Lymphatic Filariasis:
Transmission, Treatment and Elimination

Wilma Stolk

Lymphatic Filariasis:
Transmission, Treatment and Elimination

Lymfatische Filariasis:
Transmissie, Behandeling en Eliminatie

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus
Prof.dr. S.W.J. Lamberts
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
vrijdag 25 november 2005 om 11.00 uur

door

Wilhelmina Allegonda Stolk

geboren te Bunnik

Promotiecommissie

Promotor:	Prof.dr. J.D.F. Habbema
Overige leden:	Prof.dr. H.A. Verbrugh Prof.dr. M.G.M. Hunink Dr. M. Yazdanbakhsh
Copromotor:	Dr. S.J. de Vlas

Colofon

ISBN-10: 90-9019949-7
ISBN-13: 978-90-9019949-8

Lay-out: Wilma Stolk
Cover-illustration: Marloes de Vries (mooieboel@gmail.com)
Printed by: PrintPartners Ipskamp, Enschede
This thesis was printed with financial support of the J.E. Jurriaanse Stichting and the Department of Public Health, Erasmus MC, Rotterdam.

Contents

Publications reprinted in this thesis

Chapter 2: Subramanian S, Stolk WA, Ramaiah KD, Plaisier AP, Krishnamoorthy K, Van Oortmarssen GJ, Amalraj D, Habbema JDF and Das PK (2004). The dynamics of *Wuchereria bancrofti* infection: a model-based analysis of longitudinal data from Pondicherry, India. *Parasitology* **128:** 467-482.

Chapter 3: Stolk WA, Subramanian S, Van Oortmarssen GJ, Das PK and Habbema JDF (2003). Prospects for elimination of bancroftian filariasis by mass drug treatment in Pondicherry, India: a simulation study. *J Infect Dis* **188:** 1371-1381.

Chapter 4: Stolk WA, De Vlas SJ and Habbema JDF (2005). Anti-*Wolbachia* treatment for lymphatic filariasis. *Lancet* **365:** 2067-2068.

Chapter 5: Stolk WA, De Vlas SJ and Habbema JDF (2005). Advances and challenges in predicting the impact of lymphatic filariasis elimination programmes. Background paper for the WHO/TDR Scientific Working Group Meeting on Lymphatic Filariasis, May 10 –12, 2005, Geneva.

Chapter 6: Stolk WA, Ramaiah KD, Van Oortmarssen GJ, Das PK, Habbema JDF and De Vlas SJ (2004). Meta-analysis of age-prevalence patterns in lymphatic filariasis: no decline in microfilaraemia prevalence in older age groups as predicted by models with acquired immunity. *Parasitology* **129:** 605-612.

Chapter 7: Stolk WA, Van Oortmarssen GJ, Subramanian S, Das PK, Borsboom GJJM, Habbema JDF and De Vlas SJ (2004). Assessing density dependence in the transmission of lymphatic filariasis: uptake and development of *Wuchereria bancrofti* microfilariae in the vector mosquitoes. *Med Vet Entomol* **18:** 57-60.

Chapter 8: Stolk WA, Van Oortmarssen GJ, Pani SP, De Vlas SJ, Subramanian S, Das PK and Habbema JDF (in press). Effects of ivermectin and diethylcarbamazine on microfilariae and microfilaria production in bancroftian filariasis. *Am J Trop Med Hyg.*

Chapter 9: De Kraker MEA, Stolk WA, Van Oortmarssen GJ and Habbema JDF. Model-based analysis of trial data: microfilaria and worm-productivity loss after diethylcarbamazine-albendazole or ivermectin-albendazole combination therapy against *Wuchereria bancrofti.* (submitted)

1

General introduction

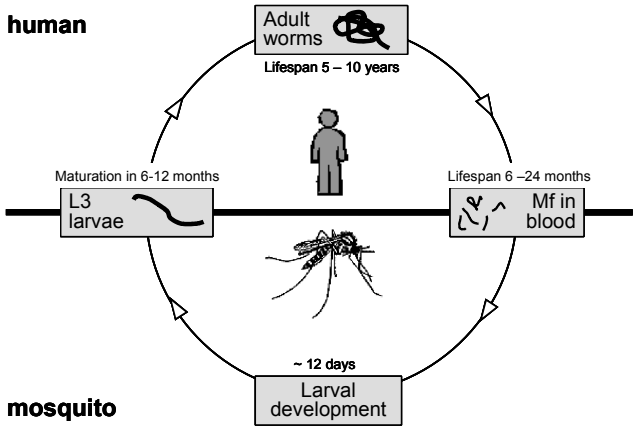
1.1 Brief introduction to lymphatic filariasis

1.1.1 Infection and disease

Lymphatic filariasis is a vector-borne parasitic disease that is endemic in many tropical and subtropical countries. The disease is caused by thread-like, parasitic filarial worms: *Wuchereria bancrofti, Brugia malayi* or *B. timori*. *W. bancrofti* is most widely spread and is responsible for more than 90% of the infections (Michael *et al.* 1996). *B. malayi* is found in several Asian countries, whereas *B. timori* is only found in Indonesia. Many different mosquito species can act as vector for transmission of lymphatic filariasis (Zagaria & Savioli 2002).

This thesis focuses on bancroftian filariasis. The life cycle of the parasite is shown in Figure 1-1. The adult worms (macrofilaria) are located in the lymphatic system of the human host, where they live for 5-10 years (Vanamail *et al.* 1996; Subramanian *et al.* 2004). During their lifespan, after mating, female worms bring millions of immature microfilariae (mf) into the blood. Some of these mf may be engorged by mosquitoes taking a blood meal. Inside a mosquito, mf develop in about 12 days into L3 stage larvae (L3). These L3 are infectious to human: they can enter the human body when a mosquito takes a blood meal. Some will migrate to the lymphatic system and develop into mature worms. Maturation takes 6-12 months (World Health Organization 1992). Mf cannot develop into adult worms without passing through the developmental stages in the mosquito. The life span of mf in the human body is estimated at 6-24 months (Plaisier *et al.* 1999).

Figure 1-1. Schematic representation of the life cycle of lymphatic filariasis, showing parasite development in the human host and vector.



Until recently, microscopic examination of peripheral blood for mf has been the only way to diagnose infection. Inconvenient night blood sampling is required, because in most areas mf only appear in the blood during the night. It is possible to find mf in day blood after provocation with the drug diethylcarbamazine (DEC), but this is less reliable. In the '90s, antigen detection tests have become available. This includes a simple card test to determine the presence of adult worm antigen in day or night blood (Weil *et al.* 1997). Antigens can be detected in nearly all microfilaraemic individuals, but also in a considerable part of the amicrofilaraemics. Using ultrasound it has now also become possible to visualize living adult worms in the male scrotum and superficial lymphatic vessels (Norões *et al.* 1996).

Lymphatic filariasis infection is chronic in nature due to the long life span of the worms and accumulation of infection over time. Many people may be infected without even knowing it, but on the long-term some people may develop severe chronic manifestations, including hydrocele and lymphoedema. These chronic manifestations are the result of accumulating, worm-induced damage in the lymphatic system. Hydrocele is an enlargement of the scrotum in males, caused by accumulation of serous fluid inside the scrotal sac, around the testicles. Hydroceles can be small and unnoticed by the patients, but can also become very large so that surgery is required. Lymphoedema is a swelling of the extremities, breasts or vulva, caused by accumulation of fluid in the subcutaneous tissue due to impaired lymph drainage. Lymphoedema sometimes progresses into elephantiasis: the skin of the enlarged body part becomes thickened, rough and hard like elephant-skin. Physical exercise and elevation of the affected body part may help to prevent progression of lymphoedema. Advanced lymphoedema and elephantiasis cannot be cured. Besides these chronic manifestations, lymphatic filariasis can cause incapacitating and painful acute episodes of lymphangitis or lymphadenitis. Such attacks can be triggered by secondary bacterial infections (Dreyer *et al.* 1999). They occur more frequently in people with lymphoedema and are an important cause of progression of the disease. Secondary infections and acute attacks can be prevented by simple measures (including hygienic measures, wearing shoes, care of small wounds), which may help to stop progression of lymphoedema (Dreyer *et al.* 2002; Shenoy 2002). Chyluria and tropical pulmonary eosinophilia are less frequently occurring manifestations of lymphatic filariasis.

1.1.2 Magnitude of the public health problem

Lymphatic filariasis is endemic in many countries in Africa, South Asia, the Pacific Islands and the Americas. Worldwide, an estimated 120 million people are affected by lymphatic filariasis, with about one third of them suffering from hydrocele or lymphoedema (Michael *et al.* 1996). Amicrofilaraemic, asymptomatic infections are not included in this estimate and the true number of affected people may even be higher. India alone accounts for about 40% of the global burden and Sub-Sahara Africa for about 37% (Ramaiah *et al.* 2000; Zagaria & Savioli 2002).

Lymphatic filariasis does not directly cause death, but its chronic manifestations are an important cause of disability and reduced quality of life. Hydrocele and lymphoedema are associated with impaired mobility and social activity, reduced work capacity, sexual dysfunction, severe psycho-social problems, stigma and bad marital prospects (Evans *et al.* 1993; Ramaiah *et al.* 1997; Ahorlu *et al.* 2001). The burden of disease in 2002 was estimated at 5.8 million disability adjusted life years (DALYs) (World Health Organization 2004). For comparison: the burden of disease for malaria and schistosomiasis was estimated at 46.5 and 1.7 million DALYs respectively.

1.1.3 Control of lymphatic filariasis

There are different ways to control lymphatic filariasis infection and to reduce the public health burden. Main strategies are treatment of the human population with anti-filarial drugs and vector control.

Treatment of human populations with antifilarial drugs has become the mainstay of lymphatic filariasis control (Ottesen *et al.* 1997). Three drugs are available for treatment of this infection: diethylcarbamazine (DEC), ivermectin and albendazole. DEC kills part of the mf and adult worms (Ottesen 1985; Norões *et al.* 1997). Ivermectin is a strong microfilaricidal drug; it probably does not kill adult worms, but may reduce their fertility (Dreyer *et al.* 1995; Plaisier *et al.* 1999). Albendazole is a broad-spectrum antiparasitic drug, which can be given in combination with DEC or ivermectin to enhance the effectiveness. Treatment with a single dose of DEC, ivermectin, or their combinations with albendazole leads to a strong reduction in mf intensity in the blood, which is usually sustained for over one year. Mass treatment programmes can be organized to treat all individuals in a community at the same time, which will lead to a strong reduction in the mean worm burden and transmission. Such mass treatment programmes aim at treating all individuals, irrespective of their infection status. This is preferred above selective treatment of infected individuals, because screening for infection is cumbersome, costly and leaves many false-negatives untreated. Mass treatment is considered safe, since side effects of treatment are mild and usually related to high intensity of infection. However, because of severe side effects, DEC cannot be used in the large parts of Africa where *Onchocerca volvulus* is endemic, and neither DEC nor ivermectin should be used in *Loa loa*-endemic areas (some African countries).

Vector control is a general term for measures that aim to reduce human-vector contact. The number of mosquitoes can be brought down by reducing the number of breeding sites for mosquitoes, killing of adult mosquitoes in houses with insecticides, or measures against mosquito larvae (chemical or biological). Bed nets and other personal protection methods can be used to reduce the number of mosquito bites (Anonymous 1994). The choice of methods depends on the local mosquito species, because species vary in their breeding, resting and feeding habits. Vector control played an important role in the control of lymphatic filariasis in the past, and is still recommended as a complementary tool in mass treatment programmes.

1.1.4 The Global Programme to Eliminate Lymphatic Filariasis

Yearly mass treatment effectively brings down the prevalence and intensity of infection. There is no non-human reservoir of *W. bancrofti* and animals also play no role in the transmission of *B. malayi* or *B. timori* infection, although brugian parasites have been found in several animal species (World Health Organization 1992; Fischer *et al.* 2004). These considerations have led to the recognition that it may be possible to eliminate lymphatic filariasis by repeated mass treatment, if it is continued sufficiently long (Centers for Disease Control 1993). In 1997, the World Health Assembly adopted a resolution, calling for the world wide elimination of lymphatic filariasis as a public health problem (World Health Organization 1997) and in 1998 the Global Programme to Eliminate Lymphatic Filariasis (GPELF) was initiated.

