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Model development, quantification and application

4.1 The LYMFASIM simulation program for modelling lymphatic filariasis and its control

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4.2 Modelling the epidemiology, transmission and control of lymphatic filariasis

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4.3 The dynamics of *Wuchereria bancrofti* infection: a model-based analysis of longitudinal data from Pondicherry, India

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4.4 Prospects for elimination of bancroftian filariasis by mass drug treatment in Pondicherry, India: a simulation study

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4.1 The LYMFASIM simulation program for modelling lymphatic filariasis and its control

Summary

The LYMFASIM modelling-framework for the transmission and control of the tropical parasitic disease lymphatic filariasis is described and its use in the context of an endemic community in North-eastern Brazil is illustrated. Lymphatic filariasis is a disease with a complex natural history with many unknowns. This complicates decision making with respect to control strategies. With LYMFASIM, a variety of hypotheses can be tested about the life-history of the parasite *Wuchereria bancrofti*, its transmission from man to man through mosquitoes, the role of the immune-system in regulating parasite numbers, the development of disease symptoms, and the effects of control measures (drug treatment or mosquito-control). The implications of alternative assumptions and uncertainty about the quantification of parameters for the effectiveness of control strategies can be investigated. Thanks to the use of stochastic microsimulation, LYMFASIM is highly flexible and can be adapted and extended as new knowledge emerges.

Introduction

Lymphatic filariasis is a parasitic disease, which affects over 70 million people mainly in Africa and South-East Asia. The large majority of patients is infected with the filarial worm *Wuchereria bancrofti*. In less than 10% of the cases (mainly in the Western Pacific) *Brugia malayi* is the causing agent [2]. Adult *W. bancrofti* worms dwell in the lymph-vessels of a patient and expel large amounts of microfilariae, which circulate with the bloodstream. Engorged by a mosquito-vector (such as *Culex quinquefasciatus*) microfilariae can develop to infective larvae which, during one of the subsequent blood meals of the mosquito, can be transmitted to another person and grow to a new adult worm. The clinical manifestations of the infection include swelling of the limbs (lymphoedema) and genitals (hydrocele), with elephantiasis as the most severe form. These chronic symptoms are often preceded and accompanied by acute attacks of filarial fevers (adenolymphangitis, ADL). Development of severe morbidity is most likely after multiple infections (cumulative exposure to worms), although it is believed that even a single infection may cause disease [3].

Control of lymphatic filariasis in an endemic community can be achieved by controlling the vector-mosquito (vector control), by reducing the parasite reservoir via chemotherapy (parasite control) or by a combination of both. Vector control aims at reduction of the rate at which persons are bitten and receive infective larvae of the parasite. This can be achieved by preventing mosquito breeding (reducing the area of stagnant polluted water), application of (biological) insecticides, and other measures to prevent man-vector contact. The two drugs currently being used against the parasite are Diethylcarbamazine

(DEC) and ivermectin. Both effectively kill the microfilariae and, depending on the dosage, also suppress the production of microfilariae [4]. Repeated treatment reduces progress of disease-symptoms and lowers the reservoir of microfilariae available to the vector, thus reducing transmission.

In spite of a large body of research, there are still important gaps in the understanding of the natural history of both infection and disease. There is a growing awareness that the broad spectrum of parasitological and clinical states (susceptible, infected with or without microfilariae in the bloodstream, symptomatic with or without microfilariae) should be considered as a dynamic system with transitions between states occurring over time [5,6]. Although it is accepted that these dynamics are largely driven by the immune system [7], the exact mechanisms remain unknown. Several hypotheses have been put forward about the role of immunological reactions in regulating the parasite and provoking disease symptoms [8,9].

Both the incomplete understanding of the disease and the complexity of the life cycle of *W. bancrofti* makes it difficult to evaluate the effectiveness of control and to arrive at a sound prognosis of the long-term impact of control-strategies. In such a situation, a quantitative model for the transmission and control of the disease can be used to gain more insight in the dynamics of infection and disease, to test hypotheses (e.g., on the role of the immune-system, the effect of treatment) against observations, and to make predictions of the (cost-) effectiveness of control strategies. Encouraged by favourable experiences with relatively simple analytical models [10,11], the LYMFASIM computer simulation model has been developed. Like earlier models, LYMFASIM aims at unravelling the processes underlying field observations, but it can also be used for making predictions of future trends under various control-scenarios. Rather than being one particular formalized description of lymphatic filariasis and its control, LYMFASIM is a modelling *framework*. By selecting from several alternative assumptions and by varying the quantification of parameters, different models can be composed and their behaviour compared.

In LYMFASIM the technique of stochastic microsimulation is employed [12]. Contrary to most analytical approaches and deterministic simulation which describe processes in the population as a whole, in microsimulation the individual is the modelling unit. In a person stochastic events occur, such as birth, acquisition and loss of parasites, onset of morbidity, and death. Persons in the population interact indirectly through biting mosquitoes that may transmit parasites. The same microsimulation technique has previously been applied in the ONCHOSIM simulation model [13] and has proven to be useful in the integrated analysis of data and the prospective evaluation of control strategies [14].

In this paper we will first present the processes included in the modelling framework. Next, a formal mathematical description and a description of LYMFASIM as a simulation tool will be provided. Finally, an example of the use of the model to reproduce a data-set, collected in the city of Recife (Brazil), and to predict the trends during control will be given.

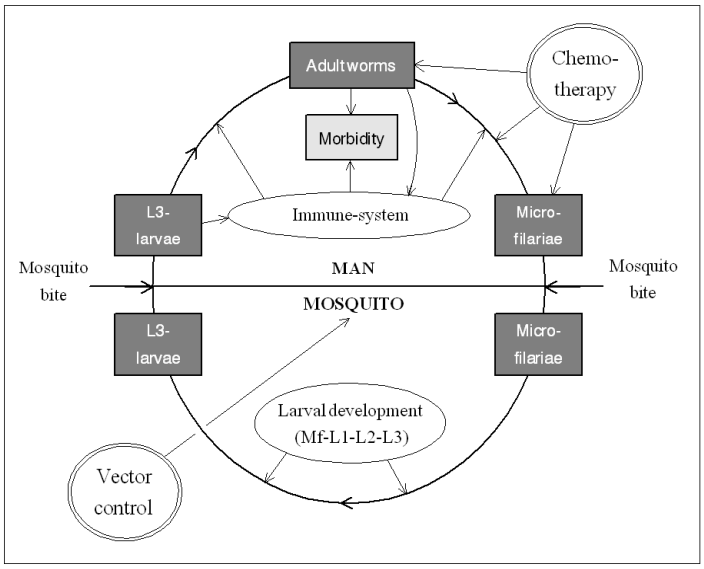

Figure 1 Schematic representation of the transmission and control of lymphatic filariasis.

Structure of the modelling framework

Figure 1 summarizes the transmission and control of lymphatic filariasis as included in the LYMFASIM modeling framework. A key-variable is the number of lymph-dwelling adult male and female *W. bancrofti* parasites in the human host (adult worms). In the presence of at least one male worm, female worms continuously produce microfilariae (Mf). Mf can be engorged by a bloodsucking mosquito vector, and a fraction may develop through two intermediate stages (L1, L2 larvae) to the stage of infective L3-larva. In LYMFASIM several assumptions can be made about how the number of L3-larvae developing in a mosquito depends on the Mf-density in the human blood consumed by the mosquito [15]. L3-larvae can be transmitted to another person during one of the subsequent bites of the mosquito. Apart from the Mf-density in the human blood, the probability that a mosquito will transmit L3-larvae also depends on the survival of the mosquito during the development from Mf to L3 [16]. Inoculated L3-larvae may develop to a new adult worm.

The average number of new adult worms a person acquires per unit of time (the force-of-infection) is proportional to the number of mosquito-bites, the average number of L3-larvae released per bite, and the probability that these larvae reach adulthood. The number of bites will vary among persons. This is partly related to age and gender (usually lowest for females and young children), but it may also depend on a number of other factors such as behaviour, attractiveness to the mosquitoes, etc. These latter unknown factors are summarized in the

'exposure-index' of a person. Since mosquito biting rates are usually very high (up to thousands per person per month) differences in L3-load between mosquitoes are ignored and at a particular moment the average number of L3-larvae released per bite is considered to be equal for all individuals. The probability that injected L3-larvae survive and become a worm depends on the effectiveness of the immune system.

Given the complexities and the unknowns with respect to the role of the immune system in regulating parasite numbers and preventing or provoking clinical symptoms, LYMFASIM must inevitably be crude and simple in this respect. As yet, no variables are included which could be directly related to observed antigen (PC-Ag) or antibody (IgG) levels. Following the ideas of Woolhouse [17], the reactivity of the immune system is assumed to depend on the "experience of infection". The reactivity against inoculated L3-larvae, leading to a reduction in the chance that they become an adult worm depends on the experience of L3-inoculation. The reactivity to worms and microfilariae is a function of the experience of worm-load and is modelled as a reduction in the Mf-production of adult worms (anti-fecundity response). Both "experiences of infection" can be thought as to be related to numbers of specific memory cells. In this concept, the immunological memory can be lost because of the turnover of these cells. In LYMFASIM this loss of immunological memory is modelled as a decay in the experience of infection in the absence of boosting (L3-inoculation, presence of worms).

Two forms of morbidity are presently considered in LYMFASIM: lymphoedema (swelling of the limbs around the affected lymphatics) and hydroceles (swelling of the scrotum). Both forms are assumed to aggravate as a result of the presence of worms. However, in accordance with Ottesen [7] lymphoedema (ultimately leading to elephantiasis) is immunologically driven and its progress depends on the combination of the number of worms and the severity of the anti-worm (anti-fecundity) reaction mentioned before.

In spite of progress in the development of field-applicable immuno-diagnostic tests for detecting *W. bancrofti* infection [18], to date the demonstration of Mf in samples of blood taken around midnight (usually between 8 pm and 2 am) is the only reliable means to determine whether and how severely a person is infected. Taking blood-samples during night is necessary because the Mf-density in capillary blood peaks around midnight. Usually a small blood-smear (20-100µl) is examined under a microscope and the number of Mf counted. Due to random variation in the Mf-density in such a drop of blood, the sensitivity of this test tends to decline with decreasing intensity of infection in a patient. In LYMFASIM both the amount of blood examined and the variability of Mf-counts between smears (i.e. the possible deviation from the true density) can be specified.

LYMFASIM considers two types of control measures: (1) vector control, and (2) parasite control through drug treatment. Vector control can be achieved by killing the larval stage of the mosquitoes through larvicides, by killing the adult mosquitoes (adulticiding), or by limiting the number of breeding sites of the mosquitoes (polystyrene beads in cess-pits). One of the indicators of the effect of vector control is the change in the number of indoor-resting or biting mosquitoes. In LYMFASIM the impact is modelled as a percentage reduction of the man-vector contact for all persons in the community.

For parasite control, two drugs are currently available: Diethylcarbamazine (DEC) and ivermectin. Both drugs (alone or in combination) cause an immediate reduction in the Mf-density and, depending on dosage, suppress the production of microfilariae following treatment [4]. Several regimens for administration of the drugs have been applied and proposed, including a twelve-days course with DEC at a dosage of 6 mg/kg body weight, DEC-medicated cooking salt, ivermectin at 400 µg/kg, etc. [19]. In LYMFASIM, the effect of each of these regimens is assumed to be a combination of an instantaneous killing of a certain (usually high) fraction of the Mf, a fraction of adult filariae killed, a certain period of reduced fertility of the (surviving) adult female parasites, and an irreversible reduction in the reproductive capacity of (some of) these female worms. Two types of treatment strategies can be specified within LYMFASIM: selective treatment of persons with a positive blood smear and mass-treatment. For mass-treatment, specific assumptions can be made about coverage (% of persons treated) and compliance (a person's tendency to participate in campaigns).

Formal description

In this section a mathematical description of the model will be given. An example of quantification of the parameters representing the endemic situation in Recife (Brazil) is provided in the Appendix. The structure of LYMFASIM in terms of variables (boxes), processes (rates, transitions; arrows), and interventions (ovals) is shown in Figure 2. The backbone of each of the models that can be defined within the LYMFASIM framework is a human population consisting of an enumerable number of persons with distinct characteristics. Persons are added through birth (depending on the number of women and their age-specific fertility) and are removed through death (depending on the life table).

Dynamics of transmission

If mosquitoes feed on a person with microfilariae in the blood, they can pick up a certain number of these Mf. Only a fraction of these Mf will develop to the stage of L3-larva. In the model we shortcut the relation between human Mf-load and infection of the vector by directly considering a mathematical relationship between the blood Mf density (at midnight) and the number of L3-larvae that will develop. Experimental data have shown that this relationship saturates at high human Mf-densities [1]. A hyperbolic function was found to describe the data adequately:

$$L3_i(t) = \frac{a \times m_i(t)}{1 + \left(a \times m_i(t)\right)/b} \qquad (1)$$

In this function m_i is the density of Mf in the blood of person i (per 20 µl capillary blood, collected around midnight), a quantifies the slope of the function at low human Mf-densities.

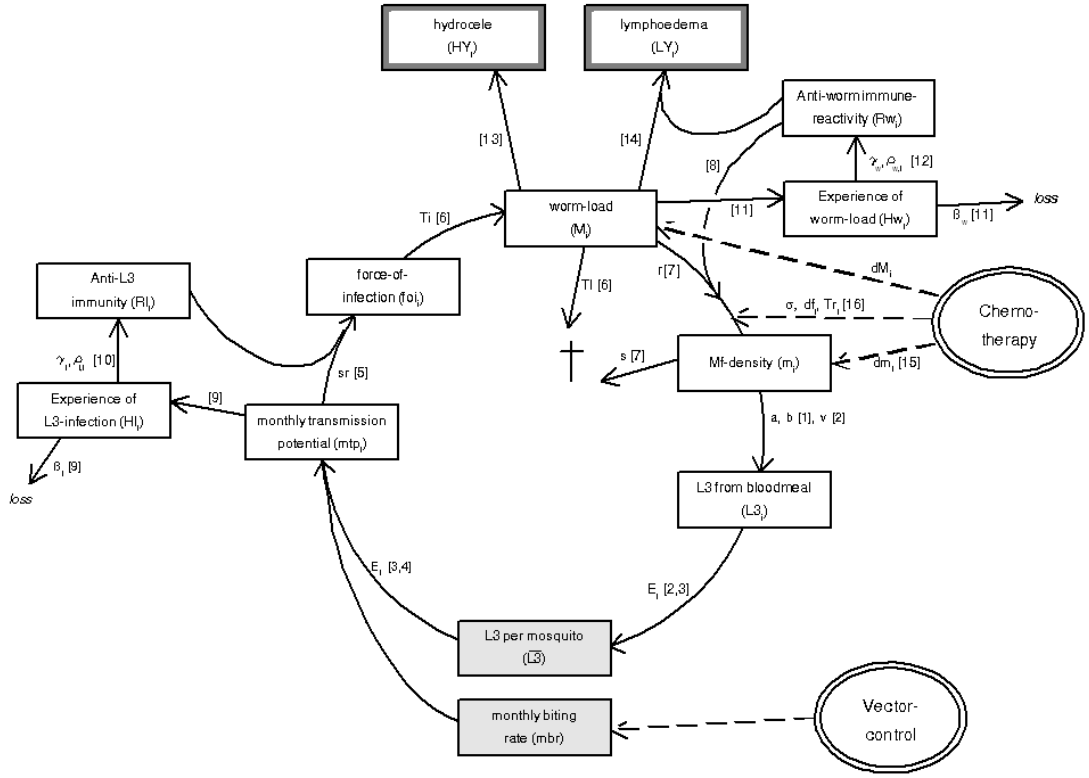

Figure 2 Flow-diagram of LYMFASIM showing the time-dependent model-variables (unshaded boxes: individual variables; shaded boxes: global variables) and their interrelation (processes; the arrows). Along the arrows, the relevant model parameters are given. Numbers within square brackets refer to the equations. Dashed arrows indicate the impact of interventions on variables and processes.

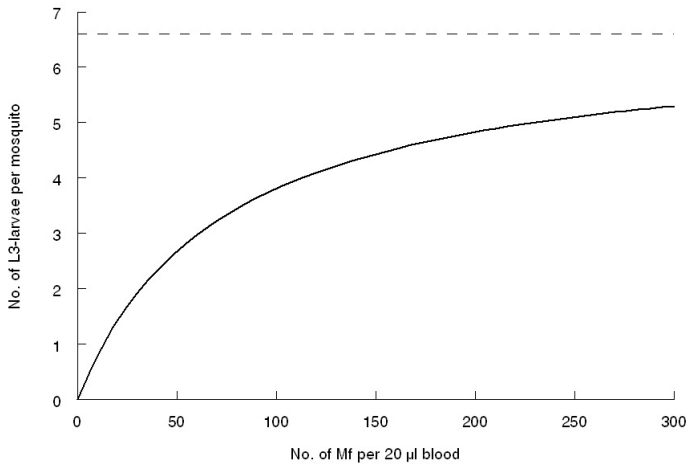

Figure 3 Example of the relationship between the human microfilariae density (per 20 µl blood) and the number of infective (L3-) larvae developing in feeding mosquitoes (see eqn. 1; a=0.09, b=6.6; based on Subramanian *et al*. 1998[1]).

The parameter *b* quantifies the saturation level for the number of L3 per mosquito at high human Mf-densities (Fig. 3). To calculate the mean number of L3-larvae carried by the mosquito-population in a given month *t*, the L3-uptake of individual mosquitoes must be weighted for the relative exposure of the persons from whom they take their blood meal:

$$\overline{L3}(t) = \frac{\sum_{i=1}^{N(t)} \left[E_i(t) \times v \times L3_i(t) \right]}{\sum_{i=1}^{N(t)} E_i(t)} \qquad (2)$$

In this expression $N(t)$ is the total population size, $L3_i(t)$ is the average number of L3-larvae resulting from a blood meal on person *i*, and $E_i(t)$ is the relative exposure of person *i*. The factor v (≤ 1) accounts for the survival of the mosquitoes in real field (i.e., non- experimental) situations and the probability that an L3-larva is released during feeding. The relative exposure of a person comprises two factors:

$$E_i = Ea_i(a,s) \times Ei_i \qquad (3)$$

The first factor quantifies the dependency of exposure on age *a* (and hence on time *t*) and sex *s*. As a rule, children are less exposed than adults and females are less exposed than males. For example, it could be assumed that *Ea* increases linearly with age until adulthood and is constant (e.g., 1.0) thereafter. The second factor, called exposure index, is a stochastic component which is assumed to vary between persons according to a continuous probability

distribution function (gamma, lognormal). For simplicity it is further assumed that a person's exposure index does not change during ones life time.

The effect of differences in exposure is twofold. The number of mosquito-bites experienced not only determines a person's contribution to the mean L3-load of the mosquitoes (eqn. 2), but at the same time the number of L3-larvae received. The monthly transmission potential *mtp* acting upon a person is defined as follows:

$$mtp_i(t) = mbr(t) \times \overline{L3}(t) \times E_i(t) \quad (4)$$

with *mbr(t)* representing the number of mosquito-bites per month for an (adult) person with relative exposure $E_i = 1$. In a situation without vector control *mbr* is assumed to be constant (and, hence, independent of population size). It is further assumed that a mosquito releases all L3-larvae during a blood meal.

Dynamics of the parasite in the human host

The force-of-infection, defined as the average number of successful infections (inoculated L3-larvae developing to adult worms) per month, experienced by person *i* equals:

$$foi_i(t) = mtp_i(t) \times sr \times \left(1 - Rl_i(t)\right) \quad (5)$$

In this expression, *Rl* is the level of immunity against L3-larvae ("resistance"): $Rl = 1$ means complete resistance, $Rl = 0$ means complete susceptibility. The constant factor *sr* ("success-ratio") is defined as the probability that an inoculated L3-larva develops to an adult worm in the absence of anti-L3 immune reactions. Successful L3-larvae will be in the stage of immature worm during a period *Ti* and live for a total period *Tl*. When in a stable situation foi_i is constant, then the expected equilibrium number of mature worms equals:

$$M_i \approx foi_i \times \left(\overline{Tl} - \overline{Ti}\right) \quad (6)$$

This equation is an approximation since in LYMFASIM discrete worm numbers are considered which are generated according to a Poisson-process (see *Simulation Methodology* section).

Since Mf-density is measured as the average number of Mf per 20 µl capillary blood at midnight, Mf-production should be defined as number of Mf produced per month in 20 µl night blood equivalents. If this Mf-production is denoted with *r*, then in case of a stable burden of female worms *F*, the expected equilibrium Mf-density (*m*) equals:

$$m_i \approx F_i \times \frac{r}{1-s} \quad (7)$$

with *s* being the monthly survival of microfilariae (and $1/(1-s)$ is the life-expectancy of Mfs). In LYMFASIM female worms can only produce Mf when the human host harbours at least one male worm. The sex ratio among L3-larvae (and hence, on average, among the worms) is assumed to be 1:1. The formula for *m* is an approximation for a stable *r*. However, one of the reasons that *r* may change is the development of an anti-adult worm immune response. In the

present version of LYMFASIM this anti-adult response is modelled as an anti-fecundity response, and the constant *r* must be replaced by the function:

$$r_i(t) = r_0 \times \left(1 - Rw_i(t)\right) \tag{8}$$

with r_0 being the Mf-production in the absence of an immune-response (constant parameter) and Rw being the level of the anti-fecundity response: $Rw = 1$ representing a situation where immune-reactions completely block Mf-production by female worms; $Rw = 0$ representing the total absence of anti-fecundity responses.

Development and loss of immune-responsiveness

The level of anti-L3 immunity (resistance $Rl_i(t)$) at time *t* is modelled as a function of the history of L3-inoculations. The following set of expressions is used:

$$Hl_i(t) = mtp_i(t) + \beta_l \times Hl_i(t-1) \quad \text{and} \tag{9}$$

$$Rl_i(t) = 1 - \exp\left[-\gamma_l \times \rho_{l,i} \times Hl_i(t)\right] \tag{10}$$

This expression implies that Rl varies between 0 (no immunity) and 1 (complete resistance) depending on the "experience of (L3-) infection" Hl (introduced by Woolhouse [17]), a parameter (γ_l) translating this experience of infection into an immune-response, and a personal factor (ϱ_l) reflecting between-person variation in the ability to develop an effective response. This latter L3-immunity index ϱ_l is modelled as a fixed characteristic, generated from a continuous probability distribution function with mean 1.0. In LYMFASIM, alternatives for the exponential function can be selected from a menu of functional relationships (e.g., a straight line truncated at 1.0). In the absence of "boosting" (i.e., no further inoculation of L3-larvae, for example, because of vector control), the immune-responsiveness may reduce. This reduction is implied by the factor β_l (immunological memory), which can be thought to represent the decay in specific memory cells or antibodies.

The expressions for the anti-adult (anti-fecundity) response are highly similar to those for the anti-L3 response:

$$Hw_i(t) = M_i(t) + \beta_w \times Hw_i(t-1) \tag{11}$$

$$Rw_i(t) = 1 - \exp\left[-\gamma_w \times \rho_{w,i} \times Hw_i(t)\right] \tag{12}$$

The major difference is that the key-variable for development of immune-reactions is a person's current and past worm-load *M*. Furthermore, β_w, γ_w and ϱ_w have a different meaning and are also thought to be independent of the corresponding factors β_l, γ_l and ϱ_l for the anti-L3 response.

Chronic morbidity

In LYMFASIM, morbidity is modelled as the consequence of cumulative exposure to adult worms. The severity of hydroceles of person *i* (men only) is directly linked to past and current worm-load:

$$HY_i(t) = HY_i(t-1) + M_i(t) \quad (13)$$

Severity HY_i is here a continuous model-variable, which has no explicit relation with any grading used in clinical observations. In LYMFASIM a person has an observable hydrocele if the severity HY_i exceeds a given threshold δ_{HY}. As yet, no differentiation into early and advanced stages is included. The progress of lymphoedema is assumed to be the result of the accumulation of immune reactions against adult worms (*Rw*). The following expression is used for the severity:

$$LY_i(t) = LY_i(t-1) + Rw_i(t) \times M_i(t) \quad (14)$$

A lymphoedema-patient is a person whose severity LY_i exceeds the threshold δ_{LY}.

Control measures

The effect of vector control is modelled as a percentage reduction of the monthly biting rate (*mbr*) during a specified period. The number of such periods and their duration and the effectiveness of each period can be chosen.

For control by (a single or repeated) drug-treatment, provision is made for 7 different administration regimes for DEC and ivermectin. These regimes are the building blocks for all kinds of different control strategies. For each simulated population treatment, one out of these 7 can be selected. An example of a list of 7 regimens [19] could be: (1) the standard 12 days course of DEC given at 6 mg/kg body weight per day; (2) a single dose of DEC (6 mg/kg); (3) an alternative dose of DEC (e.g., low dose); (4) single dose of ivermectin 400 µg/kg; (5) alternative dose of ivermectin; (6) combination of a single dose DEC (6 mg/kg) and ivermectin (400 µg / kg); (7) DEC-fortified salt, assuming that this is taken by a person during the time step of 1 month.

The effect of treatment with one of these regimes on the Mf is described as follows:

$$m_i(t+\varepsilon) = m_i(t) \times (1 - dm_i) \quad (15)$$

with $m_i(t)$ being the microfilarial load (per 20 µl of blood) at time t, treatment occurring between time t and $t+\varepsilon$, and dm_i, the fraction of Mf killed as a result of the treatment. The latter is a stochastic variable (≤ 1) which varies between persons and between treatments and which is described by a standard Pdf (beta, or gamma truncated at 1.0). Similar to Plaisier *et al.* [20] the impact on adult parasites can be twofold:

1. A fraction dM_i of them may be killed (or, equivalently, sterilized). Similar to dm_i also dM_i is a stochastic variable (≤ 1).
2. Surviving female parasites may have a temporary and/or a permanently reduced fecundity. In this case the Mf-production $r_i(t)$ of female parasites in a person i who was treated at time τ is described as follows:

$$r_i(t) = \begin{cases} r_i'(t) \times (1 - df_i) \times \left(\dfrac{t - \tau}{Tr_i} \right)^{\sigma} & for \quad t - \tau < Tr_i \\ r_i'(t) \times (1 - df_i) & otherwise \end{cases} \tag{16}$$

with $r'_i(t)$ being the Mf-production of female worms had person i not been treated (the outcome of eqn. 8), df_i (≤ 1) the irreversible reduction of fecundity caused by the treatment, Tr_i the period during which the female parasite recovers from treatment (assuming that immediately after treatment production is zero), and σ (>0) a shape parameter which determines how Mf-production increases during the recovery period Tr: $\sigma = 1$ implies linear increase; $\sigma < 1$ implies that the fastest increase occurs shortly after treatment; $\sigma > 1$ implies that recovery of productivity is mainly at the end of the period Tr (note that $\sigma \to 0$ is equivalent to $Tr \to 0$, while $\sigma \to \infty$ implies the total absence of Mf-production during Tr). Both Tr_i and df_i are stochastic variables described by a Pdf. A graphical illustration of the impact of treatment on Mf-production is provided in Figure 4.

It should be noted that all stochastic variables related to the effect of treatment (*dm*, *dM*, *df*, and *Tr*) are assumed to be independent and to be generated for each person at each treatment. Furthermore, their respective Pdfs as well as the parameter σ must be quantified for each of the drugs and administration regimes mentioned above.

Each population treatment is characterized by:

1. The drug or administration regime applied;
2. Timing (year and month). In case an epidemiological survey is simulated in the

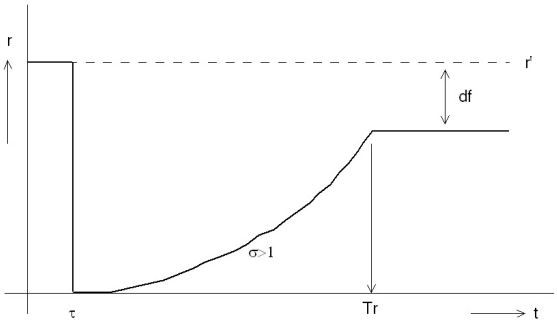

Figure 4 Illustrative example of the impact of a single drug-treatment on the rate of production *r* of microfilariae in a patient treated at time τ. See discussion of eqn. 16 for a meaning of the symbols.

same month, then this is assumed to always precede the treatment;

3. Selective versus mass treatment: in case of selective treatment, only those persons who were Mf-positive at the last survey (usually the survey in the same month) receive treatment.

In case of mass treatment:

4. Fraction of the population treated (C);
5. Compliance pattern.

Three compliance patterns are recognized:

1. Random compliance: at each round of treatment each person in the population has a random chance C to be treated;
2. Systematic compliance: each person i in the population is characterized by an invariable compliance factor co_i which is a random number between 0 and 1. A person will always be treated as long as $co_i \leq C$ and never if $co_i > C$;
3. Partially-systematic compliance: in this pattern, co_i quantifies the tendency to participate but also leaves room for random variation. To account for this tendency and at the same time to guarantee that, on average, the population coverage C will be attained, the probability for person i to participate in a round is calculated as follows:

$$co_i^{(1-C)/C} \tag{17}$$

An example of a hypothetical treatment-scenario could be: 5 years of annual mass-treatment with single dose DEC (6 mg/kg; regime 2) attaining an average coverage of 65% with random compliance, followed by 2 years of 6-monthly treatment with a combination of DEC and ivermectin (regime 6) at a coverage level of 50%. As yet, age and gender differences in compliance are not included in LYMFASIM.

Epidemiological surveys

An epidemiological survey comprises the collection of blood from the population at night and the examination of this blood for the presence of Mf. In LYMFASIM it is assumed that the whole census population is examined. It is assumed that a smear of 20 µl night-blood is collected, or a multiple of this. If this multiple is denoted with φ, and m is the Mf-density in 20 µl of blood (see eqn. 7), then the actual number of Mf counted for person i equals:

$$Mf_i \approx Negbin(\phi \times m_i, k_m) \tag{18}$$

i.e., this number is assumed to follow a negative binomial distribution with clumping factor k_m. Note that this distribution turns into a geometric distribution when $k_m = 1$ and in a Poisson distribution when $k_m \to \infty$. The resulting Mf-counts of all blood smears taken from the population can be represented in several ways: (1) the Mf-prevalence, i.e., the percentage of persons with a positive smear (at least one Mf); (2) the Mf-density, i.e., the (geometric or arithmetic) mean number of Mf per smear; and (3) the Mf-frequency distribution. The classes

of this distribution can be specified. Furthermore, survey results can be tabulated by age group and gender.

