vectors or the fraction of vectors infected. We believe that, if possible, utilization of the basic unit of measurement (the mosquito) results in the most powerful estimation of relationships and of parameters for over-dispersion.

Implications for control

The conclusion that the *W. bancrofti* - *C. quinquefasciatus* complex is of the 'limitation' type, which we draw on the basis of an experiment under natural conditions, could have important consequences for the effectiveness of control measures [5]. The estimated relationship shown in Figure 3B (which is the consequence of the saturated uptake of Mf plus the 'limitation' phenomenon) makes it clear that low human Mf densities show a relatively high capability to generate infectious mosquitoes. Hence, control should bring down and maintain Mf densities at low levels in order to sufficiently break transmission by mosquitoes. This could be one of the reasons for the difficulties to bring about a

major decline in the parasite population through 5 years of vector control in Pondicherry (1981-1985, see [37]). It was found that, although in this region the trend in prevalence and intensities of Mf continued to decline, the annual transmission index (number of infective larvae/person/year estimated from entomological biting collections) has considerably increased from 1986 onwards [38]. Similarly eradication appears to be difficult in areas where control programmes are solely aimed at reducing the parasite reservoir in the human host [5,17,39].

On the other hand, our findings do not justify firm conclusions about the implications for control. The series of observations obtained from the 13 volunteers selected for the study do not constitute a representative sample from the population of Pondicherry. During a survey in 1981 (before starting vector control, see [40]) about 10% was found to be Mf-positive on the basis of a single smear of 20 μl. Among those positive, only a small fraction (<3%) showed high counts of more than 50 Mf/smear. A re-analysis of the data for Mf uptake and L3 output after excluding patient hours with more than 50 Mf/smear resulted in estimates of c which did not significantly differ from 0; i.e., relationships which do not differ from linearity. Though this could only be concluded when assuming a positive intercept a (and again higher for L3 than for Mf), which complicates reasoning about density regulation during development from Mf to L3, this stresses that one should like to do this kind of experiments with a (large) number of patients which together reflect the distribution of Mf density in the population (see also [11,12]).

However, probably more important than density regulation of mean larval counts, is the occurrence of very high Mf uptakes and L3 outputs even for relatively low human Mf densities. Though the present experiment does not provide information on the survival chances of mosquitoes with large numbers of L3 larvae (say ≥ 8) in the field, they might play a disproportionate large role in transmission and considerably hamper the elimination of *W. bancrofti.*

Appendix

Let $W(m)$ be the hyperbolic function describing the mean Mf intake or L3 output as a function of the human Mf density m and $k(m)$ the function describing the clumping factor of the negative binomial distribution for the number of larvae in the vector as a function of m (see eqns (1) and (2); Materials and Methods section), then the parameters a, b, c, k_0, a, and β of these functions are estimated by maximizing the likelihood function:

$$L = \prod_{i=1}^{13} \prod_{h=1}^{12} \prod_{j=1}^{J_{hi}} P_{NB}\left(x = l_{hij} \mid W(m_{hi}), k(m_{hi})\right) \qquad \textbf{(A 1)}$$

with:

$P_{NB}(x \mid W,k)$ probability to find x parasites (Mf or L3) given a mean Mf uptake or L3 output of W and a clumping factor k of the negative binomial distribution:

$$P_{NB}\left(x \middle| W, k\right) = \frac{\Gamma(k+x)}{\Gamma(x+1)\Gamma(k)}\left(\frac{W}{W+k}\right)^{x}\left(1+\frac{W}{k}\right)^{-k} \qquad \textbf{(A 2)}$$

where $\Gamma(.)$ is the gamma function; J_{hi} number of mosquitoes collected in hour h (totally 12 hours) from patient i (totally 13 patients); l_{hij} number of parasites found in mosquito j caught from patient i during hour h; m_{hi} Mf density of patient i during hour h. Maximizing this likelihood function is achieved with a downhill-simplex method [41] implemented in a computer program written in C.

The expected prevalences of Figure 6A are, for each hour h, calculated as follows:

$$p_h = \frac{\sum_{i=1}^{13} J_{hi} \times P_{NB}(x>0 \mid W(m_{hi}), k(m_{hi}))}{\sum_{i=1}^{13} J_{hi}} \qquad \textbf{(A 3)}$$

with:

$$P_{NB}(x>0 \mid W,k) = 1-\left(1+\frac{W}{k}\right)^{-k} \qquad \textbf{(A 4)}$$

The expected mean Mf uptake within each hour h (Fig. 6B) is calculated as:

$$w_h = \frac{\sum_{i=1}^{13} J_{hi} \times W(m_{hi})}{\sum_{i=1}^{13} J_{hi}} \qquad \textbf{(A 5)}$$

The predicted distributions shown in Figure 4 are, for each of the considered ranges of values of human Mf densities m_{hi} (0-2, 2-16, 16-128, >128), calculated as:

$$Pr(x=l) = \frac{\sum_{i=1}^{13}\sum_{h=1}^{12} J_{hi} \times P_{NB}\left(x=l \mid W(m_{hi}), k(m_{hi})\right)}{\sum_{i=1}^{13}\sum_{h=1}^{12} J_{hi}} \qquad \textbf{(A 6)}$$

with $Pr(x = l)$ expected probability to engorge l Mf or to produce l L3 larvae.

References

1. Bryan JH, McMahon P, Barnes A. Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 3. Uptake and damage to ingested microfilariae by *Anopheles gambiae*, *An. arabiensis*, *An. merus* and *An. funestus* in east Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1990;84:265-8.
2. Bryan JH, Southgate BA. Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 1. Uptake of microfilariae. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1988;82:128-37.
3. Samarawickrema WA, Spears GF, Sone F, Ichimori K, Cummings RF. Filariasis transmission in Samoa. I. Relation between density of microfilariae and larval density in laboratory-bred and wild-caught *Aedes (Stegomyia) polynesiensis* (Marks) and wild-caught *Aedes (Finlaya) samoanus* (Gruenberg). *Annals of Tropical Medicine and Parasitology* 1985;79:89-100.
4. Pichon G. Relations mathematiques entre le nombre des microfilaires ingereese et le nombre des parasites chez differents vecteurs naturels ou experimentaux de filarioses. *Cahiers ORSTOM serie Entomologie medicale et Parasitologie* 1974;12:199-216.
5. Southgate BA, Bryan JH. Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 4. Facilitation, limitation, proportionality and their epidemiological significance. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1992;86:523-30.
6. Brengues J, Bain O. Passage of microfilariae from the stomach to the haemocoel of the vector, in the case of *Wuchereria bancrofti* in *Anopheles gambiae* A and *Aedes aegypti* and *Setaria labiatopapillosa* in *Aedes aegypti*. *Cahhiers ORSTOM, Serie Entomologie medicale et Parasitologie* 1972;10:235-49.
7. Pichon G, Perrault G, Laigret J. Rendement parasitaire chez les vecteurs de filarioses. *Bulletin of the World Health Organization* 1974;51:517-524.
8. Southgate BA. The significance of low density microfilaraemia in the transmission of lymphatic filarial parasites. *Journal of Tropical Medicine and Hygiene* 1992;95:79-86.
9. Southgate BA. Intensity and efficiency of transmission and the development of microfilaraemia and disease: their relationship in lymphatic filariasis. *Journal of Tropical Medicine and Hygiene* 1992;95:1-12.
10. Dye C. Does Facilitation Imply a Threshold for the Eradication of Lymphatic Filariasis? *Parasitology today* 1992;8:109-110.
11. Dye C. Approaches to vector control: new and trusted. 5.The epidemiological context of vector control. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1994;88:147-149.
12. Dye C, Williams BG. Non-linearities in the dynamics of indirectly-transmitted infections (or, does having a vector make a difference?). In: Dobson AP, ed. Ecology of Infectious Diseases in Natural Populations. Publications of the Newton Institute, Cambridge: Cambridge University Press, 1995: 260-279.
13. McGreevy PB, Kolstrup N, Tao J, McGreevy MM, Marshall TF. Ingestion and development of *Wuchereria bancrofti* in *Culex quinquefasciatus*, *Anopheles gambiae* and *Aedes aegypti* after feeding on humans with varying densities of microfilariae in Tanzania. *Transactions of the Royal Society of Tropical Medicine & Hygiene* 1982;76:288-96.
14. Jordan P, Goatly KD. Bancroftian filariasis in Tanganyika: a quantitative study of the uptake, fate and development of microfilariae of *Wuchereria bancrofti* in *Culex fatigans*. *Annals of Tropical Medicine and Parasitology* 1962;56:173-87.
15. Obiamiwe BA. Relationship between microfilarial density, the number of microfilaria ingested by mosquitoes and the proportion of mosquitoes with larvae. *Annals of Tropical Medicine and Parasitology* 1977;71(4):491-500.

16. Lowrie RCJ, Mark L, Eberhard ML*, et al.* Uptake and development of *Wuchereria bancrofti* in *Culex quinquefasciatus* that fed on Haintian carriers with different microfilaria densities. *American Journal of Tropical Medicine and Hygiene* 1989;41:429-435.
17. Jayasekera N, Kalpage KS, De Silva CS. The significance of low density microfilaraemia in the transmission of *Wuchereria bancrofti* by *Culex (Culex) quinquefasciatus* Say in Sri Lanka. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1991;85:250-4.
18. Subramanian S, Manoharan A, Ramaiah KD, Das PK. Rates of acquisition and loss of *Wuchereria bancrofti* infection in *Culex quinquefasciatus*. *American Journal of Tropical Medicine and Hygiene* 1994;51:244-9.
19. Kartman L. An observation on the loss of microfilariae from the mosquito host during its infected meal. *Journal of Parasitology* 1953;39:571.
20. Detinova TS. Age-grouping methods in Diptera of medical importance with special reference to some vectors of malaria. *WHO monograph series* 1962;47:1-216.
21. Clayton D, Hills M. Statistical Models in Epidemiology. 1 ed. Oxford: Oxford University Press, 1993.
22. Bryan JH, Southgate BA. Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes .2.Damage to ingested microfilarie by mosquito foregut armatures and development of filarial larvae in mosquitoes. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1988;82:138-145.
23. Failloux AB, Raymond M, Ung A, Glaziou P, Martin PMV, Pasteur A. Variation in the vector competence of *Aedes polynesiensis for Wuchereria bancrofti. Parasitology* 1995;111:19-29.
24. Wharton RH. Studies on filariasis in Malaya: field and laboratory investigations of the vectors of a rural strain of *Wuchereria bancrofti*. *Annals of Tropical Medicine and Parasitology* 1960;54:78-91.
25. Basanez MG, Remme JH, Alley ES*, et al.* Density-dependent processes in the transmission of human onchocerciasis: relationship between the numbers of microfilariae ingested and successful larval development in the simuliid vector. *Parasitology* 1995;110:409-27.
26. Das PK, Subramanian S, Manoharan A*, et al.* Frequency distribution of *Wuchereria bancrofti* infection in the vector host in relation to human host: evidence for density dependence. *Acta Tropica* 1995;60:159-165.
27. Crans WJ. Experimental infection of *Anopheles gambiae* and *Culex pipiens fatigans* with *Wuchereria bancrofti* in coastal East Africa. *Journal of Medical Entomology* 1973;10:189-93.
28. Ramachandran CP, Zaini MA. Studies on the transmission of sub-periodic *Brugia malayi* by *Aedes (Finlaya) togoi* in the laboratory. II. The development of the parasite to the infective form; the relationship between concentration of microfilariae in the vertebrate host and infection in the mosquitoes. *Medical Journal of Malaya* 1968;22:198-203.
29. Gordon RM, Lumsden WHR. A study of the behaviour of the mouth-parts of mosquitoes when taking up blood from living tissue; together with some observations on the ingestion of microfilariae. *Annals of Tropical Medicine and Parasitology* 1939;33:259-78.
30. Gubler DJ, Inui TS, Black HR, Bhattacharya NC. Comparisons of microfilaria density in blood sampled by finger-prick, venipunture, and ingestion by mosquitoes. *American Journal of Tropical Medicine and Hygiene* 1973;22:174-178.
31. Hairston NG, Jachowski LA. Analysis of the *Wuchereria bancrofti* population in the people of American Samoa. *Bulletin of the World Health Organization* 1968;38:29-59.
32. Anderson RM, May RM. Helminth infections of humans: mathematical models, population dynamics, and control. *Advances Parasitology* 1985;24:1-101.
33. Wenk P. The vector host link in filariasis. *Annals of Tropical Medicine and Parasitology* 1991;85:139-47.
34. Basanez MG, Boussinesq M, Prod'hon J*, et al.* Density-dependent processes in the transmission of human onchocerciasis: intensity of microfilariae in the skin and their uptake by the simuliid host. *Parasitology* 1994;108:115-27.
35. Anderson RM, Gordon DM. Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology* 1982;85:373-398.