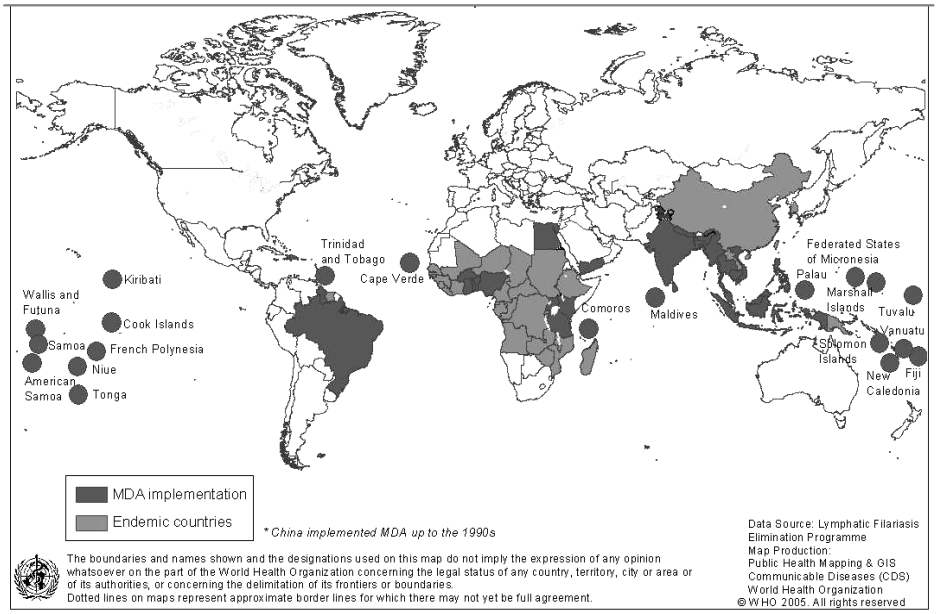
GPELF aims to eliminate the disease, and where possible to interrupt transmission, by yearly mass treatment. In addition, improved morbidity management should reduce the suffering of people with chronic disease. The recommended treatment regimen is a single dose of DEC and albendazole for countries outside Africa and a single dose of ivermectin and albendazole for African countries, where onchocerciasis may be present (Gyapong *et al.* 2005). Mass treatment is not recommended for *Loa loa*-endemic areas. Both albendazole and ivermectin are donated to the GPELF by their manufacturers (Molyneux & Zagaria 2002). In 2004, 39 countries worldwide had started mass treatment programmes to achieve the goal of elimination and this number is still growing (World Health Organization 2005). Figure 1-2 shows the endemic countries that are currently providing annual mass treatment.

1.2 Prospects of achieving elimination by mass treatment

There is a great sense of optimism that yearly mass treatment will lead to elimination of lymphatic filariasis. Based on the common assumption that adult worms live for about 5 years, it is thought that 4-6 yearly mass treatments would interrupt transmission if a sufficiently large proportion of the population receives treatment (Ottesen *et al.* 1999). The evidence base for this assumption is rather limited, though.

Lymphatic filariasis has successfully been eliminated in several areas. Active distribution of DEC and vector control have led to elimination of lymphatic filariasis from large parts of China, Malaysia, Korea and several islands of the Pacific (Ottesen 2000). In several endemic foci in Brazil, lymphatic filariasis seems to have been virtually eliminated after seven years of 6-monthly mass or selective treatment with DEC (Schlemper *et al.* 2000). However, in several other areas intensive control measures have not led to elimination of the infection. In French Polynesia recurrence of transmission occurred after cessation of a long-term mass treatment programme (Cartel *et al.* 1992). Another study from French Polynesia showed continued transmission of infection in spite of long-term intensive control programmes (Esterre *et al.* 2001). An ongoing Indian study had promising results after 6 rounds of mass treatment with DEC or ivermectin (Ramaiah *et*

Figure 1-2. Lymphatic filariasis endemic countries currently under annual mass drug administration (MDA) (as of April 2005). China already achieved basic elimination in the 90's and is now in the surveillance phase.



al. 2002; Ramaiah *et al.* 2003), but the goal of elimination was not yet achieved after respectively 9 or 8 rounds of mass treatment with DEC or ivermectin (KD Ramaiah, personal communication). Differences in outcomes of earlier control programmes and field studies may be related to the use of different treatment regimens, variation in the operational performance (e.g. the proportion of the population that received treatment), and differences in transmission dynamics between areas (e.g. related to the mosquito species responsible for transmission or characteristics of the local parasite strain).

The central question is this thesis is whether it will be possible to eliminate lymphatic filariasis by mass treatment and under what circumstances. Field experience is insufficient and not specific enough to answer this question. We will therefore address it using a mathematical model that simulates the transmission dynamics of lymphatic filariasis and can predict the long-term of interventions, while taking account of characteristics of a specific endemic setting.

1.3 Transmission dynamics

The many processes involved in parasite development and transmission were briefly described in section 1.1.1. For predicting trends in infection and the impact of control

measures, it is important to take account of density dependence and heterogeneities in these processes.

1.3.1 Density dependence

Density dependence means that the outcome of a process depends on the density of the parasite stages involved. Such density-dependent processes may impose a natural limit to the growth of the parasite population, but they also determine how easy or difficult it will be to eliminate the parasite (Dietz 1988; Duerr *et al.* 2005).

Density dependence is known to occur in the uptake and development of infection in the mosquito vector, although there are important differences between species. Let us for example consider *Culex quinquefasciatus* (the main vector in India and widespread in the world) and *Anopheles* (the main vector in Africa). In *Cx. quinquefasciatus*, the number of L3 developing in mosquitoes does not linearly increase with mf density in the blood meal, but approaches a constant value at higher mf densities (Subramanian *et al.* 1998). In other words, the proportion of mf developing into L3 declines with increasing mf uptake. Such negative density dependence is called limitation. In *Anopheles* species, the proportion of mf developing into L3 increases with mf density (Brengues & Bain 1972; Pichon *et al.* 1974; Southgate & Bryan 1992). This positive density dependence, called facilitation, occurs only at lower mf densities: at higher densities the limiting mechanisms will get the upper hand. Limitation also occurs because of reduced mosquito survival with higher parasite load (Krishnamoorthy *et al.* 2004). These density dependent processes were adequately quantified for *Cx. quinquefasciatus*, but information is lacking for most other mosquito species.

Density dependence may also occur in parasite establishment, worm maturation or survival, and mf production in the human host. This is difficult to investigate, because we cannot directly measure an individual's exposure to L3 or the number of adult worms present in the body. There may be limitation in parasite establishment due to acquisition of immunity. Evidence for this comes from animal studies, which show that previous exposure to filarial larvae protects the animals against new infection (Selkirk *et al.* 1992), but it has been difficult to prove in humans. Although immunological studies found many differences in immune responses between infected and presumably uninfected hosts, it is uncertain to what extent these differences reflect protective immunity (Ravindran *et al.* 2003). Epidemiologist found evidence for the operation of acquired immunity by studying age-patterns of filarial infection: in several locations, prevalence or intensity of infection was found to decline in older age groups, which may indicate that the older individuals have acquired immunity against infection (Woolhouse 1992; Michael & Bundy 1998). However, there may be other explanations for these observations and age-patterns have to be studied more systematically to investigate whether these patterns are common in lymphatic filariasis endemic areas.

Understanding density dependence in parasite development in vector and host is crucial for assessing the prospects of elimination. Due to density dependence,

transmission intensity is not linearly related to parasite density. Because of limiting mechanisms, transmission becomes less efficient when parasite density increases and approaches a maximum. Vice versa, transmission becomes more efficient if parasite density declines, so that the decline in transmission intensity is less than proportional. The reverse is true for facilitation, which helps for elimination. The balance between limiting and facilitating mechanisms will determine the eradicability of the infection. Part of the work in this thesis aimed to enhance our understanding of density dependent mechanisms in human host and vector.

1.3.2 Heterogeneity

Human individuals may vary with respect to their exposure to mosquitoes, susceptibility to infection, compliance to treatment, their responsiveness to treatment, etc. Because of such heterogeneities, the distribution of parasites over the population is not even: whereas some people may be uninfected, others may have high worm burdens (i.e. aggregation). The importance of heterogeneity as determinant of transmission and the persistence of infection in the human population is often overlooked. Ignoring such heterogeneities, however, may lead to overestimation of the effectiveness of population-based control measures and the probability of elimination (Duerr *et al.* 2005). The people with highest worm burdens contribute most to transmission, but also receive most new infections. The probability of male and female worms mating and the intensity of transmission are therefore higher than expected based on the average worm burden per individual. Also, to clear infection from all individuals, including the most-heavily infected, treatment may have to be continued longer than would be expected based on the average worm load per individual. Sometimes it may be useful to adapt the design of control programmes, e.g. by targeting high-risk groups (Anderson & May 1991). For predicting the impact of mass treatment, it is also important to consider individual variation in compliance and responsiveness to treatment (Plaisier *et al.* 1999; Stolk *et al.* in press).

1.4 Simulating lymphatic filariasis transmission and control

1.4.1 The LYMFASIM simulation model

Research on lymphatic filariasis at the Department of Public Health of Erasmus MC has aimed at predicting the impact of different control strategies to inform policy makers and public health authorities involved in the control of lymphatic filariasis. For this purpose, the LYMFASIM modelling framework has been developed.

LYMFASIM aims at realistic prediction of the effects of control measures. It mimics the acquisition and loss of infection in individual humans. Individuals form together a dynamic population and they interact through biting mosquitoes. The model mimics the key-processes involved in transmission of lymphatic filariasis as outlined in section 1.1.1.

Density dependence in the uptake and development of mf in mosquitoes is taken into account and acquired immunity can optionally be included in the model. Human individuals may differ with respect to their exposure to infection, immune responsiveness or compliance to treatment, so that the infection intensity varies between individuals. Adult worms, which are also simulated at the individual level, vary with respect to their life span and (optionally) the rate of mf production. A schematic representation of this part of the model is provided in Figure 2-1 in the next chapter. Other parts of the model concern the development of chronic morbidity, the efficacy of antifilarial drugs, characteristics of vector control or population treatment interventions, and the diagnosis of infection.

The design of this modelling framework reflects current knowledge about the dynamics of lymphatic filariasis. All processes and mechanisms that are relevant for transmission and control of lymphatic filariasis in the human population are described by mathematical equations. A full mathematical description of the model is provided elsewhere (Plaisier *et al.* 1998). LYMFASIM is based on the technique of stochastic microsimulation model (Habbema *et al.* 1996). This is a powerful technique that allows explicit simulation of the (chance) processes involved in transmission, taking account of heterogeneities. Probability distribution functions are used to describe heterogeneity in the human or parasite population. The same technique was used in the ONCHOSIM model for onchocerciasis (river blindness), which was earlier developed at the Department of Public Health (Plaisier *et al.* 1990; Plaisier 1996) and has been used widely for evaluation of the large-scale Onchocerciasis Control Programme in West-Africa that ran from 1975 – 2002.

LYMFASIM was developed in close collaboration with experts from research institutes from India (Vector Control Research Centre, Pondicherry) and Brazil (Centro de Pesquisas Aggeu Magalhães, Recife).

1.4.2 Simulating transmission dynamics in a specific area

As part of model development, the many processes and parameters in the model must be quantified carefully, taking account of local characteristics. The value of various biological parameters can be expected to be independent of the area under study (e.g. the adult worm or mf life span, the duration of the premature period). The value of others may depend on the mosquito species responsible for transmission (e.g. those related to the development of the parasite in the mosquito) or characteristics of the study population (e.g. demography, exposure to mosquitoes). Several parameters represent typical characteristics of the control programme under study. With respect to mass treatment programmes, this concerns for example the choice of drugs, the number and timing of treatment rounds, and the proportion of the population covered in each round. Sources of information for parameter quantification include scientific literature, expert opinion, local field observations, routinely collected statistical data, and operational data collected for evaluation of ongoing control programmes. If data are not available, additional data

may have to be collected to obtain the necessary information. The value of remaining parameters can be estimated by comparing model predictions to observed data. Such comparison is crucial for validation of the model.