Apart from the Mf count, also the clinical status of persons is determined at the survey. This results in age- and gender-specific prevalence of lymphoedema, hydrocele (men only), and total disease.

The computer simulation program LYMFASIM

Simulation methodology

A microsimulation program [12] has been developed for performing calculations and predictions with the model described in section on *Formal Description*. Microsimulation is a special kind of discrete-event simulation in which the human population is composed of a finite number of individuals, whose fates are being followed over time. Each individual has a number of characteristics (or attributes), some of which are constant (e.g., gender, exposure index (*Ei*, eqn. 3), compliance with treatment (*co*, eqn. 17)), others changing during the course of the simulation (e.g., experience of L3-inoculation (*Hl*, eqn. 9), severity of chronic disease (*HY*, *LY*, eqns. 13, 14)). One of the important changing characteristics is the number of male and female worms. Also the life-histories of these inhabitant worms are simulated explicitly.

Microsimulation is a stochastic simulation technique, in which most variables (the attributes of persons, indicated with the subscript *i* in the equations of section on *Formal Description,* and worms) are determined by or change as a result of stochastic processes. Events governed by a Poisson-process [21] are the birth of an individual (with λ = the average birth-rate) and the acquisition of a (new) worm (λ = the force-of-infection *foi*; eqn. 5). Both rates are updated at monthly intervals and are assumed to be constant within this interval. Within each month, births and new infections are generated using a time-to-next-event approach. A monthly time-step is appropriate given the duration of the pre-patent period of at least 6 months, which implies that newly acquired worms can never produce microfilariae, and hence contribute to transmission, within the time step. At birth, the age-at-death of a person is determined by generating a random variate from the life-table. The age attained by a worm (in the absence of treatment) is based on a continuous probability distribution function.

A problem of microsimulation is that attempts to estimate model parameters by fitting the model to observations is hampered by stochastic variation in the outcome (see Figure 8). As a consequence, most standard gradient-based optimization methods (e.g. Newton-Raphson) cannot be used for optimizing the Goodness of Fit. Appropriate alternatives employed in fitting the model are Response Surface methods [22] and Nelder-Mead Simplex variants [23].

On the other hand, stochastic variation reflects natural variation in real world populations, which can be very pronounced in the transmission of infectious diseases. By taking explicit account of this variation, microsimulation models can be used for assessing the range of possible outcomes of particular control strategies. For example, repeated simulation

of a control strategy aiming at complete elimination of the disease will provide an estimation of the risk of failure of such strategy [24,25].

Computer programs

The entire LYMFASIM package comprises three programs: (1) a menu-driven program for preparation of input, (2) the actual simulation program, and (3) a program for processing simulation output. All programs are written in C (Borland) for DOS and perform well on a 80386 PC with mathematical co-processor. In the simulation program both human individuals and male and female parasites are represented by a linked list of structures. Depending on the modelled level of endemicity (which determines the number of worms per person, usually between 0 and 20), in particular on PCs the amount of computer memory can

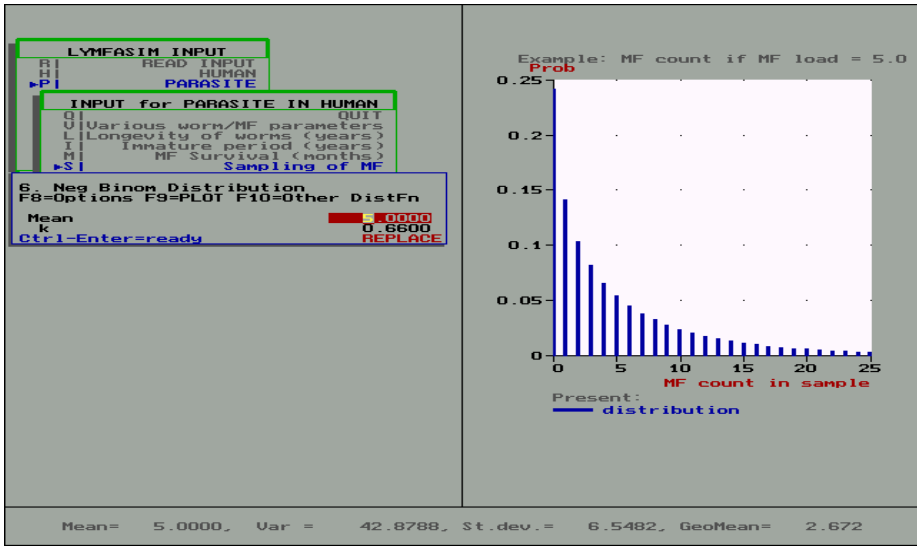

Figure 5 Example of a screen of the program for preparing input for LYMFASIM. See text for details.

become a limiting factor for the size of the simulated population (1,000 to 2,000 individuals). The simulation program also runs under UNIX and this is utilized to perform extensive series of simulations with large human populations.

The program for input-preparation has been developed to facilitate the quantification of parameters and design strategies for surveillance and control. It provides graphs for functional relationships (such as the hyperbolic-function shown in Fig. 3) and for probability distribution functions. An example of the latter is shown in Figure 5. This figure shows a screen of the program with on the left side, part of the menu-structure and a window for quantification of the sampling variation of blood smear-counts. On the right side the associated graph is shown: the probability to find a certain number of microfilariae in a smear of 20 µl taken from a person with an Mf-density $m = 5.0$, assuming that sampling variation is

described by a Negative Binomial distribution with clumping-factor k_m = 1.0 (see eqn. 18; assuming φ = 1.0).

On desired moments during the actual simulation, graphs such as the age- and gender-dependent percentage of persons with a positive blood-smear, are displayed on the screen. At the end of a run, the trend in summary statistics such as the population prevalence of chronic disease are shown. Since simulation output tends to comprise large amounts of information (many variables for each of the simulated surveys), it is accumulated in a binary file. A special program is written to either derive detailed age/gender-specific tables from this binary output or to transform it into summarizing trends which can easily be used for producing graphs (with spreadsheets or graphics programs). The tables are useful for a detailed investigation of the simulation results or for fitting the model to data. LYMFASIM offers the possibility to select specific cohorts from the simulated population, such as persons who were present at a first survey or who were Mf-positive at a first survey. Finally, it is also possible to produce output (Mf-count and disease status) for each simulated individual at each survey. This kind of output is suitable for performing statistical analyses on the simulation results.

Examples of the use of LYMFASIM

The use of LYMFASIM to reproduce a given data set (fitting of a particular model) is illustrated in Figures 6 and 7. The data represent the infection status of two filariasis endemic areas in the city of Recife (Brazil), comprising a total of 6,582 individuals [26]. Figure 6 shows for 3 age-categories the percentage of persons with a positive Mf-count based on a thick drop

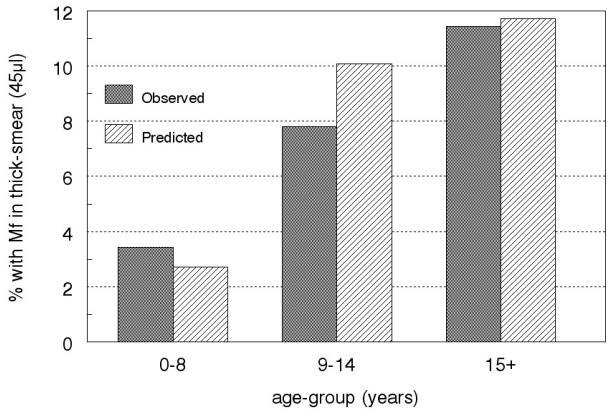

Figure 6 Age-specific prevalence of Mf in 45 µl blood. Comparison of observations in the population of Coque and Mustardinha (Recife, Brazil) and the result of a simulation with LYMFASIM. See Appendix for parameter quantification.

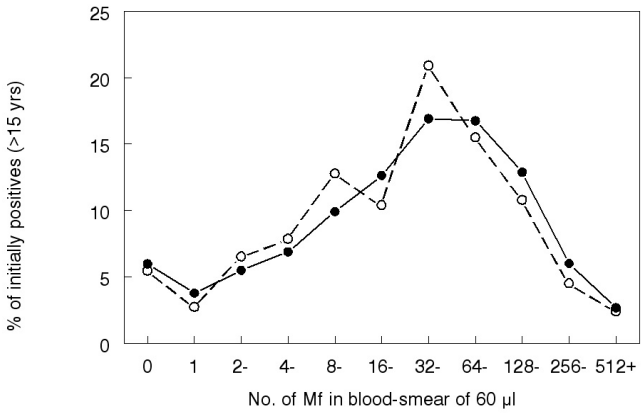

Figure 7 Number of Mf in 60 µl in those persons (aged 15 years and above) who were Mf-positive in the survey shown in Figure 6. Open circles: observations; Closed circles: simulation results. See Appendix for parameter quantification.

of 45 µl of blood (i.e. φ = 2.25; see eqn. 18) collected from 4997 individuals. It can be concluded that for the Mf-prevalence of the age-group 0 to 8 years and 15 years and older and predicted values are well in agreement. The prevalence for age groups 9 to14 years is over-estimated. Figure 7 shows for one of these age classes (≥15 years) the distribution of Mf-counts in those persons who were found to be Mf-positive in the survey reported in Fig. 6. For determining these Mf-counts, a smear of 60 µl (i.e., φ = 3) is used. The observed and simulated distributions are well in agreement, though the modus of the simulated distribution is slightly shifted to the right.

The simulation results are based on the quantification of the parameters as given in the Appendix. This quantification represents one of the estimates obtained by fitting LYMFASIM to the observations from Recife. The goodness-of-fit is based on a sum of χ^2 values for the agreement between the simulated and observed frequency distribution of Mf-counts (for the 3 age-groups of Fig. 6). To minimize the effects of random variation, the predicted distribution is obtained by simulating a population of as many as 50,000 individuals. Since LYMFASIM s.l. comprises many parameters and the data from Recife comprise only cross-sectional observations, it is impossible to arrive at a robust estimate for all these parameters. Therefore, we first identified a particular model within LYMFASIM by ignoring certain processes. In the example of Figures 6 and 7 we ignored the anti-fecundity immune response by putting $\gamma_w = 0$ (and, as a consequence $R_w = 0$; see eqn. 12). Secondly, we fixed a number of parameters which are hardly identifiable when using a cross-sectional data set. Examples are the reproductive lifespan of *W. bancrofti* which can only be estimated from longitudinal observations during transmission control [27], the variability of the exposure index

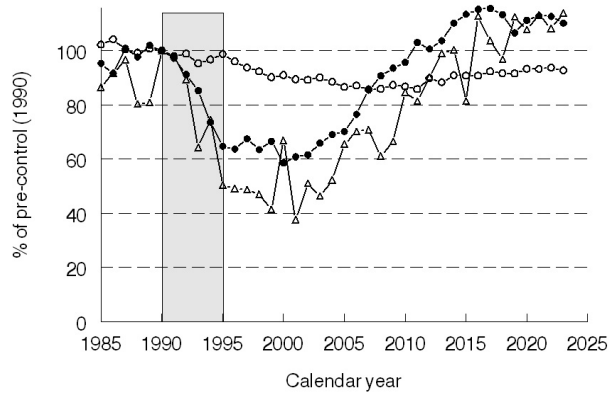

Figure 8 Prediction of the effects of 5 years of vector control (1990-1995) in the endemic situation represented by Figures 6 and 7. Trends are shown as a percentage of the situation before control (1990). Closed circles: fraction of persons with at least 1 Mf in a blood smear of 100 µl (i.e. φ=5); triangles: mean number of Mf per blood smear (only in Mf-positive persons); open circles: men with a hydrocele. See Appendix for parameter quantification.

(*Ei*, see eqn. 3) which has a similar effect on the distribution of Mf-counts as the variability in the ability to develop an anti-L3 response (ϱ_b, eqn. 10), and the success-ratio *sr* (an important determinant of the worm-load of a person; see eqn. 5) which has a similar effect on the Mf-density as the Mf-production per worm (*r*; eqn. 7). Some parameters are fixed because their estimate is based on other data sets. Examples are the life-table for the Recife population (not given in the Appendix) and the parameters *a* and *b* of the hyperbolic function for the relation between the human Mf-density and the number of infective L3-larvae developing in feeding mosquitoes (eqn. 1).

A prediction of the effect of a (hypothetical) control strategy in the situation as represented by Figures 6 and 7 is shown in Figure 8. In the simulation, this time comprising about the same population size as observed in the areas in Recife (i.e., around 5,000), it is assumed that vector control is applied in the period 1990-1995 such that during this period the biting rate is reduced with 75%. After this period, mosquitoes bite at the same frequency as before control. The trends are shown as a percentage of the situation in 1990 (the year where the control starts). Furthermore, each year a survey is simulated in which a blood smear of 100µl (φ = 5) is collected from the entire population. The Mf-prevalence (closed circles) and the mean Mf-count (triangles) in positive persons both decline during the period of control. The decline in Mf-prevalence is slightly less pronounced. This can be explained from the fact that persons may harbour more than one worm and only if all worms have died (as well as their Mf progeny) a person is Mf-negative. There is only a small decline in the

prevalence of chronic disease (in this example only men with a hydrocele) and this decline is delayed. Both are related to the fact that disease is caused by cumulative exposure to worms. Hence, be it at a lower rate, disease continues to progress and new patients emerge during the vector control period. From the year 2010 and onwards, the Mf-prevalence and Mf-count exceed the pre-control level. The explanation is that due to control and the resulting decline in L3-inoculation, persons have lost part of their anti-L3 immune responsiveness and thus have an (temporary) increased susceptibility to new infections.

Discussion

Modelling lymphatic filariasis: past, present, and future

The development and application of the LYMFASIM modelling framework is not the first attempt to approach the epidemiology of lymphatic filariasis quantitatively. An overview of such approaches is provided in review-paper of Grenfell and Michael [28]. The earliest models were catalytic models [29], which were used to calculate the age-specific fraction of persons with microfilaraemia (i.e. with Mf in the blood) on the basis of a force-of-infection and a rate of loss of infection[3]. More recently, such models have been used to estimate the age-specific rates of acquisition and loss of infection from a cohort of persons examined over a five-year period in Pondicherry (India [30]). An important extension of this analysis is the work of Bundy *et al.* [10] and Srividya *et al.* [11]. They demonstrated that, in contrast to many other infectious diseases, there is no straightforward relation between infection and disease. The age-specific prevalence of chronic disease was found to correspond well with the fraction of persons who had been microfilaraemic, but who subsequently cleared their microfilariae from the blood. This latter finding points to the possible role of the immune-system in combating the infection and at the same time provoking disease symptoms.

Based on the results obtained with these explanatory models, the need was born to develop models that could be used in planning and evaluation of control [2]. In response to this, the LYMFASIM modeling framework described in this paper was developed. A number of considerations have led to the choice for a simulation rather than an analytical approach. Most important is that, on the one hand, the envisaged models must comprise a detailed description of the epidemiology of lymphatic filariasis (dynamics of transmission through mosquitoes, regulation of parasite numbers in the human host, and the development of chronic disease) while, on the other hand, they must be used as a tool to predict the (long-term) impact of a variety of control strategies. Such models tend to become too complicated for analytic evaluation. Related to this, simulation models can be more easily extended and adapted when new knowledge emerges. This is particularly true for the stochastic microsimulation technique applied in LYMFASIM.

Further development and validation of LYMFASIM

A model for a complex system as the epidemiology and control of lymphatic filariasis is inevitably based on highly simplifying assumptions. A major problem in the design and

quantification of a model is that, while a huge amount of information is available on the life history of the parasite in the human host, information that can be readily translated into quantitative statements is still very scarce. For example, several hypotheses have been put forward about the potential role of the immune system in preventing establishment of new parasites (acquired immunity [9]) and in combating (or the opposite: tolerating [8]) established parasites. Apart from the question how likely these hypotheses are, it is very difficult to transform them in a mathematical formulation and to obtain (empirical) evidence about the values of the parameters of these formulations. A way out is to design a model structure that comprises as much as possible elements of the hypotheses and subsequently compare the model-calculations with indirect observations such as the (age-dependent) microfilarial density. A good example of this is provided by Woolhouse in his theoretical framework for the immuno-epidemiology of helminth infection [17]. His ideas have been utilized in the way in which immune reactions have been incorporated in LYMFASIM. The examples provided in Figures 6 and 7 show how parameters of an "acquired immunity model" (i.e., a model that only considers immune-reactions against invading parasites) are estimated by fitting that model to observed microfilarial counts.

It must be stressed that this is, indeed, just one example. Since acquisition (and loss) of immunity are by definition dynamic processes resulting in changes in worm-load and Mf-density over time, one should preferably estimate the relevant model parameters from a longitudinal data set. The cross-sectional data from Recife [26] not only allow for a number of different models, but also for a wide range of possible parameter-quantifications within each of these models that are still compatible with the data (i.e., result in an acceptable goodness-of-fit). For that reason, attempts are being made to fit (models defined within) LYMFASIM to the longitudinal data set collected by the Vector Control Research Centre in Pondicherry (South-India [31]). Using these data (which include observations with a 5-year interval) will help to restrict the range of possible quantification and will enable the identification of rate-parameters as the loss of immunological memory (the β's of eqns. 9 and 11).

Apart from a further validation, also the model-structure will have to be reviewed as new knowledge emerges. In its present form, LYMFASIM is mainly a model framework for explaining trends and heterogeneities in Mf-counts. As yet, fairly simple assumptions are made about the progress of chronic disease. Though these assumptions are sufficient to roughly reproduce the (cross-sectional) age-specific prevalence of lymphoedema and hydrocele, extension may be necessary to fit models to more detailed data on the grading of symptoms (early - reversible, severe, elephantiasis) or to use models for predicting the effects of treatment on morbidity. One aspect of morbidity that still has to be included in LYMFASIM is episodic adenolymphangitis (ADL). These acute attacks of filarial fever are thought to pave the way for chronic symptoms [32] and are an important reason for absenteeism at work. Inclusion of this phenomenon is essential for applying LYMFASIM in cost-effectiveness analyses of treatment strategies. Continuous efforts to improve LYMFASIM are further required with respect to the behaviour of the immune system. For

example, there is considerable evidence that children from microfilaraemic mothers have a significantly higher chance to become microfilaraemic themselves (neonatal tolerance [28,33]).

Following further validation, LYMFASIM will be used for assessing the effectiveness of chemotherapy based control strategies. Such an assessment is necessary since at least 7 different treatment regimes based on DEC and ivermectin are available (section on *Control Measures*). While all these regimes show a prominent impact on microfilarial levels, important criteria for effectiveness will be the impact of treatment on the adult filariae and the impact on morbidity. Furthermore, given the large scale of the control strategies, the costs of treatment will be a decisive factor in the choice for the best alternative. A predictive model like LYMFASIM can be a valuable tool in such cost-effectiveness considerations.

Appendix: Table showing all parameters of the basic epidemiological model (i.e. excluding parameters related to interventions) and the numerical values used in the example simulations of Figures 6, 7, and 8.

Symbol	See equation	Description	Numerical value
a	1	Slope of the relation between human Mf-density m and the no. of L3-larvae developing in biting mosquitoes	0.09
b	1	Maximum number of L3-larvae resulting from a single bite	6.6
v	2	Factor to discount for survival of mosquitoes during the development of a microfilaria to L3-larva	0.1
Ei	3	Cpdf[a] for the exposure index (mean = 1.0)	
		Family	Gamma[b]
		Shape-parameter α	1.0
Ea	3	Function relating relative exposure to age (gender-differences are ignored)	
		Family	Linear
		Exposure at birth	0.18
		Age (years) of maximum exposure	15.6
mbr	4	Monthly mosquito biting rate experienced by an average adult man	4000
sr	5	Success-ratio: probability that an L3-larva develops to a male or female worm (in the absence of an anti-L3 immune-response)	0.00028
Tl	6	Cpdf for the life-span of *W. bancrofti*	
		Family	Weibull
		Mean	10.0
		Shape-parameter α	1.0
Ti	6	Cpdf for the duration of the development from L3 to adult worm (immature period)	
		Family	Constant
		Mean	0.67
		Shape-parameter	n.a.[c]
s	7	Monthly survival of microfilariae	0.9
r_0	8	Mf-production in the absence of immune-response and in the absence of treatment	2.2
β_l	9	$(1-\beta_l)$ = Monthly loss of immunological memory for immune-responses against L3-larvae	0.986
ϱ_l	10	Cpdf for the individual variation in the ability to develop an anti-L3 immune-response (mean = 1.0)	
		Family	Gamma
		Shape-parameter α	1.3
γ_l	10	Parameter determining the strength of the immune-response at a given experience of L3-infection (Hl)	0.003
β_w	11	$(1-\beta_w)$ = Monthly loss of immunological memory for immune-responses against worms	n.a.[d]
ϱ_w	12	Cpdf for the individual variation in the ability to develop an anti-worm (anti-fecundity) immune-response	n.a.[d]
γ_w	12	Parameter determining the strength of the immune-response at a given experience of worm-load (Hw)	0[e]
δ_{HY}	13	Threshold-value for hydroceles (i.e. a hydrocele occurs as soon as $HY>\delta_{HY}$)	160
δ_{LY}	14	Threshold-value for lymphoedema (i.e. lymphoedema occurs as soon as $LY>\delta_{LY}$)	n.a.[d]
k_m	18	Clumping factor of the Negative Binomial distribution for the sampling variation of Mf-counts in a bloodsmear from an individual.	1.0

[a] Continuous probability distribution function

[b] Other possibilities: Constant (i.e. no variability), Uniform, Exponential, Weibull, Normal (zero-truncated), Lognormal

[c] n.a.=not applicable; i.e. no variation assumed or value of other parameter overrules the current parameter

[d] see value of γ_w and its implications for the outcome of eqns. 12 and 14.

[e] i.e., this type of immune-response is not considered.

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4.2 Modelling the epidemiology, transmission and control of lymphatic filariasis

Summary

Wuchereria bancrofti transmitted by *Culex quinquefasciatus* accounts for >90% of the global burden of lymphatic filariasis (LF). Recent advances in diagnostic and control tools and a better epidemiological understanding of the disease have led to hope that LF is eradicable. The World Health Organization has helped a number of member countries to launch nation-wide programmes of mass treatment with antifilarial drugs such as diethylcarbamazine, albendazole and ivermectin, for the elimination of this disease. In order to make rational decisions about control strategies, reliable predictions of the long-term impact of such treatment, and of alternative interventions, need to be made, and these can only be based on a sound, quantitative understanding of the population biology of the parasites. Mathematical models have proven valuable in gaining quantitative insights into the population dynamics of the parasites, and may be used to make credible predictions of the likely outcomes of various control strategies. This article provides an overview of the development of the relevant mathematical/statistical models and of their application in studies of the epidemiology, transmission and control of lymphatic filariasis.

Introduction

One species of filarial nematode, *Wuchereria bancrofti*, transmitted mostly by the pest mosquito *Culex quinquefasciatus*, causes >90% of the global burden of lymphatic filariasis (LF)[5]. Recent improvements in the tools available for the diagnosis and control of LF have led to increasing hope that the disease can be eliminated, at least as a public-health problem, within the next two decades. However, the complex nature of the disease and the lack of a sound, quantitative understanding of the population dynamics of *W. bancrofti* mean that a number of important operational questions remain to be answered. Mathematical models may provide the necessary quantitative framework for investigating the key issues related to the parasite's population dynamics, making credible predictions of epidemiological trends, and aiding decisions about control strategies before, during and after cessation of the mass drug administrations (MDA). There are several examples of the successful and practical application of mathematical models in disease control. Perhaps the best is the application of a simulation model in the Onchocerciasis Control Programme in the West Africa [6]. The present article is intended to provide an overview both of the development of mathematical/statistical models to facilitate studies on the transmission and control of *W. bancrofti*, and of the application of these models in the optimisation of control strategies and in predicting the outcome of the various interventions that are possible. Most of the current mathematical models fall into two broad categories: analytical and simulation.

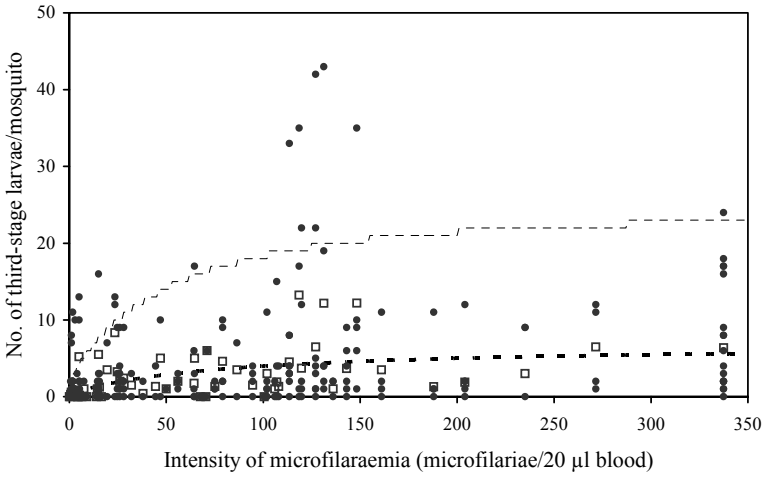

Figure 1 The relationship between the number of human-infective, third-stage larvae of *Wuchereria bancrofti* (L3) found in *Culex quinquefasciatus* that had been fed on human volunteers and the intensity of microfilaraemia in the volunteer on which each mosquito had fed. The data-points show the actual numbers of L3 (●) and the mean number for each intensity of microfilaraemia (□). The lower and upper lines indicate the predicted mean counts of L3 and the upper limits of the 95% confidence intervals, respectively. From Subramanian *et al. (1998)* [3]

Analytical models

An analytical model describes the basic characteristics of a disease using simple mathematics and is usually based on sets of differential equations that keep track of a few important variables, such as the number of infected individuals.

Modelling transmission dynamics

The capability of a mosquito to ingest microfilariae (Mf) and then support their development to the human-infective, third-stage larvae (L3) is an important determinant of transmission. Three processes determine overall vector competence: (1) the uptake of Mf from a microfilaraemic human; (2) the development of the ingested Mf to L3; and (3) the transmission of those L3 to a human host. Several experimental studies on transmission, involving various vector–parasite combinations, have been carried out in an attempt to understand the dynamics of infection in the vector. In most of these studies, statistical models which describe the relationship between the parasite load in the mosquito and the intensity of microfilaraemia in that mosquito's bloodmeal source have been produced. Some of these models focused only on the infection dynamics in the vector host [3,7,8] whereas others considered the overall success of transmission through the parasite's life-cycle [9-11]. The results

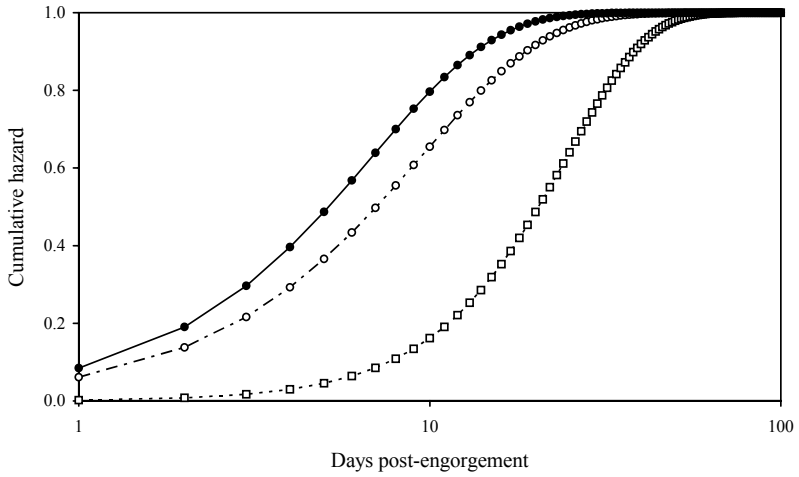

Figure 2 The cumulative hazard, as predicted in a Weibull model, for *Culex quinquefasciatus* fed on amicrofilaraemic humans (□) or humans with low (○) or high (●) *Wuchereria bancrofti* microfilaraemias of 0.33-21 and 22-440 microfilariae/20 µl blood, respectively. Derived from Krishnamoorthv *et al.(2004)* [2]

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 bloodmeal is related linearly to the intensity of microfilaraemia in the bloodmeal source. However, the relationship between the number of L3 that developed in the mosquitoes and the intensity of microfilaraemia was found to be non-linear and saturating (Fig. 1), indicating a density-dependent regulation of parasite numbers within the mosquito [3,7,8]. The key issue in all of these studies was the mechanism that regulates parasite load in the mosquitoes. Analysis of the frequency distribution of the counts of parasites/mosquito and of the age distribution of infection, in wild-caught mosquitoes, indicated that parasite-induced vector mortality occurred [12-15]. This was confirmed in experimental studies [2,16,17], when the survival distributions of mosquitoes fed on microfilaraemics were found to differ markedly from those of similar mosquitoes fed on amicrofilaraemics (Fig. 2). The results of experimental and field studies, involving *W. bancrofti* and *C. quinquefasciatus*, provide overwhelming evidence for the regulation of *W. bancrofti* larvae in *C. quinquefasciatus*[3,7,8]. Knowledge of the functional relationship between the number of L3 developed and the intensity of microfilaraemia is crucial to the development of useful mathematical models and to understanding the role that these models can play in the design and monitoring of interventions. Such quantitative knowledge is available for *C. quinquefasciatus*, but needs to be generated for the anopheline and aedine vectors involved in the transmission of *W. bancrofti* [5,18].

Modelling the dynamics of human infection and disease

There have been two analytical approaches to modelling the dynamics of infection in the human host: one which considers only the changes in infection status of individuals (microfilaraemic v. amicrofilaraemic; leading to 'prevalence models') and one which explicitly considers the intensity of the microfilaria (leading to 'intensity models').

Prevalence models. Hayashi [19] was the first to apply catalytic models, as devised by Muench [20], to describe the age-specific incidence of filarial infection and disease using cross-sectional data. In his application, Hayashi assumed that susceptible individuals can become infected and recover and those individuals who are or have been infected will always be resistant to further infection. Later, in a 'landmark' development in the epidemiology of lymphatic filariasis, Hairston and Jachowski [21] 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 and that, therefore, individuals could repeatedly switch from being amicrofilaraemic to microfilaraemic and back to amicrofilaraemic. Analyses of cross-sectional data by Hairston and Jachowski [21] provided the first estimates of the duration of the fecundic life-span 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.* [22] 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 indicates that there may be some density-dependent limitation on the prevalence of human infection. In further analyses of the same data-set from Pondicherry, attempts were made to define the relationship between microfilaraemia and the development of chronic lymphoedema in the infected population [23,24].