36. Pacala SW, Dobson AP. The relation between the number of parasites/host and host age: population dynamic causes and maximum likelihood estimation. *Parasitology* 1988;96:197-210.
37. Subramanian S, Pani SP, Das PK, Rajagopalan PK. Bancroftian filariasis in Pondicherry, south India: 2. Epidemiological evaluation of the effect of vector control. *Epidemiology and Infection* 1989;103(3):693-702.
38. Das PK, Manoharan A, Subramanian S*, et al.* Bancroftian filariasis in Pondicherry, south India--epidemiological impact of recovery of the vector population. *Epidemiology & Infection* 1992;108(3):483-93.
39. Biswas H, Sharma SP, Das M, Rao VG, Yadava RL, Narasimham MV. Filariasis control in rural areas through detection and treatment with diethylcarbamazine. *Journal of Communicable Diseases* 1989;21:272-81.
40. Rajagopalan PK, Das PK, Subramanian S, Vanamail P, Ramaiah KD. Bancroftian filariasis in Pondicherry, south India: 1. Pre-control epidemiological observations. *Epidemiology and Infection* 1989;103:685-92.
41. Nelder JA, Mead R. A simplex method of function minimization. *Computer Journal* 1965;7:308-312.

3.2 Frequency distribution of *Wuchereria bancrofti* infection in the vector host in relation to human host: evidence for density dependence

Summary

This paper uses detailed entomological data from Pondicherry to compare the microfilaria distribution in vector with that of human and also to examine the evidence for the operation of density dependence on parasite transmission. Analysis showed that the distribution in vector is similar to that in human. The expected distribution derived from the fit of a zero-truncated negative binomial to the human data, closely reflected the observed microfilaria distribution in the vector. This implies that a relatively large proportion of observed microfilaria negatives in the vector population may be *true zeros*, as observed in human, arising from the biting on true negatives (as yet uninfected, with unmated female worms or immune) in humans, rather than attributable to the blood sampling process. Further it was found that both prevalence of infection and the degree of parasite aggregation in the vector population falls significantly with parasite stage, implying the operation of density dependence, perhaps via parasite-induced vector mortality.

Introduction

A key question in the epidemiology of vector-borne diseases is whether nonlinear (density dependent) processes operate on parasite transmission from the vector population [2,3]. Broadly speaking, this can manifest itself as facilitation (an increase in infection rate over some ranges of parasite density) or limitation (a density dependent constraint on transmission)[4]. One of the main mechanisms proposed to drive limitation is parasite-induced vector mortality at high parasite densities [3].

The key observation of density dependent constraint on transmission is by Samarawickrema and Laurence [5]. They analyzed the distribution of *Wuchereria bancrofti* larvae in a natural population of the mosquito *Culex quinquefasciatus* in a region endemic for bancroftian filariasis in Sri Lanka. They equated the burden of successive stages of parasite larvae (microfilariae (Mf), L1, L2, L3) with duration of infection in the vector, and analyzed the resulting frequency distributions by fitting a lognormal distribution. After showing that the distribution of Mf in freshly blood-fed mosquitoes did not differ significantly from that in blood samples from the human population, they demonstrated that the distribution of L3 larvae in infected vectors had a significantly shorter tail than for the earlier parasite stages. They then used the results from experimental infections to interpret this relative reduction in the proportion of vectors with large burdens of older parasites in terms of the operation of parasite-induced host mortality. Samarawickrema and Laurence [5] is the only report of this potentially important observation.

In this paper we use an extensive data set collected from the field in Pondicherry, South India to examine the role of density dependent constraint on transmission. Our analysis is based on recent work, which shows that the microfilarial frequency distribution in blood samples from human populations follows a mixed Poisson distribution, reflecting both heterogeneity in microfilaraemia amongst hosts and Poisson blood sampling of a density distribution of microfilariae in the peripheral blood [6-9]. Furthermore, since a relatively large proportion of human hosts are truly parasite-negative, the observed distribution shows an excess of zeros above those expected from Poisson sampling [7,8]. Clearly, the same line of reasoning should apply to the distribution of microfilariae 'sampled' by mosquitoes [6], and we examine this possibility below.

Database and Statistical Methods

The data

The analysis is based on entomological data collected during an integrated vector control programme against bancroftian filariasis, carried out by the Vector Control Research Centre of the Indian Council of Medical Research, in Pondicherry, South India during the period 1981 to 1986 (see, [10], *for full details*). The main pre-control data set, which was collected in 1981, forms the basis of this analysis. It comprises of the results of fortnightly resting collections, which yielded a total of 18738 female *C. quinquefasciatus* vectors for parasitological dissection and age-grading, according to number of previous egg layings (parity). There were 7419 nulliparous, 7462 one parous (1P), 3294 2P, 500 3P, 57 4P and 6 5P mosquitoes; of these 2524 mosquitoes were infected with filarial larvae. Full details of the method of collecting parasitological data from the human population has been reported elsewhere [11]. Briefly 24946 human blood samples were examined for the presence of microfilaria using a stratified random sampling protocol.

Statistical analysis

Assuming that the vectors bite randomly with respect to host infection status [12], the frequency distribution of Mf in freshly-fed nulliparous mosquitoes (i.e. after their first feed) should follow a mixed Poisson distribution, with an augmented zero class. The rationale for this model which is discussed in detail by, [8] is as follows. If the mean density of Mf in the peripheral blood of infected individuals follows a continuous probability density, $h(m)$, then 'sampling' of this distribution by biting vectors (essentially a Poisson process) will lead to a Poisson mixture (such as the negative binomial or the Sichel distribution; [8] for the positive counts, with a proportion of zeros which are due purely to the sampling process (i.e., classified as negative, although the individuals bitten are Mf-positive; [6]). Since an, often large, proportion of humans will also be 'true' negatives (as yet uninfected, with unmated female worms, or immune) [7], nulliparous mosquitoes which bite them will also be negative. This then leads to a Poisson mixture

with excess zeros, which can be fitted to observed Mf distributions by a zero-truncated fit, which is restricted to the positive counts.

Grenfell *et al.*[8] found that zero-truncated Sichel and negative binomial distributions fitted the Mf distribution in human blood samples from a number of regions much better than the distributions including zeros. Here, we test whether this effect is also apparent in the distribution of parasites in the vector.

The expected distributions of later (post-Mf) stages of parasites in older hosts are likely to be much more complex, since they reflect a balance between development and death processes [13]. Because of relatively small sample sizes in some of these stages, we compare the degree of over-dispersion between stages using the variance to mean ratio [1]. The relationship between infection prevalence and parasite stage (Mf to L3) and vector age (2P and 3P) were examined using generalized linear model analysis [14]. The mean parasite intensity (parasites per mosquito) between vector age were compared using the Kruskal-Wallis non-parametric test [15].

Results

(a) Prevalence of infection with mosquito and parasite stage

The sample sizes and the prevalence of infection according to mosquito age and parasite stage are depicted in Table 1. Since the minimum time required for Mf to reach L3 stage is 10 days, it is not expected to have L3 in mosquitoes of age less than 2P (which is 8 - 10 days old). However a low prevalence of advanced stages (L2 and L3) of parasites were

Table 1 Prevalence of infection for different mosquito and parasite stages

Parasite stage	Mosquito age				
	NP	1P	2P	3P	Overall
	(7419)	(7462)	(3294)	(563)	(18738)
Mf	5.78	5.62	6.56	6.93	5.89
L1	0.94	8.23	12.20	12.26	6.16
L2	0.11	2.25	6.65	10.30	2.42
L3	0.05	0.31	3.10	7.82	0.92
Overall	6.66	14.65	23.56	28.60	13.47

Figures in parentheses are sample sizes

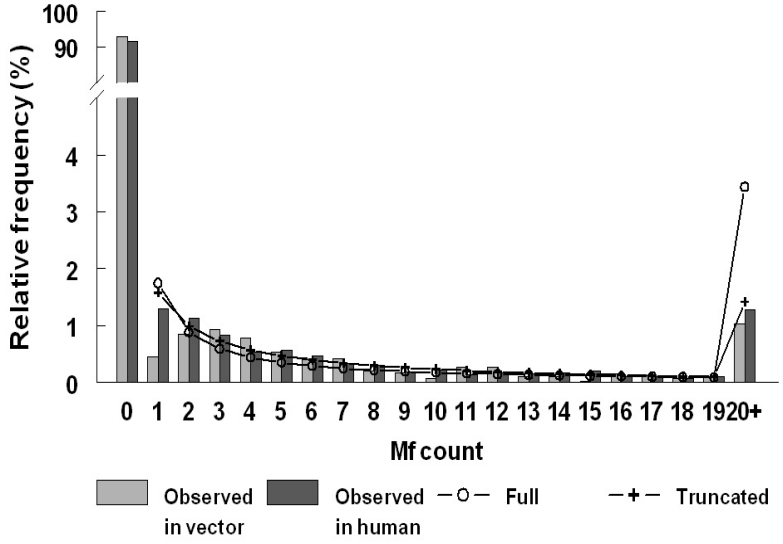

Figure 1 Observed proportional frequency distribution of Mf in humans and nulliparous fully fed mosquitoes in Pondicherry in 1981. Expected curves are derived from fits of the zero truncated and non-truncated negative binomial distribution to the human Mf distribution (see Grenfell *et al*., 1990 for details).

also recorded in NP and 1P mosquitoes. Therefore we restrict our analysis in comparing 2P and 3P mosquitoes. Analysis of variations in prevalence with parasite and mosquito stage indicates that the infection prevalence significantly decreases with parasite stage (χ^2=203.0, D.F. =3, $P < 0.0001$), and increases with mosquito age (χ^2=12.7, D.F.=1, $P < 0.05$).

(b) Patterns of aggregation in the vector population

The means and variance/mean ratio of infection intensity in the vector population, classified according to parasite stage and vector age are presented in Table 2. The overall intensity of parasite increases with vector age (χ^2=687.4, D.F. = 3, P<0.01). Although all the variance to mean ratios are significantly greater than 1 (indicating marked overdispersion in parasite numbers), there is a clear indication (Table 2), particularly in the older vectors (2P and 3P), for parasite distributions to become significantly less aggregated by larval stage.