1.4.3 Other models for lymphatic filariasis

LYMFASIM is not the only mathematical model for lymphatic filariasis. An overview of the use of different types of models in lymphatic filariasis research was recently published (Das & Subramanian 2002). Targeted models, which consider part of the processes involved in transmission, have been developed to clarify for example the role of acquired immunity (Michael & Bundy 1998; Michael *et al.* 2001), the effects of treatment on adult worm (Plaisier *et al.* 1999), or the trends in infection intensity during mass treatment (Plaisier *et al.* 2000). There is one other model, called EPIFIL, which simulates the full transmission cycle (like LYMFASIM) and is also being used to predict the long-term impact of control measures (Chan *et al.* 1998; Norman *et al.* 2000; Michael *et al.* 2004). The two models and their predictions are compared in chapter 5 of this thesis.

1.5 Objectives and research questions

The primary objective of this thesis is to quantify the parameters of the LYMFASIM model and to use the model for predicting the long-term impact of mass treatment and assessing elimination prospects. A secondary objective is to clarify some of the gaps in our knowledge of the transmission dynamics. Specific research questions are:

1. What are the prospects for elimination of lymphatic filariasis by mass treatment?
2. Does protective immunity develop after prolonged exposure to lymphatic filariasis infection?
3. How do mosquito species differ with respect to their efficiency in transmitting lymphatic filariasis infection?
4. What are the effects of DEC, ivermectin, and their combinations with albendazole, on adult worms and microfilariae?

1.6 Structure of the thesis

The work reported in this thesis can be divided into two parts.

In the first part of the thesis, we apply the LYMFASIM simulation model to Pondicherry in India, addressing research question 1 on the conditions under which lymphatic filariasis can be eliminated. Pondicherry offers an ideal starting point for our studies, because a wealth of epidemiological and entomological data is available from this area. Using these data, we quantify the model parameters (**chapter 2**). The model is subsequently used to investigate how many yearly mass treatment rounds with ivermectin

or other drugs would be required to eliminate lymphatic filariasis from Pondicherry (**chapters 3** and **4**). Our predictions are compared with published predictions from the other available model for lymphatic filariasis, EPIFIL, and advances and challenges in predicting the impact of lymphatic filariasis programmes are discussed (**chapter 5**).

In the second part of the thesis, we report studies that were done to enhance our understanding of lymphatic filariasis and to quantify specific model parameters. Research questions 2-4 are addressed in these studies. We first review age-patterns of filarial infection from India and Africa, to investigate whether acquired immunity protects older people from infection (**chapter 6**). The differences between mosquito species in their efficiency in transmitting infection are subsequently addressed, focusing on mf uptake and development of mf into L3 in *Cx. quinquefasciatus* and *Ae. polynesiensis* (**chapter 7**). Lastly, the effects of DEC or ivermectin and their combinations with albendazole are studied (**chapters 8** and **9**).

The general discussion (**chapter 10**) completes this thesis. This final chapter provides concise answers to the questions posed in the introduction, discusses remaining challenges for model-based support of lymphatic filariasis control, and lists the main conclusions and recommendations.

1.7 References

Ahorlu CK, Dunyo SK, Asamoah G and Simonsen PE (2001). Consequences of hydrocele and the benefits of hydrocelectomy: a qualitative study in lymphatic filariasis endemic communities on the coast of Ghana. *Acta Trop* **80**: 215-221.

Anderson RM and May RM (1991). *Infectious diseases of humans: dynamics and control.* Oxford, Oxford University Press.

Anonymous (1994). *Lymphatic filariasis infection and disease: control strategies. Report of a consultative meeting held at the Universiti Sains Malaysia, Penang, Malaysia (August 1994).* WHO/CTD/TDR Consultative Meeting, Penang, Malaysia, World Health Organization.

Brengues J and Bain O (1972). Passage des microfilaires de l'estomac vers l'hémocèle du vecter, dans les couples *Wuchereria bancrofti - Anopheles gambiae* A., *W. bancrofti - Aedes aegypti* et *Setaria labiatopapillosa - A. Aegypti*. *Cah ORSTOM, sér Ent méd et Parasitol* **10**: 235-249.

Cartel JL, Nguyen NL, Spiegel A, Moulia-Pelat JP, Plichart R, Martin PM, Manuellan AB and Lardeux F (1992). *Wuchereria bancrofti* infection in human and mosquito populations of a Polynesian village ten years after interruption of mass chemoprophylaxis with diethylcarbamazine. *Trans R Soc Trop Med Hyg* **86**: 414-416.

Centers for Disease Control (1993). Recommendations of the International Task Force for Disease Eradication. *MMWR Morb Mortal Wkly Rep* **42**: 1-38.

Chan MS, Srividya A, Norman RA, Pani SP, Ramaiah KD, Vanamail P, Michael E, Das PK and Bundy DA (1998). Epifil: a dynamic model of infection and disease in lymphatic filariasis. *Am J Trop Med Hyg* **59**: 606-614.

Das PK and Subramanian S (2002). Modelling the epidemiology, transmission and control of lymphatic filariasis. *Ann Trop Med Parasitol* **96**: S153-S164.

Dietz K (1988). Density-dependence in parasite transmission dynamics. *Parasitol Today* **4**: 91-97.

Dreyer G, Norões J, Amaral F, Nen A, Medeiros Z, Coutinho A and Addiss D (1995). Direct assessment of the adulticidal efficacy of a single dose of ivermectin in bancroftian filariasis. *Trans R Soc Trop Med Hyg* **89**: 441-443.

Dreyer G, Medeiros Z, Netto MJ, Leal NC, de Castro LG and Piessens WF (1999). Acute attacks in the extremities of persons living in an area endemic for bancroftian filariasis: differentiation of two syndromes. *Trans R Soc Trop Med Hyg* **93**: 413-417.

Dreyer G, Addiss D, Dreyer P and Norões J (2002). *Basic lymphoedema management: treatment and prevention of problems associated with lymphatic filariasis*. Hollis, Hollis Publishing Company.

Duerr HP, Dietz K and Eichner M (2005). Determinants of the eradicability of filarial infections: a conceptual approach. *Trends Parasitol* **21**: 88-96.

Esterre P, Plichart C, Sechan Y and Nguyen NL (2001). The impact of 34 years of massive DEC chemotherapy on *Wuchereria bancrofti* infection and transmission: the Maupiti cohort. *Trop Med Int Health* **6**: 190-195.

Evans DB, Gelband H and Vlassoff C (1993). Social and economic factors and the control of lymphatic filariasis: a review. *Acta Trop* **53**: 1-26.

Fischer P, Supali T and Maizels RM (2004). Lymphatic filariasis and *Brugia timori*: prospects for elimination. *Trends Parasitol* **20**: 351-355.

Gyapong JO, Kumaraswami V, Biswas G and Ottesen EA (2005). Treatment strategies underpinning the global programme to eliminate lymphatic filariasis. *Expert Opinion on Pharmacotherapy* **6**: 179-200.

Habbema JD, De Vlas SJ, Plaisier AP and Van Oortmarssen GJ (1996). The microsimulation approach to epidemiologic modeling of helminthic infections, with special reference to schistosomiasis. *Am J Trop Med Hyg* **55**: 165-169.

Krishnamoorthy K, Subramanian S, Van Oortmarssen GJ, Habbema JD and Das PK (2004). Vector survival and parasite infection: the effect of *Wuchereria bancrofti* on its vector *Culex quinquefasciatus*. *Parasitology* **129**: 43-50.

Michael E, Bundy DA and Grenfell BT (1996). Re-assessing the global prevalence and distribution of lymphatic filariasis. *Parasitology* **112**: 409-428.

Michael E and Bundy DA (1998). Herd immunity to filarial infection is a function of vector biting rate. *Proc R Soc Lond B Biol Sci* **265**: 855-860.

Michael E, Simonsen PE, Malecela M, Jaoko WG, Pedersen EM, Mukoko D, Rwegoshora RT and Meyrowitsch DW (2001). Transmission intensity and the immunoepidemiology of bancroftian filariasis in East Africa. *Parasite Immunol* **23**: 373-388.

Michael E, Malecela-Lazaro MN, Simonsen PE, Pedersen EM, Barker G, Kumar A and Kazura JW (2004). Mathematical modelling and the control of lymphatic filariasis. *Lancet Infect Dis* **4**: 223-234.

Molyneux DH and Zagaria N (2002). Lymphatic filariasis elimination: progress in global programme development. *Ann Trop Med Parasitol* **96 (Suppl 2)**: S15-40.

Norman RA, Chan MS, Srividya A, Pani SP, Ramaiah KD, Vanamail P, Michael E, Das PK and Bundy DA (2000). EPIFIL: the development of an age-structured model for describing the transmission dynamics and control of lymphatic filariasis. *Epidemiol Infect* **124**: 529-541.

Norões J, Addiss D, Amaral F, Coutinho A, Medeiros Z and Dreyer G (1996). Occurrence of living adult *Wuchereria bancrofti* in the scrotal area of men with microfilaraemia. *Trans R Soc Trop Med Hyg* **90**: 55-56.

Norões J, Dreyer G, Santos A, Mendes VG, Medeiros Z and Addiss D (1997). Assessment of the efficacy of diethylcarbamazine on adult *Wuchereria bancrofti in vivo*. *Trans R Soc Trop Med Hyg* **91**: 78-81.

Ottesen EA (1985). Efficacy of diethylcarbamazine in eradicating infection with lymphatic-dwelling filariae in humans. *Rev Infect Dis* **7**: 341-356.

Ottesen EA, Duke BOL, Karam M and Behbehani K (1997). Strategies and tools for the control/elimination of lymphatic filariasis. *Bull World Health Organ* **75**: 491-503.

Ottesen EA, Ismail MM and Horton J (1999). The role of albendazole in programmes to eliminate lymphatic filariasis. *Parasitol Today* **15**: 382-386.

Ottesen EA (2000). Towards eliminating lymphatic filariasis. in: *Lymphatic filariasis*. (ed. TB Nutman). London, Imperial College Press. **1:** 201-215.

Pichon G, Perrault G and Laigret J (1974). Rendement parasitaire chez les vecteurs de filarioses. *Bull World Health Organ* **51**: 517-524.

Plaisier AP, van Oortmarssen GJ, Habbema JD, Remme J and Alley ES (1990). ONCHOSIM: a model and computer simulation program for the transmission and control of onchocerciasis. *Comput Methods Programs Biomed* **31**: 43-56.

Plaisier AP (1996). *Modelling onchocerciasis transmission and control*. PhD Thesis, Erasmus University Rotterdam, Rotterdam, the Netherlands.

Plaisier AP, Subramanian S, Das PK, Souza W, Lapa T, Furtado AF, Van der Ploeg CPB, Habbema JDF and Van Oortmarssen GJ (1998). The LYMFASIM simulation program for modeling lymphatic filariasis and its control. *Methods Inf Med* **37**: 97-108.

Plaisier AP, Cao WC, van Oortmarssen GJ and Habbema JD (1999). Efficacy of ivermectin in the treatment of *Wuchereria bancrofti* infection: a model-based analysis of trial results. *Parasitology* **119**: 385-394.

Plaisier AP, Stolk WA, van Oortmarssen GJ and Habbema JD (2000). Effectiveness of annual ivermectin treatment for *Wuchereria bancrofti* infection. *Parasitol Today* **16**: 298-302.

Ramaiah KD, Kumar KN, Ramu K, Pani SP and Das PK (1997). Functional impairment caused by lymphatic filariasis in rural areas of south India. *Trop Med Int Health* **2**: 832-838.

Ramaiah KD, Das PK, Michael E and Guyatt H (2000). The economic burden of lymphatic filariasis in India. *Parasitol Today* **16**: 251-253.

Ramaiah KD, Vanamail P, Pani SP, Yuvaraj J and Das PK (2002). The effect of six rounds of single dose mass treatment with diethylcarbamazine or ivermectin on *Wuchereria bancrofti* infection and its implications for lymphatic filariasis elimination. *Trop Med Int Health* **7**: 767-774.