Intensity models. The development of models which take account of the variation seen in the intensity of microfilaraemia is an essential step in quantifying the overall transmission dynamics of filariasis. Grenfell *et al.* [25] developed a theoretical model to examine the relationship between microfilarial burdens and the prevalence of adult (macrofilarial) worms in the human host population. Their main finding was that most of those who appear amicrofilaraemic are probably 'true zeros' (arising from the absence of macrofilarial infections or the presence of adult worms of only one sex) rather than being false-negatives attributable to deficiencies in the blood-sampling process. The distribution of the intensities of microfilaraemia in those found microfilaraemic, as determined by blood sampling, should therefore be Poisson. Das *et al.* [26] applied this theoretical model to the Pondicherry data-set, to examine the effects of host age and sex on the frequency distribution of *W. bancrofti* infections in the human host. By fitting zero-truncated, negative-binomial distributions to the counts of Mf in different age-groups of humans, they showed a significant, decreasing trend in over-dispersion with age. This pattern provided indirect evidence for the operation of density-dependent constraints on the intensity of microfilaraemia. The analysis by Das *et al.* [26] 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).

Simulation models

Unlike analytical models, simulation models use recent advances in computer technology to incorporate many variables influencing the dynamics of a disease, in an attempt to produce much better predictions and explanations. Such models provide a unified framework for the quantitative description of the dynamics of transmission, infection and disease, and of the impact of control. In the modelling of LF, simulation models are perhaps more appropriate than analytical models because so little is known about the dynamics of filarial infection or of symptomatic filariasis in humans. In this respect, the inability to count adult worms in vivo is a major constraint in validating the models. Although immunological markers for infection status are being developed, microfilaraemia is still the most reliable measure of human infection in the field. Furthermore, in the absence of animal models, it is not known whether the pathology seen in the lymphatics of humans with LF is caused only by the adult worms or by a combination of the adult worms and the Mf.

The World Health Organization helped to initiate epidemiological modelling for research on LF and control of the disease in 1990. Since then, considerable progress has been made in the development, validation and application of models for studies on the transmission and control of *W. bancrofti*. Two simulation models were developed using two different approaches: (1) a stochastic, micro-simulation model based on difference equations (LYMFASIM); and (2) a deterministic, macro-simulation model based on differential equations (EPIFIL). The former was the result of collaborative work between the Vector Control and Research Centre in Pondicherry (VCRC) and the Department of Public Health, Erasmus University, Rotterdam, The Netherlands, whereas the latter resulted from collaboration between the VCRC and the Department of Parasite Epidemiology, Oxford University, Oxford, U.K. Unlike the classical catalytic models [21,22], which simply describe the gain and loss of human infection, both LYMFASIM and EPIFIL describe the adult-worm and larval dynamics for *W. bancrofti*. Both models have been explored using the longitudinal data collected from Pondicherry by staff of the VCRC [4,27,28].

The micro-simulation model: LYMFASIM

LYMFASIM simulates the life-histories of individual humans and parasites, the dynamics of the vector population and the impact of interventions based on vector control or chemotherapy or a combination of both. A typical endemic situation can be characterized using data on the demography of the population (life-tables, fertility figures) and the species and density of the vector involved. The micro-simulation technique allows LYMFASIM to take account of variation in the human populations (in terms of age, sex, exposure to mosquitoes, ability to develop immune responses, inclination to get treatment etc) and in the adult parasites (in terms of life-span, production of Mf etc). Details of treatment (coverage, frequency and duration of treatment), and surveillance (time of survey, type of survey -

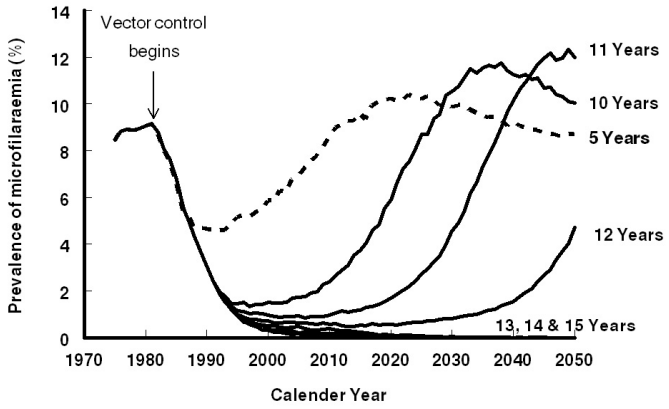

Figure 3 Application of the LYMFASIM model to predict trends in the prevalence of *Wuchereria bancrofti* microfilaraemia in relation to the duration of vector control in Pondicherry. Vector controls begins in 1981 and continues for 5, 10, 11, 12, 13, 14, or 15 years (indicated against each trend line). From Anon (2000) [1]

complete, random or cohort- and volume of blood checked) can be specified. Similarly, for vector control, the duration and effect of vector control in terms of the reduction in human–vector contact can be specified [29].

Depending on the need, the model can be used to produce a summary document or a detailed output for each individual considered. The summary output includes year-wise details of the prevalences and intensities of microfilaraemia, infection with the adult worms, and filarial antigenaemia, and of the prevalences of hydrocele and lymphoedema. In the detailed output for each person, age, sex, numbers of Mf and adult worms, number of L3 received, level of antigenaemia and disease status are available. The output of the model can be exported into any of the more common spreadsheet programmes (such as Microsoft's Excel) for further processing and presentation. Those developing the LYMFASIM model hoped to be able to use it to test hypotheses, forecast trends, predict the outcome of control strategies, optimise control interventions, and perform cost-effectiveness analyses.

LYMFASIM *for testing hypotheses.* The model parameters have recently been quantified using the detailed data-sets, on the frequency distribution of microfilarial counts and the age–prevalence and age–intensity trends in microfilaraemia, collected from a cohort of individuals in Pondicherry who were followed for 5 years [28]. While fitting the model to these data, three hypotheses on the immune mechanisms that may regulate the intensity of microfilaraemia were tested: a 'null' hypothesis (in which no immune mechanism was assumed); 'anti-L3 immunity' (in which individuals exposed to L3 antigens develop immunity that reduces the

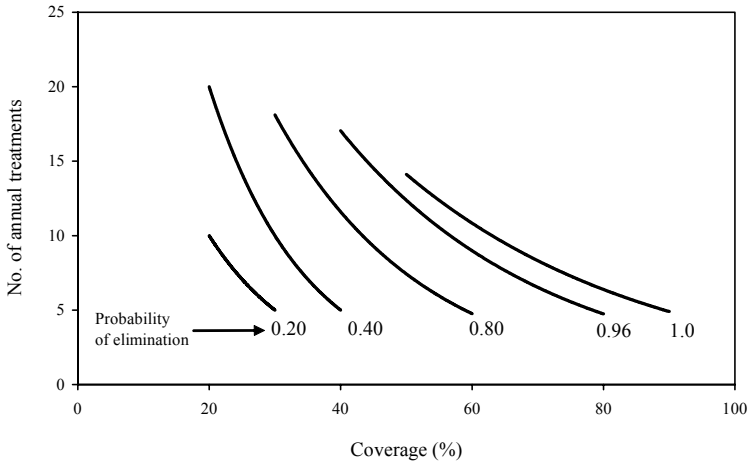

Figure 4 Application of the LYMFASIM model to predict the probability of eliminating *Wuchereria bancrofti* infection in Pondicherry, in relation to treatment coverage and the number of annual mass treatments with diethylcarbamazine. From Anon (2000) [1]

success of any L3 inoculated later); and 'anti-fecundity immunity' (in which those infected with adult worms develop immunity that causes a reduction in the production of Mf by the female worms). The fit of the model to the data was similarly good when either anti-L3 or anti-fecundity immunity was assumed, and better than when no such immune mechanism was assumed [28].

LYMFASIM *for prediction.* In LYMFASIM, the impact of transmission control (by vector or parasite control or both) can be simulated. The effect of vector control can be mimicked by specifying the duration and effect of vector control (in terms of reductions in man-biting rate of the mosquitoes). Using the Pondicherry data again, the model has been applied to determine the duration of vector control that would be required (if used as the only intervention) for the complete elimination of human infection with *W. bancrofti*. The results indicate that at least 13 years of vector control would be required to reach a zero prevalence of microfilaraemia (Fig. 3). The efficacy and effectiveness of diethylcarbamazine (DEC) in reducing the prevalence and intensity of microfilaraemia have been proved beyond doubt, both in clinical [30-32] and community trials [33-36]. It is not clear, however, how long DEC-based MDA will have to be continued to prevent recrudescences or new infections after the rounds of chemotherapy stop, or even what level of treatment coverage will be required to achieve the goal of eliminating LF as a public-health problem. The most important application of LYMFASIM is perhaps determining the duration of MDA-based control required, to achieve elimination, for a given level of endemicity, drug of choice, coverage, pattern of compliance, and frequency of treatment. Predictions based on preliminary analyses indicate that at least 90% coverage will be required to achieve the goal of elimination with five, annual, DEC-

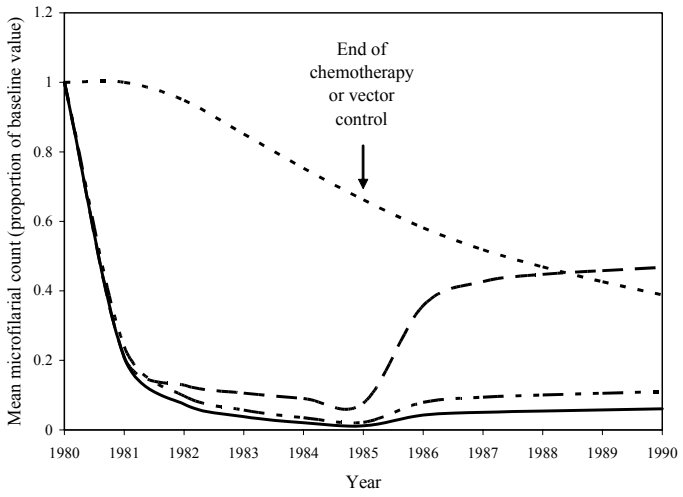

Figure 5 Application of the EPIFIL model to simulate the effects of 5 years of vector control (dotted line) or five annual mass treatments with diethylcarbamazine alone (dotted dashed), ivermectin alone (dashed) or a combination of the two drugs (solid) on the intensity of *Wuchereria bancrofti* microfilaraemia. Each intervention is assumed to have begun in 1980. From Norman *et al (2000)* [4]

based MDA, and that coverage will still have to be at least 60% if 11 such MDA are used (Fig. 4).

The macro-simulation model: EPIFIL

Those who developed EPIFIL wanted: (1) a model that could adequately describe the age-dependent patterns seen in the prevalence and intensity of human infection and in the prevalence of the clinical manifestations of such infection; and (2) a model that could accurately predict the impact of an intervention (vector control, MDA or both) on the mean prevalences and intensities of infection and disease in the target community.

Using a set of differential equations, the model describes changes by age in worm burden, intensity of microfilaraemia, immunity and prevalence of lymphatic damage, lymphoedema and hydrocele. It assumes that only the adult filarial worms cause damage to the lymphatic system and thus progression to disease. The parameters of the model were estimated using epidemiological data collected in Pondicherry [27]. The good fit of the model to these data shows that both hydrocele and lymphoedema 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 lymphoedema. The fit of the model to the data did not support the hypothesis that disease progression is immune mediated.

The model can be used to estimate probable trends in mean values (of age-specific prevalence and intensity). It does not suffer from stochastic variation and provides a quick indication of the efficacy of certain control measures. It helps users to understand the

transmission dynamics, by showing the implications of certain assumptions (e.g. about the immune system's regulation of parasite numbers) on the shape of the age–prevalence and age–intensity curves. Efforts were made to make EPIFIL a user-oriented model that can be easily linked to the user's own data-set (such as age–prevalence curves, life tables and operational indices such as the desired level of prevalence to be reached by control).

EPIFIL for prediction. The EPIFIL model has been applied to simulate the effects of four control options (5 years of vector control or five annual rounds of MDA based on DEC alone, ivermectin alone, or DEC–ivermectin) on the mean intensity of microfilaraemia over a period of 10 years (Fig. 5). The results indicate that chemotherapy has a larger short-term impact than vector control but that the effects of vector control can last longer, beyond the 5-year period of intervention [4]. Under the same compliance and treatment plans, DEC alone appeared superior to ivermectin alone in reducing community microfilarial loads, DEC or DEC–ivermectin being predicted to decrease microfilarial loads for much longer than ivermectin alone, over the 10-year period. The results indicated that, although DEC adds considerably to the benefits of ivermectin, there is relatively little benefit in combining ivermectin with DEC [4].

LYMFASIM and EPIFIL compared

There are similarities but also important differences between the LYMFASIM and EPIFIL models and between the outcomes of the models. The most important similarity is that both models describe the dynamics of the adult worms and the Mf. Compared with the classical catalytic models, which primarily describe the gain and loss of human infection, this approach is much better for testing assumptions about immune regulation and disease development [22,23]. Application of LYMFASIM or EPIFIL leads to the same conclusions: that the force of infection is age-dependent and that establishment of worms is immune regulated. The most striking difference between the models is that EPIFIL is a simple deterministic model whereas LYMFASIM is a comprehensive stochastic model. In LYMFASIM, the units of simulation are individual human hosts and parasites (micro-simulation) whereas EPIFIL is population-based (macro-simulation) and so the model outputs are individual- and population- based, respectively.

External validity of the existing models

So far, both LYMFASIM and EPIFIL have been quantified and applied using data from an urban setting such as Pondicherry. Before the models are used with data from rural areas of India and from countries other than India, further quantification and validation are required, for at least four reasons:

(1) the age-specific epidemiological patterns in rural and urban areas are expected to be different, as the establishment of infection and disease in rural areas is a relatively recent event;

(2) the relationship between human infection and mosquito infection is also expected to be different in rural areas from that in urban areas (at least in India), because the behaviour of the vectors differs;

(3) the species of mosquito acting as vector varies geographically, and in some areas more than one species is involved; and

(4) since both the models have been quantified using longitudinal data collected from a demonstration project in Pondicherry, application of the models in national control programmes, from which only cross-sectional data are usually available, needs further investigation.

Additional modelling/data analysis

Although LYMFASIM and EPIFIL render the same conclusions - that the force of human infection is age-dependent and that the establishment of the parasite in its human host is immune regulated - the mechanisms that regulate the parasite population in humans are still not clear. It is unlikely that either model will be very useful in the optimisation of control strategies or in predicting outcomes until these mechanisms are elucidated and this will not be possible without the collection and analysis of additional data.

So far, both models have been quantified and validated against data pertaining to human infection. To address several important questions that relate to public health, the disease part of each model has to be updated and quantified by including the results of recent research on the dynamics of symptomatic LF. Both the models must be improved so that aspects such as population movements (emigration and immigration), development of drug resistance in the parasite, and age-specific patterns of compliance can be considered.

Additional field research

Exposure heterogeneity

The present version of LYMFASIM indirectly quantifies the age- and sex-related exposure of humans to mosquito biting by assuming a linear, saturating relationship between the mosquito biting rate and human age and sex. Also, it is assumed that each individual differs in his or her exposure to mosquito biting according to his or her personal characteristics. The results of additional data collection and analysis may help in reducing the number of 'exposure' parameters to be estimated.

Heterogeneity in response to treatment

The results of clinical trials indicate that individuals differ in their response to treatment. It is not known whether individual response to treatment reflects parasite burden. The available field data may be utilized to explore such a relationship.

Compliance pattern

An individual's willingness to receive treatment often differs with each round of MDA. At present, a semi-systematic pattern of compliance (i.e. one that is neither systematic nor random) has been assumed. This assumption was based on observations made during ivermectin-based MDA for the control of onchocerciasis. The available field data on compliance patterns needs to be analysed and incorporated in the models.

Conclusions

LYMFASIM and EPIFIL have each been used to compare the potential effectiveness of various types and frequencies of LF-control interventions. The models should be applied to identify parameters that can be monitored, during the progress of a control programme, to assess whether the programme is achieving its targets and to allow interventions to be adapted, changed or supplemented (if and when this is considered beneficial) while the programme is running. At present, only researchers are using the models. Both models must be made more 'user-friendly', so that people in operational settings can also reap the benefit of the models and plan a more rational allocation of funds and effort in the control of LF.

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4.3 The dynamics of *Wuchereria bancrofti* infection: a model-based analysis of longitudinal data from Pondicherry, India

Summary

This paper presents a model-based analysis of longitudinal data describing the impact of integrated vector management on the intensity of *Wuchereria bancrofti* infection in Pondicherry, India. The aims of this analysis were: (1) to gain insight into the dynamics of infection, with emphasis on the possible role of immunity, and (2) to develop a model that can be used to predict the effects of control. Using the LYMFASIM computer simulation program, two models with different types of immunity (anti-L3 larvae or anti-adult worm fecundity) were compared with a model without immunity. Parameters were estimated by fitting the models to data from 5071 individuals with microfilaria-density measurement before and after cessation of a five-year vector management programme. A good fit, in particular of the convex shape of the age-prevalence curve, required inclusion of anti-L3 or anti-fecundity immunity in the model. An individual's immune-responsiveness was found to halve in ~10 years after cessation of boosting. Explanation of the large variation in Mf-density required considerable variation between individuals in exposure and immune responsiveness. The mean lifespan of the parasite was estimated at about 10 years. For the post-control period, the models predict a further decline in Mf prevalence, which agrees well with observations made 3 and 6 years after cessation of the integrated vector management programme.

Introduction

Despite availability of effective anti-parasitic treatment and other tools for control, lymphatic filariasis continues to be a major public health problem in tropical areas of Asia, Africa, the Western Pacific and parts of the Americas. More than one-third of the estimated 120 million infected people live in India [1]. There is increasing interest in applying strategies for transmission control based on mass-chemotherapy with annual single dose diethylcarbamazine (DEC), ivermectin, or a combination of either of these with albendazole [2,3]. Where feasible, vector control is recommended as an adjunct to chemotherapy based strategies [4]. Worldwide elimination of the disease as a public health problem is considered feasible [5].

To evaluate the effects of control measures, to anticipate the effectiveness of population-based interventions and to aid decision-making about control strategies, the transmission dynamics of the parasite should be well understood. Epidemiological models have proven to be valuable tools in this respect [6,7]. Various deterministic models have been used to study the dynamics of infection and disease due to *Wuchereria bancrofti* [8-16]. In

the present paper, we use the LYMFASIM [17] model, which is based on the stochastic microsimulation technique [18].

LYMFASIM offers a framework for integrating current knowledge on the dynamics of transmission. By simulating the processes and mechanisms involved in parasite development and transmission, and taking individual variation in exposure to infection into account, the model allows prediction of trends in infection prevalence and intensity over time. However, a considerable number of parameters need to be quantified. For this purpose, we use data collected by the Vector Control Research Centre (VCRC) of the Indian Council of Medical Research, for the evaluation of integrated vector management in urban Pondicherry, India [19-22]. The VCRC-database is unique in that it combines entomological and epidemiological observations and that it includes a very large sample of the population of Pondicherry (almost 25000 observations in 1981). Furthermore, the infection status of humans has been measured at 4 time points (1981, 1986, 1989, and 1992), which enables the study of longitudinal cohorts.

In this study, LYMFASIM is fitted to data for a cohort of individuals examined both in 1981 and 1986, i.e. before and after the integrated vector management programme in Pondicherry. The aim of the present analysis is 2-fold. The first objective is to provide more insight into the dynamics of lymphatic filariasis, and more specifically into the possible role of the host immune response in regulating infection. Different types of models – with and without immunity – are compared and parameters that are important for the dynamics of infection are quantified. The second objective of the study is to arrive at models that can be used to predict the effectiveness of vector control or mass treatment strategies for the control of *W. bancrofti* in Pondicherry, India. The resulting models are tested, by comparing model predictions 3 and 6 years after cessation of vector control with the actual observations.

Materials and Methods

Description of LYMFASIM

LYMFASIM is a stochastic microsimulation model for the epidemiology of lymphatic filariasis in human populations [17,18]. The model simulates the life-histories of human individuals (birth, acquisition and loss of parasites, death) and individual parasites (maturation, mating, production of Mf, death). Together, the simulated persons constitute the population of an endemic village or area. A detailed description and mathematical formulation of the model has been given in an earlier publication [17]. Here we restrict ourselves to a brief description of the model and the factors that are directly relevant to the effects of vector control. Of particular importance are the regulation of parasite density in both the vector and the human host.

Transmission and parasite dynamics

A graphical representation of the model is given in Figure 1. In this figure, the monthly force-of-infection (*foi*) indicates the number of parasites that enter the human host in a month and the proportion that develops successfully into adult worms, *sr*. The force-of-infection varies between individuals and over time; its calculation is explained below.

In the case of a constant force-of-infection, the expected equilibrium worm-load (*M*, number of mature worms) in a person is found by multiplying the force-of-infection for this person times the average reproductive lifespan (i.e. total lifespan minus duration of immature stage) of an adult parasite. The total lifespan of the worm is assumed to vary between parasites, and is described by a Weibull distribution with mean $\overline{Tl}$ and shape-parameter α_{Tl}. Estimates for the life span of *Onchocerca volvulus* (the filarial nematode species causing human onchocerciasis), indicated less than exponential variation ($\alpha_{Tl} > 1$) and hence we have fixed the value of α_{Tl} to 2.0 [23]. The duration *Ti* of the immature stage is considered to be 8 months [24]. The parasite lifespan not only determines the equilibrium worm-load, but also the rate of worm-mortality and thereby the rate at which the worm-load declines in the case of interruption of transmission.

Female adult worms produce microfilariae (Mf), provided that the human host harbours at least one adult male parasite, assuming a totally polygamous system in *W. bancrofti*. The Mf production is equal to r_0 per month per 20 μl blood per female parasite in the absence of an anti-fecundity immune response, but is reduced when the human hosts develop such a response (see below). The simulated true Mf-density, *m*, in a person is expressed in terms of the average number per 20 μl peripheral blood taken for diagnosis and is updated monthly using the number of Mf produced by each female worm per month. Mf-mortality is governed by a monthly survival fraction $s = 0.9$ for the Mf [25]. The variability (between blood samples within a host) in the actual (discrete) number of Mf counted in the smear is described by a negative binomial distribution with a mean equal to the true Mf-density in an individual and a parameter of dispersion k_m. Overdispersion will be smaller (k_m larger) when a larger volume of blood is examined (due to increased sensitivity). Due to intra-host and observer variability in Mf counts, false Mf-negative cases (count=0) can occur.

Based on experimental data, the relation between the Mf density *m* in a human and the number of L3 that will develop in *Culex quinquefasciatus* mosquitoes, the principal vector of *W. bancrofti* in Pondicherry, feeding on such a person (L3 from blood meal) is described by the following hyperbolic function [26],

$$L3 = \frac{\varphi m}{1 + \zeta m} \qquad (1)$$

with parameter values in Table 1.

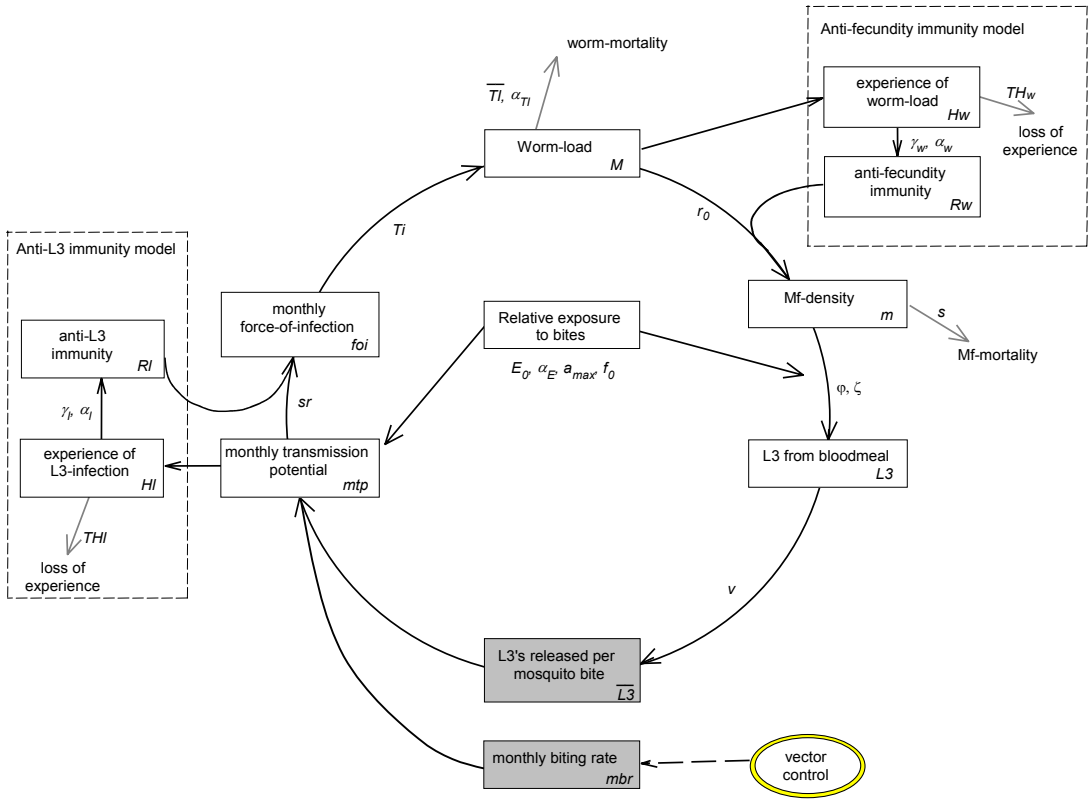

Figure 1 Schematic representation of LYMFASIM. Immune-regulation is optional in LYMFASIM. The shaded boxes are entomological variables; these variables do not vary between individuals. The unshaded boxes are human variables; the index *i* indicates that the variable may differ between individuals. Along the arrows, the symbols of relevant parameters are shown.

Table 1 Description of state variables and parameters of LYMFASIM with values compiled from field observations, experiments and the literature (expressed in months unless otherwise stated)

Parameter/Variables		Value (95% CL)	Source
mbr	Monthly biting rate	2200 per person per month	See Figure 2
v	Fraction of the L3 larvae, resulting from a single blood meal, that is relaeased by a mosquito	0.1	Fixed[1]
φ	Proportion of Mf (in 20 μl blood) developing to the L3 stage within the mosquito vector as Mf density tends to zero	0.09 (0.04– 0.24)	Subramanian *et al.* (1998)
ζ	Severity of density-dependent limitation of L3 output within the mosquito vector	0.013 (0.0025-0.0510) per microfilaria	Subramanian *et al.* (1998)
α_{Ti}	Shape-parameter for the Weibull-distribution describing the variation in the adult parasite lifespan	2.0	Fixed[2]
Ti	Duration of the immature stage of the parasite in the human host	8 months	WHO (1992)
s	Proportion of Mf surviving per month	0.9	Plaisier *et al.* (1999)
H_w	Cumulative experience of worm-load, which is a determinant of the duration of immunological memory (*THw*, see Table 2)	State variable	N.A.
R_w	Level of anti-fecundity immune response, which is a function of strength of anti-fecundity immune response (γ_w) and individual ability to elicit such a response (α_w)	State variable	N.A.
H_l	Cumulative experience of L3-infection, which is a determinant of the duration of immunological memory (*THl*, see Table 2)	State variable	N.A.
R_l	Level of anti-L3 immune response, which is a function of strength of anti-L3 immune response (γ_l) and individuals ability to elicit such a response (α_l)	State variable	N.A.

[1] Both v and sr (see Materials and Methods section) are linear multiplication factors in the same sequence of calculations. Only sr is estimated by model fitting.

[2] A value of $\alpha_{Ti} = 1$ means an exponential distribution. This is often (implicitly) assumed in mathematical models. Estimates for the lifespan of *Onchocerca volvulus* suggest less variation ($\alpha_{Ti} > 1$).

N.A. – Not applicable.

This relationship saturates at φ / ζ at high human Mf densities and has an initial slope of φ. Because of this saturation, the development of the parasite in the vector is one of the density regulation mechanisms in the transmission of the parasite.

The number of L3-stage larvae released per mosquito bite ($L3$) depends on this relationship between Mf-density in human and L3 developing in a mosquito, and also on a number of mosquito-related factors, such as the survival of the mosquitoes between the uptake of microfilariae and the development to the L3-stage under natural conditions, the fraction of mosquitoes that is potentially infectious (i.e. taking into account that some mosquitoes never had a blood meal before), and the probability that a L3-larva will actually be released during the act of biting. These mosquito-related factors have been combined in the factor *v*. Since *v* and *sr* are linear multiplication factors in the same sequence of calculations, we decided to arbitrarily fix the proportion *v* at 0.1, and estimate *sr*. The average number of infective larvae L3 released per mosquito-bite is calculated as a population average, by weighting each person with his or her relative exposure.

An individual's relative exposure to bites depends on his/her age, but there is also inter-individual variability. We assume the following relation between age and exposure: at birth a person has a relative exposure of E_0, and thereafter it increases linearly until age a_{max} at which a maximum exposure is reached, which remains at this level for the remainder of life. The variation in mosquito bites between individuals is captured by a personal 'exposure index'. This exposure index is assumed to be a life-long characteristic of a person. Its value is randomly selected from a gamma probability distribution with mean = 1 and shape-parameter α_E. This gamma-distribution allows for persons to have low or very low relative exposure, but it does not allow for zero exposure. We therefore consider an additional parameter, the fraction f_0 of persons that is never exposed to the bites of mosquitoes. We assume that males and females are equally exposed to mosquito bites.

The monthly transmission potential (*mtp*) is defined as the number of incoming L3 larvae per person per month, which varies between individuals and over time. The transmission potential is calculated as the product of the average monthly biting rate (*mbr*, number of mosquito-bites per month for an adult person), the relative exposure to bites of this person, and the average number of infective L3 released per mosquito-bite into a human host. Only a fraction of the larvae that entered the human body will survive the larval stages and develop into mature adult worms. This brings us back to the monthly force-of-infection, which depends on the monthly transmission potential, on the proportion *sr* of inoculated larvae that will survive the L3 and L4 stages, and on the individual's level of immunity to L3-larvae, which may vary between 0 (no immunity) and 1 (full immunity, no larva will survive).