Table 2 Parasite intensity and variance to mean ratio for different mosquito and parasite stages

Parasite stage	Mosquito parity									
	NP		1P		2P		3P		Overall	
	Mean	Ratio	Mean	Ratio	Mean	Ratio	Mean	Ratio	Mean	Ratio
Mf	0.570	25.601[a]	0.540	32.356[b]	0.740	48.811[c]	0.570	32.288	0.590	33.260
L1	0.072	16.260[a]	0.590	18.124[b]	0.830	15.013[c]	0.960	21.094[d]	0.440	17.310
L2	0.004	7.000	0.110	16.324	0.330	12.859[c]	0.520	9.139[d]	0.120	13.650
L3	0.005	20.000	0.007	4.430	0.110	8.205[c]	0.230	5.850[d]	0.030	8.330
Overall	0.650	24.986	1.250	24.820	2.010	27.985	2.280	19.571	1.180	25.640

All the variance to mean ratios are significantly greater than 1 (chi-square test (variance to mean ratio) for agreement with a Poisson series [1]. 'Ratio' indicates the variance to mean ratio; Ratios joined by matching superscripts indicate that the corresponding distributions are significantly different from each other at the 95% probability level (Kolmogorov-Smirnov two sample test (Sokal & Rohlf, 1981)).

(c) Microfilarial distributions in humans and vectors

Figure 1 compares the observed relative frequency of Mf in the human and (nulliparous fully fed) vector populations of Pondicherry in 1981. Though the distributions are apparently similar in all counts, they differ significantly at 5% level (χ^2=57.7; D.F.=18; $P < 0.001$). The overall microfilarial prevalence was 8.38% in the human population and 7.11% in nulliparous full-fed vectors.

Since we are interested in comparing the effects of blood sampling process by mosquitoes with that of human blood sampling, we restrict ourselves to compare the observed distribution of Mf in mosquito with the expected distributions arising from the fits of negative binomial probability model to zero-truncated and non-truncated human Mf distribution. Figure 1 also shows fits of the full (estimated parameter values:μ=3.62±1.2; k=0.019 ± 0.0004; goodness of fit χ^2 = 397.0, D.F. = 83, $P < 0.001$) and zero- truncated (estimated parameter values:μ=6.62±0.36; k=0.34 ± 0.03; goodness of fit χ^2 = 83.4, D.F. = 49, $P < 0.001$) negative binomial distribution to human Mf counts. Comparison with counts in the vector population shows that the zero-truncated fit similarly slightly over-estimates the observed counts. However, the non-truncated fit to the human data does not accord with the observed distribution for the vector. In particular, the non-truncated fit is far more aggregated than the observations, with a much longer tail of high counts.

Discussion

The prevalence and intensity of infection declines with increasing age of the parasite but increases with age of the vector. Since the parasite cannot either multiply or be transferred from one mosquito to another, the increase in both prevalence and intensity could only be due to accumulation of infection as the vector age increases. The reduction in prevalence of infection with increasing age of the parasite could be either due to density dependent parasite loss or mosquito mortality or combination of both. If there were a loss of parasite due to mortality in mosquitoes one would expect differential survival rate between infected and uninfected mosquitoes. However due to complex host-parasite interactions it is difficult to estimate the survival rate of the mosquitoes based on the parity alone [16]. It is further evident from the presence of advanced stages of parasites in the NP and 1P mosquitoes, which again might be due to delay in oviposition as reported earlier [16,17].

Further, examination of the degree of parasite aggregation in the vector population indicates that it falls significantly with parasite stage as evidenced from variance to mean ratio. In particular, L3 distributions are markedly less aggregated than earlier larval distributions as reported elsewhere [5]. This implies the operation of density dependence, perhaps via parasite-induced vector mortality. Such reduction in parasite aggregation may be influenced either by sampling or by sampling outliers of heavily infected mosquitoes and therefore the results need to be viewed cautiously. However, in

the present study, the smallest sample size dealt with was 563 and hence sampling variation is not likely to be the major problem. Therefore the reduction in parasite aggregation is due to presence of heavily infected mosquitoes (outliers) in the sample. Therefore the decline in aggregation with the increasing age of parasite could be attributed to mortality of heavily infected mosquito.

Samarawickrema and Laurence [5] also observed a similarity between the distributions of Mf in human and vector, apart from the 1 Mf category for the vectors, which was under-represented, probably due to errors in counting. The finding that the non-truncated fit of the human Mf distribution [7,8] does not accord with observed Mf distribution in vector indicates that a proportion of mosquitoes are uninfected due to bites on true Mf negatives in the human population. Our analysis also shows that the relatively similar Mf frequency distributions in humans and vectors are better described by zero truncated mixed Poisson distributions (such as the negative binomial), probably because both mosquitoes and parasitologists take blood from a proportion of negative hosts [7].

The dynamics of infection in the vector host is complex because both the acquisition and loss of infection are continuous processes as the vector can loose or gain infection during subsequent blood feeding. The relationship is further complicated by the different rates of survival of both parasite and mosquito. The successful development of the ingested Mf to become an infective larvae (parasite yield), is an essential component of the transmission success of this parasite. Quantification of the effects of parasitism in the vector host needs modelling of these complexities.

References

1. Elliot JM. Some methods for the statistical analysis of samples of benthetic invertebrates. Ambleside: Freshwater Biology Association, 1977.
2. Dietz K. Density-Dependence in Parasite Transmission Dynamics. *Parasitology today* 1988;4:91-97.
3. Dye C. Does Facilitation Imply a Threshold for the Eradication of Lymphatic Filariasis? *Parasitology today* 1992;8:109-110.
4. Southgate BA, Bryan JH. Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 4. Facilitation, limitation, proportionality and their epidemiological significance. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1992;86:523-30.
5. Samarawickrema WA, Laurence BR. Loss of filarial larvae in a natural mosquito population. *Annals of Tropical Medicine and Parasitology* 1978;72:561-5.
6. Park CB. Microfilaria density distribution in the human population and its infectivity index for the mosquito population. *Parasitology* 1988;96:265-71.
7. Das PK, Manoharan A, Srividya A, Grenfell BT, Bundy DA, Vanamail P. Frequency distribution of *Wuchereria bancrofti* microfilariae in human populations and its relationships with age and sex. *Parasitology* 1990;3:429-34.
8. Grenfell BT, Das PK, Rajagopalan PK, Bundy DA. Frequency distribution of lymphatic filariasis microfilariae in human populations: population processes and statistical estimation. *Parasitology* 1990;3:417-27.
9. Medley GF, Sinden RE, Fleck S, Billingsley PF, Tirawanchai N, Rodriquez MH. Hetrogeneity in patterns of malarial occyst infections in the mosquito vector. *Parasitology* 1993;106:441-449.
10. Rajagopalan PK, Das PK. The Pondicherry Project on Integrated Disease Vector Control. (Filariasis Control Demonstration Project). Pondicherry: Vector Control Research Centre, 1987.
11. Rajagopalan PK, Das PK, Subramanian S, Vanamail P, Ramaiah KD. Bancroftian filariasis in Pondicherry, south India: 1. Pre-control epidemiological observations. *Epidemiology and Infection* 1989;103:685-92.
12. Sasa M. Human filariasis. A global survey of epidemiology and control. London and Tokyo, 1976.
13. Tallis GM, Leyton MK. Stochastic models of populations of helminthic parasites in the definitive host. *Journal of Theoretical Biology* 1966;13:251-260.
14. Baker D, Nelder JA. Numerical Algorithm Group: GLIM version 3.77: User Manual. Oxford, 1978.
15. Sokal RR, Rohlf FJ. Biometry. San Francisco: W.H. Freeman & Company, 1981.
16. Subramanian S, Manoharan A, Ramaiah KD, Das PK. Rates of acquisition and loss of *Wuchereria bancrofti* infection in *Culex quinquefasciatus*. *American Journal of Tropical Medicine and Hygiene* 1994;51:244-9.
17. Rajagopalan PK, Kazmi SJ, Mani TR. Some aspects of transmission of *Wuchereria bancrofti* and ecology of the vector *Culex pipiens fatigans* in Pondicherry. *Indian Journal of Medical Research* 1977;66:200-15.

3.3 Rates of acquisition and loss of *Wuchereria bancrofti* infection in *Culex quinquefasciatus*

Summary

Rates of acquisition and loss of parasitic infection in the vector host of *Wuchereria bancrofti* were estimated using a new method. The age of parasitic infections was estimated from the abdominal condition, determined on the basis of Sella's stages of blood digestion and ovary development, and parity of the vector host and larval stages of the parasite. A negative exponential relationship between age and number of *W. bancrofti* infections provided estimated daily losses of 25.3% and 33.2% of the initial infection from the resting and biting populations, respectively, of the mosquito *Culex quinquefasciatus*. The rates per day of acquisition of infection estimated from resting and biting female *C. quinquefasciatus* were found to be 9.6% and 8.6%, respectively. The mean minimum developmental time for *W. bancrofti* infective larvae estimated from the natural resting and biting populations were 8.9 ± 1.41 (± SEM) and 8.33 ± 0.85 days, respectively, which was somewhat less than the duration observed under laboratory conditions. Comparisons of the estimates (mean age, rates of acquisition and loss of infections) based on the resting and biting data suggest that the rates are independent of the method of collecting mosquitoes.

Introduction

Estimation of the age of infection in vectors is important in determining the developmental period of the parasite larvae and the dynamics of transmission. Krafsur and Garrett-Jones had observed that the developing *Wuchereria bancrofti* infections in unfed blood-seeking *Anopheles funestus* can be classified into five age groups, namely, three, six, nine, 12, and 15 days, since this mosquito species is assumed to take a blood meal at three-day intervals [1]. Based on this assumption, they determined the age of filarial infections and subsequently estimated the probability of the vector host becoming infected with the filarial parasite. However, the interval between successive blood feedings to repletion by the mosquito may be prolonged due to either partial blood meals or the time lost between oviposition and blood feeding or delay in oviposition. In such instances, age of the mosquito or age of infection estimated based on the fixed duration of the interval between two successive blood meals may not reflect the true age. Although partial blood feeding is not common in *Culex quinquefasciatus* [2], a delay in oviposition due to filarial infection and a consequent lack of urgency in feeding is well known [3]. Determination of the physiologic age based upon the parasite stage may provide a more accurate estimation of mosquito age or age of infection provided that the development of the parasite is not prolonged by its high density in the vector host

(density-dependent effect). However, since the developmental time is dependent upon the number ofingested microfilariae [4], it may not be appropriate to use the parasite stage alone as a criterion for age determination. Therefore, we propose a method of assigning age to infection by considering parity, abdominal conditions (determined based on Sella's stages of blood digestion and ovary development [5]), and parasite stages in the vector. This assigned age of parasitic infection was then used to estimate the rates of acquisition and loss of infection in the vector mosquitoes.

Materials and Methods

Study area

The study area in Pondicherry is located at 11°45'-12°15'N and 79°35'-80°E in southern India on the coast of the Bay of Bengal. It has an area of 59.38 km² and a population of 272, 250 (1981 census). During the study period in 1981, the mean monthly temperature ranged from 31.5°C in June to 25.5°C in February. Total annual rainfall was 1,099 mm and the relative humidity ranged from 69 to 86%. Pondicherry is endemic for bancroftian filariasis and the prevalence of microfilaremia was 8.48% in 1981. The density of *C. quinquefasciatus*, the principal vector of bancroftian filariasis, was also found to be high in the study area.