Ramaiah KD, Vanamail P, Pani SP and Das PK (2003). The prevalences of *Wuchereria bancrofti* antigenaemia in communities given six rounds of treatment with diethylcarbamazine, ivermectin or placebo tablets. *Ann Trop Med Parasitol* **97**: 737-741.

Ravindran B, Satapathy AK, Sahoo PK and Mohanty MC (2003). Protective immunity in human lymphatic filariasis: problems and prospects. *Med Microbiol Immunol (Berl)* **192**: 41-46.

Schlemper BR, Jr., Steindel M, Grisard EC, Carvalho-Pinto CJ, Bernardini OJ, de Castilho CV, Rosa G, Kilian S, Guarneri AA, Rocha A, Medeiros Z and Ferreira Neto JA (2000). Elimination of bancroftian filariasis (*Wuchereria bancrofti*) in Santa Catarina state, Brazil. *Trop Med Int Health* **5**: 848-854.

Selkirk ME, Maizels RM and Yazdanbakhsh M (1992). Immunity and the prospects for vaccination against filariasis. *Immunobiology* **184**: 263-281.

Shenoy RK (2002). Management of disability in lymphatic filariasis--an update. *J Commun Dis* **34**: 1-14.

Southgate BA and Bryan JH (1992). Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 4. Facilitation, limitation, proportionality and their epidemiological significance. *Trans R Soc Trop Med Hyg* **86**: 523-530.

Stolk WA, van Oortmarssen GJ, Pani SP, Subramanian S, Das PK and Habbema JDF (in press). Effects of ivermectin and diethylcarbamazine on microfilariae and microfilaria production in bancroftian filariasis.

Subramanian S, Krishnamoorthy K, Ramaiah KD, Habbema JDF, Das PK and Plaisier AP (1998). The relationship between microfilarial load in the human host and uptake and development of *Wuchereria bancrofti* microfilariae by *Culex quinquefasciatus*: a study under natural conditions. *Parasitology* **116**: 243-255.

Subramanian S, Stolk WA, Ramaiah KD, Plaisier AP, Krishnamoorthy K, Van Oortmarssen GJ, Dominic Amalraj D, Habbema JD and Das PK (2004). The dynamics of *Wuchereria bancrofti* infection: a model-based analysis of longitudinal data from Pondicherry, India. *Parasitology* **128**: 467-482.

Vanamail P, Ramaiah KD, Pani SP, Das PK, Grenfell BT and Bundy DA (1996). Estimation of the fecund life span of *Wuchereria bancrofti* in an endemic area. *Trans R Soc Trop Med Hyg* **90**: 119-121.

Weil GJ, Lammie PJ and Weiss N (1997). The ICT filariasis test: a rapid-format antigen test for diagnosis of bancroftian filariasis. *Parasitol Today* **13**: 401-404.

Woolhouse ME (1992). A theoretical framework for the immunoepidemiology of helminth infection. *Parasite Immunol* **14**: 563-578.

World Health Organization (1992). Lymphatic filariasis: the disease and its control. Fifth report of the WHO Expert Committee on Filariasis. *World Health Organ Tech Rep Ser* **821**: 1-71.

World Health Organization (1997). Elimination of lymphatic filariasis as a public health problem - resolution of the executive board of the WHO (WHA50.29). Geneva, Switzerland, Fiftieth World Health Assembly.

World Health Organization (2004). The World Health Report 2004. Changing history. Geneva, Switzerland, World Health Organization.

World Health Organization (2005). Global Programme to Eliminate Lymphatic Filariasis - Progress report for 2004. *Wkly Epidemiol Rec* **80**: 202-212.

Zagaria N and Savioli L (2002). Elimination of lymphatic filariasis: a public-health challenge. *Ann Trop Med Parasitol* **96 Suppl 2**: S3-13.

2

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

S. SUBRAMANIAN, W. A. STOLK*, K. D. RAMAIAH, A. P. PLAISIER*, K. KRISHNAMOORTHY, G. J. VAN OORTMARSSEN*, D. AMALRAJ, J. D. F. HABBEMA* and P. K. DAS

*Vector Control Research Centre, Indian Council of Medical Research, Pondicherry, India, and *Department of Public Health, Erasmus MC, University Medical Center Rotterdam, the Netherlands*

Parasitology (2004) 128, 467–482

Abstract

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 5-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 microfilaria density required considerable variation between individuals in exposure and immune responsiveness. The mean life span of the parasite was estimated at about 10 years. For the post-control period, the models predict a further decline in microfilaraemia 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 (Michael *et al.* 1996). 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 (Ottesen *et al.* 1997; Ottesen *et al.* 1999). Where feasible, vector control is recommended as an adjunct to chemotherapy based strategies (Ottesen & Ramachandran 1995). Worldwide elimination of the disease as a public health problem is considered feasible (World Health Organization 1997).

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 (Anderson & May 1985; Isham & Medley 1996). Various deterministic models have been used to study the dynamics of infection and disease due to *Wuchereria bancrofti* (Hairston & Jachowski 1968; Subramanian *et al.* 1989b; Vanamail *et al.* 1989; Rochet 1990; Day *et al.* 1991b; Srividya *et al.* 1991; Das *et al.* 1994; Michael *et al.* 1998; Michael *et al.* 2001b). In the present paper, we use the LYMFASIM (Plaisier *et al.* 1998) model, which is based on the stochastic microsimulation technique (Habbema *et al.* 1996).

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 needs 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 (Rajagopalan *et al.* 1989; Subramanian *et al.* 1989a; Das *et al.* 1992; Manoharan *et al.* 1997). 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 two-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.

Material and Methods

Description of LYMFASIM

LYMFASIM is a stochastic microsimulation model for the epidemiology of lymphatic filariasis in human populations (Habbema *et al.* 1996; Plaisier *et al.* 1998). The model simulates the life-histories of human individuals (birth, acquisition and loss of parasites, death) and individual parasites (maturation, mating, production of microfilariae (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 (Plaisier *et al.* 1998). 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.

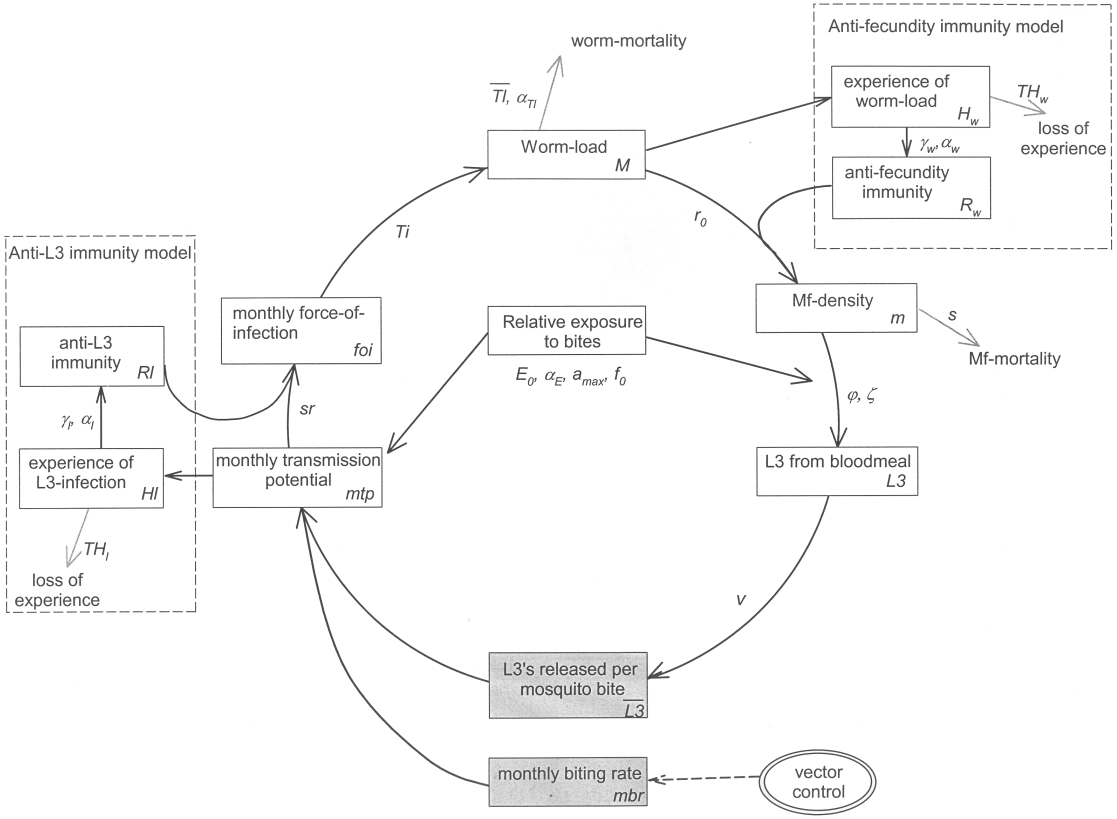
Transmission and parasite dynamics

A graphical representation of the model is given in Figure 2-1. 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 life span (i.e. total life span minus duration of immature stage) of an adult parasite. The total life span of the worm is assumed to vary between parasites, and is described by a Weibull distribution with mean *Tl* and shape-parameter α_{Tl}. Estimates for the life span of *Onchocerca volvulus* (another 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 (Plaisier *et al.* 1991). The duration *Ti* of the immature stage is considered to be 8 months (World Health Organization 1992). The parasite life span 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 mf, provided that the human host harbours at least one adult male parasite, assuming a totally polygamous system in *W. bancrofti*. The mf

Figure 2-1. 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 value of these variables may differ between indivdiuals. Along the arrows, the symbols of relevant parameters are shown.



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 (Plaisier *et al.* 1999). 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 bloodmeal) is described by the following hyperbolic function (Subramanian *et al.* 1998),

$$L3 = \frac{\varphi m}{1+\zeta m} \qquad (2\text{-}1)$$

with parameter values in Table 2-1. 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 mf 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 bloodmeal 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's contribution by his/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.

Table 2-1. 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/Variable		Value (95%CI)	Source
mbr	Monthly biting rate	2200 per person per month	See Figure 2-2
v	Fraction of the L3 larvae, resulting from a single blood meal, that is released 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 mf	(Subramanian *et al.* 1998)
α_{Ti}	Shape-parameter for the Weibull-distribution describing the variation in the adult parasite life span	2.0	Fixed[2]
Ti	Duration of the immature stage of the parasite in the human host	8 months	(World Health Organization 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 (TH_w, see Table 2-2)	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 (TH_l, see Table 2-2)	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 individual ability to elicit such a response (α_l)	State variable	N.A.

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

[2] A value of α_{Ti} =1 means an exponential distribution. This is often (implicitly) assumed in mathematical models. Estimates for the life span of the closely related parasite species *Onchocerca volvulus* suggest less variation (α_{Ti} >1).

N.A. – not applicable, mf – microfilaria

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 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.* (1991a, b) 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.* (1995) 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 (Helmy *et al.* 2000). Several other epidemiological studies also provide indirect evidence for the possible role of acquired immunity in regulating filarial infections (Vanamail *et al.* 1989; Das *et al.* 1990; Bundy & Medley 1992; Michael & Bundy 1998; Michael *et al.* 2001b). However, the above field observations (Day *et al.* 1991a, b; Beuria *et al.* 1995) corroborate the evidence from laboratory studies on cat-*Brugia pahangi* (Denham *et al.* 1972; 1983; Grenfell *et al.* 1991; Michael *et al.* 1998; Devaney & Osborne 2000) and jird-*Acanthocheilonema viteae* (Eisenbeiss *et al.* 1994; Bleiss *et al.* 2002) 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 mf and progress of disease (Maizels & Lawrence 1991). Whether and to what extent the adult worms or the mf 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 Figure 2-1), and is analogous to Woolhouse's (1992) '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,

Table 2-2. 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 the boundaried of the 95% confidence interval (CI) for the duration of the immunological memory and success ratio, and are the estimates for the strength of the immune-response corresponding to lower and upper boundaries of the duration of immunological memory.