Immune-regulation of parasite numbers

In LYMFASIM we assume two mechanisms for the working of the immune system on the dynamics of the parasite: anti-L3 immunity and anti-fecundity immunity. Anti-L3

Table 2 Parameters of LYMFASIM describing the transmission dynamics of *Wuchereria bancrofti* in humans and their estimated values arising from the fit of models with and without immunity. (Units are in years unless otherwise stated. The sign '-'denotes parameters that are not included in a particular model. Values in parentheses are 95% confidence boundaries for the duration of the immunological memory, success ratio and the estimates for the strength of the immune-response corresponding to lower and upper boundaries of the duration of immunological memory)

Parameter and description		Numerical value estimated (95% CL)		
		Anti-L3 immunity model	Anti-fecundity immunity model	No immunity model
sr	Success ratio: fraction of inoculated L3-larvae developing to an adult male or female worm in the absence of immune-regulation (x10^{-3})	1.03 (0.66 - 1.36)	0.42 (0.34 - 2.07)	0.58
E_0	Relative exposure at birth	0.26	0.40	0.41
a_{max}	Age at which maximum exposure is reached	19.1	21.3	19.0
α_E	Shape-parameter for the gamma-distribution describing individual variation in exposure (mean = 1)	1.13	1.14	0.93
f_0	Fraction of the population not exposed to mosquito bites	-	-	0.64
Tl	Mean lifespan of the adult parasite in the human host	10.2	11.8	9.1
k_m	Overdispersion parameter of the Negative Binomial distribution describing the variation in Mf counts in bloodsmears for an individual	0.35	0.35	0.33
r_0	No. of Mf produced per female parasite per month per 20 μl peripheral blood in the absence of immune-reactions and in the presence of at least 1 male worm	0.61	4.03	0.58
γ_l	Strength of the anti-L3 immune-response (x10^{-5})	5.89 (8.55 – 4.65)	-	-
α_l	Shape-parameter for the gamma-distribution describing individual variation in the ability to develop an anti-L3 immune-response	1.07	-	-
THl	Duration of immunological memory: period in which strength of anti-L3 immune response is halved in the absence of boosting by L3	9.60 (5.0 - 18.3)	-	-
γ_w	Strength of the anti-fecundity immune-response	-	0.026 (0.042 – 0.025)	-
α_w	Shape-parameter for the gamma-distribution describing individual variation in the ability to develop an anti-fecundity immune-response	-	1.07	-
THw	Duration of immunological memory: period in which strength of anti-fecundity immune response is halved in the absence of boosting by adult worms	-	11.2 (5.0 – 16.7)	-

immunity is triggered by exposure to L3-antigens and reduces the success of inoculated L3-larvae to mature in the human body. This mechanism is proposed on the basis of work by Day *et al.* [13,27] who found, among people followed for one year, an increase in antibodies to the L3 surface mainly in subjects aged 20 years and older i.e. subjects with the longest history of L3-inoculation. Beuria *et al.* [28] also found an age-specific increase in the presence of antibodies and further concluded that antibody levels were highly variable between individuals. Further, a recent study showed that the prevalence of antibodies to L3 surface antigens was higher among amicrofilaraemic persons with or without antigenaemia than in subjects with microfilaraemia [29]. Several other epidemiological studies also provide indirect evidence for the possible role of acquired immunity in regulating filarial infections [10,15,16,30,31]. However, the above field observations [13,27,28] corroborate the evidence from laboratory studies on cat-*Brugia pahangi* [32-36] and jird-*Acanthocheilonema viteae* [37,38] models that immunity acts against re-infection.

Anti-fecundity immunity reflects that prolonged presence of adult parasites may cause a breakdown in tolerance to the parasites, resulting in clearance of microfilariae and progress of disease [39]. Whether and to what extent the adult worms or the microfilariae are the target of this response is not yet clear. In the model we assume that the immune response causes a reduction in Mf production.

The modelling of these two types of immunity is similar (see Fig. 1), and is analogous to Woolhouse's [40] 'larval antigens, anti-larval response (LL)' and 'adult worm antigens, anti-egg response (AE)' models. In the following, those parameters referring to the anti L3-immunity and the anti-fecundity models are denoted by, respectively, attaching a suffix *l* or *w* to the corresponding symbols. Cumulative 'experience' (H) of, respectively, L3 infection (H_l) and adult worm infection (H_w) determines the level of immunity, R_l and R_w. Loss of experience is governed by THl and THw, the half-life (in years) of experience of infection in the absence of boosting. The factor γ ('strength of immunity') translates the experience of infection into an immune response (γ_l and γ_w). The immune responsiveness levels R_l and R_w vary between individuals according to a gamma-probability distribution with mean 1 and shape-parameters α_l and α_w. A list and definitions of the model variables and parameter values for which we use external sources (observations, experiments, and literature), or for which we simply fixed the value within plausible ranges is given in Table 1. Table 2 summarises the parameters estimated from fitting the models to the Pondicherry data.

Model quantification

The population of Pondicherry in 1981 is simulated by quantifying the life-table and human fertility from statistics for that year [41]. The values for the monthly biting rate (*mbr*, see Fig. 2) during the vector management programme were estimated from fortnightly collection of human landing mosquitoes in one site in Pondicherry [42]. The *mbr* was used to assess the seasonal effect on vector population and also to monitor the impact of integrated vector management. Entomological observations indicated that the vector

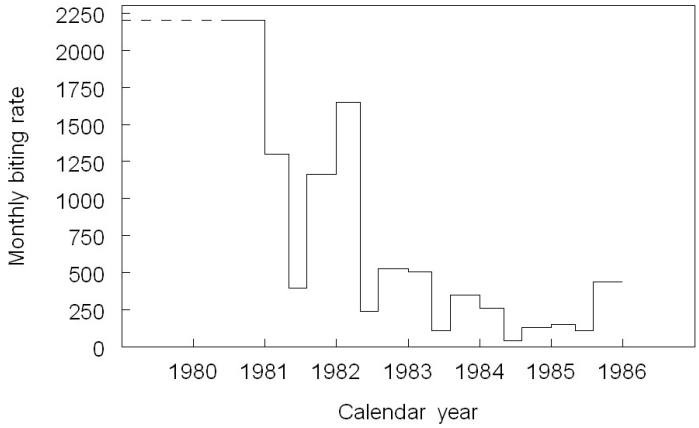

Figure 2 Observed monthly biting rate in Pondicherry over the period 1980 – 1986.

management programme has achieved a large reduction in transmission but did not achieve a total interruption [42]: within two years the annual infective biting rate was reduced by 86% and in four years by 94%; the average annual infective biting rate during the programme period was 45, compared to 228 prior to its start (80% reduction). Assuming that the observed pre-control monthly biting rate is representative for the situation in Pondicherry prior to the year 1981, we fixed the monthly biting rate at 2200 per adult person for the period before the start of vector management and after its cessation.

Simulations are always started 150 years before 1981 in order to ensure an equilibrium age-composition of the human population and a dynamic equilibrium for the parasite population. The two types of immune response are considered in separate models. The parameters for the anti-L3 immunity are estimated by assuming that there is no anti-fecundity immunity, and vice versa. Adding the possibility of no immune-regulation, we have three versions of the full LYMFASIM model: anti-L3 immunity model, anti-fecundity immunity model, and no-immunity model.

Data

Epidemiological data are from the five-year Integrated Vector Management programme in Pondicherry. Surveys were carried out right before and after the completion of the programme (in 1981 and 1986). Details of sampling design and parasitological data collection are given by Rajagopalan *et al.* [19] and Subramanian *et al.*[20]. Mf-counts in 20 μl blood smears for both 1981 and 1986 are available for a cohort of 5071 persons. To enable a comparison of simulation results with the observations, the longitudinal data are

represented as age-specific cross-tabulations of the Mf-count in 1981 versus the Mf-count in 1986 (Table 3). Data on overall Mf prevalence in 1989 and 1992 [22] are used for a first validation of the model.

Goodness-of-fit

Simulation results from the three models are compared with data for each of the cells in Table 3. The agreement between observed and simulated data is assessed by the following statistic,

$$X^2 = \sum_{a,i,j} \frac{(O_{aij} - C_a E_{aij})^2}{C_a E_{aij}(1+C_a)} \qquad (2)$$

with: O_{aij}: Observed no. of persons in age-class *a* (3-7, 8-10, etc.) of whom the Mf-count in 1981 was in class *i* (0, 1-5, or >5 Mf per smear) and the Mf-count in 1986 was in class *j*. E_{aij}: See O_{aij}, for the simulated population. C_a: O_a/E_a, with O_a total observed and E_a total simulated no. of persons in age-class *a*.

In some age-classes, cells with *i* and *j* combinations have been merged to ensure that they comprise at least 5 observed individuals. The factor $(1+C_a)$ in the denominator accounts for the stochastic variation in the simulated population (i.e., the 'expected' number is derived from a finite simulated population; with increasing simulation size, C_a approaches zero).

A *P*-value for the goodness-of-fit is calculated by assuming that X^2 follows a χ^2-distribution with D.F.= 42 for models with anti-L3 or anti-fecundity immunity, and D.F.= 44 for the model without immunity. The number of degrees of freedom is derived from the number of cells in Table 3 (72), minus the number of cells combined with other cells to ensure a minimum of 5 persons in each (combined) cell (12), minus the number of age-groups (8), minus the number of parameters to be estimated on the basis of the data (10 for the immunity models and 8 for the model without immunity). *P*-values >0.05 are taken to indicate a satisfactory agreement between estimations and observed data.

Due to the stochastic nature of the various processes involved in the model, the simulation output will be subject to random variation and will only represent an estimate of the true outcomes of the model. As a compromise between random variation and computing time for each version of the model (no immunity, anti-L3 or anti-fecundity immunity) a maximum of 1500 simulation runs was carried out and the total number of individuals per simulation run is approximately 50,000.

As a result of variability in simulation output the standard estimation procedures (e.g. maximum likelihood estimation) are not applicable. Instead parameters in Table 2 are estimated by minimizing X^2 in Eqn. (2) through a downhill-simplex routine [43]. For the immunity models, a 95%-CI was determined for the immunological memory (parameters *THl* and *THw*) and for the success ratio (parameter *sr*) following the method of Plaisier *et*

Table 3 Cross-tabulation of the observed frequencies of *Wuchereria bancrofti* microfilarial counts in 1981 and 1986 by age-group, in Pondicherry, India

Age in 1981 (Years)	Mf-count in 1981	Mf-count in 1986		
		0	1-5	6+
3-7	0	693	13	11
	1-5	7	2	3
	6+	6	4	4
8-10	0	560	10	6
	1-5	12	6	2
	6+	11	3	5
11-14	0	616	22	10
	1-5	28	6	2
	6+	17	9	8
15-19	0	462	18	9
	1-5	27	6	5
	6+	20	6	12
20-29	0	709	18	15
	1-5	34	7	10
	6+	24	10	18
30-39	0	594	15	7
	1-5	29	6	2
	6+	8	1	6
40-49	0	451	6	5
	1-5	16	5	3
	6+	9	1	9
50+	0	366	8	8
	1-5	14	1	3
	6+	5	4	3
All ages	0	4451	110	71
	1-5	167	39	30
	6+	100	38	65

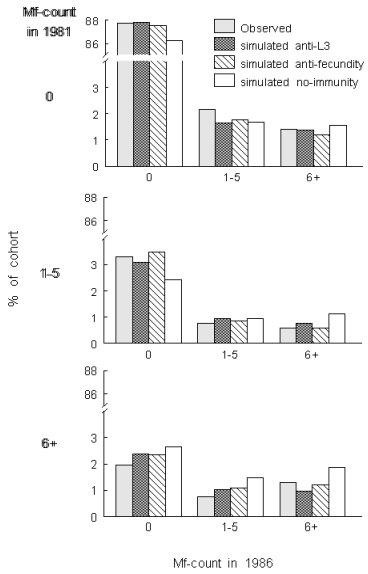

Figure 3 Observed and simulated distributions for the number of Mf per blood smear in the IVM programme in 1981 and 1986. The upper graph shows the percentages of persons that were Mf-negative in 1981 and that showed 0, 1-5 or ≥6 Mf per blood smear in 1986. The middle graphs apply to persons with 1-5 Mf in 1981, etc. Values are shown for all age-classes combined. The simulation outcomes of the 3 models with and without immunity are standardized to the age-distribution of the observed cohort.

al. [44]. Starting from the best-fitting values of these parameters, alternative lower and higher values are tested and the other parameters re-estimated. Those values that result in a X^2-difference of approximately 3.84 (95th percentile of a χ^2-distribution with D.F.= 1) are considered to be the CI-boundaries.

Results

Goodness-of-fit of models with and without immunity

Table 2 gives a complete list of the estimated parameters and their values in the different models. The two immunity models and the model without immunity have all been fitted to the cross-tabulated 1981 and 1986 Mf-counts of the people in vector management area (Table 3 and Fig. 3). Figure 3 shows the observed and predicted Mf distributions before (1981) and after (1986) vector control. Results in terms of age-specific prevalence, incidence and loss of infection are shown in Figure 4. The goodness-of-fit was satisfactory for both the anti-L3 (X^2=49.5; D.F.=42; P=0.20) and the anti-fecundity immunity model (X^2=48.8; D.F.=42; P=0.22); no good agreement with the data was obtained for the model without immunity (X^2=117.9; D.F.=44; $P < 0.001$).

The model without immunity had difficulty in fitting the relatively low pre-control Mf prevalence; a prevalence of 8.5% could only be reproduced by assuming that nearly two-thirds of the population was not exposed (f_0=0.64), which is very unlikely given the ubiquity of the mosquito vector, *C. quinquefasciatus*. Also, this model failed to reproduce the observed decline in Mf prevalence after the age of 20 (Fig. 4).

The two models with immunity show a satisfactory fit to the low overall Mf prevalence and the age-specific data on prevalence, incidence and loss-of-infection (Fig. 4). For this fit, a long immunological memory of about 10 years is needed for both the anti-L3 and the anti-fecundity model. The values of the other parameters in Table 2 will be addressed in the discussion section.

Prevalence of Mf and adult worms

Figure 5 compares the prevalence of adult (male or female) worms for the immunity models with the Mf prevalence. In both models, the worm-prevalence (dashed line) is much higher than the Mf-prevalence as determined by a blood-smear (solid line). For the anti-L3 immunity model, the main reason for this difference is the presence of single-sex infections (Fig. 5A). Production of Mf will only occur in hosts that harbour at least one female and one male worm. The percentage of persons that satisfy this condition is depicted in Fig. 5A (dot-dashed line). The difference between the proportion of people harbouring both male and female worms and the simulated Mf prevalence is mainly caused by the occurrence of negative counts at low Mf-densities because of the variability of the number of Mf counted in a blood-smear of 20 µl. The difference between adult worm-prevalence and Mf-prevalence is larger for the anti-fecundity immunity model (Fig. 5B), which is caused by the anti-fecundity response.

Confidence intervals

In estimating the confidence boundaries for the duration of immune responsiveness (*THl* and *THw)*, the remaining parameters listed in Table 2 were re-estimated for each value of the duration, optimising the goodness of fit. The sensitivity of the remaining parameter values to the value of the duration of immunological memory is as follows. The strength of immunity (parameters γ_l and γ_w) decreases approximately hyperbolically with increasing memory duration, indicating that the strength and duration compensate for each other in a multiplicative way. The values for other parameters (data not shown) listed in Table 2 remained virtually unchanged. The 95% CI indicates that neither a very short (under 5 years) nor a very long (over 18 years) duration of immunity is in agreement with the data and that the duration of immunity does not differ significantly between the two types of immunity. We also determined the confidence intervals for the success ratio (Table 2).

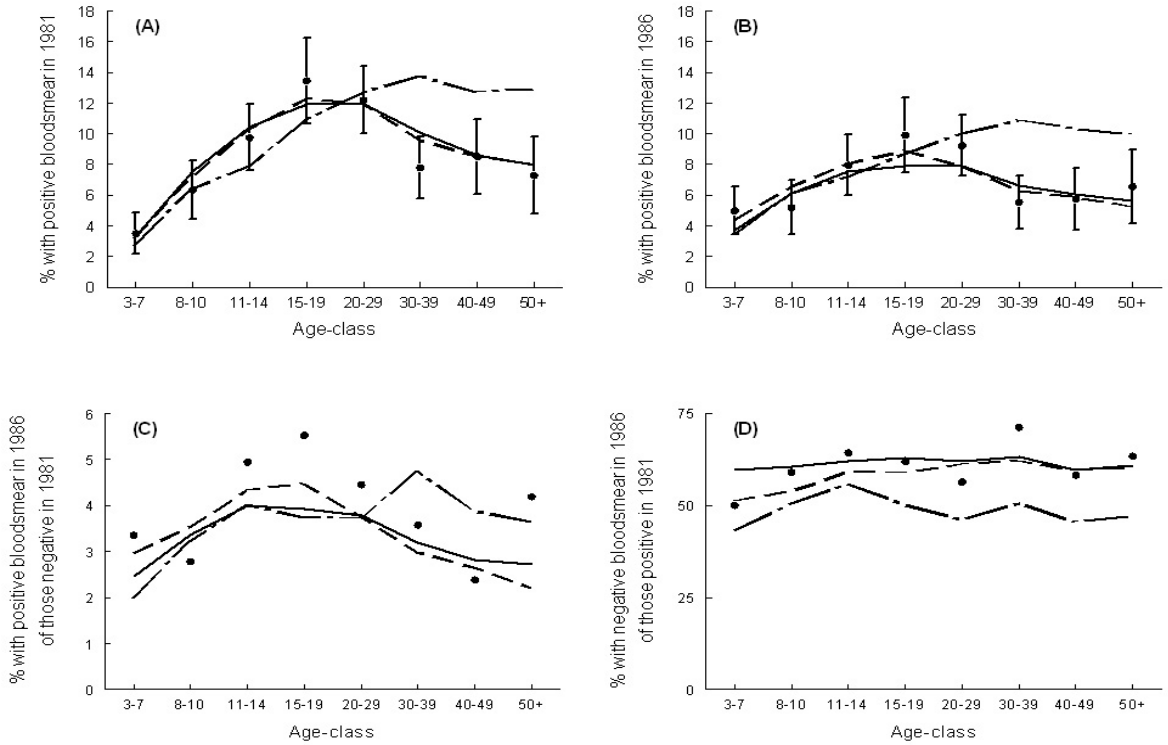

Figure 4 Observed (dots) and simulated age-specific Mf-prevalence (in 1981 and 1986, A & B respectively), incidence of infection (% of Mf-negatives in 1981 that were positive in 1986, C) and loss of infection (% of Mf-positives in 1981 that were Mf-negative in 1986, D). The solid line is the prediction with anti-L3 immunity model, the dashed line applies to anti-fecundity immunity model, the dot-dashed line to model with no-immunity and the bars are 95% confidence limits for the prevalence calculated using normal approximation to binomial distribution.

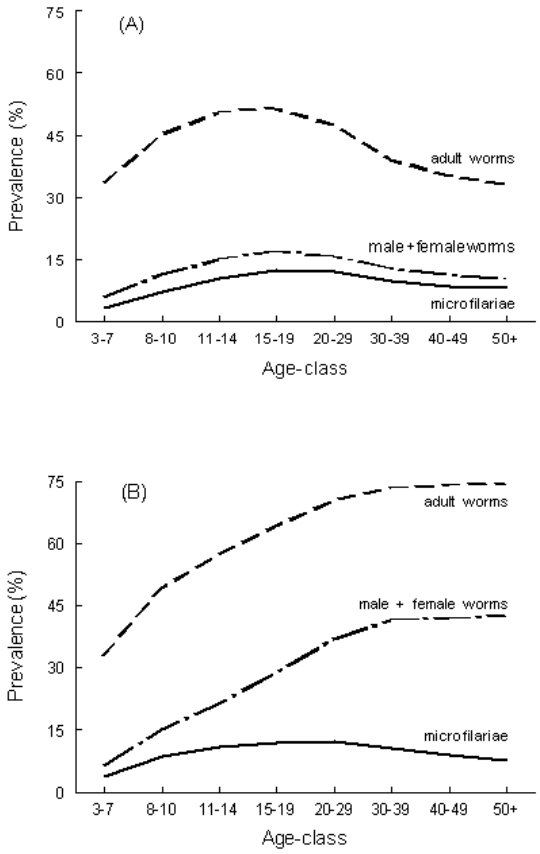

Figure 5 Simulated age-specific Mf-prevalence (in 1981; solid line), prevalence of persons with at least one adult worm (dashed line) and prevalence of persons with at least one male and at least one female worm (dot-dashed line). Predictions of the models with anti-L3 (A) and anti-fecundity immunity (B).

Long-term predictions

In order to explore the predictive validity of the immunity models, the trends in prevalence after cessation of the vector control are also assessed (Fig. 6). The observations (circles) are for the entire surveyed population in 1981, 1986, 1989 and 1992 in the integrated vector management area. The predicted prevalence is standardized to the age-distribution in the 1981 population. Both models predict the continuing down-going trend during the first few years after cessation of vector control, but the anti-L3 immunity

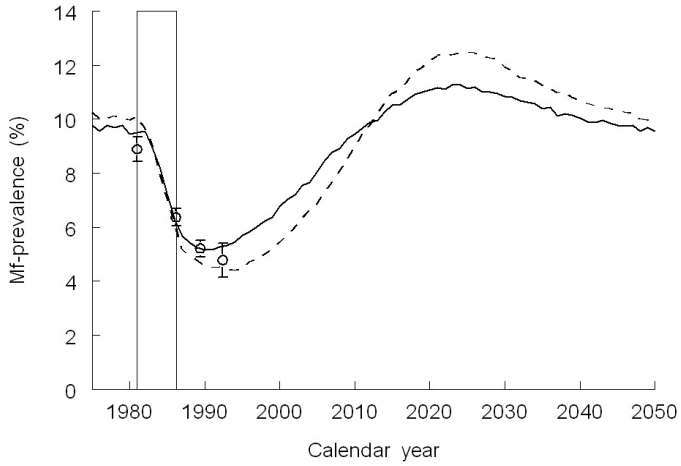

Figure 6 Predicted and observed trend in the Mf-prevalence (% of persons with a positive blood smear). Lines: predicted Mf-prevalence for the anti-L3 immunity model (solid line) and anti-fecundity immunity model (dashed line). Circles: observed prevalence levels in 1981 (8.9%), 1986 (6.4%), 1989 (5.2%) and in 1992 (4.8%). Window bar highlights the duration of the integrated vector management programme (1981-1986). Bars are 95% confidence limits calculated using normal approximation to binomial distribution.

gives the most accurate prediction. There are no data to check the long-term model prediction of a rapid increase in prevalence.

Discussion

In this paper we analysed longitudinal data describing the impact of a 5-year integrated vector management programme on the intensity and prevalence of *W. bancrofti* infection in Pondicherry, India. The analysis helped us to gain further insight into the dynamics of the parasite in the human host. Emphasis was put to arrive at plausible estimates for the duration of immunological memory. Further, the analysis rendered a model that can be used for evaluation and prediction of the effects of vector management and other control measures including mass chemotherapy.

Immune-regulation of parasite numbers

Immune regulation in lymphatic filariasis is complex [45,46], and it is not yet known how the immune system regulates parasite density in the human host. To cover this uncertainty, we considered two immunity-models that have been proposed for helminth infection by Woolhouse [40], i.e. anti-L3 and anti-fecundity immunity.

Immune regulation appeared essential in describing the observed Mf-distribution in Pondicherry. A model without immunity failed to explain the decreasing prevalence levels in older age groups. Our conclusions on the role of acquired immunity critically depend on the ability of the models to explain the observed age-specific data. As was shown in previous studies, models with immunity can reproduce a peak in the age-prevalence curve [40,47]. The position of the peak-age, its height, and the declining trend after the peak depend on the present and past transmission intensity, the worm lifespan, the strength of the immune response and the duration of the immunological memory [6].

The data from Pondicherry did not allow us to distinguish between the two types of immunity: both models could reproduce the observed data on Mf prevalence and intensity equally well. The anti-L3 type of immunity was found compatible with cross-sectional data from Pondicherry and other areas [15,16,27,28,48], and is supported by data from animal infection experiments [34,35,49]. The anti-fecundity immunity assumption has not previously been applied in lymphatic filariasis, and it remains to be seen whether it could also explain the results obtained in the above-mentioned studies.

Because the two immunity models predicted different age-specific patterns of adult worm-prevalence, an indication of their suitability to mirror observations could be obtained by comparing predicted adult worm prevalence with observed prevalence of circulating filarial antigen. The latter reflects the presence of live adult worms by detecting the presence of their excretory /secretory antigens. The Pondicherry dataset does not include data on antigenaemia, since this test was not available at the time of data-collection, but several other studies present age-specific data on Mf and antigen prevalence [50-56]. These studies generally show a much higher prevalence of antigenaemia than of microfilaraemia, although the patterns of Mf and antigen prevalence by age are more or less similar. These observations are more consistent with the results of the anti-L3 immunity model than with the results of the anti-fecundity immunity model (Fig. 5).

Our analysis suggests that the decay of immunity after interruption of transmission is slow: it takes about 10 years to reduce the "experience of infection" by 50%. How this translates into levels of herd immunity depends on the pre-control level of immunity in the population and the variation between individuals [6].

Alternative explanations of a convex pattern of infection intensity by age are possible, such as a decrease in exposure to infection in older groups [47,57] or mechanisms that reduce the probability of an incoming larva to develop into mature adult worms at older ages [35]. These alternative mechanisms have not been examined in this paper, since most studies have stressed the possible role of acquired protective immunity [13,27,28,39,58-61].

Lifespan

Our analysis also yielded an estimate of the lifespan of *W. bancrofti* in the human host. The mean lifespan of *W. bancrofti* in the human host was estimated to vary between 10 and 12 years in the present study, including the 8-month immature period. These estimates lie within the range of previous estimates, which varied from 8 to 15 years [8,62-67], but is about

twice as high as the estimate by Vanamail *et al.* [10,68], which was based on the same data. The reason for our longer lifespan estimate is that we took the possibility of false negative counts in 1981 and 1986 into account. What naively is counted as loss or acquisition of infection between 1981 and 1986 is often the consequence of false negative counts. By neglecting the possibility of false-negatives, Vanamail *et al.* [10,68] estimated a short duration in view of the observed high frequency of apparent loss and acquisition of infections.

Individual variation

A good fit of the immunity models to the data was achieved only by assuming considerable between-person variability in exposure to the vector and in immune response, and by allowing for sampling variation in the number of Mf counted in a 20 μl night blood sample at a given true Mf-density [69]. A significantly worse fit is obtained if one of these sources of variation is ignored.

The existence of exposure variation has been demonstrated by a study in Egypt, which revealed a positive association between the presence of microfilaraemia and residing in houses located near vacant land where *Culex* biting rates were higher [70]. Recent results also suggest wide inter-individual variation in exposure to mosquito bites, as measured by matching mosquito blood meals with human blood samples using the polymerase chain reaction (PCR) technique [71]. Our estimate suggests that the monthly biting rate in Pondicherry for individuals aged ≥20 years could vary between 100 and 4000.

Mf-counts are highly variable between smears from an individual. This variability in Mf counts can result from several sources: variations in blood sampling time [69,72], short-term variation in Mf-density [73-75], sampling variability [30,69,72,76-80], and variability in counting Mf. An important implication is that the false negative rate is a function of the Mf-density (the mean of the distribution of Mf in an individual). In terms of our estimated k_m (0.33) value, and for persons with (true) mean densities of 5, 10 and 20 Mf/20 μl, the probability of finding (false) zero Mf-counts according to the negative binomial distribution would be 40, 32 and 26%, respectively ($p(0) = [1 + m / k_m]^{-k_m}$). Analysis of Mf frequency distributions among human populations in Pondicherry showed that about 5% of the Mf-negatives were in fact false-negatives, and that the proportion of false negatives varied between 5 and 20% for different age-classes [30]. Thus, the potential for false negative counts may be considerable [79].

Long-term predictions

The model predictions are in agreement with the observations during the first few years after cessation of control, especially those of the anti-L3 immunity model (Fig. 6). In a sensitivity analysis, it appeared that the post-control results could also be predicted with a slightly longer immunological memory for the anti-L3 model and a shorter memory for the anti-fecundity model. Otherwise, the predictions become inaccurate. Because entomological observations suggest that stopping the integrated vector management

programme has resulted in a return of the vector to pre-control densities [21], we assumed that from 1986 onwards the *mbr* returned to the pre-control level of 2200 bites/adult/month. The most striking difference between the models is the more pronounced decline and subsequent increase in Mf prevalence predicted by the anti-fecundity immunity model. Both models predict that about 25 years after cessation of vector control the Mf-prevalence would reach the pre-control level of 1981. After this period the prevalence continues to increase beyond the pre-control level returning to this level after about 65 years. Although long-term predictions with a model that is based on 5 years of observations should be considered with caution, the predictions do illustrate the impact of loss of immunity by showing this (damped) oscillation. The higher peak with the anti-fecundity immunity model is not surprising if we realize that, as a result of a reduced transmission, many persons will have lost all their worms and, hence, boosting will be completely interrupted in these persons. Also, the reduced transmission may result in a much longer period before a newborn child acquires his/her first worm, i.e. the moment that the build-up of immunity starts. In anti-L3 immunity model, boosting (rate of inoculation of L3-larvae) is not interrupted but reduced to lower values and this reduction applies to all individuals in the population.

Model validation and generalisation

The next step in the development of LYMFASIM is to validate the fitted models. Necessarily, the model is a simplified representation of reality and several aspects related to transmission of infection in a dynamic population have not been considered, such as mobility of the human and vector population or focality of transmission.

We focussed on the role of acquired immunity in regulating infection intensity in the human host. Two alternative immunity models were in agreement with the longitudinal data from Pondicherry. To assess the validity of these models and their implication for the role of immunity, it is necessary to test the models against independent data sets from a range of endemic areas. Such a study is also necessary because of the different epidemiological patterns observed in Pondicherry and in other areas. In Pondicherry, the prevalence and intensity curves depict a convex relationship with age (monotonic increase over the age range 0-20 years and a declining trend in adults). In many places, though, the age-prevalence curves are better described by a saturating non-linear pattern (increasing in children until a stable prevalence is reached at adult age, see for example, [50,81,82-86]). While the convex-pattern is suggestive of the role of acquired immunity or a decrease in exposure with increasing age, the saturating non-linear pattern could merely reflect the balance between gain and loss of infection due to natural death of parasites or age-dependent exposure levels until at adult age the exposure level is constant [57].