Field collection

Resting and biting adult *C. quinquefasciatus* females collected in 1981 by the Filariasis Control Demonstration Project were used for this analysis. Resting mosquitoes were collected every two weeks and weekly biting collections were made from 17 and five fixed sites, respectively in the study area of the Pondicherry urban agglomeration. A detailed description of the collection, dissection, and parasite identification has been previously reported [6].

Method of assigning age to infection

Because the analysis depends upon the accurate assignment of age to a particular infection, which is the number of days the parasite has been in the vector, it is essential to provide detailed information about the methods adopted for this analysis. Krafsur and Garret-Jones have achieved this by sampling only the unfed mosquitoes when they seek to bite human indoors [1]. They determined the age of infections by age grading the developmental stages of filariae into five distinct age groups. This method is not suitable for most of the data because the sampled resting population consists of unfed (UF), full-fed (FF), semigravid (SG) and gravid (G) mosquitoes (of various age classes with different cohorts of infection); thus, the mosquito age may not coincide with the parasite age. Furthermore, classification of the parasite into five distinct age groups is not feasible when the sample size is large; thus, the mortality rate based on three age groups of the parasite, as stated by Krafsur and Garret-Jones [1], may provide a less reliable estimate of

the age of infection. We propose the following technique for determining the age of a parasite infection.

Field-collected mosquitoes were examined externally and sorted according to the gonotrophic classification of Sella's stage [5] (1=UF; 2 and 3=FF; 4, 5, and 6=SG; and 7=G). Female mosquitoes were dissected to determine the physiologic age based on the follicular relics, the number of follicular dilatations, and the infection status of the mosquitoes. It is well known that the number of blood meals agree with the number of ovipositions in *C. quinquefasciatus* [7]. The average durations of the first and second gonotrophic cycles were five and three days, respectively [8]. Thus, the age of a mosquito was determined by adding the duration for each abdominal condition (based on Sella's stage as 1 = 0-2 days old; 2 and 3 = 0.5-2.5 days old; 4-6 = 2.5-4.5 days old; and 7 = 3-5 days old for parous/nulliparous mosquitoes) with physiologic age determination based on the number of dilatations.

Algebraically, the exact age m of a mosquito can be represented as follows. Let (k+i) and k be the duration of the gonotrophic cycle in days, for the first and subsequent blood feedings, respectively, and let d denote the number of days needed to reach a particular Sella's stage (based on the appearance of the abdominal condition). The age m of the mosquito is then calculated as $m = (k+i)+kf+d$, if $f = 0$ then $k = 0$, or $m = k(f+1)+i+d$, where f and i are the number of dilatations and the period from emergence to first blood feeding, respectively.

Similarly, the age of the parasite present in the infected mosquito (age of the parasitic infection) can be approximated by considering the stage duration of the parasite under laboratory conditions. Under laboratory conditions, the minimum stage durations of *W. bancrofti* larvae were estimated to be one, four, and four days from microfilaria (Mf) to the first larval stage (L1), from L1 to L2, and from L2 to L3, respectively [3]. Because parasite development in the mosquito is continuous and density dependent, the age of an infection must be determined on the basis of parasite stage, abdominal conditions, and the number of dilatations observed in a mosquito, and can be represented as follows. Let p be the apparent age of the parasite based on the stage of the parasite present in the vector. The age of infection, I, for a mosquito is then calculated as follows. If $p < d$, $I = d-2$, for $f = 0$ and $I = d$ otherwise. For $f \geq 1$, $I = p$ if $p \leq (f-1)k + d$, $I = fk + d$, if $(f-1)k + d < p \leq fk + d$, and $I = m$ otherwise.

When multiple infections were observed, the number of cohorts of infections were determined based on the stages of the parasite present in a mosquito. For example, Mf and L1, Mf and L2, Mf and L3, L1 and L2, and L2 and L3 were considered double infections, and the combination of three or four stages were considered triple or quadruple infections.

The mean parasite age of each larval stage was estimated for both resting and biting mosquitoes. A plot of the number of mosquitoes infected with various larval stages of the parasite (number of infections, Y) and the estimated mean age of the respective larval stages of the parasite (X) suggests a negative exponential relationship of the form

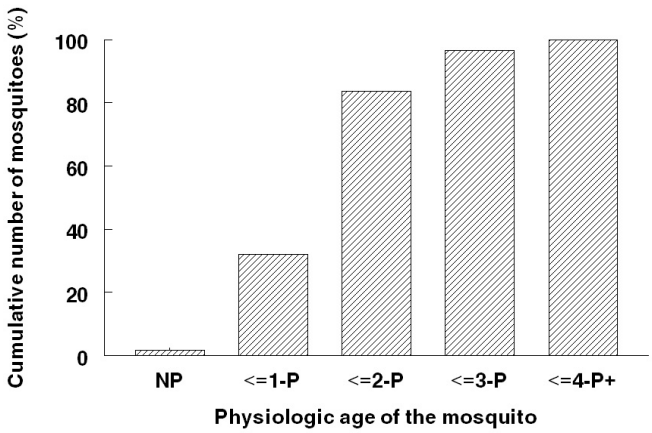

Figure 1 Cumulative number of *Culex quinquefasciatus* mosquitoes showing older infections (second- and third-stage larvae) according to physiologic age. NP = nulliparous; P = parous.

given by $Y = ae^{-bX}$, for which the appropriate linear transformation is $\ln Y = \ln a - bX$. The slope of this regression, b, is the average rate of loss of infection per day and the intercept, a, is the expected number of infections on day zero.

Results

A total of 16,865 resting female *C. quinquefasciatus* mosquitoes were dissected to determine the physiologic age and infection status of the mosquitoes. There were 10,130 parous mosquitoes (60.1%) comprised of 6,615 (39.2%) individuals with one ovariole dilatation, 3,006 (17.8%) with two, 449 (2.7%) with three, and 60 (0.4%) with four or more dilatations. Among the resting population, 2,205 (13.1%) were found to be infected for any stage of the parasite. Single, double, triple, and quadruple infections were observed in 1,924, 252, 25, and four mosquitoes, respectively. Most (87.3%) of the infected population harbored a single infection. Approximately 32% of the advanced stages of infections (L2 or L3 stage) were observed in nulliparous or one parous mosquitoes, and 84% were observed in two parous or less than two-parous mosquitoes. In other words, most of the Mf and L1 reached the L2 and L3 stages before the mosquitoes completed their third gonotrophic cycle. This shows a lack of correlation between parasite stage developmental time and gonotrophic cycle of the mosquito (Fig. 1).

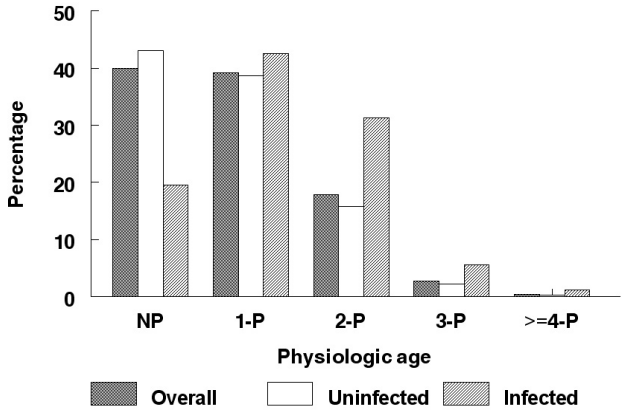

Figure 2 Age distribution of infected, uninfected, and overall resting adult female *Culex quinquefasciatus*. NP=nulliparous; P=parous.

Mortality rates of infected and uninfected vectors

The distribution of overall (infected plus uninfected), infected, and uninfected populations according to parity conditions are shown in Figure 2. Although the survival rates of the uninfected and overall populations decreased in an exponential pattern, the frequency of the infected population increased steadily up to the age when the mosquitoes become parous for the first time and decreased thereafter. While the physiologic age structure of the uninfected population reflected the overall distribution, the infected population varied significantly from both the uninfected and overall patterns (Fig. 2). The parous rate among the infected population (80.5%) is significantly higher than that of the uninfected population (57.0%) ($\chi^2 = 442.53$, degrees of freedom [D.F.] = 1, $P < 0.0001$). This is due to the fact that the probability of acquiring infection increases with blood meal frequency and so also parity. In other words, the proportion of nulliparous mosquitoes was lower in the infected population than in the uninfected one. Because the probability of acquiring infection increases with blood meal frequency, it is likely to collect number of parous infected than nulliparous infected. The parous rate calculated based on the infected population is an overestimation that has been reflected in the above comparison. Therefore, a comparison of the mortality rates of the infected and uninfected populations indicates that it would be misleading to conclude that the infected population is not being affected by the parasitic invasion. However, mortality

Table 1 Frequency distributions of the mean and SEM age (days) for each stage of the parasite (*Wuchereria bancrofti*) and the probability of significance indicating a difference in the estimates calculated from resting and biting *Culex quinquefasciatus* populations

Parasite stage	Resting			Biting			P†
	n	Mean	SEM	n	Mean	SEM	
Mf	928	1.40	0.03	15	0.40	0.16	0.00
L1	1024	2.62	0.03	91	2.23	0.11	0.00
L2	410	5.63	0.03	31	5.52	0.09	0.32
L3	157	8.95	0.11	12	8.33	0.25	0.14

Mf = microfilaria; L = larval.

† Based on Student's *t*-test for independent samples.

rates can be estimated from the proportion of infected mosquitoes surviving to support filarial development to a particular stage [9]. Assuming that mosquitoes harboring stages L1, L2, and L3 have survived at least one day, those harboring stages L2 and L3 have survived at least five days, and those harboring L3 alone have survived at least nine days, the estimated rates of daily mortality are 36.8%, 18.7%, and 13.3%, respectively, based on Mf to L1, L1 to L2, and L2 to L3.

Rates of acquisition and loss of infections

Frequency distributions of the age of infections for Mf, L1, L2, and L3 stages of the parasite were determined. The mean minimum and standard error of age were estimated for each stage of the parasite (Table 1). The frequency of infections was correlated with the estimated mean minimum age corresponding to each stage of the parasite. The regression of the number of *W. bancrofti* infections on age has resulted in the following equation: $\ln Y = 7.3861 - 0.2532 X$.

The strong negative correlation between age and number of infections ($r = -0.9800$) suggests that infections decrease with age (Figure 3). If a constant proportion of mosquitoes is acquiring parasites, one would expect an accumulation in number of infections with age. In reality, the number of infections decreases with age. This decrease could be due to mosquito mortality, to density-dependent loss in the parasite itself, or to both. The regression coefficient ($b = -0.2532 \pm 0.0363$) was found to be significantly different from zero ($t = 6.97$, D.F. = 2, $P = 0.0199$).