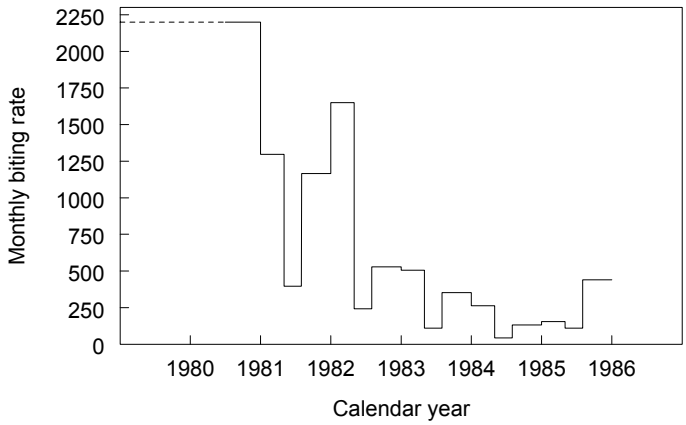
		Numerical value estimated (95% CI)		
Parameter and description		Anti-L3 immunity model	Anti-fecundity immunity model	No immunity model
sr	Success ratio: fraction of inoculated L3-larvae developing to an adult male or female worm in the absence of immune-regulation ($x10^{-3}$)	1.03 (0.66 - 1.36)	0.42 (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	—	—
TH_l	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	—
TH_w	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)	—

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 TH_l and TH_w, 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 used external sources (observations, experiments, and literature) or for which we simply fixed the value within plausible ranges, is given in Table 2-1. Table 2-2 summarizes 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 (Registrar General of India and Census Commissioner 1981). The values for the monthly biting rate (*mbr*, see Figure 2-2) during the vector management programme were estimated from fortnightly collection of human landing mosquitoes in one site in Pondicherry (Ramaiah *et al.* 1992). 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 management programme has achieved a large reduction in transmission but did not achieve a total interruption (Ramaiah *et al.* 1992): within 2 years the annual infective biting

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



rate was reduced by 86% and in 4 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.* (1989) and Subramanian *et al.* (1989a). 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 2-3). Data on overall mf prevalence in 1989 and 1992 (Manoharan *et al.* 1997) 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 2-3. The agreement between observed and simulated data is assessed by the following statistic,

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

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.

Table 2-3. 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

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 2-3 (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 50000.

As a result of variability in simulation output the standard estimation procedures (e.g. maximum likelihood estimation) are not applicable. Instead parameters in Table 2-2 are estimated by minimizing X^2 in Equation 2-2 through a downhill-simplex routine (Nelder & Mead 1965). For the immunity models, a 95%-CI was determined for the immunological memory (parameters TH_l and TH_w) and for the success ratio (parameter *sr*) following the method of Plaisier *et al.* (1995). 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

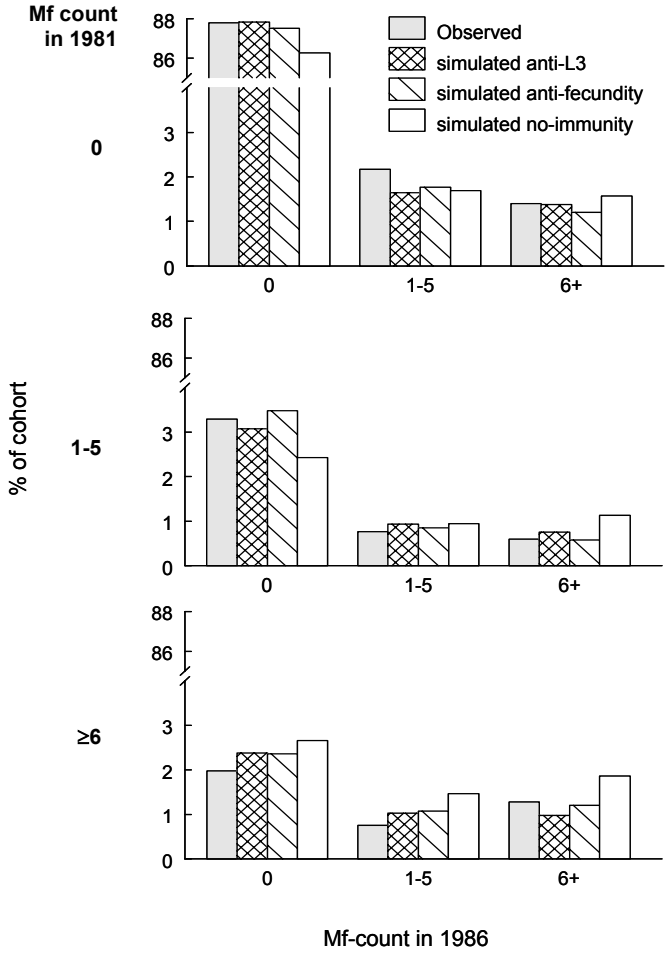
Goodness-of-fit of models with and without immunity

Table 2-2 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 the integrated vector management area (Table 2-3 and Figure 2-3). Figure 2-3 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 2-4. 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 (Figure 2-4).

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 (Figure 2-4). 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 2-2 will be addressed in the discussion section.

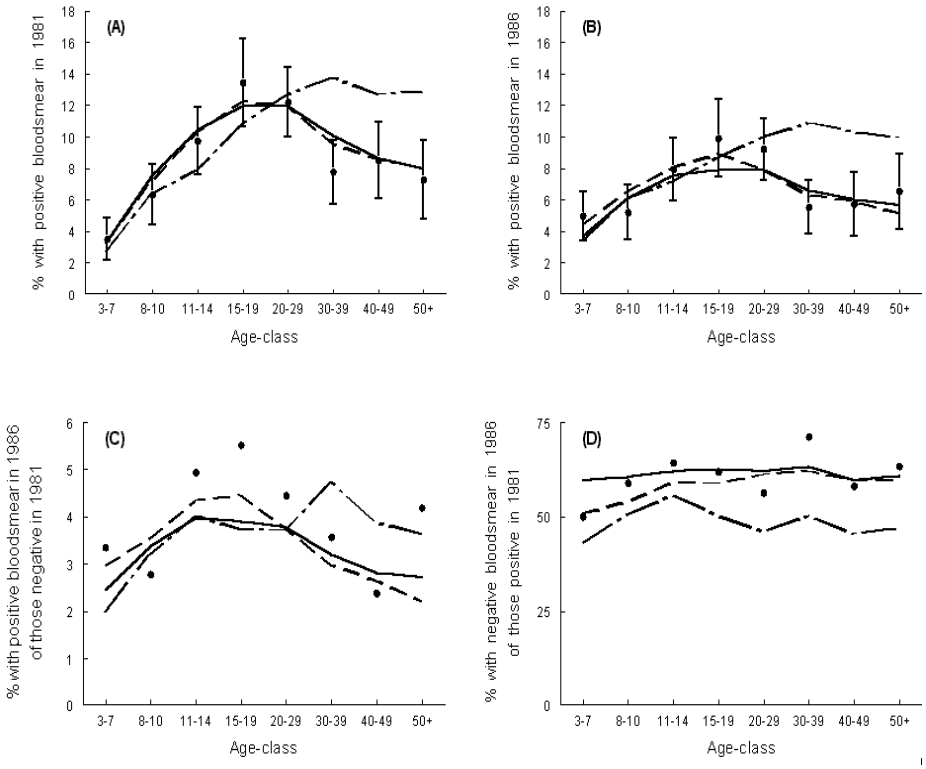
Figure 2-3. Observed and simulated distributions for the number of mf per blood smear in the integrated vector management programme in 1981 and 1986. The upper graph shows the percentages of persons that were mf-negative in 1981 and that showed 0, 1-5 or ≥6 mf per blood smear in 1986. The middle graphs 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.



Prevalence of mf and adult worms

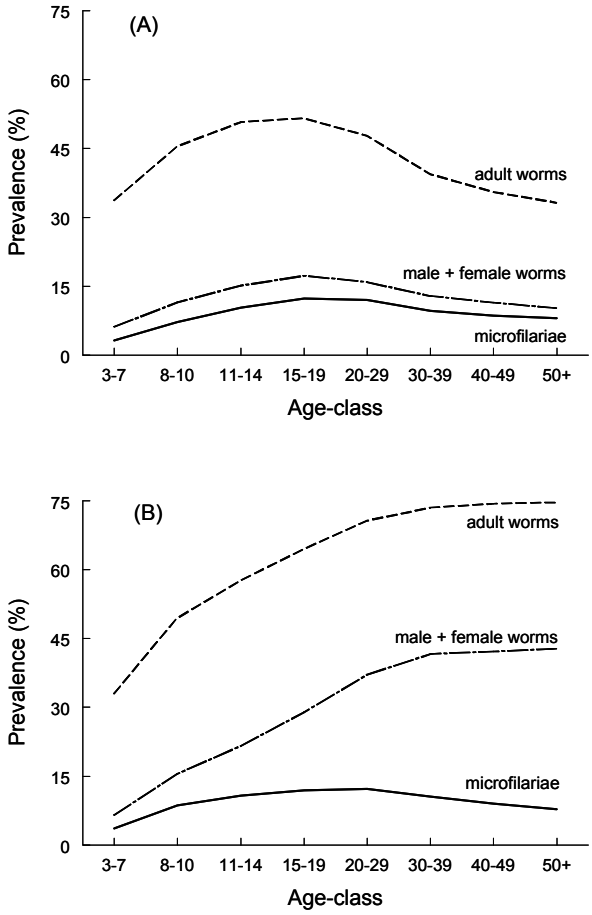
Figure 2-5 compares the prevalence of adult (male or female) worms for the immunity models with the mf prevalence. In both models, the worm-prevalence (dashed line) is much higher than the mf prevalence as determined by a blood-smear (solid line). For the

Figure 2-4. 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.



anti-L3 immunity model, the main reason for this difference is the presence of single-sex infections (Figure 2-5A). 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 Figure 2-5A (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 (Figure 2-5B), which is caused by the anti-fecundity response.

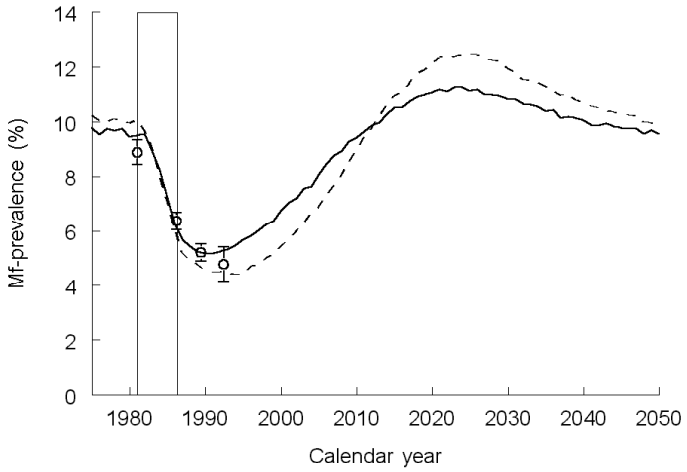
Figure 2-5. Simulated age-specific mf-prevalence (in 1981; solid line), prevalence of persons with at least one adult worm (dashed line) and prevalence of persons with at least one male and at least one female worm (dot-dashed line). Predictions of the models with anti-L3 (A) and anti-fecundity immunity (B).



Confidence intervals

In estimating the confidence boundaries for the duration of immune responsiveness (TH_l and TH_w), the remaining parameters listed in Table 2-2 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

Figure 2-6. 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.



for each other in a multiplicative way. The values for other parameters listed in Table 2-2 remained virtually unchanged (data not shown). 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 2-2).

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 (Figure 2-6). 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 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 (Piessens 1981; Ottesen 1992), 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 (1992), 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 (Fulford *et al.* 1992; Woolhouse 1992). 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 life span, the strength of the immune response and the duration of the immunological memory (Anderson & May 1985).

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 (Day *et al.* 1991a; Beuria *et al.* 1995; Chan *et al.* 1998; Michael & Bundy 1998; Michael *et al.* 2001b), and is supported by data from animal infection experiments (Grenfell *et al.* 1991; Denham *et al.* 1992; Michael *et al.* 1998). 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 (Lammie *et al.* 1994; Ramzy *et al.* 1994; Chanteau *et al.* 1995; Itoh *et al.* 1999;

Sunish *et al.* 2001; 2002; Weerasooriya *et al.* 2002). 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 (Figure 2-5).

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 (Anderson & May 1985).