Application of LYMFASIM to other areas would demand adaptation to the local epidemiological situation, taking differences in the vector-parasite combination and individual heterogeneity in exposure to mosquito biting into account. *C. quinquefasciatus* is

the principal vector of *W. bancrofti* infection in Pondicherry. The non-linear saturating relationship between numbers of *W. bancrofti* L3 developed in *C. quinquefasciatus* and human Mf-density is one of the important regulating mechanisms considered in LYMFASIM. Therefore application of LYMFASIM to other areas where the vector or parasite species are different would require re-quantification of this relationship. If the parasite species is the same but the vector is different, most of the parameters describing the dynamics of parasite in human (success ratio, Mf-production, variation in smear count, lifespan of the parasite) may not differ very much. Heterogeneity in exposure to mosquito biting is expected to vary between areas, and hence would have to be re-quantified.

Conclusion

In order to explain the dynamics of *W. bancrofti* infection in Pondicherry, immune regulation and inter-individual variations in both exposure and immunity are necessary. Our analyses rendered quantified models that can be used to prospectively evaluate the effectiveness of various control strategies. Indeed, the models have already been used to simulate the effects of mass treatment programmes in Pondicherry and to assess the probability of elimination in relation to population coverage and the number of treatment rounds [87]. The robustness of the model in other situations has yet to be assessed, as the urban Pondicherry epidemiological pattern may not be applicable.

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4.4 Prospects for elimination of bancroftian filariasis by mass drug treatment in Pondicherry, India: a simulation study

Summary

LYMFASIM, a microsimulation model for transmission and control of lymphatic filariasis, was used to simulate the effects of mass treatment, in order to estimate the number of treatment rounds necessary to achieve elimination. Simulations were performed for a community that represented Pondicherry, India, and that had an average precontrol microfilariae (Mf) prevalence of 8.5%. When ivermectin was used, 8 yearly treatment rounds with 65% population coverage gave a 99% probability of elimination. The number of treatment rounds necessary to achieve elimination depended to a large extent on coverage, drug efficacy, and endemicity level. Changing the interval between treatment rounds mainly influenced the duration of control, not the number of treatment rounds necessary to achieve elimination. Results hardly changed with alternative assumptions regarding the type of immune mechanism. The potential impact of mass treatment with a combination of diethylcarbamazine and albendazole is shown under different assumptions regarding its efficacy. Human migration and drug resistance were not considered. Results cannot be directly generalized to areas with different vector or epidemiological characteristics. In conclusion, the prospects for elimination of bancroftian filariasis by mass treatment in Pondicherry seem good, provided that the level of population coverage is sufficiently high.

Introduction

Lymphatic filariasis currently affects >128 million individuals worldwide, with 43 million people suffering from chronic lymphedema or hydrocele [3,4]. In 1997, the 50th World Health Assembly passed a resolution to eliminate lymphatic filariasis as a public health problem [5]. The main strategy for reaching this goal is interruption of transmission, through annual mass treatment with antifilarial drugs, combined with individual management of patients, to improve the condition of individuals suffering from chronic disease due to infection [6].

Mass treatment aims at reducing the microfilariae (Mf) load in the population, thereby reducing both Mf-uptake by mosquitoes and transmission of infection. Several studies have shown that mass treatment with a single dose of diethylcarbamazine, ivermectin, or a combination of these drugs leads to a strong reduction in the prevalence and intensity of Mf [7-14]. Although the results of community-based trials are promising, the number of treatment rounds in these studies is usually limited. Therefore, it is uncertain whether continuation of mass treatment would lead to elimination. In a *Wuchereria bancrofti*–positive locality in Brazil, lymphatic filariasis was virtually eliminated after 7 years

of 6-monthly mass treatment [15], whereas in French Polynesia transmission continued despite long-term intensive control [16,17].

In view of the worldwide initiation of programs to eliminate lymphatic filariasis, it is crucial to have an indication of the number of treatment rounds necessary to achieve elimination. To get a first indication, we used the mathematical simulation model LYMFASIM, which simulates the dynamics and transmission of lymphatic filariasis [18]. The model had been quantified previously to mimic the life cycle of *W. bancrofti* transmitted by *Culex quinquefasciatus* and to represent the endemic situation in Pondicherry, India [19]. In the present study, using the same model quantification, we simulated the effects of mass-treatment programs and assessed how the probability of elimination depends on the population coverage and the number of treatment rounds.

Predicting the impact of mass treatment requires quantitative estimates of the efficacy of treatment, distinguishing between the killing of Mf, the killing of adult worms, and a permanent or temporary fecundity reduction in the surviving female worms. As yet, such estimates have only been published for ivermectin [2]. Therefore, we focused our analysis on the impact of mass treatment with ivermectin (200-μg/kg body weight).

In our baseline analysis, we calculated the probability that elimination could be achieved by mass treatment with a 200-μg/kg dose of ivermectin, and we predicted how many treatment rounds would be necessary to achieve a 99% probability of elimination. In a sensitivity analysis, we assessed the impact of uncertainty in estimates of efficacy of treatment. We also tentatively predicted how many treatment rounds would be necessary to achieve elimination when the population was treated with either a higher, 400-μg/kg dose of ivermectin or with the combination of diethylcarbamazine and albendazole, which is currently recommended for use in mass treatment in India. For mass treatment with a 200-μg/kg dose of ivermectin, we further investigated how the results change when variation in efficacy of treatment is taken into account, and we studied the impact of changes in the interval between treatment rounds and in transmission intensity.

Methods

LYMFASIM

LYMFASIM simulates the transmission and control of *W. bancrofti* in a dynamic population over time. A detailed description of the structure of the model has been published elsewhere [18]. Here we restrict ourselves to a brief description. (A more detailed description of the basic transmission model is provided in Section 4.3, which includes a complete list of parameters and their quantification. Also see Appendix B for details of demographic parameters and their quantification).

The transmission model. LYMFASIM is based on stochastic microsimulation. The model simulates life histories of human individuals, which, considered together, constitute a dynamic population that, because of the birth and death of individuals, changes over time.

During their lifetimes, individuals gain and lose infections. Human individuals differ with respect to exposure to mosquitoes, age at death, ability to develop immune responses, inclination to participate in mass treatment, and responsiveness to treatment. Consequently, infection intensity varies between humans.

Transmission is mimicked by modeling both exposure to mosquitoes and the life cycle of the parasite. Exposure to mosquitoes increases with the age of the human host, until maximum exposure is reached at ~20 years of age. The model mimics uptake of Mf by biting mosquitoes, the development of Mf to L3 in the vector, the release of L3 larvae when a mosquito bites, the development of L3 larvae into adult worms in the human host, and the Mf production by adult female worms after mating.

Both the development of parasites and their fecundity in human individuals can be influenced by host immune responses. In the present study, we consider two alternative immune mechanisms -anti-L3 immunity and antifecundity immunity. Anti-L3 immunity is triggered by incoming L3 larvae and reduces the probability that incoming larvae develop into adult worms; antifecundity immunity is triggered by the presence of adult worms and reduces the Mf production per female worm. In the absence of boosting, both types of immunity diminish, which can be interpreted as loss of immunological memory.

The predicted Mf prevalence in the model is based on Mf counts for all individuals in the population. Mf counts reflect values that would have been measured by microscopic examination of a 20-μl smear of finger-prick blood taken at night; sampling variation in individuals' Mf counts is taken into account and may result in false negatives.

Elsewhere, we have reported the quantification of the basic transmission model for Pondicherry, India, where *W. bancrofti* is transmitted by *C. quinquefasciatus* and where the precontrol Mf prevalence is ~8.5% [19]. As far as possible, this quantification was based on knowledge from the literature, observed data, and expert opinion: for example, the mosquito-bite rate for an adult human was assumed to be 2200/month [19], the demographic parameters were directly quantified on the basis of census data [1], and the average Mf life span and the duration of the prepatent period of adult worms were assumed to be 10 and 8 months, respectively [2,20]. For 2 variants of the model -one including anti-L3 immunity and the other including antifecundity immunity -values for biological parameters that could not be directly quantified were estimated by fitting the model to longitudinal data from urban Pondicherry (a model without immunity could not be fitted to these longitudinal data); in this way, in both variants of the model, the life span of adult worms was estimated to be >10 years, and the half-life for immunological memory in the absence of boosting was estimated to be ~10 year. The 2 model quantifications that were obtained for Pondicherry when either anti-L3 immunity or antifecundity immunity was assumed were used in the present study.

Simulation of the effects of mass treatment. The effectiveness of mass treatment depends on the assumed efficacy of the treatment regimen. Quantitative estimates of the efficacy of treatment with ivermectin are taken from a meta-analysis by Plaisier *et al.* [2]. This meta-

Table 1 Quantification of efficacy of different treatment regimens used in the baseline simulation experiment and sensitivity analysis

Treatment	Mf killed	Fecundity reduction	Adult worms killed
Baseline simulation experiment			
Ivermectin (200 μg/kg)[a]	1	0.77	-
Sensitivity analysis			
Uncertainty in fecundity reduction, 200-μg/kg dose of ivermectin			
95 % Confidence interval			
Lower boundary[b]	1	0.64	-
Upper boundary[b]	1	0.85	-
Minimum estimate[c]	1	0.39	-
Maximum estimate[d]	1	0.91	-
400 μg/kg dose of ivermectin[a]	1	0.92	-
Combination: diethylcarbamazine plus albendazole[e]			
1	1	-	0.50
2	1	-	0.75

Note: Data are decimal fractions. Mf, microfilariae
[a] Estimate from meta-analysis assuming an Mf lifespan of 1-year[2]
[b] An Mf lifespan of 1 year is assumed [2]
[c] An Mf lifespan of 2 years is assumed [2]
[d] An Mf lifespan of 6 months is assumed [2]
[e] Assumptions are as explained in the LYMFASIM subsection in the main text.

analysis used a simple deterministic simulation model to analyze trends in Mf density and to estimate efficacy of treatment. The results suggest that a 200-μg/kg dose of ivermectin kills virtually all Mf and also irreversibly reduces net Mf production in treated individuals. Such a reduction in net Mf production could result from different mechanisms -for example, the killing of fertile adult worms or a fecundity reduction in the female worms; the simple model cannot distinguish between these different mechanisms. Because a macrofilaricidal effect could not be demonstrated for ivermectin [21], we assume that the irreversible productivity loss is due to a permanent fecundity reduction in the female worms. The quantitative estimates of this fecundity reduction were found to depend to a large extent on assumptions regarding Mf life span [2]. In our baseline simulations, we use the point estimate for efficacy of a 200-μg/kg dose of ivermectin that is obtained under the assumption of a 1-year Mf life span; in our sensitivity analysis, we consider a range of

other quantifications, which take into account the uncertainty in this estimate (see Table 1).

The meta-analysis does not provide insight into the amount of variation in efficacy of treatment. In our baseline quantification for a 200-μg/kg dose of ivermectin, we assume that efficacy of treatment is constant. In the sensitivity analysis, we consider the impact of variation in fecundity reduction that is caused by treatment. We assume that this variation is described by a β distribution with a mean of 0.77 (equal to the constant efficacy in our baseline quantification) and an SD of 0.2. Variation occurs randomly either between treatments (inter-treatment variation) or between individuals (inter-individual variation). In inter-treatment variation, the proportion of the fecundity reduction is randomly drawn from the β distribution whenever someone is treated, independent of the individual being treated. In inter-individual variation, the per-treatment proportion of the fecundity reduction is randomly drawn from the β distribution for each individual, but an individual will always have the same response; consequently, treatment may always have poor efficacy in some individuals but complete efficacy in others.

Ivermectin alone probably will not be used in mass-treatment programs in India; for this region, a combination of diethylcarbamazine and albendazole is recommended [22]. Evidence of the efficacy of this combination regimen is still limited [23-29], and quantitative efficacy estimates are not yet available. However, this combination is expected to have macrofilaricidal effect: the macrofilaricidal efficacy of diethylcarbamazine has been proven [30-32] and may be further enhanced by albendazole, which, when given in high doses, seems to have macrofilaricidal efficacy of its own [33]. We assumed that such a treatment kills a constant proportion - either 50% or 75% - of (male and female) adult worms and kills 100% of Mf.

The effectiveness of mass treatment also depends on both the population coverage and individuals' compliance with treatment over time. Population coverage is defined as the percentage of the total population that receives treatment and is assumed to be the same in all treatment rounds, although not always the same individuals are treated. We assume a "partial systematic" compliance pattern [18]. Each individual has a certain inclination to attend mass-treatment programs: some persons will attend most treatment rounds, others hardly any; a random mechanism determines whether the individual actually attends. This mechanism was found to give a fair representation of the attendance pattern in a mass-treatment program for onchocerciasis in Asubende, Ghana [34].

Simulation experiments

Each simulation starts with a "warming-up" period, during which the population grows to an average size of ~3700 persons and a more or less stable endemic situation develops. After this warming-up period, mass treatment is introduced into the simulation. Since LYMFASIM is a stochastic model, repeated simulations never give exactly the same

results, even when the input is exactly the same. When the model quantifications for Pondicherry are used, the approximate variation in precontrol prevalence (just before the first treatment) is 4%–11%, whereas10% of the simulations may produce values that are more extreme. Similarly, the effects of mass treatment may differ between runs. To deal with this stochastic variation in the output, large series of runs are performed, and standard statistical techniques are used to analyze the simulation results.

In a baseline-simulation experiment, we assessed the effectiveness of yearly mass treatment with a 200-μg/kg dose of ivermectin and compared the outcomes of the 2 immunity variants of the model -anti-L3 immunity and antifecundity immunity. A large series of simulation runs (n=5550) is performed for each of the 2 immunity variants. Within each series of runs, we varied the population coverage (10%–100%) and the number of treatment rounds (1,2,…,15) and kept all other assumptions the same. We stored the simulation results for further analysis, recording for each run the precontrol Mf prevalence and whether infection was eliminated (i.e., zero Mf prevalence 40 years after the first round of mass treatment). In some simulation runs, infection disappeared by chance during the warming-up period; when the precontrol Mf prevalence was ≤1.0%, a run was excluded from further analysis.

In a sensitivity analysis, we performed a number of series of 5550 simulation runs, using different assumptions. First, we studied the impact of uncertainty regarding the estimated fecundity reduction after treatment with a single dose of 200−μg/kg dose of ivermectin. Next, we investigated the impact of assuming random or inter-individual variation in responsiveness to treatment. We also explored the effectiveness of mass treatment with a higher, 400−μg/kg dose of ivermectin and with a combination of diethylcarbamazine and albendazole. In addition, the impact of changing the interval between subsequent treatment rounds to 6 months or 2 years was studied. Last, we assessed the impact of transmission intensity or endemicity level. In the model, endemicity is largely determined by the monthly biting rate: a higher biting rate results in higher transmission intensity and, consequently, in higher prevalence and greater intensity of infection. We changed the mosquito-bite rate of 2200/person/month by ±10% and ±25% -that is, to1650, 1980, 2420, and 2750.

Statistical analysis

The results of each series of simulation runs were analyzed by means of the Statistical Package for the Social Sciences program(SPSS version 9), by logistic regression, to predict the probability of elimination in relation to the population coverage and the number of treatment rounds. For the question at hand, the resulting statistical model can be regarded as a summary of the relation between LYMFASIM input and output. Because the simulated precontrol Mf prevalence varied between runs and may confound the relationship, we included this term in the logistic-regression equation. To determine which variables and interaction terms had to be included in the equation, we fitted several alternative equations to results from our baseline-simulation experiment. We considered

different transformations for the population coverage and for the number of treatment rounds, with the condition that the resulting equation would describe a continuous increase in the probability of elimination with higher population coverage and with a larger number of treatment rounds. The most parsimonious model that gave a good fit to the simulation results of our baseline-simulation experiment is given in equation (1); the fit of the equation could not be improved by including higher-order terms (likelihood-ratio test). The following logistic-regression equation was used to analyze and summarize all simulations results, with the βs being estimated separately for each series of runs:

$$Y = \beta_0 + \beta_1 prev + \beta_2 c + \beta_3 \ln(n) + \beta_4 c \ln(n) \qquad (1)$$

where Y is the logit transformation of the probability that elimination will not be achieved in a simulation, β_0–β_4 are the estimates of the coefficients in the regression model, "prev" is the precontrol Mf prevalence, c is the population coverage, and n is the number of treatment rounds.

To check whether the resulting logistic-regression models adequately summarize simulation results in our baseline-simulation experiment, we compared the predicted probability of elimination by logistic regression against the proportion of 100 repeated simulation runs that resulted in elimination: for each combination of population coverage (40%, 50%, 65%, 80%,and 90%) and number of treatment rounds (2,4,…,12), we performed 100 runs with exactly the same input and calculated the 95% confidence interval (95%CI) for the proportion of runs resulting in elimination [35].

The logistic-regression equations were numerically solved by Microsoft Excel Solver, to find the population coverage and the number of treatment rounds that give a 1% probability that elimination would not be achieved -or, equivalently, a 99% probability of elimination. A precontrol Mf prevalence of 8.5% was entered into the formula; this was the average precontrol prevalence from the simulations in our baseline-simulation experiment, which corresponds to the observed precontrol Mf prevalence in Pondicherry [36]. Only when we analyzed the impact of endemicity level did we use the average Mf prevalence of the series of simulations for a specific monthly biting rate and immunity model.

Results

Baseline-simulation experiment

Figure 1 shows the probability of elimination after yearly mass treatment with a single 200-μg/kg dose of ivermectin, for both the anti-L3 variant of the model and the antifecundity variant of the model. The corresponding regression equations are given in Appendix A. The predictions of logistic regression matched well with the results of 100 repeated runs, for several combinations of population coverage and number of treatment

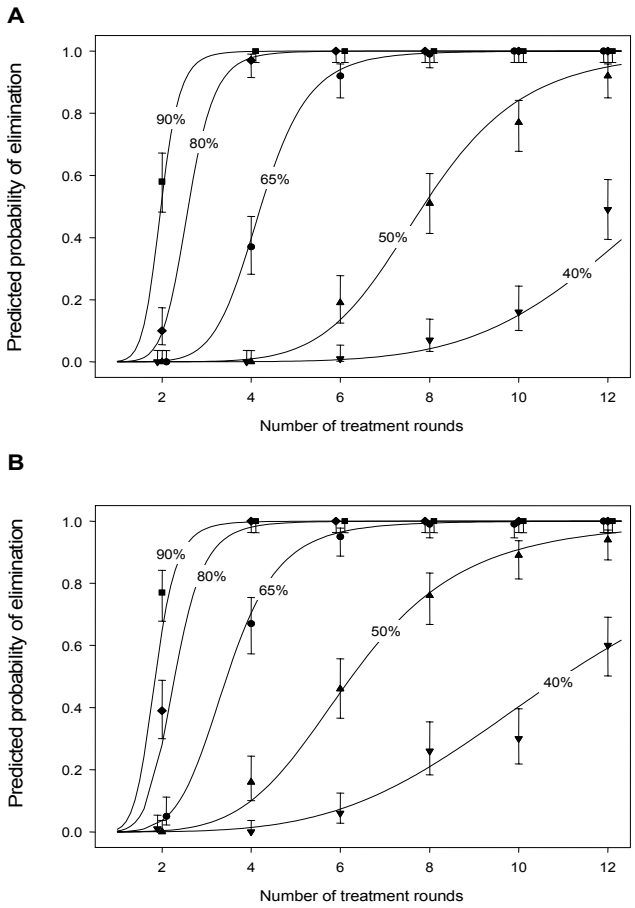

Figure 1 Probability of elimination, in relation to the population coverage and the number of yearly rounds of mass treatment with a 200-μg/kg dose of ivermectin, for the anti-L3 (A) and antifecundity (B) variants of the model. The curves indicate the probability of elimination as predicted by the logistic regression model (see Appendix A). Each symbol indicates the proportion of 100 repeated runs that resulted in elimination for a specific combination of each population-coverage proportion (40%[▼], 50%[▲], 65%[●], 80%[◆] and 90%[■] and 2, 4, 6, 8, 10, and 12 yearly treatment rounds; the vertical bars indicate the 95% confidence intervals. To be able to differentiate these confidence intervals for different population-coverage levels when curves overlap, several points have been displayed slightly to either the right or the left of the exact number of treatment rounds.

rounds. With high population-coverage levels of 80%–90%, a few rounds of mass treatment already give a high probability of elimination. When the population coverage in each treatment round is low (40%–50%), many rounds of mass treatment will be

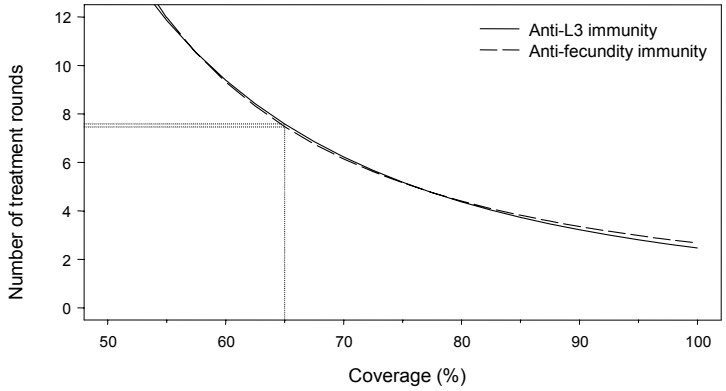

Figure 2 Number of yearly treatment rounds, with a 200–μg/kg dose of ivermectin, and the population coverage that are necessary to achieve a 99% probability of elimination under baseline assumptions for anti-L3 (*unbroken line*) and antifecundity (*broken line*) immunity. The "drop lines" (i.e., the fainter, intersecting horizontal and vertical lines) indicate the number of treatment rounds that would be necessary to achieve a 99%probability of elimination, when the population coverage is 65%, calculated by solving the regression equations of Appendix A.

necessary to achieve a high probability of elimination. Inspection of Figure 1A and 1B shows that the results for the anti-L3 variant were not much different from those for the antifecundity variant.

The number of yearly treatment rounds, with a 200-μg/kg dose of ivermectin, and the population coverage that are necessary to achieve a 99% probability of elimination are shown in Figure 2. For both the anti-L3 variant of the model and the antifecundity immunity variant of the model, the predicted probability of elimination has reached 99% after 8 rounds of mass treatment with ivermectin when coverage is 65%.

Sensitivity analysis

The results of the sensitivity analysis, for a population coverage of 65%, are summarized in Figure 3. We found differences between the 2 types of immunity and have presented the results separately for the 2 models. The horizontal lines in the figures represent the results of the baseline simulations. The symbols indicate the number of treatment rounds necessary to achieve a 99% probability of elimination, under alternative assumptions.

The estimated number of treatment rounds was strongly influenced by uncertainty in the estimated fecundity reduction. In the best case, 7 treatment rounds were sufficient; in the worst case, 15 or 16 treatment rounds were necessary to achieve elimination,

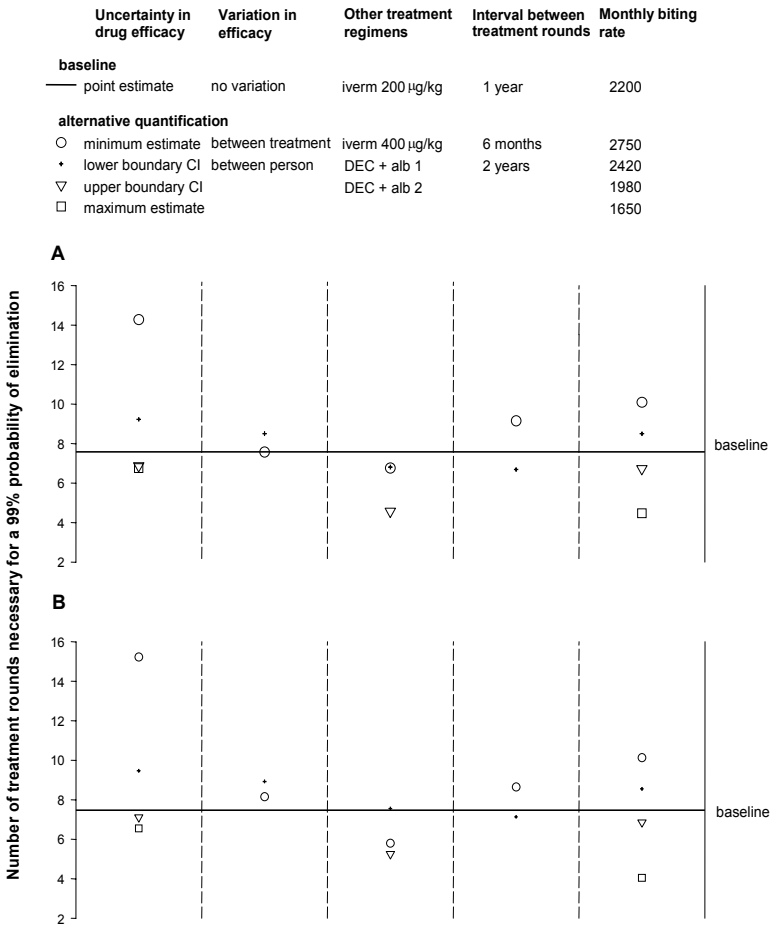

Figure 3 Sensitivity analysis of the number of mass-treatment rounds necessary to achieve a 99% probability of elimination, when the population coverage is 65%, for the anti-L3 (*A*) and antifecundity (*B*) immunity models. The horizontal lines indicate the baseline situation and correspond to the values indicated by the drop lines in Figure 2. The symbols indicate the number of treatment rounds necessary to achieve elimination when one of the assumptions is changed; the way in which assumptions were changed is noted at the top of each column. For quantifications of treatment efficacy (pertaining to the "Uncertainty in drug efficacy," Variation in efficacy," and "Other treatment regimens" sections of the figure), see Table 1; for corresponding values for precontrol Mf prevalence, see the "Sensitivity Analysis" subsection in the main text. alb, albendazole; CI, 95% confidence interval; DEC, diethylcarbamazine.

depending on the type of immunity. The results were somewhat less favorable when variation in the efficacy of treatment was taken into account, and this was especially true when the response to treatment in some individuals was systematically lower response than that in others (i.e., inter-individual variation).

The number of treatment rounds was reduced when more effective treatment regimens were used. With a higher dose of ivermectin, the total number of treatment rounds necessary was reduced by 1 or 2, respectively, when anti-L3 immunity or antifecundity immunity was assumed. For combination treatment, the impact clearly depended on the assumed macrofilaricidal efficacy. When 50% of adult worms were killed by combination treatment, this treatment regimen did not give much better results than did a 200-μg/kg dose of ivermectin. However, when 75% of worms were killed, the number of treatment rounds necessary to achieve elimination was reduced to 5 or 6.

Reducing the interval between subsequent treatment rounds resulted in a small increase in the total number of treatment rounds necessary to achieve a 99% probability of elimination, although the total duration of the mass-treatment program was reduced. Increasing the interval to 2 years resulted in a slight reduction in the necessary number of treatment rounds.

The right-hand column of Figure 3 shows the impact of endemicity level when the mosquito-bite rate of 2200/person/month was varied by ±10% and ±25% -that is, to 1650, 1980, 2420, and 2750. The corresponding average precontrol Mf prevalence levels were 5.5%, 7.6%, 9.2%, and 10.0% when anti-L3immunity was assumed and were 4.7%, 7.4%, 9.5%, and 10.5% when antifecundity immunity was assumed. Endemicity appeared to have a strong impact on the total number of treatment rounds necessary to achieve interruption of transmission: it was much more difficult to achieve elimination when endemicity levels were higher and much easier when they were lower.

Discussion

We used LYMFASIM to assess the prospects for elimination of lymphatic filariasis by mass treatment and to determine the number of treatment rounds necessary to achieve a 99% probability of elimination. Simulations were performed for a community with 8.5% precontrol Mf prevalence, reflecting the endemic situation in Pondicherry, India.

Determinants of the probability of elimination

Coverage. Our baseline-simulation experiment concerned mass treatment with a 200-μg/kg dose of ivermectin, a treatment regimen for which evidence-based estimates of efficacy are available [2]. The number of treatment rounds necessary to achieve elimination was found to depend to a large extent on the population coverage. When the population coverage in each treatment round was 65%, 8 yearly rounds of mass treatment gave a 99% probability of elimination, for both types of immunity; however, when the population coverage in each treatment round was low (40%–50%), many more yearly

rounds of mass treatment were necessary. Data from a large-scale mass treatment program in Tamil Nadu, India, showed that a population-coverage level of 65% is realistic in rural areas but that low population coverage, ~40%, occurs in urban areas [37]; clearly, for successful control, the population coverage in urban areas should be improved.

Efficacy of treatment. The estimated number of treatment rounds necessary to achieve a 99% probability of elimination depended to a large extent on assumptions regarding efficacy of treatment: with 65% population coverage, the estimates ranged from 7 to 10 when the 95%CI for the estimated fecundity reduction for an Mf life span of 1 year was taken into account; the estimates ranged from 6 to 15 when we used the more extreme, minimum and maximum estimates of fecundity reduction. The high level of uncertainty in estimates of efficacy of treatment hampers accurate prediction of the impact of mass treatment. More-precise estimates of efficacy of treatment are needed. It will not be easy to get better estimates, however, because the adult-worm burden in the human body cannot be measured directly.

Variation in efficacy of treatment. The amount of variation in efficacy of treatment influences the impact of mass treatment; and, especially when there is systematic inter-individual variation in efficacy of treatment, results may be less favorable. To clear infection in individuals who always have a poor response to treatment, more treatments are necessary, compared with what is necessary in individuals who have a better response to treatment. As yet, there is not much evidence regarding the extent of inter-individual variation in responsiveness to treatment.