Assuming that the expected zero-day old infections ($\ln a = 7.39$, $a = 1{,}613$) are a fraction of the resting population, the probability of a mosquito becoming infected in a

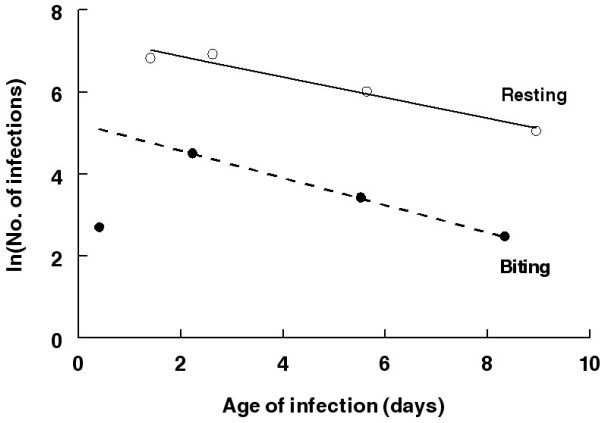

Figure 3 Regression of log number of infections on age for both resting and biting *Culex quinquefasciatus*. Circles are observed and lines are predicted.

single feeding can be estimated as 0.0956 (1,613/16,865). This figure, however, may be an underestimate because 52.4% of the mosquitoes (as is evident from the stages of blood digestion and ovary development) took a blood meal more than one day ago. Therefore, by considering only fully fed, resting mosquitoes (8,020 are presumed to have taken their blood meal less than a day before), the probability of acquiring infection is 0.2011 (1613/8020).

The regression coefficient, an estimate of the rate of loss of infection from the infected vector population, indicated that an average of approximately 25% of the initial infections were lost. This is due either to mortality of larvae during the course of development from Mf to L3 and/or mortality of the infected mosquitoes. A similar analysis was carried out, based on the data of biting female *C. quinquefasciatus*, to test the validity of the results obtained with the resting population. Comparison of the mean ages of infections between resting and biting populations revealed significant differences only for Mf and L1 ($P < 0.05$) (Table 1). Regression of the number of infections on age based on a sample of 2,217 biting female mosquitoes provided a significant negative exponential relationship (Figure 3) (r = -0.99997, t = 129.14, D.F. = 1, $P = 0.0049$) and is given by the equation ln Y = 5.2532 - 0.3316 X.

The estimated rates of acquisition and loss of infection based on the intercept and slope are 8.62% and 33.2%, respectively. The slopes of the two regressions based on the resting and biting populations were not different, suggesting that the estimated loss of

infection based on the proposed method is independent of the two populations (resting and biting) (t = 1.57, D.F. = 3, *P* = 0.2144).

Discussion

Although the present method of assigning age to infection to mosquitoes is manually cumbersome, it could be computerized to handle large samples. Furthermore, we are confident in assigning age accurately to infection by considering the parity and abdominal conditions of the mosquito and parasite age. Lastly, the method is applicable to mosquitoes that are sampled either while resting or biting, or by trapping. On the other hand, the method of Krafsur and Garret-Jones [1] is applicable only to mosquitoes that are sampled while biting and whose mean duration of each gonotrophic cycle is expected to be constant. However, many studies have reported that oviposition of infected *C. quinquefasciatus* females was considerably prolonged when compared with normal colony mosquitoes and concluded that delayed egg laying and subsequent prolongation of the gonotrophic cycle could be the reason for the presence of advanced filarial stages in physiologically younger mosquitoes [3,10,11].

Although partial blood meals may be sufficient for acquiring pathogens, they are insufficient for ovarian development [12]. In this study, it has been observed that approximately 1.8% of the matured infections occurred in nulliparous mosquitoes, 30.2% in one-parous mosquitoes, and 51.9% in two-parous mosquitoes. This is contrary to the assumption that age of infection coincides with the duration of the gonotrophic cycle; thus, the age of infection alone cannot be used for estimating the mortality of an infected population, as had been done by Krafsur and Garret-Jones [1]. However, differences in parasite strains and vector species may be responsible for the lack of synchronization in infection and the gonotrophic cycle of the mosquitoes observed in this study.

Based on the infection status of the resting population, the estimated probability of *C. quinquefasciatus* becoming infected with *W. bancrofti* ranged between 9.6% and 20.1%, which is higher than that reported for *An. funestus* (5.8--8.7%) with *W. bancrofti* infection [1]. The proportion of the human population with patent microfilaremia in 1981 in Pondicherry was 8.48%, which is lower than the estimated prevalence of microfilaremia (9.6--20.1%) in the vector population. In this study, however, the infection acquisition of the resting population did not vary greatly from that of the biting population (8.62%), thereby validating our proposed method of assigning age to infection.

The slope of the regression of log number of infections on age based on the two populations (resting and biting) provided an estimate of the rate of loss of infection. The slopes did not differ significantly, suggesting that the proposed method is independent of the method of sampling mosquitoes. The rate of loss of *W. bancrofti* infection by *C. quinquefasciatus* observed in this study is higher than that reported for *An. funestus* (16.46% by Krafsur and Garrett-Jones [1] and 15.6% by Gillies and Wilkes [13]). The rate of loss of infection estimated from the frequency distribution of L2- and L3-infected *C. quinquefasciatus* mosquitoes was 27.6%. This loss was attributed to the

density-dependent mortality of the parasites during the development of larvae from L1 to L2 and L3 and the mortality of the infected vector population (Vector Control Research Centre, Unpublished data). Samarawickrema and Laurence [14] observed a decrease in parasite density during development and attributed this reduction to the increased mosquito mortality that is dependent on the density of parasite infection. A comparison of these results suggests that the rate of loss in our study might be an underestimate because it includes loss of all stages of filarial infections.

Estimates of natural survival are often based on the parity and infection status of vectors [15,16]. A comparison of the rates of mortality based on the infected and uninfected populations could not be made due to the difference in the age structure of these populations. This difference, which influenced the parous rate of these two populations, is due mainly to the fact that the parous mosquitoes are more abundant in the infected population. However, estimates of daily mortality based on filarial infection decreased with increasing age of the parasite, which contradicts the earlier results of increasing mortality with age [15]. Nathan [17] estimated daily survival rates based on the method suggested by Laurence [9] and using the data of resting and biting populations, observed differences in mortality rates between the results of these two populations. Comparison of these findings suggest that survival rates based on the age of filarial infection method are biased towards the age of the parasite if the parasite stage duration and the physiologic age of the mosquito did not coincide with each other.

Although mosquitoes of all ages in a population are equally exposed to the risk of acquiring microfilaria infection, the probability is relatively higher in parous mosquitoes (since they have completed at least one blood feeding) than in nulliparous ones. This study shows that most of the infected vectors harbor a single cohort of infection. The decrease in the number of infections with age is likely due to increased vector mortality as a result of parasitism [18,19]. It may be partly due to the escape of infective larvae during the subsequent blood feeding by the mosquito. Thus, the involvement of parasite-related vector mortality cannot be ruled out, as has been reported elsewhere in other vector species [1]. However, since our study did not take into account parasite density or distribution, it may be worthwhile to analyse the parasite stage frequency distributions for delimiting the stage and threshold level of parasite that would be responsible for parasite-induced host mortality.

References

1. Krafsur ES, Garrett Jones C. The survival in nature of *Wuchereia*-infected *Anophels funestus* Giles in North-eastern Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1977;71:155-60.
2. Ramaiah KD, Das PK. Non-involvement of nulliparous females in the transmission of bancroftian filariasis. *Acta Tropica* 1992;52:149-53.
3. Rajagopalan PK, Kazmi SJ, Mani TR. Some aspects of transmission of *Wuchereria bancrofti* and ecology of the vector *Culex pipiens fatigans* in Pondicherry. *Indian Journal of Medical Research* 1977;66:200-15.
4. Lindsay SW. The migration of infective larvae of *Brugia pahangi* within the mosquito *Aedes aegypti*. *Parasitology* 1986;92:369-78.
5. Detinova TS. Age-grouping methods in Diptera of medical importance with special reference to some vectors of malaria. *WHO monograph series* 1962;47:1-216.
6. Ramaiah KD, Das PK. Seasonality of adult *Culex quinquefasciatus* and transmission of bancroftian filariasis in Pondicherry, south India. *Acta Tropica* 1992;50:275-83.
7. Rajagopalan PK. Population dynamics of *Culex pipiens fatigans*, the filariasis vector, in Pondicherry - Influence of climate and environment. *Procedings of the Indian National Science Academy* 1980;B46:745-751.
8. Subra R. Biology and control of *Culex quinquefasciatus* Say 1823 (Diptera, Culicidae) with special reference to Africa. 1980;WHO/VBC/80.781.
9. Laurence BR. Natural mortality in two filarial vectors. *Bulletin of the World Health Organization* 1963;28:229-234.
10. Samarawickrema WA. A study of the age composition of natural populations of *Culex pipiens fatigans* Widemann in Relation to the Transmission of Filariasis due to *Wuchereria bancrofti* (Cobbold) in Ceylon. *Bulletin of the World Health Organization* 1967;37:117-137.
11. Joe LK, Hudojo, Wijono, Amaliah S. *Wuchereria bancrofti* infection in Djakarta, Indonesia. A study of some factors influencing its transmission. *Indian Journal of Malariology* 1960;14:339-352.
12. Harwood RF, James MT. Entomology in Human and Animal Health. 7 ed. New York: Macmillan Publishing Co., Inc., 1979.
13. Gillies M, Wilkes T. A study of the age composition of populations of *Anophels funestus* Giles in north-eastern Tanzania. *Bulletin of Entomological Research* 1965;56:237-262.
14. Samarawickrema WA, Laurence BR. Loss of filarial larvae in a natural mosquito population. *Annals of Tropical Medicine and Parasitology* 1978;72:561-5.
15. Davidson G. Estimation of the survival rate of Anopheline mosquitoes in nature. *Nature* 1954;174:792-793.
16. De Meillon B, Hayashi S, Sebastian A. Infection and reinfection of *Culex pipiens fatigans* with *Wuchereria bancrofti* and the loss of mature larvae in blood-feeding. *Bulletin of the World Health Organization* 1967;36:81-90.
17. Nathan MB. Bancroftian filariasis in coastal North Trinidad, West Indies: intensity of transmission by *Culex quinquefasciatus*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1981;75:721-30.
18. Wharton RH. Studies on filariasis in Malaya: the efficiency of *Mansonia longipalpis* as an experimental vector of *Wuchereria malayi*. *Annals of Tropical Medicine and Parasitology* 1957;51:422-39.
19. Lavoipierre MMJ. Studies on the host parasite relationships of filarial nematodes and their arthropod host. II The arthropod as a host to the nematode: a brief appraisal of our present knowledge, based on a study of the more important literature from 1878 to 1957. *Annals of Tropical Medicine and Parasitology* 1958;52:326-45.

3.4 Vector survival and parasite infection: the effect of *Wuchereria bancrofti* on its vector *Culex quinquefasciatus*

Summary

This paper investigates a cohort of 2187 laboratory reared *Culex quinquefasciatus* fed on 69 human volunteers, including 59 persons with different levels of *Wuchereria bancrofti* microfilariae and 10 without microfilaria. Mosquitoes were followed until death. Mosquito survival was analysed in relation to the level of microfilaria in the human and larval count in the dead mosquito. Vector mortality during the extrinsic incubation period (12 days post-engorgement) was significantly higher in mosquitoes fed on microfilaraemic volunteers (50%) than in those fed on amicrofilaraemics (29%). Both the percentage infected and the geometric mean parasite density was significantly higher among mosquitoes which died before 13 days (45% infected and 10 larvae per infected mosquito) than those surviving beyond 13 days (39% and 2.2), suggesting a parasite loss of more than 80% during the extrinsic incubation period. A large proportion (62%) of the mosquitoes that died during the early phase of parasite development were infected (36% in low, 26% in medium and 90% in high human Mf-density). Survival analysis showed that the parasite load in mosquitoes and the human Mf-density for a given parasite load are independent risk factors of vector survival. Overall, the hazard of dying was found to be 11-15 times higher among mosquitoes fed on microfilaraemic volunteers than those fed on amicrofilaraemics. The hazard doubles for every increase of about 60-70 parasites in the vector. As a consequence of the parasite-induced reduction in vector survival, the transmission success of the parasite is reduced. The implication of the results on control/elimination of lymphatic filariasis using mass-drug administration is discussed.