Alternative explanations of a convex pattern of infection intensity by age are possible, such as a decrease in exposure to infection in older groups (Fulford *et al.* 1992; Duerr *et al.* 2003) or mechanisms that reduce the probability of an incoming larva to develop into mature adult worms at older ages (Michael *et al.* 1998). These alternative mechanisms have not been examined in this paper, since most studies have stressed the possible role of acquired protective immunity (Simonsen 1985; Bosshardt *et al.* 1991; Day *et al.* 1991a; b; Maizels & Lawrence 1991; Beuria *et al.* 1995; Simonsen & Meyrowitsch 1998; King 2001).

Life span

Our analysis also yielded an estimate of the life span of *W. bancrofti* in the human host. The mean life span 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 (Jachowski *et al.* 1951; Conn & Greenslit 1952; Manson-Bahr 1959; Leeuwin 1962; Nelson 1966; Hairston & Jachowski 1968; Mahoney & Aiu 1970), but is about twice as high as the estimate by Vanamail *et al.* (1989; 1996), which was based on the same data. The reason for our longer life span 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.* (1989; 1996) 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 (Sasa 1976). 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 (Gad *et al.* 1994). 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 (Michael *et al.* 2001a). 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 (Sasa 1976; Simonsen *et al.* 1997), short-term variation in mf density (Rachou 1954, 1955; Pichon *et al.* 1981), sampling variability (Southgate 1974; Sasa 1976; Pichon *et al.* 1980; Park 1988; Das *et al.* 1990; Grenfell *et al.* 1990; Dreyer *et al.* 1996; Simonsen *et al.* 1997), 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 (Das *et al.* 1990). Thus, the potential for false negative counts may be considerable (Grenfell *et al.* 1990).

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 (Figure 2-6). 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 (Das *et al.* 1992), 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 generalization

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, (Kumar & Chand 1990; Kar *et al.* 1993; Gyapong *et al.* 1994; Kumar *et al.* 1994; Lammie *et al.* 1994; Meyrowitsch *et al.* 1995; Kazura *et al.* 1997). 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 (Duerr *et al.* 2003).

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 parasites in human (success ratio, mf production, variation in smear count, life span 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 (Stolk *et al.* 2003). The robustness of the model in other situations has yet to be assessed, as the urban Pondicherry epidemiological pattern may not be applicable.

Acknowledgements

We are greatly indebted to the late Dr Vijai Dhanda, former Director of Vector Control Research Centre, Pondicherry for his encouragement and support. S. Subramanian received support from the special programme UNDP/World Bank/WHO for Research and Training in Tropical Diseases (ID: 920743 and 950247).

References

Anderson RM and May RM (1985). Herd immunity to helminth infection and implications for parasite control. *Nature* **315**: 493-496.

Beuria MK, Bal M, Dash AP and Das MK (1995). Age-related prevalence of antibodies to infective larvae of *Wuchereria bancrofti* in normal individuals from a filaria-endemic region. *J. Biosci.* **20**: 167-174.

Bleiss W, Oberlander U, Hartmann S, Adam R, Marko A, Schonemeyer A and Lucius R (2002). Protective immunity induced by irradiated third-stage larvae of the filaria *Acanthocheilonema viteae* is directed against challenge third-stage larvae before molting. *J Parasitol* **88**: 264-270.

Bosshardt SC, McVay CS, Coleman SU and Klei TR (1991). *Brugia pahangi*: circulating antibodies to adult worm antigens in uninfected progeny of homologously infected female jirds. *Exp Parasitol* **72**: 440-449.

Bundy DA and Medley GF (1992). Immuno-epidemiology of human geohelminthiasis: ecological and immunological determinants of worm burden. *Parasitology* **104**: S105-119.

Chan MS, Srividya A, Norman RA, Pani SP, Ramaiah KD, Vanamail P, Michael E, Das PK and Bundy DA (1998). Epifil: a dynamic model of infection and disease in lymphatic filariasis. *Am J Trop Med Hyg* **59**: 606-614.

Chanteau S, Glaziou P, Plichart C, Luquiaud P, Moulia-Pelat JP, N'Guyen L and Cartel JL (1995). *Wuchereria bancrofti* filariasis in French Polynesia: age-specific patterns of microfilaremia, circulating antigen, and specific IgG and IgG4 responses according to transmission level. *Int J Parasitol* **25**: 81-85.

Conn HC and Greenslit FS (1952). Filariasis residuals in veterans with report of a case of microfilaremia. *Am J Trop Med Hyg* **59**: 606-614.

Das PK, Manoharan A, Srividya A, Grenfell BT, Bundy DA and Vanamail P (1990). Frequency distribution of *Wuchereria bancrofti* microfilariae in human populations and its relationships with age and sex. *Parasitology* **101**: 429-434.

Das PK, Manoharan A, Subramanian S, Ramaiah KD, Pani SP, Rajavel AR and Rajagopalan PK (1992). Bancroftian filariasis in Pondicherry, south India--epidemiological impact of recovery of the vector population. *Epidemiol Infect* **108**: 483-493.

Das PK, Srividya A, Pani SP, Ramaiah KD, Vanamail P and Dhanda V (1994). Cumulative exposure and its relationship with chronic filarial disease in bancroftian filariasis. *Southeast Asian J Trop Med Public Health* **25**: 516-521.

Day KP, Gregory WF and Maizels RM (1991a). Age-specific acquisition of immunity to infective larvae in a bancroftian filariasis endemic area of Papua New Guinea. *Parasite Immunol* **13**: 277-290.

Day KP, Grenfell B, Spark R, Kazura JW and Alpers MP (1991b). Age specific patterns of change in the dynamics of *Wuchereria bancrofti* infection in Papua New Guinea. *Am J Trop Med Hyg* **44**: 518-527.

Denham DA, Ponnudurai T, Nelson GS, Rogers R and Guy F (1972). Studies with *Brugia pahangi*. II. The effect of repeated infection on parasite levels in cats. *Int J Parasitol* **2**: 401-407.

Denham DA, McGreevy PB, Suswillo RR and Rogers R (1983). The resistance to re-infection of cats repeatedly inoculated with infective larvae of *Brugia pahangi*. *Parasitology* **86**: 11-18.

Denham DA, Medeiros F, Baldwin C, Kumar H, Midwinter IC, Birch DW and Smail A (1992). Repeated infection of cats with *Brugia pahangi*: parasitological observations. *Parasitology* **104**: 415-420.

Devaney E and Osborne J (2000). The third-stage larva (L3) of *Brugia*: its role in immune modulation and protective immunity. *Microbes Infect* **2**: 1363-1371.

Dreyer G, Pimentael A, Medeiros Z, Beliz F, Moura I, Coutinho A, de Andrade LD, Rocha A, da Silva LM and Piessens WF (1996). Studies on the periodicity and intravascular distribution of *Wuchereria bancrofti* microfilariae in paired samples of capillary and venous blood from Recife, Brazil. *Trop Med Int Health* **1**: 264-272.

Duerr HP, Dietz K and Eichner M (2003). On the interpretation of age-intensity profiles and dispersion patterns in parasitological surveys. *Parasitology* **126**: 87-101.

Eisenbeiss WF, Apfel H and Meyer TF (1994). Protective immunity linked with a distinct developmental stage of a filarial parasite. *J Immunol* **152**: 735-742.

Fulford AJ, Butterworth AE, Sturrock RF and Ouma JH (1992). On the use of age-intensity data to detect immunity to parasitic infections, with special reference to Schistosoma mansoni in Kenya. *Parasitology* **105**: 219-227.

Gad AM, Feinsod FM, Soliman BA, Nelson GO, Gibbs PH and Shoukry A (1994). Exposure variables in bancroftian filariasis in the Nile Delta. *J Egypt Soc Parasitol* **24**: 439-455.

Grenfell BT, Das PK, Rajagopalan PK and Bundy DA (1990). Frequency distribution of lymphatic filariasis microfilariae in human populations: population processes and statistical estimation. *Parasitology* **101**: 417-427.

Grenfell BT, Michael E and Denham DA (1991). A model for the dynamics of human lymphatic filariasis. *Parasitol Today* **7**: 318-323.

Gyapong JO, Magnussen P and Binka FN (1994). Parasitological and clinical aspects of bancroftian filariasis in Kassena-Nankana District, upper east region, Ghana. *Trans R Soc Trop Med Hyg* **88**: 555-557.

Habbema JDF, De Vlas SJ, Plaisier AP and Van Oortmarssen GJ (1996). The microsimulation approach to epidemiologic modeling of helminthic infections, with special reference to schistosomiasis. *Am J Trop Med Hyg* **55**: 165-169.

Hairston NG and Jachowski LA (1968). Analysis of the *Wuchereria bancrofti* population in the people of American Samoa. *Bull World Health Organ* **38**: 29-59.

Helmy H, Weil GJ, Faris R, Gad AM, Chandrashekar R, Ashour A and Ramzy RM (2000). Human antibody responses to *Wuchereria bancrofti* infective larvae. *Parasite Immunol* **22**: 89-96.

Isham V and Medley G, Eds. (1996). *Models for infectious human diseases. Their structure and relation to data.* Series: Publications of the Newton Institute (no. 6). Cambridge, Cambridge University Press.

Itoh M, Weerasooriya MV, Gunawardena NK, Mudalige MP, Samarawickrema WA and Kimura E (1999). *Wuchereria bancrofti* antigenaemia in Sri Lanka. *Trop Med Int Health* **4**: 207-210.

Jachowski LA, Otto GF and Wharton JD (1951). Filariasis in American Samoa 1. Loss of microfilaria in the absence of continued reinfection. *Proceedings of the Helminthological Society of Washington* **18**: 25-28.

Kar SK, Mania J and Kar PK (1993). Prevalence of lymphatic nodule in a bancroftian endemic population. *Acta Trop* **55**: 53-60.

Kazura JW, Bockarie M, Alexander N, Perry R, Bockarie F, Dagoro H, Dimber Z, Hyun P and Alpers MP (1997). Transmission intensity and its relationship to infection and disease due to *Wuchereria bancrofti* in Papua New Guinea. *J Infect Dis* **176**: 242-246.

King CL (2001). Transmission intensity and human immune responses to lymphatic filariasis. *Parasite Immunol* **23**: 363-371.

Kumar A and Chand SK (1990). Prevalence of *Wuchereria bancrofti* infection in some coastal villages of Ganjam, Orissa. *J Commun Dis* **22**: 209-212.

Kumar A, Dash AP and Mansing GD (1994). Prevalence of filariasis in rural Puri, Orissa. *J Commun Dis* **26**: 215-220.

Lammie PJ, Hightower AW and Eberhard ML (1994). Age-specific prevalence of antigenemia in a *Wuchereria bancrofti*- exposed population. *Am J Trop Med Hyg* **51**: 348-355.

Leeuwin RS (1962). Microfilaraemia in Surinamese living in Amsterdam. *Trop Geogr Med* **14**: 355-360.

Mahoney LE, Jr. and Aiu P (1970). Filariasis in Samoan immigrants to the United States. *Am J Trop Med Hyg* **19**: 629-631.

Maizels RM and Lawrence RA (1991). Immunological tolerance: the key feature in human filariasis? *Parasitol Today* **7**: 271-276.

Manoharan A, Das PK, Keerthiseelan VB and Ramaiah KD (1997). Trend of *Wuchereria bancrofti* infection in Pondicherry urban agglomeration after the withdrawal of a five year vector control programme. *J Commun Dis* **29**: 255-261.

Manson-Bahr P (1959). The story of Filaria bancrofti. A critical review. Part I. Early history. *J Trop Med Hyg* **62**: 53-61.

Meyrowitsch DW, Simonsen PE and Makunde WH (1995). Bancroftian filariasis: analysis of infection and disease in five endemic communities of north-eastern Tanzania. *Ann Trop Med Parasitol* **89**: 653-663.

Michael E, Bundy DA and Grenfell BT (1996). Re-assessing the global prevalence and distribution of lymphatic filariasis. *Parasitology* **112**: 409-428.

Michael E and Bundy DA (1998). Herd immunity to filarial infection is a function of vector biting rate. *Proc R Soc Lond B Biol Sci* **265**: 855-860.