Treatment regimen. The prospects for elimination obviously depend on the treatment regimen used. A single 400-μg/kg dose of ivermectin is more effective than a lower, 200-μg/kg dose [2,38,39]; indeed, with 65% population coverage, the number of treatment rounds necessary to achieve elimination could be reduced by 1 or 2 when the higher dose is used. Currently, a combination of diethylcarbamazine and albendazole is recommended for use in mass treatment in India [22]. Quantitative estimates of the efficacy of diethylcarbamazine plus albendazole are not yet available. To predict the possible impact of mass treatment with this combination regimen, we used 2 plausible, alternative quantifications, which differed in terms of macrofilaricidal efficacy. If a single treatment would kill 50% of adult worms and all Mf that are present in a human host, then mass treatment with diethylcarbamazine plus albendazole is approximately as effective as mass treatment with a 200–μg/kg dose of ivermectin. This may be unexpected, because treatment with ivermectin, which reduces Mf production by 77%, initially may result in a stronger reduction in transmission intensity. However, because male worms are not affected by ivermectin, recrudescence of transmission may occur more easily after treatment with ivermectin than after treatment with the combination regimen, which is assumed to kill both male and female worms. If combination treatment would kill 75% of the worms, the goal of elimination could be achieved in 5 or 6 rounds, with 65% population coverage.

Important potential benefits of using a combination of 2 drugs with different working mechanisms include (1) a reduction in the number of people with no or poor response to treatment and (2) a reduction in the risk that parasites develop resistance against treatment. Furthermore, albendazole (like ivermectin) also has an effect on other parasitic diseases as well, which may lead to additional public health benefits and may enhance compliance with the mass-treatment program [40,41].

Treatment interval. The inter-treatment interval influences the number of treatment rounds necessary to achieve elimination, through several mechanisms. Giving the same number of treatments within a shorter period causes a more rapid decline in transmission intensity, which tends to increase the probability of elimination. This effect is counteracted by a higher number of (preexisting and new) worms that survive during the control program and remain fertile, resulting in a higher level of residual transmission and a lower probability of elimination. In our simulations, this relates to male worms that are never affected by ivermectin and to female worms that, by chance, escape treatment. These opposing mechanisms influence the number of treatment rounds necessary to achieve elimination. This number further depends on the immune status of the population, which, in turn, is related to the effectiveness and duration of control. When coverage was 65%, the number of treatment rounds necessary to achieve elimination was lowest for a 2-year interval; however, both for practical reasons and for reduction of the total duration of the program, a 1-year interval may be preferable.

Endemicity level. A very important determinant of the number of treatment rounds necessary to achieve elimination is the precontrol endemicity level. In our baseline-simulation experiment, precontrol Mf prevalence was, on average, 8.5%. We investigated the impact that endemicity level has on the prospects for elimination, by varying the monthly biting rate. A higher monthly biting rate results in a higher prevalence of infection, a higher precontrol worm load, and a higher probability that any residual transmission will cause recurrence of infection. Compared with the large variation in Mf prevalence levels that occurs in the field, the 4.5%–10.5% prevalence range considered in the sensitivity analysis is relatively small; nonetheless, it resulted in a big difference in the number of treatments necessary to interrupt transmission (4–10 rounds, with65% population coverage).

Model variants. All analyses were performed with 2 variants of the model, with different assumptions regarding the type of immune regulation. Although several studies have suggested that acquired immunity plays a role in lymphatic filariasis [19,42-45], the human immune response against this infection is not fully understood. With regard to the estimated number of treatment rounds necessary to achieve elimination, we found small differences between the 2 models, but the main conclusions did not change.

Pattern of attendance. An important threat to the effectiveness of mass treatment is the existence of a group of individuals who never attend the mass-treatment program and therefore continue to contribute to transmission of lymphatic filariasis in the population.

This has not been investigated in the present study, but it has been clearly presented in a previous model exercise [34]. It is very likely that some people will systematically miss treatment, because of either refusal, absence, or ineligibility.

Elimination

The way in which elimination is defined influences the results of our analysis. In the literature, the term "elimination" has been used to denote complete absence of an infectious agent, absence of transmission, absence of specific clinical manifestations caused by infection, or control of clinical manifestations such that an infection is no longer regarded as a public health problem [46]. The present study considers elimination of transmission, which we have operationalized as zero Mf prevalence 40 years after the start of control, with Mf positivity determined in each individual in the population by a 20-μl-thick smear of blood drawn by finger prick. We assessed Mf prevalence after a 40-year period because this interval allows transmission to decline slowly after cessation of control. Zero Mf prevalence does not always imply absence of infection, because individuals may still carry single-worm or single-sex infections and because Mf tests may give false-negative results. It is extremely unlikely that this residual infection would cause recrudescence. The simulated Mf prevalence shows a continuous decline after cessation of control, before finally reaching zero, indicating that the overall Mf load already had been brought below the threshold level necessary to sustain transmission. In the Pacific Islands, where filariasis is transmitted by *Aedes* mosquitoes, recrudescence of infection has been found to occur <2 years after mass treatment, although Mf prevalence had been reduced to almost zero [47]; this fast recrudescence probably is due to the high efficiency of *Aedes* in transmitting infection at low Mf densities.

With our definition of elimination, we have provided a minimum estimate of the efforts necessary to achieve local elimination. If elimination is to be achieved sooner or if programs are aimed at elimination of infection rather than at interruption of transmission, mass treatment will have to be continued for a longer period.

Underlying assumptions

The numerical results of our analyses depend on a number of underlying assumptions concerning both the circumstances under which control programs are carried out and the effectiveness of these programs. First, the simulated community is geographically isolated: there is no human migration into or out of the endemic area, and there is no mosquito invasion from other areas. The impact of these factors depends on several aspects, including the rates of human migration and mosquito invasion, whether control programs cover the outside population, the endemicity level in the outside population, the biting rate, and the efficiency of vectors in the transmission of infection. Elimination obviously becomes more difficult when there is human immigration or mosquito invasion from endemic areas. Second, we have assumed that the endemic situation had been stable before the start of control efforts and that the biting rate is constant over time. An

increasing trend in either endemicity level or biting rate will make it more difficult to achieve elimination, and vice versa. Third, we have assumed that mosquitoes homogeneously mix with the human population, although some human individuals may be bitten more frequently than others. In practice, because of the limited flight range of mosquitoes, transmission may be more focal, and there may be geographical sub areas with higher vector density, transmission, and infection intensity. Foci of more-intense transmission may be found, for example, in the proximity of breeding sites [48].To eliminate lymphatic filariasis from these foci, mass treatment would have to be continued longer than would be expected on the basis of the overall prevalence in the community.

We have assumed that efficacy of treatment does not depend on the number of times that an individual has previously been treated. Furthermore, the possible existence of either parasites that are resistant to treatment or development of resistance in the parasite population has not been taken into account. In practice, these assumptions may not hold.

Generalizability

The model used in the present study was quantified for Pondicherry, India. Differences in the vector species, in the parasite strain, and in the prevalence and intensity of the infection in the population limit the generalizability of the results of our simulation. Mosquito species differ with respect to the proportion of engorged Mf developing into infectious L3 larvae, efficiency in transmission of infection to the human host, and survival in the presence or absence of parasites [49]. In Pondicherry, *W. bancrofti* infection is transmitted by *C. quinquefasciatus*. This parasite-vector complex shows "limitation" -that is, a decreasing yield of L3 with increasing Mf uptake by the mosquito [50]. The effectiveness of control strategies maybe different when the number of L3 larvae developing per engorged microfilariae either is proportional to or increases with Mf uptake (i.e., when there is either proportionality or facilitation). Differences between parasite strains -for example, with respect to either life span or Mf production- also may influence the number of treatment rounds necessary to achieve elimination. For areas with the same vector-parasite combination, our sensitivity analysis of the monthly biting rate may give some indication of the efforts necessary to achieve elimination of filariasis in areas with higher or lower endemicity levels; however, in this case, too, generalizability is limited, because of demographic differences between populations in different areas, differences in heterogeneity in exposure to mosquito bites, and differences in individuals' inclinations to comply with mass treatment.

Prospects for elimination

Because factors such as human migration and resistance were not considered in the present study, our results should be regarded with caution; nonetheless, the prospects for elimination of lymphatic filariasis by mass treatment in Pondicherry, India, are positive. Our predictions show that elimination is very likely after 8 rounds of mass treatment with

ivermectin, provided that population-coverage levels are sufficiently high (i.e., ≥ 65%). The number of treatment rounds necessary to achieve elimination depends, to a large extent, on coverage, efficacy of the treatment regimen, and endemicity level. Although the results in Pondicherry cannot simply be generalized to other areas, qualitatively our conclusions are applicable in other situations with the same vector-parasite complex.

Appendix A: *Logistic regression equations for the baseline-simulation experiment*

Logistic regression analysis of results from our baseline simulation experiment, in which we simulated the impact of mass treatment with a 200-μg/kg dose of ivermectin, yielded the following equations: for anti-L3 immunity, y=17.98+0.70prev-19.45c-3.74ln(n)-6.31cln(n); for antifecundity immunity, y=9.29+0.59prev-10.56c-1.35ln(n)-7.11cln(n). Results are shown in Figures 1 and 2. The logistic regression analysis was based on simulations with up to 15 rounds of mass treatment; extrapolation of results to more treatment rounds is not warranted.

Appendix B: Quantification of demographic parameters

Age-group (years)	Proportion of the initial population per age-group (%)		Life table (probability to be dead at upper limit of agegroup) [a]	Fertility (birth rate per female) [a]
	M	F		
0-4	6.5	6.5	0.096	0.000
5-9	5.8	5.8	0.105	0.000
10-14	5.1	5.1	0.112	0.000
15-19	5.1	5.1	0.121	0.075
20-24	7.2	7.2	0.136	0.254
25-29	6.5	6.5	0.151	0.222
30-39	5.1	5.1	0.188	0.096
40-49	4.3	4.3	0.244	0.013
40-89	4.3	4.3	1.000	0.000

[a] quantified according to the lifetable and fertility rates of the 1981 census in Pondicherry [1]

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5

General discussion

Introduction

The major objective of the present work was to develop, quantify and validate an epidemiological simulation model for lymphatic filariasis transmission and control. For this purpose, we carried out an in-depth analysis of an extensive dataset generated from an integrated vector management (IVM) programme in Pondicherry, India [1]. The conclusions derived from this epidemiological analysis will have relevance to evaluating any vector control programme and in understanding the parasite epidemiology. Especially the ease or difficulty of transmission of infections between human and vector is an important determinant of elimination of LF infection. Therefore experimental and field studies were carried out to have a better understanding of the transmission dynamics of the parasite in the vector.

This General Discussion begins with summarizing how our epidemiological analyses (Section 5.1) contributed to the epidemiological evaluation of the impact of vector control. The contribution of entomological studies to understanding the transmission and dynamics of the parasite in the vector is discussed in Section 5.2. Section 5.3 deals with how the experimental and field studies contributed to the development and quantification of the LYMFASIM simulation model and how the application of LYMFASIM contributed to understanding the dynamics of infection in the human host and the evaluation of the impact of vector control. Section 5.4 presents the state of the art of LYMFASIM model and its usefulness for prospective evaluation of control programmes. The future of LYMFASIM, in terms of further quantification, refinement and validation of the disease module, its usefulness for cost-effective analysis, and in aiding decision making is discussed in Section 5.5. Section 5.6 discusses the answers to the research questions stated in Section 1.6. Finally, Section 5.7 concludes the entire work with a summary of the conclusions and recommendations that could be useful to enhance more widespread application of LYMFASIM as a decision tool in operational settings.

5.1 Epidemiological evaluation of vector control

Vector control had been the strategy of choice for lymphatic filariasis control for several years in many endemic countries [1-8]. The filaria control activities in India, the largest endemic country in the world, were limited only to urban areas. The activities mainly consist of conventional recurrent anti-larval measures such as application of insecticides to vector breeding habitats and anti-parasite measures through detection and treatment of microfilaraemic individuals with diethylcarbamzaine (DEC)[9]. However, the conventional methods achieved only limited success and have some limitations. Hence, as an alternative, an Integrated Vector Management (IVM) programme that focuses on environmental improvement was tested in one part of Pondicherry. For comparison purpose, the conventional anti-larval measures were implemented in the other part (see Section 1.4, Figure 4 for more details).

We started the evaluation of the epidemiological impact of IVM programme (1981-1985) by analyzing the changes in age specific prevalence and intensity of Mf in human population from pre-control (1980) to post-control period (1986; Section 2.1). The analyses proved that the age-specific prevalence at young ages, and not overall prevalence, is an important indicator of effectiveness of short-term (5 years) vector control measures. This is not surprising because both the prevalence and intensity of infection in the human host depends on the rate of gain and loss of infection. In the age groups with constant level of prevalence and intensity, rate of gain and loss of infection would be more or less in equilibrium. However, in the age groups with an increasing trend in prevalence, vector control is expected to result in more noticeable change in prevalence, owing to reduction in rate of gain of infection. And indeed, we observed significant reductions in prevalence of Mf only in population of age classes less than 30 years in IVM area and in two age classes (12-14 and 25-29 years) in the comparison area. However, the decrease in Mf-density (No. of Mf per infected person in 20 μl of peripheral blood) between pre- and post-control was not significant in any age group in either area. The results imply that prevalence of Mf, particularly in younger and adolescent age classes, is a better indicator of the effectiveness of vector control than Mf-density. In contrast, in the onchocersiasis control programme (OCP) in West Africa, intensity of microfilariae in individuals older than 20 years CMFL (Community Microfilaria Load) was shown to be the most sensitive and meaningful indicator of the effectiveness of vector control [10]. The longer duration of vector control in OCP (8 years, which is nearer to mean life span of the parasite ~10 years), might possibly have resulted in reduction of parasite reservoir and therefore CMFL. Lack of evidence for mechanisms regulating *Onchocerca volvulus* infection in human could have favoured the use of CMFL as a sensitive indicator of effectiveness of vector control.

The decline in overall prevalence from pre- to post-control period was significant in both IVM (28% reduction) and conventional (17%) programme areas. However, such reductions were not significantly different between the two programme areas. Change in

prevalence is to be apparent in children aged ≤5 years where, with perfect vector control, no new cases should occur. Reduction in transmission is reflected on the prevalence of Mf in children (aged 0-5 years) who were born during the period of control (Section 2.1). The prevalence of Mf declined from 23 to 2 per 1000 examined (91% reduction) in that age class in the IVM area and from 18 to 4 per 1000 (64% reduction) in the conventional programme area. The reductions, however, were not significantly different between the two areas due to the lowest prevalence in this age class, even when infection was endemic and uncontrolled in 1981. Thus assessment of the relative impact of two control programmes was difficult to detect in this age group. However, prevalence when measured in the younger population (<20 years) showed that the decline in the IVM area was significantly greater than the decline in comparison area. Since the period of vector control is shorter than the lifespan of the parasite, no reduction in adult worm load, Mf-production and hence in Mf-intensity is expected. Comparison of observed Mf-intensity (no. of Mf per person per 20 μl of peripheral blood) between pre- and post control periods indicated this situation in both areas. Reductions in overall prevalence were also not significantly different between the two areas. Since impact of vector control will not be realized in adult age class (≥20 years) where the prevalence is stable or tends to decline, combining the data for younger and adult population has obscured the effect of IVM over conventional method of control. Thus evaluation based on overall reduction in prevalence would underestimate the impact of IVM. Whereas by restricting the analysis to a subgroup of the population, where the prevalence increases rapidly with age, the impact of vector control was found to be better in the IVM area than in the conventional programme area.

The withdrawal of the IVM, implemented over five years, and later switching over to the conventional programme lead to a slow resurgence of the vector population in the IVM and also the conventional programme areas [11]. The slow, instead of a rapid, resurgence was mainly because of longer lasting impact of the environmental management/modification (e.g. construction of underground drainages etc.) activities of the IVM programme that contributed to the reduction of breeding sources. We assessed the impact of recovery of the vector population on human infection (Section 2.2) in 1989, three years after cessation of IVM. Inspite of vector resurgence in IVM and comparison areas, the prevalence of Mf continued to decline in the IVM area and the Mf-density did not change significantly in either area. The rate of gain of infection increased only marginally in the IVM area (0.0126 in 1981-86 to 0.0142 per year in 1986-89, 13% increase), but noticeably in the comparison area (0.0094 in 1981-86 to 0.0158 per year in1986-89, 68% increase). The rate of loss of infection increased between 1981-86 and 1986-89 in both IVM (0.196 to 0.409 per year, 109% increase) and comparison (0.215 to 0.361 per year, 68% increase) areas. The increased loss of infection during the post-control period (1986-89) in both areas might be related to the reduced transmission during control period (1981-86), which is possible due to longer-lasting reduced mosquitogenic conditions brought about through better inter-sectoral collaboration of

various departments and/or the effect of selective DEC treatment of detected Mf-carriers (prevalence of Mf, 6.4%) at the end of control.

The nearly unchanged intensity of Mf (per 20 µl peripheral blood) in the IVM area during and even three years after cessation of IVM might be due to the effect of IVM on transmission. The IVM has reduced the rate of gain of infection and thereby increased the number of true Mf-negatives in the population. Other explanations could be related to higher sampling variability in Mf-counts or perhaps enhanced productivity of the surviving female worms. In contrast in the comparison area, no change in intensity could be explained by the increased rate of gain of infection.

The general conclusion emerging from the evaluation of the impact of vector control is that (i) Mf prevalence in younger age groups is a more sensitive indicator of the effectiveness of vector control than Mf intensity (ii) the impact is better in the IVM area than in the conventional programme area (iii) the changes in parasitological parameters did not correspond with the changes in transmission parameters because these occur at different times, and (iv) the nearly unchanged intensity of infection immediately and 3 years after cessation of control in both areas appears to be a function of the dynamics of infection in both human and vector populations.

5.2 Dynamics of infection in the vector

The recovery of the vector population, though did not produce a discernible increase in the prevalence of Mf during post IVM period, has contributed to an increase in transmission potential (see Fig. 2, Section 2.2). The increased transmission potential suggests that the IVM programme has not lowered the parasite reservoir below the transmission threshold. This leads to the question whether the increased transmission potential will change the course of downtrend in prevalence and if so how many years after the cessation of IVM? To answer such questions it is crucial to gain a quantitative understanding of the parasite population dynamics in human and vector. The thesis, by addressing the following questions through a series of transmission studies, contributed to the knowledge on the dynamics of *W. bancrofti* infection in *C. quinquefasciatus*: Is thereany regulation of parasite population in the vector? If so, does parasite regulation occur via 'excess' vector mortality? What would be the implications of such regulatory mechanisms to transmission control?

Parasite regulation

Experimental transmission studies have identified three types of regulatory mechanisms: facilitation -an increase in L3 yield, number of L3 per Mf-ingested, over some ranges of Mf-uptake as is seen in Anopheline vectors; limitation - a density dependent constraint on L3 yield as is seen in *Culex*, *Aedes* and *Mansonia* vector species; and proportionality - constant rate of L3 yield at all levels of Mf-uptake. The phenomena of 'facilitation' and 'limitation' may be the result of a combination of a variety of vector related factors such as interaction of the ingested Mf with cibarial teeth, peritrophic membrane, clotting agents and digestive enzymes acting on the blood meal, cellular reactions in the epithelium of the gut and vector defences. The limitation phenomenon would make the geographical spread of the infection relatively easy compared to facilitation and allows persistence of transmission even when control programmes reduce Mf-prevalence and intensity to very low level [12-15]. This phenomenon has important implications for control/elimination programmes and its relevance to *C. quinquefasciatus* needs validation under natural conditions [16].

Relationships of human Mf-density with uptake and development of Mf

The relation between the Mf-density in human and the number of Mf-ingested, and infective larvae (L3) developed in the vector mosquito are important determinants of the transmission success of the parasite between human and mosquito, and Section 3.1 deals with quantitative relationships of these processes using the data from the semi-experimental study. Both the processes showed non-linear saturating relationships with human Mf-density demonstrating the existence of density regulating mechanism in the vector. This non-linearity in *W. bancrofti* Mf-uptake by *C. quinqufasciatus* is in agreement

with many other studies involving different vectors and *W. bancrofti* combinations [17-20] as well as results from a meta analysis of various experimental studies [21]. However, the result differs from the linear relationship found in various other experimental studies [22-25]. The variable relationships are reported to be related to difference in study designs: variations in sample size, methods used for sampling Mf in human and mosquito and range of human Mf-densities [21].

The quantitative relationship, established in this study, between human Mf-density and number of L3 developed per mosquito was direct input for LYMFASIM, as one of the important density regulating mechanisms of the parasite (Section 3.1). Further, while confirming the existence of 'limitation' phenomenon for *W. bancrofti - C. quinquefasciatus* combination under natural conditions, the estimated relationships provided an estimate of the level of saturation for the number of Mf ingested (28.9 per mosquito) and L3 developed (6.6 per mosquito) at high human Mf-densities. In Section 3.2 by analysing data collected from different mosquito catching sites in the IVM area, we have obtained strong evidence of density-dependence: degree of parasite aggregation in the vector population declines with parasite stage. The finding from field study corroborates our "limitation" type results of the experimental study (Section 3.1).

Parasite-induced vector mortality

One of the mechanisms responsible for limitation is parasite-induced vector mortality at high parasite densities. Samarawickrema and Laurence [26], while observing similarity between the distributions of Mf in humans and mosquitoes, noticed a reduction in the tail of the distribution of infective stage (L3) compared to tail of the distribution of Mf in mosquitoes. They attributed the reduction in tail of the L3-distribution to excess mortality of heavily infected mosquitoes. A previous study [27] and our analyses in Section 3.3 also indicate that the survival of *C. quinquefasciatus* infected with *W. bancrofti* declines with an increasing number of infections.

In Section 3.4, this finding was confirmed through an experimental transmission study in which a cohort of laboratory reared *C. quinquefasciatus* were fed on volunteers with or without *W. bancrofti* Mf. Significantly higher vector mortality was observed during the extrinsic incubation period (12 days post-engorgement) in mosquitoes fed on volunteers with Mf (50%) than those fed on without Mf (29%). A large proportion (62%) of the mosquitoes that died during the early phase of parasite development were infected (30% in low and 90% in high Mf-density), which caused a parasite loss of more than 90%. Modelling the survival of mosquitoes in relation to level of microfilaria in the human and larval count in the dead mosquito showed that the parasite load in mosquitoes and the human Mf-density for a given parasite loads 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 doubled 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. Further, our experimental study (Section 3.4) concurs with field observation (Section 3.2) that most of the vector mortality occurs in the early stage of parasite development, i.e. between Mf and L1 stage.

Our observation that density-dependence also occurs in the field, as observed elsewhere in experimental transmission studies in Sri Lanka [22,] in India [28,29], suggests that even if mass treatment programmes reduce the Mf-load to a very low level, transmission is likely to continue. This implies that the present day elimination programmes should either increase the duration and coverage of mass treatment programmes or consider the possibility of including vector control measures as a supplement to few rounds of mass drug administration.

5.3 Modelling dynamics of infection in human

The need for a simulation model

Direct measurement of the worm burden in the human host would help in understanding the dynamics of infection and hence in assessing the impact of control programmes. Despite promising advances in immunodiagnosis [30-32] and ultrasound scanning [33,34], microfilaria in the blood is still the only reliable measure of current infection. Since microfilaria counts are highly aggregated, evaluation of control programmes through Mf surveys often results in some amount of uncertainty. Therefore in order to have a better understanding of the population dynamics of the parasite and for making long-term predictions of the effectiveness of control strategies, a comprehensive modelling framework is required. Section 4.1 provides a description of the simulation model, LYMFASIM, along with mathematical formulations. The model can be used to test a variety of hypotheses about the parasite life history, its transmission, the role of the immune system in parasite regulation, dynamics of infection and disease, and to make prospective evaluations of the control strategies. Subsequently, the data and outcomes of the studies in Chapters 2 and 3 were utilized to quantify and apply LYMFASIM to lymphatic filariasis transmission and control. In the following sections, I will discuss how the LYMFASIM model was applied to test hypotheses related to mechanisms that regulate the parasite number in humans, its contribution to increased understanding of the dynamics of infection in the human host, its application to predict the impact three and six years after cessation of IVM programme in Pondicherry and to assess the prospects for elimination of LF by mass drug administration (MDA).

Parasite regulation in human

As an important step in understanding the dynamics of infection in human population, simple catalytic models were applied to estimate the rates of gain and loss of infections. The estimates showed an age-dependent decline in the rates of gain of infection [35,36]. This was consistent with evidence from field [37-41] and laboratory studies [41-49] that suggest the possible role of acquired immunity in regulating the parasite population. The above result is also substantiated by the convex nature of both the age-prevalence and age intensity curves (Fig. 5, Section 1.4), which is in accordance with the observations in human hookworm [50] and *Schistosoma haemotobium* [51] infections for the possible role of acquired immunity in shaping epidemiological patterns.

LYMFASIM was applied to describe the impact of IVM programme on the intensity of *W. bancrofti* infection in a cohort of individuals in Pondicherry (Section 4.3). While applying, emphasis was on hypotheses related to immune mechanisms in human. Three models were considered for the mechanism regulating parasite numbers in human: (i) anti-L3 immunity caused by prolonged exposure to L3-antigens, leading to diminished

success of inoculated L3-larvae to become adult, (ii) anti-fecundity immunity caused by the cumulative presence of adult worms leading to reduction in Mf-production and (iii) total absence of immunity (no-immunity). We found that immune regulation (antigen originated from L3 or worm) is essential to describe the observed convexity in age-prevalence curve and the relatively low Mf-prevalence and that the immunity is long lasting but not life-long (95% confidence interval for half-life are 5-18 and 5-17 years respectively for anti-L3 and anti-fecundity immunity models). Second, the analysis rendered validated models that can be used for long-term predictions of the effects of vector control and chemotherapeutic interventions using DEC or ivermectin etc. Third, we concluded that variation in observed Mf-load in and between humans is, in addition to natural factors such as individual variation in exposure to mosquito bites and immune responses, to a large extent due to sampling variability in Mf-count in blood smear.

Of the three models, the two immunity models explained the observations equally well compared to a model with no immunity. In order to salvage the no-immunity model one has to assume that exposure to infection declines at older ages [52,53] or the existence of mechanisms which reduce the chance of an incoming larva to develop into mature adult worms at older ages [41]. However, most studies provide evidence for a role of acquired protective immunity [37-39,54-59]. Earlier inference about immunity was based on cross sectional observations [60,61]. We have added longitudinal and cohort-based evidence.

Worm load

The detailed simulation results also enabled us to have an idea of the worm load in the human host. The pre-control worm-load (male + female worms) calculated by anti-L3 immunity model is 0.82 per person and 3.1 per Mf-positive person. For anti-fecundity immunity model these numbers are 1.9 and 3.7 respectively. The higher worm load estimated by the anti-fecundity immunity model is due to the regulation mechanism, which reduces the Mf-output of the parasites. Hence, this model indicates that many Mf-negative persons may carry adult parasites. The estimated mean load of reproducing female worms were 4 [62] and 6.5 [35] per microfilaraemic patient. Both estimates are higher than ours, which is reasonable because these estimates are derived from microfilaraemic patients.

Worm life span

The mean life span of *W. bancrofti* in human is estimated at 10.2 and 11.8 years for anti-L3 immunity model and anti-fecundity immunity model respectively. The corresponding fecundic life span (a period in which female worms capable of producing Mf) for these estimates, after subtracting the presumed immature duration of the parasite (8 months), are approximately 9.5 and 11.1 years respectively for the two models. Both these estimates are about twice as high as the earlier estimate (5.4 years) obtained from the same data set [36,63]. The main reason for our higher estimate is that in LYMFASIM we corrected

for false negative counts during pre- and post-IVM periods. The apparent loss or gain of infection between pre- and post IVM period is often the consequence of false negative counts, and more loss and gain implies a shorter life-span. Our present estimates fall within the range of 8-15 years reported elsewhere for *W. bancrofti* [35].

Microfilaria density and prevalence

The number of productive female worms and the recent Mf-production of these worms determine the Mf-density in a person. The most important parameters of both these variables (number of female worms and Mf-production) are success-ratio of injected L3 larvae (sr) and the basic Mf-production (r_o) respectively. While the estimates given in Table 2 (Section 4.3) are those that minimized X^2, it can be argued that the product of $sr \times r_0$ matters more than the values of the individual parameters and that the alternative quantifications would also result in an acceptable fit when they leave the product unchanged. There are however limits to this argument. Very low values of *sr* combined with high values for r_o will result in very low worm-loads which, given the requirement of regular mating of worms (in LYMFASIM ensured by the presence of at least 1 adult male), precludes the establishment of a stable endemic situation. Very high values of *sr* combined with a very low per-worm Mf-production (r_0) on the other hand will lead to a high mean worm load and this tends to lift the Mf-prevalence to much higher values than the ~10% in our observed cohort. The low values for *sr* estimated for both models suggest a fairly low efficiency for the transmission of *W. bancrofti*. This is in agreement with the work of Hairston and De Meillon [64] who used a reversible catalytic model [65] for the analysis of data from Burma. They estimated the efficiency of transmission, defined as the probability that an L3-stage larva in a mosquito causes or enhances microfilariaemia in an individual, at ~0.00006. Comparing this figure with our parameter-estimates is difficult. In terms of LYMFASIM, this efficiency is composed of the product $sr \times v$ (0.0001 for anti-L3 immunity model and 0.00004 for anti-fecundity immunity model), the Mf-production of the worms, the regulatory role of the immune system, and the sensitivity of the blood sampling for detecting microfilariaemia.

Reproducing a Mf-prevalence of less than 10% using a model without immune-regulation could only be achieved by making the assumption that more than 64% of the population is excluded from transmission (f_0=0.64). This is not only unlikely given the ubiquity of the vector mosquito *C. quinquefasciatus*, but it also conflicts with the presence of disease-symptoms in the Mf-negative population. Observations in the population of Pondicherry revealed that the relative risk of parasite carriers developing disease (any manifestation) was only marginally higher compared to amicrofilaremic persons (1.18) [66]. A study comprising many other regions had the same result [67].

Long-term evaluation of vector control

The model predictions of the impact of vector control are in agreement with observations for the first few years after stopping vector control. On the long term both immunity models predicted that the trend in prevalence would reverse to the pre-control level about 25 years after cessation of control in 1985 (see Fig. 6 in Section 4.3). The most striking difference between the models is the more pronounced decline and subsequent increase in Mf prevalence predicted by the anti-fecundity immunity model. The model predictions also illustrate the impact of reduced transmission on level of immunity via damped oscillations in predicted trends. The higher peak in prevalence predicted by the anti-fecundity immunity model could be explained in terms of loss of immunity resulting from natural death of existing worms as well as delay in mounting immune response by new born children due to reduced level of transmission. In the anti-L3 immunity model boosting is not completely interrupted, since the IVM has only reduced the level of transmission. Therefore loss of anti-L3 immunity is not expected to be as fast as in anti-fecundity immunity. Our predictions in Section 4.3 were based on point estimates for the duration of immune response and the parameters describing the functional relationship between human Mf-density and L3 larvae in the vectors.