Introduction

The survival of the vector *Culex quinquefasciatus* is an important determinant of the transmission dynamics of the human filarial parasite *Wuchereria bancrofti*. For successful transmission of the parasite, the vector must survive longer than the 12 days it takes the parasite to develop to the stage of infective larva. Not all infected mosquitoes survive that long. Apart from meteorological factors and age, parasite load and parasite stage distribution influence the survivorship of an infected mosquito. In a previous experimental transmission study we observed that the relationship between *W. bancrofti* microfilarial (Mf) load in the human host and uptake of Mf by *C. quinquefasciatus* and parasite development was non-linear and saturating, which is suggestive of density-dependent regulation of parasite in the vector [1]. Further, the yield of L3 larvae (ratio of L3 to Mf ingested) decreased with increasing Mf-uptake by *C. quinquefasciatus* ('limitation'). The main mechanisms proposed to drive limitation are increased mortality of the larvae (loss of parasites) and parasite-induced vector mortality particularly at high

parasite densities [2-6]. Both experimental studies [1,7-10] and field studies [6,11] found excess-mortality among heavily infected mosquitoes. In field studies, it is difficult to distinguish the effect of vector-age from the effect of parasite burden on the survival of mosquitoes because the wild population is a mixture of members with different ages. Most of the experimental studies were designed to quantify the relationship between infection in the human and uptake by the vector, and not to assess the risk of parasite load on the survival of infected/uninfected vector population. Therefore, an experimental study was carried out in which laboratory reared *C. quinquefasciatus* mosquitoes were allowed to engorge blood from human volunteers with known *W. bancrofti* Mf-density. The results of the analysis of the data on mosquito survival, parasite density in the volunteers, and parasite counts in dead mosquitoes are reported in this paper. The analysis focuses on the relationship of the survival of the mosquitoes to microfilarial load in human volunteers and larval counts in the dead mosquitoes.

Materials and Methods

Parasitological and entomological procedures

Male and female human volunteers were randomly selected from a list of Mf-carriers from a blood sample survey conducted in and around Pondicherry. Informed consent was obtained from all volunteers. Newly emerged female *C. quinquefasciatus* mosquitoes from the laboratory-reared colony were used. Female mosquitoes were starved for 48 h before engorgement. Prior to the feeding experiment three 20 μl blood smears were taken from each of the Mf-carriers by the finger prick method. One arm up to the elbow of the volunteer was exposed for 30 min between 20.00 h and 21.00 h to the mosquitoes kept in a cage to engorge. Immediately after the feeding, three more blood smears were collected from the volunteers. All volunteers were given a full course of diethylcarbamazine following the experiment.

Fully-fed female mosquitoes were caught, counted and released into another cage. Mosquitoes were maintained on raisin and water till their death at a controlled room temperature of 28-30 °C and relative humidity of 80-85%. Each day, dead mosquitoes were removed and dissected to assess the infection load. The number of parasites in relation to the stage was recorded separately for abdomen, thorax and head. To assess the natural survival, another batch of female mosquitoes was fed on amicrofilaraemic persons (determined on the basis of 3 blood smears) and was also followed up until death.

Statistical methods

Mosquito survival: The mosquitoes were classified into 4 groups according to human Mf-density: 0, 0.3-7.0, 7.0-21.0 & 21.0-440 per 20 μl of peripheral blood (Table 1). The classification of mosquitoes fed on Mf-volunteers was made in such a way that the percentile distributions of the specimens in each Mf-density category were approximately equal: 28.6, 33.8 & 37.6% respectively fed on low, medium & high Mf-density categories.

The geometric mean was used to express the parasite load in infected mosquitoes fed on different Mf-density categories. The 95% asymmetric confidence intervals for the means were used to compare the difference in mean larval loads within or between Mf-density categories. The chi-square heterogeneity test was used to compare the difference in proportion infected between time periods. The generalised Wilcoxon test (Breslow) was used to compare the survival distributions of different Mf-density categories. Since the observed survivorship curves for mosquitoes fed on low (0.3–7.0) and medium Mf-density (7.0-21.0) categories appear to be overlapping with each other (Fig. 1) these two categories were pooled for fitting survival models and hence all the mosquitoes were classified under three Mf-density categories: 0, 0.33-21.0 & >21.0-440 per 20 μl of peripheral blood.

Using the observed survival times, the survivor function $S(t)$, can be estimated. Since there is no censoring, the Kaplan-Meier estimate of $S(t)$ will be the same as that of the empirical survivor function and life-table method [12]. The empirical survival distributions for each of the Mf-density category were used to determine a suitable baseline hazard function. Visual inspection suggested that the hazards in the different Mf-categories are not proportional (when the log-cumulative hazard - i.e. log(-log $S(t)$) - is plotted against log t the lines are far from being parallel) [12]. Therefore a (non-proportional) Weibull hazard model was fitted to the data. For a mosquito that fed on a person in Mf-category j (j∈0,1,2) and that had parasite load x at death, it is assumed that the hazard $h_j(t;x)$ of dying at time t follows a Weibull probability distribution with cumulative hazard:

$$H_j(t;x) = \lambda_j(x)\, t^{\gamma_j} \qquad (1)$$

with shape parameter γ_j (>0) and a scale parameter $\lambda_j(x)$ (>0) which depends on the parasite load x in a mosquito. The log-cumulative hazards will be parallel if the shape parameters γ_j are equal between human Mf-categories. The scale parameter is assumed to depend on the human Mf-category and to increase exponentially with increasing parasite load x:

$$\lambda_j(x) = e^{\alpha_j + \beta x} \qquad (2)$$

with α_j, logarithm of the ratio of the hazard (relative risk, RR) for a mosquito feeding on a human in Mf-category j, to that of a mosquito feeding on a human in Mf-category 0; β, change in the logarithm of the hazard ratio for a unit increase in the number of parasites it harbours.

For the special case of γ_j=1, the hazard takes a constant value λ_j and the mosquito survival has an exponential distribution. For other values of γ_j, the hazard of dying increases or decreases monotonically with time.

Table 1 Summary of dissection results of mosquitoes fed in humans with different Mf-density

Particulars of human volunteers/dead mosquitoes	Human Mf-density per 20 μl blood				
	0 Mf	0.3-7.0	7.0-21.0	21.0-440	Total > 0
	(Zero)	(Low)	(Medium)	(High)	(Low+Medium+High)
Number of human volunteers	10	15	17	27	59
Number of mosquitoes that engorged blood	440	500	591	656	1747
% Engorged on Mf-volunteers	NA	29%	34%	37%	100%
Number (and %) of mosquitoes died in					
0-2 days (Mf)	13 (3%)	39 (8%)	57 (10%)	109 (17%)	205 (12%)
3-12 days (L1, L2 & L3)	114 (26%)	185 (37%)	179 (30%)	307 (47%)	671 (38%)
≤ 12 days	127 (29%)	224 (45%)	236 (40%)	416 (64%)	876 (50%)
≥ 13 days (L3)	313 (71%)*	276 (55%)*†	355 (60%)*†	240 (37%)*	871 (50%)*
Number (and %) infected among dead in					
0-2 days (Mf)	NA	14 (36%)[a]	15 (26%)[a]	98 (90%)[a]	127 (62%)[a]
2-12 days (L1, L2 & L3)	NA	29 (16%)[b]	41 (23%)[a]	201 (65%)[b]	271 (40%)[b]
≤ 12 days	NA	43 (19%)[b]	56 (24%)[a]	299 (72%)[b]	398 (45%)[a]
≥ 13 days (L3)	NA	43 (16%)[b]	122 (34%)[b]	173 (72%)[b]	338 (39%)[b]
All together	NA	86 (17%)*	178 (30%)*	472 (72%)*	736 (42%)
Geometric mean number of parasites /positive mosquito dying in§					
0-2 days (Mf)	NA	2.6 (1.7-3.2)	1.7 (1.3-2.0)	43.7 (33.0-50.4)	21.8 (15.9-25.6)
2-12 days (L1, L2 & L3)	NA	1.7 (1.4-1.9)	1.9 (1.5-2.1)	10.1 (8.7-10.9)	6.5 (5.6-6.9)
≤ 12 days	NA	1.9 (1.6-2.1)	1.9 (1.5-2.0)	16.3 (13.9-17.7)	9.6 (8.2-10.3)
≥ 13 days (L3)	NA	1.2 (1.1-1.3)	1.6 (1.4-1.7)	3.1 (2.7-3.3)	2.2 (1.9-2.3)
All together	NA	1.6 (1.4-1.7)	1.7 (1.5-1.8)	8.9 (7.8-9.5)	4.8 (4.3-5.1)

The chi-square heterogeneity test was used to compare percentage infected/dead between time periods or between Mf-density categories. Percentages with same superscripts (a, b) within a column indicate that they do not differ significantly at $P < 0.05$

* The difference in percentages between 0 Mf, low, medium and high Mf-density category is significant at $P < 0.05$.

† Difference in percentages is not significant at $P < 0.05$.

§ Values in parentheses are 95% asymmetric confidence intervals for the geometric means.

NA Not applicable

The parameters α_j, β and γ_j are estimated from the observed parasite loads $\mathbf{x}_{ij}$ and times of death $\mathbf{t}_{ij}$ by maximizing the following likelihood function for the 3 parasite load categories ($j \in 0, 1, 2$) and the n_j mosquitoes (i=1,..n_j) in each of these load categories:

$$\prod_{j=0}^{2} \prod_{i=1}^{n_j} f_{ij}(t_{ij}, x_{ij}) \qquad (3)$$

with $f_{ij}(t)$ being the Weibull probability density function of the survival distribution $S_{ij}(t)$ which is given by:

$$f_{ij}(t) = e^{\alpha_j + \beta x} \gamma_j t^{\gamma_j - 1} e^{-\left(e^{\alpha_j + \beta x} t^{\gamma_j}\right)} \qquad (4)$$

Maximizing the likelihood function was achieved using the GLIM software package and a modification of its associated library macro *Weibull* [13].

Results

Human volunteers and mosquitoes

A total of 69 human volunteers participated in this experiment, including 10 amicrofilaraemic persons and 59 microfilaraemic carriers. Out of the 59 microfilaraemic individuals, 15 were categorized as having low Mf-density (ranging from 0.3-7.0 per 20 μl of peripheral blood), 17 as having medium Mf-density (7.0-21.0) and the other 27 individuals having high Mf-density (higher than 21.0; the highest average Mf count in 6 smears of 20 μl was 440). A total of 2187 mosquitoes were full-fed on these volunteers: 440 on amicrofilaraemics and the remaining were fed on low, medium and high microfilaraemic individuals.

Infection in mosquitoes

Table 1 shows the summary of dissection results of the mosquitoes that died on different days post engorgement (p.e.). The proportion infected among mosquitoes fed on humans in the high Mf-density category was significantly higher (72%) than among those fed on humans in the low (17%) or medium (30%) Mf-density categories ($P < 0.005$).