Michael E, Grenfell BT, Isham VS, Denham DA and Bundy DA (1998). Modelling variability in lymphatic filariasis: macrofilarial dynamics in the *Brugia pahangi*--cat model. *Proc R Soc Lond B Biol Sci* **265**: 155-165.

Michael E, Ramaiah KD, Hoti SL, Barker G, Paul MR, Yuvaraj J, Das PK, Grenfell BT and Bundy DA (2001a). Quantifying mosquito biting patterns on humans by DNA fingerprinting of bloodmeals. *Am J Trop Med Hyg* **65**: 722-728.

Michael E, Simonsen PE, Malecela M, Jaoko WG, Pedersen EM, Mukoko D, Rwegoshora RT and Meyrowitsch DW (2001b). Transmission intensity and the immunoepidemiology of bancroftian filariasis in East Africa. *Parasite Immunol* **23**: 373-388.

Nelder JA and Mead R (1965). A simplex method of function minimization. *Computer Journal* **7**: 308-313.

Nelson GS (1966). The pathology of filarial infections. *Helminth Abstract* **35**: 311-336.

Ottesen EA (1992). The Wellcome Trust Lecture. Infection and disease in lymphatic filariasis: an immunological perspective. *Parasitology* **104**: S71-79.

Ottesen EA and Ramachandran CP (1995). Lymphatic filariasis infection and disease: control strategies. *Parasitol Today* **11**: 129-131.

Ottesen EA, Duke BOL, Karam M and Behbehani K (1997). Strategies and tools for the control/elimination of lymphatic filariasis. *Bull World Health Organ* **75**: 491-503.

Ottesen EA, Ismail MM and Horton J (1999). The role of albendazole in programmes to eliminate lymphatic filariasis. *Parasitol Today* **15**: 382-386.

Park CB (1988). Microfilaria density distribution in the human population and its infectivity index for the mosquito population. *Parasitology* **96**: 265-271.

Pichon G, Merlin M, Fagneaux G, Riviere F and Laigret J (1980). Étude de la distribution des numerations microfilariennes dans les foyers de filariose lymphatique. *Tropenmed Parasitol* **31**: 165-180.

Pichon G, Rivière F, Thirel R, Chebret M, Tetuanui A and Toudic A (1981). Étude statistique de la variabilité caractérisant les mesures de la microfilarémie capillaire. *Bull Soc Pathol Exot Filiales* **74**: 525-532.

Piessens WF (1981). Lymphatic filariasis in humans: an immunologic maze. *Ann Intern Med* **95**: 778-779.

Plaisier AP, van Oortmarssen GJ, Remme J and Habbema JD (1991). The reproductive lifespan of *Onchocerca volvulus* in West African savanna. *Acta Trop* **48**: 271-284.

Plaisier AP, Alley ES, Boatin BA, Van Oortmarssen GJ, Remme H, De Vlas SJ, Bonneux L and Habbema JD (1995). Irreversible effects of ivermectin on adult parasites in onchocerciasis patients in the Onchocerciasis Control Programme in West Africa. *J Infect Dis* **172**: 204-210.

Plaisier AP, Subramanian S, Das PK, Souza W, Lapa T, Furtado AF, Van der Ploeg CPB, Habbema JDF and Van Oortmarssen GJ (1998). The LYMFASIM simulation program for modeling lymphatic filariasis and its control. *Methods Inf Med* **37**: 97-108.

Plaisier AP, Cao WC, van Oortmarssen GJ and Habbema JD (1999). Efficacy of ivermectin in the treatment of *Wuchereria bancrofti* infection: a model-based analysis of trial results. *Parasitology* **119**: 385-394.

Rachou RG (1954). Daily variation in microfilaraemia due to *Wuchereria bancrofti* in 27 infected persons observed for 28 consecutive days. *Revista da Brasileira Malariol Doencas Tropical* **6**.

Rachou RG (1955). Variation in microfilaraemia of *Wuchereria bancrofti* in 25 carriers in Santa Catarina examined for 52 weeks. *Revista da Brasileira Malariol E Doencas Tropical* **7**: 209-224.

Rajagopalan PK, Das PK, Subramanian S, Vanamail P and Ramaiah KD (1989). Bancroftian filariasis in Pondicherry, south India: 1. Pre-control epidemiological observations. *Epidemiol Infect* **103**: 685-692.

Ramaiah KD, Das PK, Arunachalam N, Rajavel AR and Paily KP (1992). Observations on population density of *Culex quinquefasciatus* and transmission indices of Bancroftian filariasis during and after Integrated Vector Management strategy. *J Commun Dis* **24**: 173-184.

Ramzy RM, Hafez ON, Gad AM, Faris R, Harb M, Buck AA and Weil GJ (1994). Efficient assessment of filariasis endemicity by screening for filarial antigenaemia in a sentinel population. *Trans R Soc Trop Med Hyg* **88**: 41-44.

Registrar General of India and Census Commissioner (1981). Census of India 1981, Series-30 and series-32, Pondicherry. (ed., Controller of Publications, Civil Lines, New Delhi.

Rochet MJ (1990). A simple deterministic model for bancroftian filariasis transmission dynamics. *Trop Med Parasitol* **41**: 225-233.

Sasa M (1976). *Human filariasis. A global survey of epidemiology and control*, University Park Press.

Simonsen PE (1985). *Wuchereria bancrofti* in Tanzania: immune reactions to the microfilarial surface, and the effect of diethylcarbamazine upon these reactions. *Trans R Soc Trop Med Hyg* **79**: 852-858.

Simonsen PE, Niemann L and Meyrowitsch DW (1997). *Wuchereria bancrofti* in Tanzania: microfilarial periodicity and effect of blood sampling time on microfilarial intensities. *Trop Med Int Health* **2**: 153-158.

Simonsen PE and Meyrowitsch DW (1998). Bancroftian filariasis in Tanzania: specific antibody responses in relation to long-term observations on microfilaremia. *Am J Trop Med Hyg* **59**: 667-672.

Southgate BA (1974). A quantitative approach to parasitological techniques in Bancroftian filariasis and its effect on epidemiological understanding. *Trans R Soc Trop Med Hyg* **68**: 177-186.

Srividya A, Pani SP, Rajagopalan PK, Bundy DA and Grenfell BT (1991). The dynamics of infection and disease in bancroftian filariasis. *Trans R Soc Trop Med Hyg* **85**: 255-259.

Stolk WA, Subramanian S, Oortmarssen GJ, Das PK and Habbema JD (2003). Prospects for elimination of bancroftian filariasis by mass drug treatment in Pondicherry, India: a simulation study. *J Infect Dis* **188**: 1371-1381.

Subramanian S, Pani SP, Das PK and Rajagopalan PK (1989a). Bancroftian filariasis in Pondicherry, south India: 2. Epidemiological evaluation of the effect of vector control. *Epidemiol Infect* **103**: 693-702.

Subramanian S, Vanamail P, Ramaiah KD, Pani SP, Das PK and Rajagopalan PK (1989b). A simple deterministic model for host-parasite relationship in *Wuchereria bancrofti* infection & its relevance to parasite regulation in human host. *Indian J Med Res* **89**: 411-417.

Subramanian S, Krishnamoorthy K, Ramaiah KD, Habbema JDF, Das PK and Plaisier AP (1998). The relationship between microfilarial load in the human host and uptake and development of *Wuchereria bancrofti* microfilariae by *Culex quinquefasciatus*: a study under natural conditions. *Parasitology* **116**: 243-255.

Sunish IP, Rajendran R, Satyanarayana K, Munirathinam A and Gajanana A (2001). Immunochromatographic test (ICT) for estimation of true prevalence of bancroftian filariasis in an endemic area in southern India. *Trans R Soc Trop Med Hyg* **95**: 607-609.

Sunish IP, Rajendran R, Mani TR, Munirathinam A, Tewari SC, Hiriyan J, Gajanana A and Satyanarayana K (2002). Resurgence in filarial transmission after withdrawal of mass drug administration and the relationship between antigenaemia and microfilaraemia--a longitudinal study. *Trop Med Int Health* **7**: 59-69.

Vanamail P, Subramanian S, Das PK, Pani SP, Rajagopalan PK, Bundy DA and Grenfell BT (1989). Estimation of age-specific rates of acquisition and loss of *Wuchereria bancrofti* infection. *Trans R Soc Trop Med Hyg* **83**: 689-693.

Vanamail P, Ramaiah KD, Pani SP, Das PK, Grenfell BT and Bundy DA (1996). Estimation of the fecund life span of *Wuchereria bancrofti* in an endemic area. *Trans R Soc Trop Med Hyg* **90**: 119-121.

Weerasooriya MV, Gunawardena NK, Itoh M, Qiu XG and Kimura E (2002). Prevalence and intensity of *Wuchereria bancrofti* antigenaemia in Sri Lanka by Og4C3 ELISA using filter paper-absorbed whole blood. *Trans R Soc Trop Med Hyg* **96**: 41-45.

Woolhouse ME (1992). A theoretical framework for the immunoepidemiology of helminth infection. *Parasite Immunol* **14**: 563-578.

World Health Organization (1992). Lymphatic filariasis: the disease and its control. Fifth report of the WHO Expert Committee on Filariasis. *World Health Organ Tech Rep Ser* **821**: 1-71.

World Health Organization (1997). Elimination of lymphatic filariasis as a public health problem - resolution of the executive board of the WHO (WHA50.29). Geneva, Switzerland, Fiftieth World Health Assembly.

3

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

W. A. STOLK, S. SUBRAMANIAN*, G. J. VAN OORTMARSSEN, P. K. DAS*, and J. D. F. HABBEMA

*Department of Public Health, Erasmus MC, University Medical Center Rotterdam, the Netherlands, and *Vector Control Research Centre, Indian Council of Medical Research, Pondicherry, India*

J Infect Dis (2003) 188, 1371-1381

Abstract

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 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 (Michael *et al.* 1996; Michael & Bundy 1997). In 1997, the 50th World Health Assembly passed a resolution to eliminate lymphatic filariasis as a public health problem (World Health Organization 1997). 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 (Ottesen *et al.* 1997).

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 (Laigret *et al.* 1980; Balakrishnan *et al.* 1992; Kimura *et al.* 1992; Bockarie *et al.* 1998; Meyrowitsch & Simonsen 1998; Gyapong 2000; Das *et al.* 2001; Ramaiah *et al.* 2002). 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 (Schlemper *et al.* 2000), whereas in French Polynesia transmission continued despite long-term intensive control (Cartel *et al.* 1992; Esterre *et al.* 2001).

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 (Plaisier *et al.* 1998). 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 (Subramanian *et al.* 2004). 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 (Plaisier *et al.* 1999). 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 (Plaisier *et al.* 1998). Here we restrict ourselves to a brief description. A more detailed description of the basic transmission model is provided in chapter 2 of this thesis, and in Appendix B of the electronic publication on the website of the Journal of Infectious Diseases.

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% (Subramanian *et al.* 2004). 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 per month (Subramanian *et al.* 2004), the demographic parameters were directly quantified on the basis of census data (Registrar General of India and Census Commissioner 1981), 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 (World Health Organization 1992; Plaisier *et al.* 1999). 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.* (1999). This meta-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 (Dreyer *et al.* 1995), 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 (Plaisier *et al.* 1999). 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 3-1).

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 beta distribution with a mean of 0.77 (equal to the constant

Table 3-1. 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 [a]	1	0.64	-
Upper boundary [a]	1	0.85	-
Minimum estimate [b]	1	0.39	-
Maximum estimate [c]	1	0.91	-
400-µg/kg dose of ivermectin [a]	1	0.92	-
Combination: diethylcarbamazine plus albendazole [d]			
1	1	-	0.50
2	1	-	0.75

NOTE. Data are decimal fractions. Mf, microfilariae, - Effect is not considered.
[a] Estimate from meta-analysis, assuming an mf lifespan of 1 year (Plaisier *et al.* 1999).
[b] Estimate from meta-analysis, assuming an mf lifespan of 2 years (Plaisier *et al.* 1999).
[c] Estimate from meta-analysis, assuming an mf lifespan of 6 months (Plaisier *et al.* 1999).
[D] Assumptions are as explained in the LYMFASIM subsection in the main text.

efficacy in our baseline quantification) and a SD of 0.2. Variation occurs randomly either between treatments (intertreatment variation) or between individuals (interindividual variation). In intertreatment variation, the proportion of the fecundity reduction is randomly drawn from the beta distribution whenever someone is treated, independent of the individual being treated. In interindividual variation, the per-treatment proportion of the fecundity reduction is randomly drawn from the beta 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 (Ottesen 2000). Evidence of the efficacy of this combination regimen is still limited (Ismail *et al.* 1998; Shenoy *et al.* 1999; Dunyo *et al.* 2000a, b; Shenoy *et al.* 2000; Ismail *et al.* 2001; Dunyo & Simonsen 2002), 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 (Ottesen 1985; Figueredo-Silva *et al.* 1996; Norões *et al.* 1997) and may be further enhanced by albendazole, which, when given in

high doses, seems to have macrofilaricidal efficacy of its own (Jayakody *et al.* 1993). 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 (Plaisier *et al.* 1998). 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 (Plaisier *et al.* 2000).