(i) Duration of immune responsiveness

The impact of immune response on the parasite population was further explored by predicting trends in prevalence of Mf corresponding to the 95% confidence interval of the duration of immunological memory. For this purpose the LYMFASIM model parameters were re-estimated for the given upper and lower limits of 95% CI for the duration of immune responsiveness. This alternative quantification has been used to predict the long-term impact of IVM in Pondicherry (Fig. 1). The effect of alternative quantifications for the duration of immunity is limited in these predictions (but may have been very important had the IVM programme been extended or had reductions in vector intensity been larger). For the anti-L3 immunity model, the steepest post-control rise in prevalence occurs with the assumption of short-lived immunity. This makes sense because the higher rate of loss of immunological memory makes that many people will become susceptible for infection. The post-control trends predicted with the different quantifications of anti-fecundity model are less straightforward. The only clear relation is between the immunological memory and the height of the peak reached 35 – 40 years post-control. An explanation is that in LYMFASIM the consequence of a long-lasting immunological memory not only results in a slow decay in immune-responsiveness but also in a longer period of boosting required to re-establish immunity. Hence, the build-up phase takes longer which explains the higher peak.

The conclusion that immune regulation is an essential component for describing the observed Mf-distribution in the human population has important implications for the control of lymphatic filariasis by chemotherapy. A long immunological memory implies a slower rate of decline in the level of immune response but also requires a longer period of

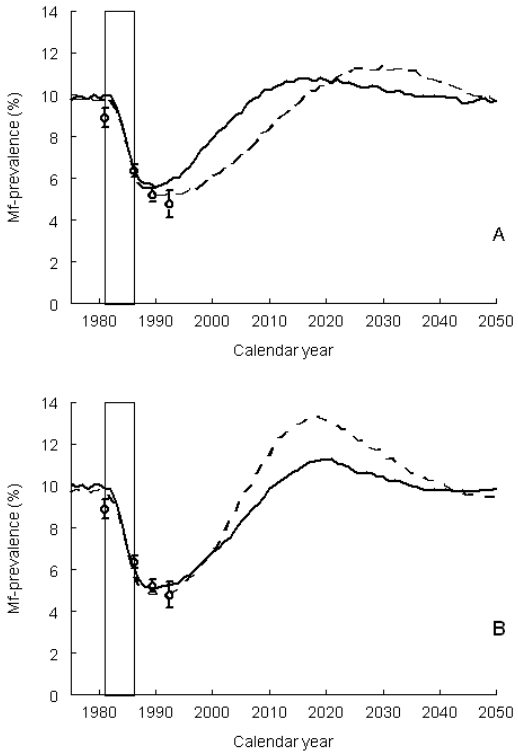

Figure 1 Trends in observed (open circles with error bars) and predicted prevalence of Mf based on short (solid line) and long immunological (dotted line) memories of both anti-L3 immunity (A) and anti-fecundity immunity models (B). Vertical bar represents the period of integrated vector management programme in Pondicherry.

boosting to re-establish immunity. Therefore if mass treatment programme is implemented at a level less than that required for eliminating parasite reservoir, it has the potential to significantly reduce the level of immunity and this would enhance the establishment of new infections in high endemic areas where no vector control measure is in operation. This is another 'limitation' mechanism with similar implications as observed in transmission studies.

(ii) Strong regulation of parasite in vector

The database from the experimental transmission study reported in Section 3.1 allowed us to obtain an alternative quantification of the relationship between human Mf-density and number of L3-larvae per mosquito. The alternative quantification is based on the upper 95% confidence limit for L3-output. This quantification results in a much steeper rise in the L3 load even at low human Mf-densities (strong density regulation in the vector, Fig.

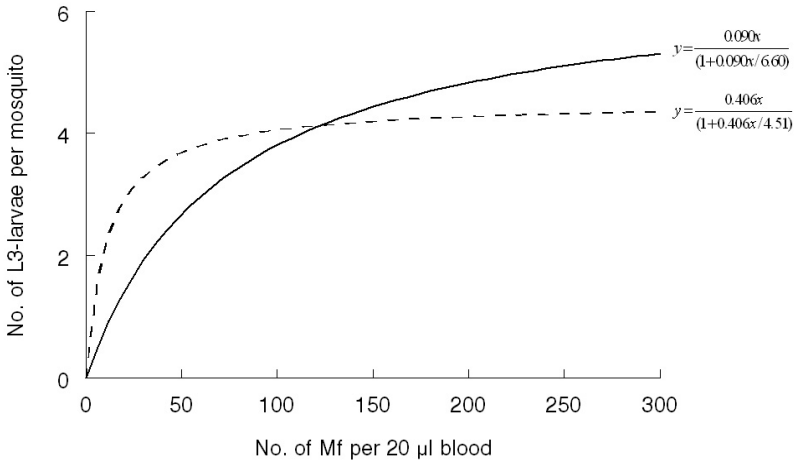

Figure 2 Estimated relationship between the density of microfilariae (x) in the peripheral blood of the human host (measured by the no. of micrpfi;ariae in a blood smear of 20 µl and the average number of L3-larvae (y) developing in feeding mosquitoes. The solid line represents the quantification of this relationship that rendered the best fit to the observations. The dashed line represents one of the 95% confidence limits of the function parameter that determine the efficiency of mosquitoes to develop L3-larvae after feeding on low-density Mf-carriers.

2). The implication of this alternative quantification in predicting the long-term impact of vector control was explored with the anti-L3 immunity model, by simulating the long-term impact of the 5 years IVM programme in Pondicherry. Figure 3 shows the predicted trend in prevalence of Mf at the beginning and 3 years after cessation of the programme. The post-control trend suggests that a strong parasite regulation results in a faster recrudescence of infection and that the prevalence stabilized at a lower level than a weak regulation. The faster recrudescence can be explained by a higher transmission efficiency of mosquitoes when the level of immunity in the population is in declining phase. The low-level stabilization is due to the higher level of boosting of anti-L3 immunity. This in turn has a negative effect on the force of infection.

Prospects for elimination of LF by MDA

LYMFASIM was used to estimate the number of rounds of MDA required to achieve elimination by mass DEC or ivermectin administration. Our predictions based on a very pessimistic criterion (i.e. elimination can be achieved if the prevalence is less than 0.1% immediately 5 years after stopping MDA) indicated that at least 90% coverage would be

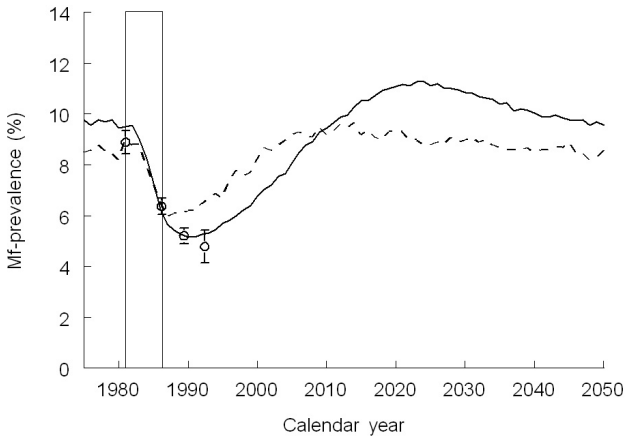

Figure 3 Observed (open circles with error bars) and predicted trends (lines) in Mf-prevalence based on anti-L3 model. The solid line represents the predictions based on the point estimates of the parameters of hyperbolic function describing relationship between human Mf-density and no. of L3 per mosquito and the dotted line is based on 95% confidence limits of the parameters in the above relationship (see Fig. 2). Vertical bar represents the period of integrated vector management programme in Pondicherry.

necessary to achieve elimination with five, annual, DEC-based MDA, and that coverage will still have to be at least 60% if 11 such MDA are used (Section 4.2).

Adopting a very optimistic criterion (i.e. zero Mf-prevalence 40 years after the start of control) our model predictions indicated that at least 65% coverage will be required to achieve elimination with 8, annual, ivermectin (200 μg/kg body weight)-based MDA (Section 4.4). The number of rounds of MDA necessary to achieve elimination depended to a large extent on coverage, drug efficacy, and level of endemicity. However, changing the interval between rounds of MDA mainly influenced the duration of control, not the number of rounds of MDA necessary to achieve elimination (Section 4.4).

The difference in predicted duration of control between DEC and ivermectin depends on the elimination criterion and on the reliability of the estimates of the efficacy of DEC or ivermectin in killing Mf or adult worms and its permanent or temporary effect on the fecundity of female worms. So far such estimates of efficacy are available only for ivermectin[68]. For DEC more-precise estimates are needed for accurate prediction of the impact of DEC, as our present estimates are based on a single clinical trial.[69].

5.4 State of the art of LYMFASIM

LYMFASIM, present status

Several stages can be recognized in the process of model development: (i) problem identification (ii) investigation of knowledge (iii) model design (iv) model quantification and validation (v) prediction and optimisation (vi) decision making and (vii) transfer of simulation program for end users [70]. The work presented in the thesis covers (i)-(iv) stages of model development and partly stage (v). The problems in evaluating vector control programme had (Chapter 2) clearly highlighted the need for a model for understanding the dynamics of infection and predicting the long-term impact of vector control programme (*problem identification*). Considerable time has been spent on gaining knowledge by quantifying relationships using the existing data. The transmission link between human and mosquitoes was quantified using experimental studies both in laboratory and in the field. The dynamics of infection in the vector population, particularly the effect of parasite load on the survival of infected mosquitoes, provided confirmatory evidences for the mechanism that regulate the parasite population in the vector (*investigation of knowledge*, Chapter 3). The model has been designed in close collaboration with field epidemiologists and experts in the relevant disciplines. For this purpose a number of informal meetings and workshops have been made in the beginning as well as during the process of model development [71]. Thus interaction of experts from various disciplines both from lab and field helped us to design model in such a way that it can be utilised to test hypotheses, to make long-term predictions of the effects of control programmes, and to perform cost-effectiveness analysis of various control strategies (*model design).* Much effort has been made to quantify the present version of LYMFASIM through use of the model to evaluate the impact of vector control programme in Pondicherry. At this stage of model development considerable amount of time has been spent on testing hypotheses and quantifying model parameters related to dynamics of infection in human (*quantification and validation*, Chapter 4). Finally the model has been used to make long-term predictions of the effects of vector control in Pondicherry (*prediction).* As part of *optimization*, the model has already been applied to optimize control strategies based on mass treatment with DEC or ivermectin or a combination of DEC and albendazole (Sections 4.3 & 4.4). The prospects for elimination of filariasis have been studied in relation to a number of operational characteristics (duration, coverage and compliance with annual mass treatment, level of endemicity).

LYMFASIM and current knowledge

Models can become effective tools only if there is a continuous feedback from the end users to the architects of the model. In applied modelling it is essential to update the model as and when new knowledge emerges. Section 4.2, reviews the development of

mathematical/statistical models and of their application in studies of epidemiology, transmission and control of LF. Further, the validity of the currently available simulation models (LYMFASIM and EPIFIL) for other endmic situations and the need for additional data analysis/field research are discussed. In the following sections I will briefly discuss the validity of the present structure and quantification of the model in the light of current knowledge, focusing on those aspects that are known to be crucial for understanding the dynamics of infection (Chapter 3 & 4).

Vector-parasite combination

In the present version of LYMFASIM the relationship between human and vector infection is quantified for *W. bancrofti* transmitted by *C. quinquefasciatus,* the predominant parasite-vector combination and responsible for more than 50% of total infections worldwide [72]. As stated earlier this vector-parasite combination is of the 'limitation' type, i.e. a decreasing yield of L3 with increasing Mf uptake by the mosquito. For applying the model in areas like Africa, where, apart from *C. quinquefasciatus*, other vectors are also involved in the transmission of *W. bancrofti*, and the vector-parasite combination is of 'proportionality' or 'facilitation' type (L3-larvae developing per engorged microfilariae is proportional or increases with Mf uptake) [16], the model needs to be re-quantified (Section 4.2).

Parasite and human immune mechanism

The immune mechanisms that we considered and tested in the thesis are not the only ones that are possible. For example, it may be possible that both anti-L3 and anti-fecundity responses can act simultaneously, whereas they were used one by one in the current model. Since no evidence is available for the coexistence of both mechanisms, this assumption could not be tested. Further, recent analysis of age-prevalence data from India and Africa suggests that the prevalence increases in children until at adult age and thereafter it remains stable. The stable age-prevalence in adult age class indicates the natural balance between gain and loss of infection and suggests no necessary role of immune mechanisms. This is in contrary to the convex age-prevalence pattern observed in urban Pondicherry, which suggested the possible role of acquired immunity in combating infections (in addition to natural loss) and our model based analysis also confirmed that immune mechanisms are essential to describe the observations in Pondicherry. Therefore application of LYMFASIM to other endemic situations will have to tackle the role of immune mechanisms again.

Exposure heterogeneity

Our estimate of the parameter describing variation in exposure to vector bites between individuals of a community (shape parameter of the gamma distribution) has been indirect, i.e. not based on direct measurement. The estimated value was very close to 1.0

(approximating an exponential distribution), suggesting a large *heterogeneity* in exposure to mosquitoes. This means that few individuals receive a large number of bites and many individuals receive only few bites. To our knowledge no in-depth study has been carried out to quantify the exposure variability between individuals for mosquito bites and hence risk of infection. However, a recent study has shown that a person's exposure to mosquito is age-dependent [73]. Gad [74] has reported significant association between Mf-prevalence and location of house near vacant land where *Culex* biting rates are higher. Inter-individual variation in skin secretions and skin temperature has been reported to be partially responsible for heterogeneity in exposure to the onchocerciasis vector *Simulium venustum* [75]. On the other hand if exposure is assumed to be homogeneous (i.e. every one is equally exposed to mosquito biting and have the same chance of being infected) then the consequence of this on the predicted prevalence of Mf could have important implications for the control of lymphatic filariaisis. As Dietz [76] pointed out in a theoretical paper that if parasite regulation occurs in both human and vector then in areas where exposure is homogeneous, interruption of transmission would be much easier than in areas where the exposure is more heterogeneous. This is because a heterogeneous exposure would result in lower threshold for transmission interruption than the one for a homogeneous exposure. This implies that relatively much control effort is required in areas, where the exposure is heterogeneous than that in areas where the exposure is homogeneous.

5.5 Future Work

Robustness of LYMFASIM

The quantification of model parameters for Urban Pondicherry suggests considerable uncertainty in the estimates. One must be cautioned in applying the model to evaluate control programmes in other endemic situations. Of particular importance are the uncertainty involved in the mechanisms regulating parasite numbers in the human host and the type of vector parasite relationships (proportionality, facilitation, and limitation). The former issue can be resolved in two ways: (1) attempt to reduce uncertainty by testing the model in different endemic situations, and (2) try to translate uncertainty in possible/plausible ranges of the parameter estimates (if necessary based on expert opinions) and look at the consequences of these ranges for the model predictions.

Estimation of worm burden using new diagnostic tools

Our knowledge of the processes that determine the number of parasites in the human host is incomplete. One of the reasons is the lack of proper diagnostic tools for measuring the adult worm burden. With the new diagnostic tools based on immunological markers [30-32] it is now possible to at least have a qualitative measurement of the presence of adult worm. The present version of LYMFASIM can simulate output on both qualitative and quantitative measurements of the adult worm burden and is therefore able to incorporate this new diagnostics, and to estimate the diagnostic test properties from an analysis of data from appropriate studies.

Migration

Presently the model can be used to simulate an endemic situation in a closed population. Movement of infected persons from endemic to non-endemic areas and vice versa can influence the outcomes of control strategies. This is particularly important in the context of the current worldwide campaign against filariasis. Control programmes will have difficulties to achieve the target of elimination when there is considerable immigration from areas, which are not covered by the control programme. In order to account for human migration the demography part of LYMFASIM needs to be enlarged.

Dynamics of acute and chronic diseases

At present the model explicitly considers the natural history of hydrocele and lymphoedema/ elephantiasis as two separate forms of chronic morbidities; no provision is, as yet, made for acute morbidities. The development of hydrocele is modelled as a function of worm load and lymphoedema is assumed to be a consequence of a persons's worm load in combination with immunological reactions targeted against the worms

(anti-fecundity immunity). So far no attempt has been made to quantify this part of the model. As far as morbidity is concerned LYMFASIM is in only a formative stage. This is mainly because of incomplete knowledge on the relation between dynamics of infection and immunity and disease. Recently Dreyer and colleagues [77] proposed a dynamic model for bancroftian filariasis by integrating findings from clinical research studies. The study makes distinction between pathogenesis of chronic hydroceles and chronic lymphoedema: worms alone are sufficient to induce hydroceles, but chronic lymphoedema develops only when the lymphatic system is damaged by two factors acting in concert: filarial worms and secondary bacterial infections. Since these propositions are essentially based on cross-survival or short follow-up clinical research data, studies with many more years of follow up will be required to fully understand the natural course of infection and disease. However, the former proposition (hydrocele development), which has already been incorporated in LYMFASIM, can be tested with observations. As far as the second proposition is concerned LYMFASIM needs to be adapted.

Refinement of chemotherapy module

In the present version of LYMFASIM the chemotherapy part of the module has an option for specifying the overall mass treatment coverage and pattern of compliance (i.e, random, systematic, and neither random nor systematic). The module needs to be refined to include age- and sex-specific data on coverage. Efforts are already made on to quantify and validate the compliance model with the observations from one of the VCRC conducted community drug trials in India.

Application of the model to other areas

Application of LYMFASIM to other endemic situations depends on the (i) availability of epidemiological data for fitting base-line situations and (ii) vector-parasite combination. Though it would be ideal to quantify the model to different endemic situations, it requires considerable amount of time, skilled manpower and, above all, demands suitable entomological and epidemiological data. On the other hand efforts are being made to adopt the model to different endemic situations by investigating demographic and epidemiological patterns for different regions of the world.

LYMFASIM for decision support

The present version of LYMFASIM will be more useful and become a tool for decision support, when LYMFASIM is equipped with a costing module also. Efforts have been made to transform LYMFASIM into a user friendly and readily applicable package in such a way that workers and programme managers for monitoring and evaluation of control programmes can use it.

5.6 Answers to the research questions

In the introduction of this thesis we formulated nine research questions. In the different chapters of the thesis and in the discussion thus far, these questions have been addressed. We will now answer the questions one by one. Justification for the answers is in one or more chapters of this thesis.

1. **Is the integrated vector management a better method than the conventional vector control programme to control transmission of filariasis?**

   Yes, the integrated vector management (IVM) programme has brought a significant reduction in prevalence in the younger population (< 30 years) in which a constant rate of increase was observed prior to control (Section 2.1). Additional benefits were reductions in general mosquito nuisance, which could have minimised the negative health impact of other vector borne diseases. The results were better than in the control arm which applied conventional vector control.

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?**

   Prevalence measured in the younger (<20 years) population is a better indicator than overall prevalence or intensity of Mf for assessing the relative impact of vector control. It is difficult to detect changes in epidemiological parameters in children aged < 5 years (Section 2.1 & 5.1).

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?**

   The recovery of the vector population, 3 years after cessation of IVM, signifies an increasing risk of transmission (Section 2.2). But the Mf-prevalence continued to decline. The changes in entomological parameters are expected to influence epidemiological parameters with a delay, reflecting the time-scale of the transmission cycle.

4. **Is there any regulation in the processes of *W. bancrofti* Mf-uptake by, and larval development, within the vector host *C. quinquefaciatus*?**

   Evidence from meta-analysis of experimental studies and our field study (Section 3.2), led us to infer that there is no or little evidence for the existence of regulation mechanism in the process of *W. bancrofti* Mf-uptake by *C. quinquefasciatus*. However, our experimental (Section 3.1) and field (Section 3.2-3.3) studies provide strong evidence that regulation occurs during the process of larval development between Mf and L1. L3-yield declines with increasing Mf-uptake, which confirms the existence of 'limitation' mechanism in this vector-parasite combination in nature (Section 3.1).

5. **Does parasite regulation occur through parasite-induced vector mortality?**

Our field (Section 3.3) and experimental transmission studies (Section 3.4) provided quantitative evidence that regulation of *W. bancrofti* in *C. quinquefasciatus* occurs via 'excess' mortality of vectors. Most of the vector mortality occurs in the early stage of parasite development, i.e. between Mf and L1 stage (Section 3.3 & 3.4).

6. Is regulation of parasite numbers in humans immune mediated?

The good fit of the immunity models (anti-L3 or anti-fecundity) to the observed convexity in age-specific epidemiological patterns of *W. bancrofti* infection in Pondicherry suggested that regulation of parasite numbers in human is acquired immunity mediated (Section 4.3). However, the anti-L3 and anti-fecundity mechanisms of immunity that we considered and tested are not the only ones that are possible.

7. What would be the impact of human immune mechanisms regulating *W. bancrofti* infections on lymphatic filariasis control programmes?

When transmission is reduced /completely interrupted the level of immune response will decline. This is applicable to the annual mass drug administration programmes, which aim at elimination of infection by interrupting transmission. The number of rounds of MDA should be adequate for elimination of parasite reservoir. Stopping after too few rounds of MDA, in the absence of vector control, would lead to rapid resurgence of infections, because it has the potential to significantly reduce the level of immunity and thereby increase the level of susceptibility in the population (Section 4.3 & 5.3).

8. What could be the long-term impact of IVM programme in Pondicherry?

The downward trend in prevalence beyond the period of IVM is predicted to last for about 10 years. Model outcomes suggest that subesequently the trend would drift upwards and reach the pre-control level about 25 years later in the absence of any control measure. The up-trend would be due to loss of immunity resulting from reduced transmission during the IVM period and increased intensity of transmission due to recovery of the vector population (Section 4.3 & Section 5.3).

9. What are the prospects for elimination of LF by mass drug administration programmes?

The prospects for elimination of LF in Pondicherry appears to be certain with five, annual, DEC-based MDA provided the population coverage is above 90% and that the coverage should be at least 60% if 11 such MDA are used (Section 4.2). For ivermectin (200 μg per kg body weight)-based MDA, at least 8 annual rounds are necessary to achieve elimination with 65% coverage. These predictions largely depend on the estimates of drug efficacy and the elimination criterion used for determining the duration of control (Section 4.4.).

5.7 Conclusions and recommendations

1. Prevalence of Mf measured in younger population is a better indicator of the impact of vector control than overall prevalence or intensity of Mf.
2. An integrated vector management programme can achieve a sustained control of *C. quinquefasciatus* breeding in an urban environment. Reduction in mosquito numbers has important subsidiary benefits: reducing the general mosquito nuisance and minimizing the negative health impact of other mosquito related diseases.
3. Better knowledge on the transmission dynamics of the parasite in vector and human is important to make a sound prognosis of long-term impact of control programmes.
4. There is 'no density-dependence' in the uptake of *W. bancrofti* Mf by *C. quinquefasciatus*. 'Density-dependence' occurs during the larval development between Mf and L1. L3-yield declines with increasing Mf-uptake, confirming existence of 'limitation' mechanism in the field conditions. Parasite-induced vector mortality is an important regulation mechanism in the vector.
5. Simulation aided analysis suggested that the observed epidemiological pattern in Pondicherry is immune mediated (anti-L3 or anti-fecundity), with a long lasting but not life-long immunity.
6. The model predicted downward trend in Mf-prevalence agreed well with observations made 3 and 7 years after cessation of IVM in Pondicherry. The downtrend is likely to continue for 4 more years, which appears reasonable given that the estimated mean lifespan of *W. bancrofti* is about 10 years.
7. Prospects for elimination of LF by MDA is certain with 5 and 11 annual DEC provided the coverage is at least 90% and 60% respectively. For ivermectin (200 μg per kg body weight) at least 8 rounds of MDA is necessary to achieve elimination with 65% coverage. The predictions critically depend on the estimates of drug efficacy and the criterion used for declaring elimination.
8. Considerable variations between blood samples in a person, between persons' variability in exposure to vector mosquito and in immune response contribute to the large observed variability in Mf-density in the population.
9. A consequence of the 'limitation' phenomenon is that interruption of transmission is difficult. Hence, present-day chemotherapy based control programmes should also consider the possibility of taking appropriate measures to check vector proliferation.
10. The relation between numbers of *W. bancrofti* L3 developed in *C. quinquefasciatus* and human Mf-density is one of the important regulating mechanisms considered in LYMFASIM. Application of LYMFASIM to other areas, where the vector or parasite species are different, would require re-quantification of this relationship.

11. In the current version of LYMFASIM, hydrocele and lymphoedema/ elephantiasis are the two forms of chronic morbidity. So far no attempt has been made to quantify this part of the model. Since available evidence suggests that development of hydrocele depends on worm load, LYMFASIM can be used to test this hypothesis with observations.

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Summary

Human lymphatic filariasis (LF) is mainly caused by three species of nematodes: *Wuchereria bancrofti*, *Brugian malayi* and *Brugian timori*. More than 100 mosquito species transmit lymphatic filariasis. Lymphoedema and elephantiasis in men and women and hydrocele only in men are the chronic manifestations causing disfigurement of body parts. The disease impairs mobility, social life, educational and marriage prospects and also the marital relations and sexual life. *W. bancrofti* transmitted by *Culex quinquefasciatus* is the most widely prevalent vector-parasite combination and is the focus of the thesis.

More than 1.1 billion people living in 73 countries of tropical and subtropical regions of the world are exposed to the risk of LF infection and about 128 million people are infected. India alone contributes more than one-third of 'at risk' and infected population in the world. Until recently, control of LF has received poor attention because of lack of information on the disease burden and feasible control tools and strategies. However, recent research findings on socio-economic impact of the disease and development of new diagnostic and control tools have given hope that LF is one among six communicable diseases that can be eradicated. In 1997 the 50th World Health Assembly passed a resolution to eliminate LF from the globe by 2020. The global LF elimination strategy to reach this goal is (i) interruption of transmission 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 pledged elimination programmes. With the worldwide initiation of elimination campaign it is crucial to have an idea of the positive impact of various control strategies on the epidemiology and disease burden. Such judgements are far from easy in LF, since the disease incidence is largely determined by cumulative exposure to parasites over a period of many years. Further the outcome of the control/elimination programmes depends on a multitude of factors viz., efficiency of vectors, parasite epidemiology, immunity of hosts, drugs efficacy and treatment coverage of communities. To aid decision-making about control/elimination strategies and to evaluate prospectively the long-term effects of control/elimination measures, it is therefore, important to develop epidemiological models, which describe the full transmission cycle, morbidity, and control options.

This thesis contributes knowledge through quantification of the population dynamics of *W. bancrofti* in the vector and human and uses 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.

Epidemiological indicators of vector control

Earlier studies which attempted to evaluate the effectiveness of vector control, showed no measurable impact on overall prevalence and intensity of Mf. While evaluating a 5-years integrated vector management (IVM) programme in Pondicherry, we found that prevalence of Mf in younger age groups could be a better indicator of the effectiveness of vector control than 'intensity' of infection, which is highly variable between persons (**Section 2.1**). Evaluation of prevalence levels in the juvenile population suggested that the impact is better in the IVM area than in conventional programme area and that assessment will be biased if evaluation is done without a comparison area. Evaluation after recovery of the vector population indicated that the changes in epidemiological parameters did not correspond with the changes in transmission parameters because they occur at a different time scale (**Section 2.2**). We conclude on the basis of the epidemiological evidence that the IVM programme has reduced the level of transmission and hence weakened the stability of the parasite population in the human host. Such a reduction in transmission, on the other hand, might have increased the level of susceptibility in humans. So it is not clear whether or not the vector recovery after cessation of the IVM has enhanced the transmission success of the parasite. Thus our epidemiological analyses, while indicating uncertainties in evaluating long-term impact of control programmes, suggested the need for a modeling framework to unravel the population dynamics of the parasite in human and vector in order to have a sound prognosis of the long-term impact of control programmes.

Vector-parasite interaction

The goal of the present day worldwide elimination programme is to interrupt transmission through mass drug administration. The likelihood of achieving this goal largely depends on the dynamics of the parasite in humans and mosquitoes and the ease or difficulty of transmission between these two hosts. Of particular importance is the mechanism regulating the parasite numbers in the vector host. In **Chapter 3**, we tried to elucidate this aspect through a combination of semi-experimental and field transmission studies. Both semi-experimental (**Section 3.1**) and field studies (**Section 3.2-3.3**) concur with each other in that the yield of L3-larvae declines with increasing Mf-uptake and hence corroborated the finding from laboratory studies that the *W. bancrofti* - *C. quinquefasciatus* combination is of the 'limitation' type. Further, by modeling the survival experience of a cohort of mosquitoes, exposed to microfilaraemic and amicrofilaraemic volunteers, we found that in *C. quinquefasciatus* regulation of *W. bancrofti* occurs via 'excess' mortality of vectors (**Section 3.4**). Thus we conclude that development of *W. bancrofti* larvae in *C. quinquefasciatus* is 'density-dependent' under natural settings, which has important implications for the control of lymphatic filariasis. Even at low Mf-densities, with a high vector biting rates, there can still be a considerable level of transmission. Therefore if elimination of infection were the aim of control programme

this would require concerted effort and long-lasting commitment to reduce the Mf-load in the community to near zero. Apart from gaining valuable insights into the dynamics of infection in the vector, our experimental transmission study provided a quantitative relationship between human and vector infections. This has become an important input for the subsequent development and quantification of the LYMFASIM simulation model.