In the high Mf-density category, the percentage of mosquitoes with parasitic infection was the same among those that died within 12 days p.e. compared to those that died after 12 days p.e. (72%, $P > 0.05$). A similar comparison among the mosquitoes fed on volunteers with low Mf-density also did not show a significant difference between those which died within 12 days or in the latter period (19% vs. 16%) although the difference (24% vs. 34%) was significant for those fed on persons with medium Mf-density. The percentage infected was found to be significantly higher among mosquitoes that died within 12 days compared to the 12-day survivors (45% vs. 39%, $P < 0.005$).

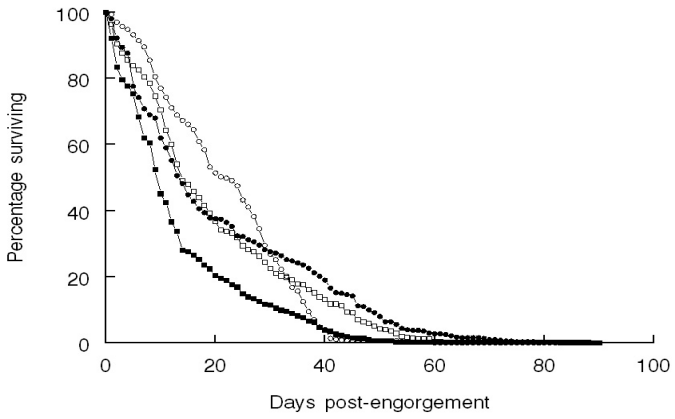

Figure 1 Observed survival function of *Culex quinquefasciatus* mosquitoes fed on human volunteers in four different Mf density categories: No Mf (open circles), 0.3-7.0 Mf (closed circles), 7.0-21.0 Mf (open squares), and 21.0-440 Mf (closed squares) per 20 μl blood.

Parasite load in mosquitoes

Table 1 also shows the geometric mean parasite load in positive mosquitoes (parasite density) and its 95% asymmetric confidence interval. The overall parasite density was significantly higher among those fed on humans with high Mf-density category (8.9) compared to low (1.6) or medium (1.7) (95% CI do not-overlap). The parasite density was much higher in mosquitoes that died on days 0-2 than in mosquitoes that survived into the later periods, especially in mosquitoes that fed on humans with high Mf-load, (non-overlapping CI). Mosquitoes, which died within 12 days, had an average 10 larvae per infected mosquito, compared to 2.2 for mosquitoes dying after 12 days. This decline in parasite load with duration of post-engorgement can be caused by selective mortality of highly infected mosquitoes but may also be related to loss of parasites during development.

Mosquito survival in relation to human Mf-density

Of the mosquitoes fed on humans with high Mf-density, only 37% survived 12 days compared to 55%, 60% and 71% of those fed in the low, medium and amicrofilaraemic persons respectively (Table 1). The difference in survival between those fed in microfilaraemic (50%) and amicrofilaraemic persons (71%) is highly significant ($P < 0.0001$). Figure 1 gives the observed survival functions for the mosquitoes, by level of Mf-density of the human volunteers. The sharp crossing of the survival curves strongly suggests non-proportional hazards, between the four groups: the survival sharply declines

Table 2 Parameter estimates and their standard errors (S.E.) for the Weibull survival models, if shape parameters γ_j are different between Mf density classes

	Human Mf-density class		
Parameter	0	0.3-21.0	21.0-440
γ_j	1.92	1.22	1.25
α_j (S.E.)	-6.15 (0.05)	-3.78 (0.06)	-3.44 (0.06)
β (S.E.)		0.0102 (0.0005)	

after 30 days for the uninfected mosquitoes. This was confirmed in a formal analysis, (see Materials and Methods section) which did not reject proportionality of the mortality hazards for mosquitoes that fed on humans in the low, medium and high Mf density categories ($P > 0.05$), but with a non-proportional hazard when compared to mosquitoes that engorged blood from Mf negative persons ($P < 0.05$). Further, the survivorship curves overlap for the two intermediate Mf-categories (low & medium), suggesting that their survival distributions are not different. Therefore, in the following analyses, the survival distributions for these Mf-categories were combined and hence there are only three Mf-density categories: 0, 0.3-21.0 and 21-440 Mf per 20 μl of peripheral blood.

Fitting Weibull hazard models

We first considered models in which the shape-parameter γ has the same value for the 3 Mf-classes. When we assume exponentiality (i.e $\gamma_j = 1$ in eqn. (4)), we get a very poor fit to the data of Figure 2 (open circles). The maximum likelihood estimate of a common shape-parameter γ equals 1.32, indicating a moderately increasing hazardous effect of mosquito age. When allowing for different shape-parameters for each Mf-class, the fit to the data improves significantly (Table 2, difference in deviance 90.0 for 2 D.F. $P < 0.0001$). See Figure 2 for comparison of simulated and observed survival. The estimated values of the shape parameter γ are almost equal for the 2 non-zero Mf categories, close to exponential (≈1.2), suggesting that the mortality hazard is nearly constant with mosquito age. The shape parameter for mosquitoes that fed on amicrofilaraemic individuals is significantly higher (γ=1.92), indicating that the mortality hazard strongly increases with mosquito age. In the mosquitoes that fed on microfilaraemic individuals the modest effect of mosquito age occurred combined with a pronounced effect of parasite load on mortality already during the early stage of parasite development.

The cumulative hazard of dying of a mosquito is estimated to increase by a factor of e^{β} (≈1.01) for every unit increase in parasite load. Hence, for mosquitoes that fed on

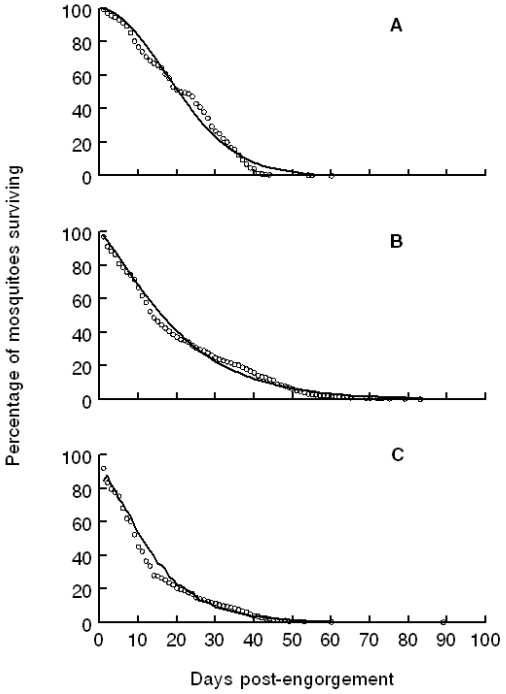

Figure 2 Observed (symbols) and expected (lines) survival of mosquitoes that fed on humans in three different Mf density categories: (A) No Mf, (B) 0.3-21.0 Mf, and (C) 21.0-440 Mf per 20 μl blood. Expected survival is given for the model in which the shape parameter γ differs between these three Mf density classes, and is calculated as the average of the expected survival of the mosquitoes, which in turn depends on the human mf-density category and the parasite load of the mosquito.

low and high Mf-density persons respectively and that harbour 100 parasites, the model predicts that 74% and 87% will die within 12 days p.e., compared to 25% for mosquitoes fed on persons without microfilariae.

Discussion

This paper reports the analysis of survival times under laboratory conditions of a cohort of *C. quinquefasciatus* mosquitoes fed on *W. bancrofti* uninfected and infected human volunteers. We obtained quantitative results on the effect of parasite density on the survival and hence on the transmission potential of the infected vectors.

Infection and parasite load in dead mosquitoes

Further analysis of the results in Table 1 show that a considerable fraction (17%, 127 out of 736) of mosquitoes with any stage of the parasite died in less than 2 days post-engorgement. This fraction was higher (21%, 98 out of 472) among those exposed to humans with high Mf-load. Mosquitoes dying in days 0-2 have a much higher parasite load than those dying in later days. This decrease in parasite load during the early phase of parasite development is in agreement with our field study in which we have found evidence for density-dependent regulation between Mf and L1 stage (Subramanian *et al*.., unpublished observations).

For mosquitoes that fed on Mf-positive persons, both the percentage infected and the geometric mean parasite density were much higher among mosquitoes that died before 13 days than those surviving beyond 13 days (45% *vs*. 39% infected, with on average 10 *vs*. 2.2 larvae per infected mosquito). This suggests a parasite loss of more than 80% during the extrinsic incubation period as a consequence of 13% excess mortality among infected vectors. This result is close to our earlier semi-experimental study in which we found that 64% of mosquitoes that died before 12 days were infected *vs*. 42% of mosquitoes dying later, with 73.1 and 10.0 larvae per positive mosquito (arithmetic means), a reduction of 91% [1] as well as other field transmission studies: 10.3 to 2.6 larvae per positive mosquito, 75% reduction [11]. The relatively higher mosquito mortality in the semi-experimental [1] study can be attributed to the added effect of age, which was controlled in the present lab experiment by using mosquitoes of the same age. These results support the conclusions derived from both experimental and field studies that 'limitation' phenomenon for *W. bancrofti* (parasite) and *C. quinquefasciatus* (vector) complex might be due to increased mortality of heavily infected mosquitoes [1,3,4,6,11,14-16].

Loss of parasites

Rapid formation of crystals and peritrophic membrane has been proposed as a barrier for the migration of Mf from the gut into the haemocele or through the epithelial cells in the vector. Loss of parasites immediately after feeding could be related to the presence and shape of the cibarial and pharyngeal armatures in the vectors. McGreevy *et al.* [17] have reported that *Anopheles farauti* and *Anopheles gambiae*, which have well developed cibarial armatures, killed 36-96% of the ingested Mf. Bryan & Southgate [18] observed 57-60%, 33-51%, 39-56% and 25% loss of *W. bancrofti* Mf ingested by *An. gambiae*, *An. arabiensis*, *An. melas* and *An. funestus* respectively. In *C. quinquefasciatus*, having a poorly developed cibarial armature, the loss of Mf was reported to be only 6% [17]. However, large proportions of the larvae die in the mid-gut or during their development in the thorax. Jordan & Goatly [19] reported considerable loss (55-99%) in ingested Mf during their development to infective larvae and showed many Mf in the dejecta of mosquito 2-3 days post-engorgement. Although the present study was not aimed at examining the mechanisms of parasite elimination, we did not observe melanized or degenerated larvae in mosquitoes that died during our observation.

Mosquito survival and life expectancy

Filarial parasites are known to influence the survival of vectors. Field studies indicate that *W. bancrofti* infection not only reduces the survival [19-22] and the fecundity of *C. quinquefasciatus* but also increases the duration of gonotrophic cycle [15]. Subramanian *et al.* [6] reported that the survival of wild *C. quinquefasciatus* infected with *W. bancrofti* declined by 25-33% during development from Mf to L3. Crans [7] observed a 2-fold increase in mortality rate of *C. quinquefasciatus* fed on persons harbouring *W. bancrofti* compared to mosquitoes fed on normal individuals. Parasite-related reduction in survival has also been reported for *Simulium* vectors infected with *Onchocerca volvulus* [23], *Aedes polynesiensis*, *Anopheles funestus* and *C. quinquefasciatus* infected with *W. bancrofti* [24]. The present study also provides supporting evidence of parasite-induced mortality and quantifies the effect of parasite load on vector survival. The effect of parasitic infection alone was examined using mosquitoes with same age. Mortality due to age during parasite development was corrected from the cohort of mosquitoes fed on amicrofilaraemic individuals, to reflect the excess mortality in vector mosquitoes due to parasite infection alone.