Development of the LYMFASIM simulation model

Difficulties involved in assessing effectiveness of vector control and its long-term impact by standard epidemiological methods, implicated the need for a comprehensive modeling framework (**Chapter 2**). In response to this and considering number of objectives in mind, the Department of Public Health, Erasmus University Rotterdam in collaboration with the Vector Control Research Centre, Pondicherry and the CpqAM in Recife Brazil developed the LYMFASIM simulation model for lymphatic filariasis transmission and control. A detailed description of LYMFASIM and its mathematical formulations are given in **Section 4.1**. LYMFASIM is a stochastic microsimulation model, in which the individual is the basic modeling unit. Like its predecessor ONCHOSIM, a model for onchocerciasis transmission and control from the Department of Public Health, Erasmus University Rotterdam, LYMFASIM is a hybrid of two simulation modeling techniques: (i) stochastic micro-simulation and (ii) deterministic macro-simulation. The stochastic micro simulation modeling technique is used to model the life histories of individual human and parasite. Events in life histories of an individual person such as birth, death, exposure to mosquito biting, acquisition and loss of worms, ability to elicit immune response, number of Mf that would appear in peripheral blood and inclination to participate in a mass treatment programme and response to treatment are governed by probability distributions. Similarly individual parasite's life span is generated from probability distribution. All the events in an individual person are updated at monthly intervals. The simulated individuals together constitute a dynamic endemic community that changes over time due to birth and death processes. A deterministic macro simulation modeling technique was used to mimic the uptake of Mf by biting mosquitoes, the development of Mf to L3 in the vector, the release of L3 larvae when a mosquito bites, the development of L3 larvae into mature worms in the human host, and the Mf production by mature female worms after mating.

LYMFASIM is a modeling framework, in which it is possible to test hypotheses about various processes that shape the dynamics of infection and disease. For example considerable uncertainties exist about the mechanisms playing a role in the dynamics of infection in the human host. The development of parasites and their fecundity in human individuals can be influenced by host immune responses. Several alternative mechanisms can be tested by modeling the process that involves human immune response, worm load and fecundity.

Initially LYMFASIM was applied to describe the epidemiological trends in North-eastern Brazil (**Section 4.1**). Since the model parameters were quantified using cross-

sectional data, a number of quite different assumptions related to human immune mechanism were found to be compatible with the observations. Therefore attempts were made later to estimate the model parameters using longitudinal data from Pondicherry, India.

Population dynamics of *Wuchereria bancrofti* in human

LYMFASIM was applied to simulate the impact of 5 years of vector control on the intensity of *W. bancrofti* infection in a cohort of persons in Pondicherry (**Section 4.3**). In fitting LYMFASIM three hypotheses about the human immunology were tested: (i) anti-L3 immunity, triggered by accumulated L3-antigens, leading to reduction in success of inoculated L3 larvae to become adult, (ii) anti-fecundity immunity, originated from cumulative presence of adult worms, leading to reduction in Mf-production and (iii) total absence of immunity. For each model, parameters were estimated by comparing the simulated outcomes with observations. The two immunity models were equally good in describing the observations compared to a poor fit of a model with no-immunity. The immunity models helped to gain insights in the population dynamics of *W. bancrofti* infection in the human host. Firstly, immune mechanism (antigen from L3 or worm) was found to be an essential component to describe observed convexity in age-specific epidemiological patterns of *W. bancrofti* infection in Pondicherry. Secondly, the model provided estimates for a number of parameters, which are not directly observable from data. Of particular importance are the duration of immune response and lifespan of the parasite. The estimated duration of immune responses were 5-17 and 5-18 years respectively for anti-L3 and anti-fecundity immunity models, suggesting that immunity is long lasting but not life-long. The mean lifespan of *W. bancrofti* in the human host was estimated at 10-12 years. Thirdly, we inferred that the variation in observed Mf-load in and between persons is to a considerable extent due to sampling variability in Mf counts in blood smear, in addition to personal differences in exposure to mosquito bites and in eliciting immune response. Lastly, the analysis provided model quantifications that are useful to predict long-term effectiveness of the IVM programme in Pondicherry as well as helpful to aid decision-making about local control policies in the present global elimination context.

Forecasting long-term impact of vector control

In an attempt to explore the validity of fitted models, LYMFASIM was used to evaluate the long-term impact of the IVM programme in Pondicherry (**Section 4.3**). The two model variants of LYMFASIM (anti-L3 and anti-fecundity) predicted prevalences 3 and 5 years after cessation of IVM programme, which were found to be in agreement with the observed continuing downward trend. There are no data to check the validity of long-term model predictions, namely that prevalence would reverse to the pre-control situation about 25 years after cessation of IVM. Although one should be cautious in applying a

model, which was quantified on the basis of, 5 years longitudinal follow up of individuals; the predictions nicely illustrate the impact of loss of immunity by damped oscillations. As a result of reduced transmission many persons would have lost all their worms, which would reduce level of anti-fecundity immunity in the absence of boosting. Since transmission is not completely interrupted, rate of loss of immunity for anti-L3 immunity model could be lower than that of anti-fecundity model, which explains why the peaks for the two models occur at different time points.

Prospects for LF elimination by MDA

As an operational application of LYMFASIM, the *two model variants* (anti-L3 and anti-fecundity immunity) were used to predict the number of rounds of MDA necessary to achieve the goal of elimination. Assuming a very pessimistic criterion for elimination (i.e. elimination can be achieved if the prevalence is less than 0.1% immediately after stopping MDA) the model predictions showed that at least 90% coverage would be required to achieve elimination with five, annual, DEC-based MDA, and that coverage will still have to be at least 60% if 11 such MDA are used (**Section 4.2**). On the other hand, assuming a very optimistic elimination criterion (i.e. zero Mf-prevalence 40 years after the start of control), the model predictions indicated that at least 65% coverage will be required to achieve elimination with 8, annual, ivermectin (200 μg/kg body weight)-based MDA (**Section 4.4**). The number of rounds of MDA necessary to achieve elimination depended to a large extent on coverage, drug efficacy, and level of endemicity. However, changing the interval between rounds of MDA mainly influenced the duration of control, not the number of rounds of MDA necessary to achieve elimination (**Section 4.4**).

Concluding remarks

The thesis has contributed knowledge on various aspects of epidemiology, population dynamics and control of lymphatic filariaisis. Epidemiological analyses indicated that prevalence of Mf in younger population is a better indicator of effectiveness of vector control than overall prevalence or intensity of Mf. Assessment based on this indicator clearly demonstrated the superiority of the integrated vector management programme over conventional method of control in Pondicherry. Further, the analyses threw light on the difficulties involved in assessing the impact of recovery of the vector population on the basis of epidemiological statistics: the changes in entomological parameters would reflect on epidemiological parameters after many more years. A better understanding of the population of dynamics of the parasite in the vector and in humans would aid sound prognosis of the long-term impact of control programmes. Our field and experimental transmission studies agree with each other that larval development in the vector is density-dependent. The main conclusion that emerged from our simulation model based analysis of longitudinal data is that observed epidemiological patterns in Pondicherry are immune mediated. Considerable variations between persons in exposure to vector biting

and eliciting immune response are found to be plausible explanations for the observed variability in Mf-density in the population. Simulation results also helped in unraveling the factors influencing the impact of vector control on the dynamics of *W. bancrofti* infection in human. The long life span of the parasite and long duration of immune response are important determinants of the dynamics of recrudescence of new infection after cessation of control and hence may influence the dynamics of control programmes. Model predicted trend in prevalence agreed with observations made during pre- and post control periods as well as three and seven years after cessation of control. Assuming that the vector population has returned to the pre-control level, predictions beyond the period of observations suggested that prevalence would reach the pre-control level 25 years after cessation of control.

Finally, a start has been made to apply LYMFASIM for addressing problems related to operational issues in mass-chemotherapy based elimination programmes. In view of the global elimination programmes, the model has already been applied to estimate the number of treatment rounds and coverage required to achieve the goal of elimination with DEC or ivermectin. It is also planned to prospectively evaluate the impact of various control strategies involving a combination of drugs such as DEC, ivermectin, albendazole. WHO initiated proposals is coming up to apply the model to identify key indicators for stopping intervention and to verify absence of infection. However, to use LYMFASIM in operational settings for monitoring and evaluation of control programmes further studies would be required to develop models representing regions with different demographic and epidemiological patterns.

Samenvatting

Humane lymfatische filariasis (LF) wordt veroorzaakt door nematoden, met als belangrijkste drie soorten: *Wuchereria bancrofti*, *Brugian malayi* and *Brugian timori*. De infectie wordt door meer dan honderd verschillende soorten muggen overgebracht. De belangrijkste ziekteverschijnselen zijn lymfoedeem, elefantiasis en, alleen in mannen, hydrocele. Deze kunnen uiteindelijk leiden tot misvormingen van lichaamsdelen. De ziekte bemoeilijkt de mobiliteit, het sociale leven, opleidingsmogelijkheden, huwelijkskansen, en ook de huwelijksrelaties en sexuele relaties.

Dit proefschrift richt zich op de meest voorkomende vector-parasiet combinatie: de parasiet *W. bancrofti* die wordt overgebracht door de mug *Culex quinquefasciatus*.

In de tropische en subtropische delen van de wereld lopen meer dan 1.1 miljard mensen in 73 landen risico op LF infecties, en ongeveer 128 miljoen mensen zijn geïnfecteerd. Rond eenderde van de risicopopulatie van geïnfecteerde mensen leeft in India. Tot voor kort kreeg bestrijding van LF weinig aandacht vanwege het gebrek aan kennis over de omvang van de ziektelast en over geschikte manieren om de ziekte te bestrijden en de daarbij te volgen strategie. Recent is meer duidelijkheid verkregen over de sociaal-economische invloed van de ziekte. Het beschikbaar komen van nieuwe methoden voor diagnostiek en behandeling heeft de verwachting gewekt dat LF een van de zes infectieziekten is die kan worden uitgeroeid. In 1997 is tijdens de 50e World Health Assembly een resolutie aangenomen om vóór 2020 LF wereldwijd te elimineren. De strategie om dit doel te bereiken is (1) onderbreking van de transmissie door jaarlijkse behandeling van de bevolking met medicijnen tegen de volwassen worm en/of tegen de door deze geproduceerde microfilariae, en (2) vermindering en preventie van het door de ziekte veroorzaakte lijden en van de handicaps.

In het afgelopen decennium zijn in verschillende landen, waaronder India, pilotstudy's en grootschalige bestrijdingsprogramma's van start gegaan. Nu bestrijding wereldwijd is begonnen is het van het grootste belang om inzicht te hebben in het verwachte effect van verschillende mogelijke bestrijdingsstrategieën op de epidemiologie en op de ziektelast in de populatie. Het schatten van de effecten van bestrijding van lymfatische filariasis is verre van eenvoudig, onder meer omdat de incidentie van de ziekte afhangt van de cumulatieve blootstelling aan infecties gedurende een periode van vele jaren. Bovendien is het effect van bestrijdingsprogramma's afhankelijk van een groot aantal factoren, zoals de efficiëntie van de muggen in het overbrengen van de infecties, de ontwikkeling van de parasieten, de immuniteit van de gastheren (mug, mens), de effectiviteit van geneesmiddelen, en de dekkingsgraad die bereikt kan worden bij het behandelen van de bevolking. Epidemiologische modellen, waarin de transmissiecyclus, de ziektelast en de strategieën voor de bestrijding van de ziekte worden beschreven, kunnen een belangrijk hulpmiddel zijn bij de besluitvorming over bestrijdingsprogramma's en bij de evaluatie van hun resultaten, inclusief de mogelijkheid van eliminatie van lymfatische filariasis.

Dit proefschrift draagt bij aan de kennis over lymfatische filariasis, allereerst via schatting en kwantificering van het verloop van *W. bancrofti* infecties in de mug en in de mens, waarna deze kennis gebruikt wordt bij de ontwikkeling en toepassing van een model voor evaluatie van strategieën gericht op bestrijding en eliminatie van lymfatische filariasis. Dit LYMFASIM model, dat in belangrijke mate gebaseerd is op de resultaten van een onderzoeksproject voor geïntegreerde bestrijding van de vector dat plaatsvond in Pondicherry, India, kan worden gebruikt voor prospectieve evaluatie van de effectiviteit van verschillende controlestrategieën die zijn gebaseerd op de beschikbare medicijnen.

Epidemiologische indicatoren voor het effect van bestrijding van de vector

Bij eerder onderzoek waarin getracht werd om het effect van bestrijding van de vector (de mug) te bepalen kon geen meetbaar effect op de totale prevalentie en intensiteit van microfilariae (Mf) in mensen worden vastgesteld. Bij de evaluatie van het vijf jaren durende geïntegreerde vector management (IVM) project in Pondicherry vonden we dat de prevalentie van Mf in de jongste leeftijdsgroepen een betere indicator voor de effectiviteit zou kunnen zijn dan de intensiteit van de infectie, die sterk varieert tussen personen (**Sectie 2.1**). Evaluatie van de niveaus van de prevalentie in jongeren suggereerde dat het effect van de bestrijding in het IVM project sterker is dan in gebieden waar het gangbare bestrijdingsprogramma plaatsvond, maar ook dat de inschatting van het effect vertekend zal zijn wanneer er geen controlegebied wordt gebruikt. Evaluatie na het herstel van de vector populatie liet zien dat de veranderingen in de epidemiologische parameters niet samenvallen met veranderingen in de transmissie parameters: er zit een aanzienlijk tijdsverschil tussen beide trends (**Sectie 2.2**). Op basis van de epidemiologische gegevens concluderen we dat het IVM programma heeft geleid tot een afname van het transmissieniveau, en dat dit daarmee de stabiliteit van de parasieten populatie in de menselijke gastheren heeft verzwakt. Een dergelijke daling van de transmissie kan echter hebben geleid tot een toename van de vatbaarheid voor nieuwe infecties. Het is dus niet duidelijk of het herstel van de vectorpopulatie na het einde van het IVM programma mogelijk heeft geleid tot verhoogde vatbaarheid van de mensen. De onzekerheden in onze epidemiologische analyses suggereren dat een modelmatige aanpak vereist is voor het ontrafelen van de dynamische processen rond de parasiet in de mens en in de vector, om daarmee vervolgens verantwoorde voorspellingen te kunnen maken over de lange termijn effecten van bestrijdingsprogramma's.

Interactie tussen vector en parasiet

Het huidige wereldwijde eliminatieprogramma heeft tot doel om de transmissie te onderbreken via grootschalige toediening van medicijnen. De kans op realisatie van deze doelstelling hangt in hoge mate af van de dynamiek van de parasiet in de mens en in de mug, en van het gemak waarmee transmissie van de parasiet tussen de twee gastheren

plaatsvindt. De regulerende mechanismen in de vector zijn hierbij van bijzonder belang. In **hoofdstuk 3** hebben we via een combinatie van veldonderzoek en laboratoriumonderzoek gepoogd meer duidelijkheid over deze mechanismen te verkrijgen. De veldstudie gaf aan dat er geen dichtheidsafhankelijke regulatie optreedt bij de opname van de parasiet door de mug (**Sectie 3.2**). Dit is niet in overeenstemming met ons eerdere onderzoek (**Sectie 3.1**) en met een aantal andere experimentele studies. Een recente meta-analyse van diverse experimentele data bevestigt de uitkomst van ons veldonderzoek. Echter, bij zowel de experimentele onderzoeken (**Sectie 3.1**) als de veldstudies (**Secties 3.2-3.3**) is gevonden dat bij hogere opname van Mf door de mug er een afname is van de proportie larven dat het L3 stadium bereikt , wat de uitkomst van laboratoriumstudies bevestigt waarbij werd geconcludeerd dat de combinatie *W. bancrofti* - *C. quinquefasciatus* tot het 'limitatie' type behoort. Verder vonden we via het modelleren van de survivalgegevens van een cohort van muggen die waren blootgesteld aan Mf-positieve of Mf -negatieve vrijwilligers, dat de regulatie van *W. bancrofti* larven in *C. quinquefasciatus* optreedt via oversterfte van geïnfecteerde muggen (**Sectie 3.4**). We concluderen hieruit dat de ontwikkeling van *W. bancrofti* larven in *C. quinquefasciatus* onder natuurlijke omstandigheden dichtheidsafhankelijk is. Dit heeft belangrijke implicaties voor bestrijding van lymfatische filariasis. Zelfs bij lage Mf dichtheid in mensen kan bij een hoge frequentie van muggenbeten een aanzienlijk niveau van transmissie bestaan. Een bestrijdingsprogramma wat tot doel heeft om de infecties te elimineren om daarmee de Mf last in de bevolking tot nul reduceren, zou dan een zeer grote en langdurige inspanning kunnen vereisen.

Behalve inzicht in de dynamiek van infecties in de vector, leverde onze experimentele transmissie studie ook een kwantificering op van de relatie tussen de infecties in de mens en in de mug. Dit is een belangrijk gegeven geworden bij de ontwikkeling en kwantificering van het LYMFASIM simulatie model.

De ontwikkeling van het LYMFASIM simulatie model

De moeilijkheden die optreden bij het gebruik van standaard epidemiologische methoden voor het bepalen van de effectiviteit van vector bestrijding op korte en lange termijn, leidden tot de conclusie dat er behoefte bestond aan een uitgebreid en flexibel model (**Hoofdstuk 2**). Daarom is op de afdeling Maatschappelijke Gezondheidszorg (MGZ) van het Erasmus Medisch Centrum in Rotterdam, in samenwerking met Vector Control Research Centre in Pondicherry (India) en het CPqAM in Recife (Brazilië), het LYMFASIM simulatiemodel voor de transmissie en bestrijding van lymfatische filariasis ontwikkeld. In **Sectie 4.1** wordt een gedetailleerde beschrijving gegeven van LYMFASIM, inclusief de wiskundige formules voor de gebruikte relaties tussen modelvariabelen en voor de kansverdelingen. LYMFASIM is een stochastisch simulatiemodel waarin het individu (mens of volwassen worm) de basiseenheid in de simulatie vormt. LYMFASIM is vergelijkbaar met het eerder op MGZ ontwikkelde ONCHOSIM model voor rivierblindheid, en gebruikt evenals ONCHOSIM een combinatie van twee

simulatietechnieken: stochastische microsimulatie voor de levensloop van individuele mensen en parasieten, en deterministische macrosimulatie voor de overige processen. Gebeurtenissen in een levensgeschiedenissen van individuele mensen, zoals geboorte, sterfte, infectie door een Mf die zich ontwikkelt tot een volwassen worm en verlies (door sterfte) van wormen, relatieve blootstelling aan infecties, werking van het immuunsysteem tegen infecties, het al dan niet deelnemen aan controleprogramma's voor behandeling van infecties, en het effect van een behandeling, worden bepaald aan de hand van trekkingen uit bijbehorende kansverdelingen. De toestand van elke persoon wordt elke maand bijgewerkt, en samen vormen deze personen een dynamische endemische populatie die in omvang verandert op basis van geboorte en sterfte van individuen. Deterministische submodellen worden gebruikt voor de opname van Mf door stekende muggen, de ontwikkeling van Mf tot L3 larve in de mug, de overdracht van deze larven naar een mens gedurende een muggensteek, en de ontwikkeling van een fractie van deze larven tot volwassen wormen (waarvan vervolgens het geslacht en de levensloop via microsimulatie wordt bepaald). Ook de Mf productie van de volwassen vrouwelijke wormen wordt op deterministische wijze bepaald.

Met behulp van LYMFASIM is het mogelijk om hypotheses over de verschillende processen die een rol spelen bij lymfatische filariasis te toetsen tegen beschikbare epidemiologische en entomologische data. Er bestaat bijvoorbeeld aanzienlijke onzekerheid over de mechanismen die het verloop van infecties in een mens beïnvloeden. Zo zou de ontwikkeling en de vruchtbaarheid van parasieten beïnvloed worden door het immuunsysteem. Verschillende mechanismen hiervoor kunnen worden getoetst.

In eerste instantie is LYMFASIM gebruikt voor het beschrijven van epidemiologische trends in Noordoost Brazilië (**Sectie 4.1**). Omdat cross-sectionele data werden gebruikt voor de kwantificering van de modelparameters, bleek dat er aan aantal zeer verschillende combinaties van modelveronderstellingen over immuniteit in overeenstemming te zijn met deze data. Daarom werd in een vervolgstudie gebruik gemaakt van longitudinale data, en wel uit Pondicherry, India.

De dynamiek van *Wuchereria bancrofti* infecties in de menselijke populatie

Met behulp van het LYMFASIM model is het effect gesimuleerd van vijf jaren bestrijding van muggen op de intensiteit van *W. bancrofti* infecties in een cohort van personen in Pondicherry (**Sectie 4.3**). Bij het fitten van het model aan de data van deze studie zijn 3 alternatieve hypotheses over de immuunprocessen in mensen getoetst: (i) anti-L3 immuniteit tegen L3 larven die bij een muggensteek in de mens worden gebracht, teweeggebracht door accumulatie van L3-antigenen; (ii) anti-vruchtbaarheid immuniteit, ontstaan uit de geaccumuleerde aanwezigheid van volwassen wormen, en waarbij vrouwtjeswormen minder of geen Mf produceren; (iii) in het geheel geen immuniteit. Voor elk van deze modelvarianten zijn de parameters geschat door gesimuleerde

uitkomsten te vergelijken met de waarnemingen. De twee varianten met immuniteit waren ongeveer even goed in het reproduceren van de waarnemingen, terwijl de variant zonder immuniteit een veel slechtere fit te zien gaf. De modelvarianten met immuniteit waren in staat om de waargenomen convexe curven voor de leeftijdsspecifieke epidemiologische kengetallen in Pondicherry te beschrijven. Ook werden schattingen verkregen voor parameters die niet direct te observeren zijn, bijvoorbeeld voor de duur van de immuunrespons en voor de levensduur van de worm. De geschatte duur van de immuunrespons was 5-17 jaar voor het anti-L3 model en 5-18 jaar voor het anti-vruchtbaarheid model, wat suggereert dat de immuunrespons langdurig, maar niet levenslang, in stand blijft. De gemiddelde levensduur van de volwassen worm werd op 10-12 jaar geschat. Volgens het model kan de waargenomen variatie in aantallen Mf binnen personen en tussen personen voor een groot deel worden toegeschreven aan steekproefvariatie bij deze waarnemingen, en verder ook aan verschillen tussen personen in de blootstelling aan muggenbeten en in hun immuunrespons. Tenslotte leverde de analyse het kwantificering op van parameters die nuttig zijn bij het voorspellen van de lange termijn effecten van het IVM programma in Pondicherry, en die ook kunnen worden gebruikt voor ondersteuning van de besluitvorming over lokale controle strategieën in het kader van het huidige wereldwijde eliminatieprogramma.

Het voorspellen van de lange termijn effecten van bestrijding van de vector.

In een poging om de validiteit van de gefitte modellen te onderzoeken is LYMFASIM gebruikt om de lange termijn effecten van het IVM programma in Pondicherry te evalueren (**Sectie 4.3**). De twee modelvarianten (anti L3 immuniteit en anti vruchtbaarheid immuniteit) voorspelden prevalenties voor drie en vijf jaar na het stoppen van het IVM programma die in overeenstemming bleken met de waargenomen continu dalende trend. Er zijn nog geen data voor het toetsen van de validiteit van de lange termijn voorspelling dat de prevalentie ongeveer 25 jaar na het stoppen van het IVM programma zal zijn teruggekeerd naar het niveau vóór de start van het IVM. Hoewel we voorzichtig moeten zijn met het toepassen van een model dat is gekwantificeerd op basis van (slechts) vijf jaar longitudinale follow-up gegevens, illustreren de voorspellingen aardig het verlies van immuniteit via de gedempte oscillaties. Ten gevolge van de afgenomen transmissie zullen veel personen al hun wormen zijn kwijtgeraakt, wat de anti vruchtbaarheid immuniteit zal doen afnemen in afwezigheid van reactivering. Omdat de transmissie niet volledig is onderbroken, zou de afname van de immuniteit lager kunnen zijn in het anti L3 immuniteit model, wat verklaart waarom de pieken in de twee modellen op verschillende tijdstippen plaatsvinden.

De vooruitzichten voor eliminatie van Lymfatische filariasis via massa behandeling

Bij wijze van een operationele toepassing van LYMFASIM zijn de twee model varianten (anti L3 en anti vruchtbaarheid) gebruikt voor het voorspellen van het aantal ronden massa behandeling dat nodig zou zijn om de doelstelling van eliminatie van lymfatische filariasis te bereiken. We gebruikten een zeer pessimistisch criterium voor eliminatie (namelijk dat eliminatie zou kunnen worden bereikt als onmiddellijk na het stoppen van massa behandeling de prevalentie minder dan 0.1% is), en vonden dat een dekkingsgraad van tenminste 90% vereist zou zijn om eliminatie met vijf jaarlijkse ronden van DEC behandeling te realiseren, en dat de dekkingsgraad tenminste 60% dient te zijn bij elf behandelingsrondes (**Sectie 4.2**). Bij een meer optimistisch criterium voor eliminatie, namelijk volledige afwezigheid van infecties na 40 jaren sinds de start van de bestrijding, voorspelt het model dat acht jaarlijkse behandelingsrondes met ivermectine (200 μg/kg lichaamsgewicht) nodig zullen zijn (**Sectie 4.4**). Het aantal behandelingronden dat vereist is hangt sterk af van de dekkingsgraad, van de veronderstelde werkzaamheid van de medicijnen, en van het niveau van de endemiciteit. Verandering van het tijdsinterval tussen behandelingen heeft nauwelijks invloed op het aantal benodigde ronden, maar wel op de totale duur van het bestrijdingsprogramma (**Sectie 4.4**).

Conclusie

Dit proefschrift draagt bij aan de kennis van verschillende aspecten van de epidemiologie, de dynamiek, en de bestrijding van lymfatische filariasis. Epidemiologische analyse liet zien dat de Mf prevalentie in jongeren en betere indicator is voor de effectiviteit van bestrijding van de vector dan de totale prevalentie of intensiteit van Mf. Toepassing van deze indicator toonde dat in Pondicherry de aanpak via een geïntegreerde vector management project te prefereren is boven conventionele bestrijdingsmethoden. De analyses verduidelijkten de moeilijkheden bij het bepalen van het effect van de terugkeer van de vectorpopulatie naar het oorspronkelijke niveau op basis van epidemiologische data: de veranderingen in entomologische parameters hebben pas na een ruim aantal jaren een duidelijk effect op de epidemiologische parameters. Beter inzicht in de populatiedynamiek van de parasiet, zowel in de vector als in de mens zal bijdragen aan het maken van betrouwbare voorspellingen van de lange termijn effecten van bestrijdingsprogramma's. Zowel onze veldstudies als onze experimentele transmissiestudies laten een dichtheidsafhankelijkheid zien in de ontwikkeling van de larven in de mug. De hoofdconclusie van de analyse van de longitudinale data met het LYMFASIM model is dat de epidemiologische patronen in Pondicherry beïnvloed zijn door immuniteit. Aanzienlijke verschillen tussen personen in de blootstelling aan muggenbeten en in het opwekken van immuunreacties bieden een plausibele verklaring voor de variatie in Mf tellingen in de populatie. De simulatieresultaten hielpen ook bij het ontwarren van de factoren die de het effect van vector bestrijding op het patroon van *W*.

bancrofti infecties in mensen beïnvloedt. De lange levensduur van de volwassen worm en de lange duur van immuunreacties zijn belangrijke determinanten voor het hernieuwd optreden van infecties na het stoppen van een controleprogramma. De door het model voorspelde trend in de prevalentie komt overeen met de waarnemingen voorafgaand aan en tijdens de bestrijding van de vector, en ook na drie en zeven jaar na het stoppen ervan. Als we aannemen dat de vector populatie na afloop van de vector controle is teruggekeerd naar hetzelfde niveau als voor de start van de bestrijding, dan voorspelt het model dat de prevalentie pas na 25 jaar op het oorspronkelijke niveau zal zijn teruggekeerd.

Tenslotte is er een begin gemaakt met de toepassing van LYMFASIM voor het beantwoorden van problemen rond operationele vraagstellingen bij eliminatieprogramma's die op massabehandeling met chemotherapie zijn gebaseerd. Ten behoeve van het wereldwijde eliminatieprogramma is het model gebruikt voor het schatten van het aantal behandelingsronden en het deelnamepercentage dat vereist is om de eliminatiedoelstelling te halen met gebruik van DEC of ivermectin. Het ligt in de bedoeling om op dezelfde wijze ook strategieën te evalueren waarbij combinaties van middelen als DEC, ivermectin, en albendazole worden gebruikt. Door de WHO zijn voorstellen gedaan om het model te gebruiken om criteria te bepalen voor het stoppen van zulke bestrijdingsprogramma's en voor het verifiëren van de afwezigheid van infecties in een populatie. Echter, gebruik van LYMFASIM voor het bewaken en evalueren van bestrijdingsprogramma's in een operationele omgeving, in gebieden met afwijkende demografische en epidemiologische patronen en kenmerken, zal aanpassing van de model kwantificatie en zonodig additioneel onderzoek vergen.

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Curriculum vitae

Subramanian Swaminathan was born on January 10, 1957 at Melasengalmedu, Tamil Nadu, India. He did his bachelor's and master's degrees in Statistics from the Annamalai University, India in 1978 and 1980, respectively. In 1981 he joined the Gandhigram Institute for Rural Health and Family Welfare Trust, Dindigul, India as a Research Assistant under the Integrated Development Project for Improved Rural Health. From 1982 to 1988 he was a Statistical Assistant in the Vector Control Research Centre (VCRC), Pondicherry, India. During this period he was involved in the Filariasis Control Demonstration Project sponsored by Indian Council of Medical Research. Since 1988, he has been working as Technical Officer in the same institution. He was a faculty for the WHO supported M.Sc., Medical Entomology course (1985-1997) conducted by VCRC affiliated to the Central University, Pondicherry, India.

As part of Ph.D. programme he was trained in the development, quantification and application of mathematical simulation model under the supervision of Prof. Dr. J.D.F. Habbema, Dr. Anton P. Plaisier and Dr. Gerrit van Oortmarssen, Department of Public Health, Erasmus University, Rotterdam. His Ph.D. programme was supported (1993-1998) by WHO/TDR research training division. He also pursued the Master of Science programme in Health Services Research, supported and offered by the Netherlands Institute for Health Sciences (NIHES), Rotterdam.

He was a co-investigator of the WHO sponsored project (1996-2000) on Operational application of the LYMFASIM simulation model for comparative assessment of chemotherapy strategies in *Wuchereria bancrofti* endemic areas in India and Tanzania. He was also involved in the design, development and operational application of LQAS technique for monitoring coverage and compliance of DEC mass drug administration for lymphatic filariasis (2001-2002). Presently he is a co-investigator in the WHO/TDR sponsored projects on (i) Developing model variants and methods for use of LYMFASIM in planning and evaluation of LF control in different regions and (ii) Feasibility of lymphatic filariasis elimination using mass annual DEC single dose supplemented with DEC fortified salt in endemic clusters.

He is married to Mrs. Ramadevi and has two sons: Praveen Kumar and Preetish.

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