The percentage of mosquitoes dying before 13 days (during the extrinsic period) was significantly higher among those fed on microfilaraemic persons (50%) than on amicrofilaraemic (29%), accounting for an excess mortality of 21%. The excess mortality is likely to be a consequence of the level of infection in mosquitoes, which in turn is directly related to the human Mf-density. The overall mortality risk for mosquitoes fed on Mf-carriers was estimated (e^{α}) to be 11-15 times higher than for those fed on amicrofilaraemic persons. Further, the mortality risk was found to be increased by the level of parasite in a mosquito. For example, among those exposed to microfilaraemic persons, if 100 is the average number of parasite supported per positive mosquito up to 10 days of p.e., the mortality is estimated to be about 70-80% compared to 30-45% for those fed on Mf-volunteers but without any parasite at the time of death. The absence of parasites, in the latter group of mosquitoes, could be due to either density-dependent mortality of the parasites or failure of these mosquitoes to ingest Mf, which increases with decrease in Mf-density in the peripheral blood [1]. As a consequence of parasite-induced reductions in survival, the life expectancy of infected mosquitoes was reduced significantly with increasing number of parasites acquired, leaving less chance to transmit infection. Saporu [10] by fitting both the Cox's proportional hazard model, and the Weibull accelerated failure time model to survival times of the onchocerciasis vector *Simulium damnosum* showed that a higher Mf-uptake reduced the survival of vector by a factor of 1.3 times compared to normal fly.

Methodological issues

In estimating the survival function in mosquitoes we have used the human Mf-density as a factor and the parasite load in dead mosquitoes as a covariate. Instead, an estimate of the Mf-uptake (obtained by dissecting a sample of mosquitoes) could have been used as a co-variate or a factor as had been done for the onchocerciasis vector *Simulium damnosum*

[10]. We consider that this would lead to problems in accuracy of the estimation of survival function because the validity of an estimate of the Mf-uptake depends on the number of mosquitoes dissected. The factors, which could influence Mf-uptake, include spatial and temporal distribution of Mf in human blood, duration of feeding, amount of blood imbibed by the mosquitoes and their efficiency to ingest Mf. Further, a fraction of the mosquitoes have to be sacrificed at the initial stage itself to assess the Mf-uptake by mosquitoes.

An important assumption in the survival model is that the parasite load as observed in dead mosquitoes influences the mortality hazard during the total lifespan of the mosquito. Thus, loss of parasites is not taken into account. Such loss would mean that the hazard is relatively high in the first days, and later on lower.

Survival after 25 days

A close look at the observed survival functions in Figure 1 shows that the Mf-carrier fed mosquitoes have over the whole range an approximately exponential survival distribution, while the mosquitoes fed on uninfected persons have an accelerating mortality hazard after day 24, eventually crossing the survival curves of the Mf-carrier fed mosquitoes. This is a puzzling phenomenon. On the one hand it could be that long-term survival in Mf-carrier fed mosquitoes is enhanced by the parasite symbiont (*Wolbachia*), facilitating in this way the possibility of finding a new host. On the other hand the survival of the mosquitoes fed on uninfected persons suddenly deteriorates. But, it is difficult to see how environmental problems could have caused such excess mortality as all the batches of mosquitoes were reared and maintained under the same laboratory conditions. Experimental infection studies with parasite symbiont *Wolbachia* may be useful in understanding the survival phenomenon of infected vectors.

Implications for control

The conclusion that the survival of *C. quinquefasciatus* infected with *W. bancrofti* declines with increasing parasite load has important implications for the control/elimination of lymphatic filariasis through mass drug administration (MDA) programmes. MDA with either single drug (DEC/ivermectin) or two drug (DEC/ivermectin+albendazole) regimens and DEC fortified salt distribution have been recommended for the control/elimination of filariasis[25]. These strategies are aimed at interruption of transmission by liquidating parasite load in the community. Following each round of mass treatment the parasite (Mf) density in the human host is reduced gradually and thereby the quantum of Mf available for the vector mosquitoes and the number of infective larvae for transmission is reduced. However, as a consequence of reduction in parasite load in the community, parasite-induced vector-mortality will be reduced and hence the vector will facilitate (instead of 'limitation') the successful development of the larvae. Since in any endemic area only 5-10% of the Mf-carriers harbour high Mf-counts [4,26], and this percentage will fall into low Mf-category following MDA. The present study

showed that 55 and 60% of the mosquitoes fed on low and medium Mf-categories survived beyond 12 days. Further 46 and 94% of the Mf ingested by these mosquitoes developed into L3, whereas the corresponding figures for the mosquitoes fed on high Mf-category is 37% and 7%. Comparison of the above results suggest that the low Mf-density carriers would remain a potential threat for total interruption of transmission following repeated MDA as has been reported elsewhere [27-29]. The threat of low Mf density carriers can be controlled either by increasing the duration and coverage of MDA or by including vector control measures as an adjunct following five rounds of MDA, which is expected to reduce the Mf prevalence below 1%.

References

1. Subramanian S, Krishnamoorthy K, Ramaiah KD, Habbema JDF, Das PK, Plaisier AP. The relationship between microfilarial load in the human host and uptake and development of *Wuchereria bancrofti* microfilariae by *Culex quinquefasciatus*: a study under natural conditions. *Parasitology* 1998;116:243-255.
2. Brengues J, Bain O. Passage of microfilariae from the stomach to the haemocoel of the vector, in the case of *Wuchereria bancrofti* in *Anopheles gambiae* A and *Aedes aegypti* and *Setaria labiatopapillosa* in *Aedes aegypti*. *Cahhiers ORSTOM, Serie Entomologie medicale et Parasitologie* 1972;10:235-49.
3. Das PK, Subramanian S, Manoharan A*, et al.* Frequency distribution of *Wuchereria bancrofti* infection in the vector host in relation to human host: evidence for density dependence. *Acta Tropica* 1995;60:159-165.
4. Dye C, Williams BG. Non-linearities in the dynamics of indirectly-transmitted infections (or, does having a vector make a difference?). In: Dobson AP, ed. Ecology of Infectious Diseases in Natural Populations. Publications of the Newton Institute, Cambridge: Cambridge University Press, 1995: 260-279.
5. Pichon G, Prodhon J, Riviere F. Filariasis: excessive dispersal of parasites and excessive infection of the ivertebrate host. *Entomologie Medicale et Parasitologie* 1980;18(1):27-47.
6. Subramanian S, Manoharan A, Ramaiah KD, Das PK. Rates of acquisition and loss of *Wuchereria bancrofti* infection in *Culex quinquefasciatus*. *American Journal of Tropical Medicine and Hygiene* 1994;51:244-9.
7. Crans WJ. Experimental infection of *Anopheles gambiae* and *Culex pipiens fatigans* with *Wuchereria bancrofti* in coastal East Africa. *Journal of Medical Entomology* 1973;10:189-93.
8. Failloux AB, Raymond M, Ung A, Glaziou P, Martin PMV, Pasteur A. Variation in the vector competence of *Aedes polynesiensis for Wuchereria bancrofti*. *Parasitology* 1995;111:19-29.
9. Maeda R, Kurihara T. The effect of age of *Aedes togoi* on the transmission of *Brugia malayi* in the laboratory. *Japanese Journal of Sanitary Zoology* 1980;31(4):277-281.
10. Saporu FW. Analysis of survival data for *Simulium damnosum* using the regression method. *Annals of Tropical Medicine and Parasitology* 1993;87:563-9.
11. Samarawickrema WA, Laurence BR. Loss of filarial larvae in a natural mosquito population. *Annals of Tropical Medicine and Parasitology* 1978;72:561-5.
12. Collett D, ed. Modelling Survival Data in Medical Research. 1 ed. Madras: Chapman & Hall, 1994.
13. Crawley MJ. Methods in Ecology. Oxford: Blackwell Scientific Publications Ltd, 1993.
14. Pichon G. Relations mathematiques entre le nombre des microfilaires ingereese et le nombre des parasites chez differents vecteurs naturels ou experimentaux de filarioses. *Cahiers ORSTOM serie Entomologie medicale et Parasitologie* 1974;12:199-216.
15. Rajagopalan PK, Kazmi SJ, Mani TR. Some aspects of transmission of *Wuchereria bancrofti* and ecology of the vector *Culex pipiens fatigans* in Pondicherry. *Indian Journal of Medical Research* 1977;66:200-15.
16. Subramanian S, Plaisier AP, Ramaiah KD, Van Oortmarssen GJ, Habbema JDF, Das PK. Density dependent development of *Wuchereria bancrofti* microfilariae in *Culex quinquefasciatus*: Analysis of data from 17 sites in Pondicherry, India. *(unpublished observations).*
17. McGreevy PB, Bryan JH, Oothuman P, Kolstrup N. The lethal effects of the cibarial and pharyngeal armatures of mosquitoes on microfilariae. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1978;72:361-8.
18. Bryan JH, Southgate BA. Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes .2.Damage to ingested microfilarie by mosquito foregut armatures and development of filarial larvae in mosquitoes. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1988;82:138-145.

19. Jordan P, Goatly KD. Bancroftian filariasis in Tanganyika: a quantitative study of the uptake, fate and development of microfilariae of *Wuchereria bancrofti* in *Culex fatigans*. *Annals of Tropical Medicine and Parasitology* 1962;56:173-87.
20. Das M. Vectors of filaria with special reference to India. *Journal of Communicable Diseases* 1976;8:101-9.
21. Nathan MB. Bancroftian filariasis in coastal North Trinidad, West Indies: intensity of transmission by *Culex quinquefasciatus*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1981;75:721-30.
22. Samarawickrema WA. A study of the age composition of natural populations of *Culex pipiens fatigans* Widemann in Relation to the Transmission of Filariasis due to *Wuchereria bancrofti* (Cobbold) in Ceylon. *Bulletin of the World Health Organization* 1967;37:117-137.
23. Basanez MG, Townson H, Williams JR, Frontado H, Villamizar NJ, Anderson RM. Density-dependent processes in the transmission of human onchocerciasis: relation between microfilarial intake and mortality in the simuliid vector. *Parasitology* 1996;113:331-355.
24. Pichon G, Prodhon J, Riviere F. Heterogeneous nature of the ingestion of blood-inhabiting parasites by their vectors: quantitative description and interpretation. *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences, D* 1980;290(14):1011-1013.
25. Ottesen EA, Ramachandran CP. Lymphatic filariasis infection and disease: control strategies. *Parasitology today* 1995;11:129-131.
26. Das PK, Manoharan A, Srividya A, Grenfell BT, Bundy DA, Vanamail P. Frequency distribution of *Wuchereria bancrofti* microfilariae in human populations and its relationships with age and sex. *Parasitology* 1990;3:429-34.
27. Basu BC, Rao SS. Studies on filariasis transmission. *Indian Journal of Medical Research* 1939;27:233-49.
28. Jayasekera N, Kalpage KS, De Silva CS. The significance of low density microfilaraemia in the transmission of *Wuchereria bancrofti* by *Culex (Culex) quinquefasciatus* Say in Sri Lanka. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1991;85:250-4.
29. Krishnaswami AK, Pattanayak S, Raghavan NGS. The susceptibility of *Culex fatigans* to different densities of Microfilariae bancrofti. *Indian Journal of Malariology* 1959;13:153-7.