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%, whereas 10% 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 ivermectin. Next, we investigated the impact of assuming random or interindividual 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, to 1650, 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 a 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 3-1; 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 β's being estimated separately for each series of runs:

$$Y = \beta_0 + \beta_1 prev + \beta_2 c + \beta_3 \ln(n) + \beta_4 c \ln(n) \qquad (3\text{-}1)$$

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 (Newcombe & Altman 2000).

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 (Rajagopalan *et al.* 1989). 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 3-1 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 the Appendix. The predictions of logistic regression matched well with the results of 100 repeated runs, for several combinations of population coverage and number of treatment 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 necessary to achieve a high probability of elimination. Inspection of Figure 3-1 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 3-2. 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 3-3. 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.

Figure 3-1. 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). Each symbol indicates the proportion of 100 repeated runs that resulted in elimination for each combination of population-coverage proportion (40% [▼], 50% [▲], 65% [●], 80% [◆], and 90% [■]) and yearly treatment rounds (2, 4, 6, 8, 10, and 12); 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.

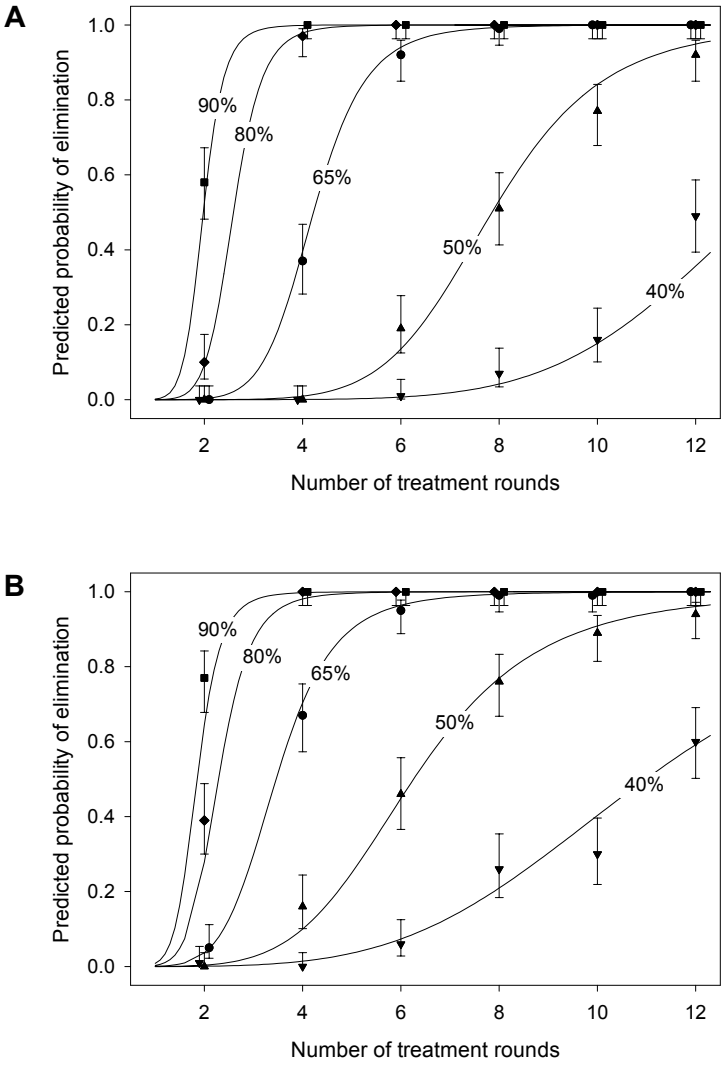
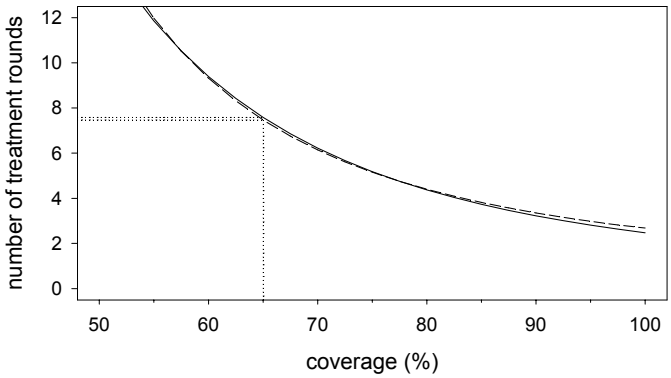


Figure 3-2. Number of yearly treatment rounds, with a 200-µg/kg dose of ivermectin, and the population coverage that are necessary to achieve a 99% probability of elimination under baseline assumptions for anti-L3 (unbroken line) and antifecundity (broken line) immunity. The "drop lines" (i.e., the fainter, 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 the Appendix.

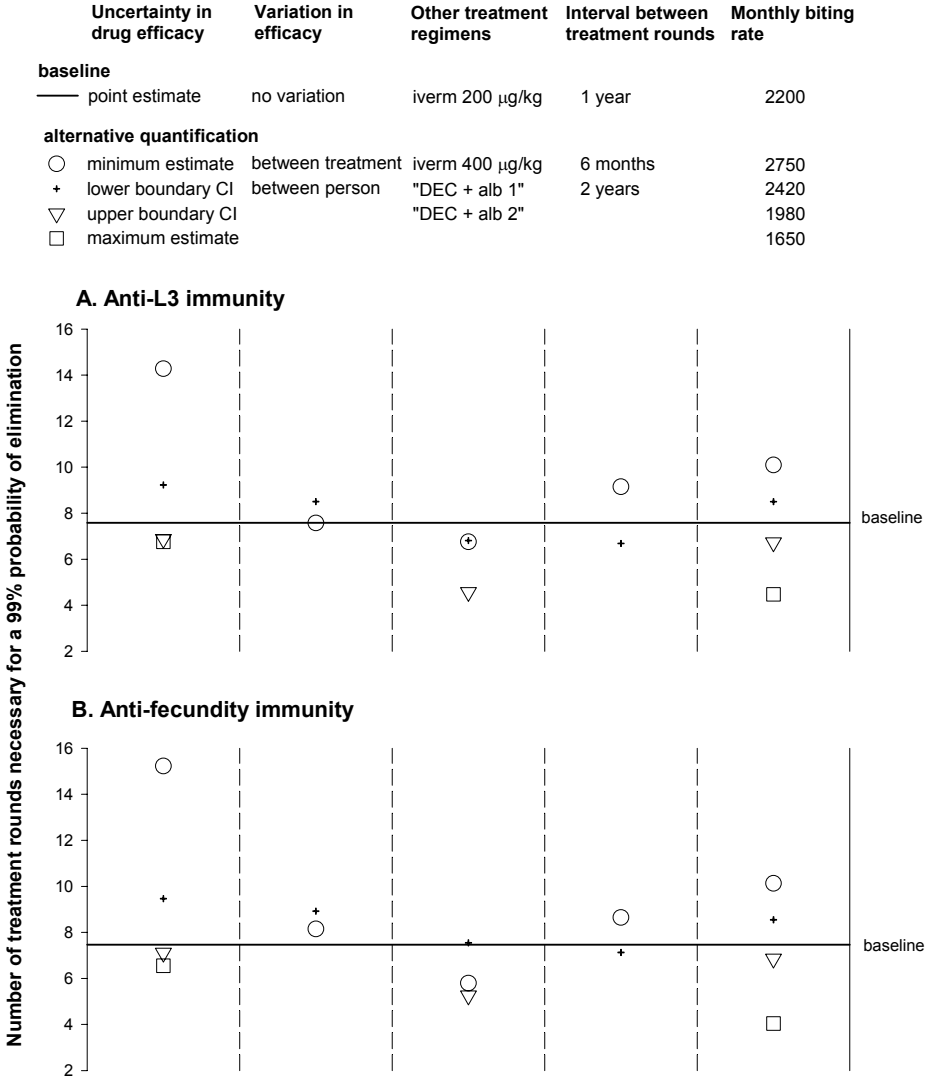


The estimated number of treatment rounds was strongly influenced by uncertainty in the estimated fecundity reduction. In the best case, 7 treatment rounds were sufficient; in the worst case, 15 or 16 treatment rounds were necessary to achieve elimination, depending on the type of immunity. The results 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 than that in others (i.e., interindividual 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.

Figure 3-3. 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 3-2. 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 3-1. For precontrol Mf prevalence levels corresponding to alternative monthly biting rates, see the "Sensitivity Analysis" subsection in the main text. Abbreviations: alb, albendazole; CI, 95% confidence interval; DEC, diethylcarbamazine; iverm, ivermectin.



The right-hand column of Figure 3-3 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-L3 immunity 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.

Baseline Simulation Experiment

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 (Plaisier *et al.* 1999). 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 (Ramaiah *et al.* 2000); 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 interindividual 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 interindividual 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 (Cao *et al.* 1997; Plaisier *et al.* 1999; Brown *et al.* 2000); 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 (Ottesen 2000). 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 (Ottesen *et al.* 1999; Horton *et al.* 2000).

Treatment interval. The intertreatment 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, with 65% 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 (Day *et al.* 1991; Steel *et al.* 1996; Michael & Bundy 1998; Michael 2000; Subramanian *et al.* 2004), 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 (Plaisier *et al.* 2000). 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 (Centers for Disease Control 1993). 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 (Ichimori 2001); 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 subareas with higher vector density, transmission, and infection intensity. Foci of more-intense transmission may be found, for example, in the proximity of breeding sites (Gad *et al.* 1994). 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 (Southgate 1992). 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 (Subramanian *et al.* 1998). The effectiveness of control strategies may be different when the number of L3 larvae developing per engorged mf 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.

Acknowledgements

We thank Anton Plaisier for his work in the development and application of LYMFASIM.

Appendix

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, $Y = 17.98 + 0.70\ prev - 19.45\ c - 3.74 \ln(n) - 6.31\ c \ln(n)$;
- for antifecundity immunity, $Y = 9.29 + 0.59\ prev - 10.56\ c - 1.35 \ln(n) - 7.11\ c \ln(n)$.

Results are shown in Figures 3-1 and 3-2. 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.

References

Balakrishnan N, Ramaiah KD and Pani SP (1992). Efficacy of bi-annual administration of DEC in the control of bancroftian filariasis. *J Commun Dis* **24**: 87-91.

Bockarie MJ, Alexander ND, Hyun P, Dimber Z, Bockarie F, Ibam E, Alpers MP and Kazura JW (1998). Randomised community-based trial of annual single-dose diethylcarbamazine with or without ivermectin against *Wuchereria bancrofti* infection in human beings and mosquitoes. *Lancet* **351**: 162-168.

Brown KR, Ricci FM and Ottesen EA (2000). Ivermectin: effectiveness in lymphatic filariasis. *Parasitology* **121**: S133-146.

Cao WC, Van der Ploeg CP, Plaisier AP, van der Sluijs IJ and Habbema JD (1997). Ivermectin for the chemotherapy of bancroftian filariasis: a meta-analysis of the effect of single treatment. *Trop Med Int Health* **2**: 393-403.

Cartel JL, Nguyen NL, Spiegel A, Moulia-Pelat JP, Plichart R, Martin PM, Manuellan AB and Lardeux F (1992). *Wuchereria bancrofti* infection in human and mosquito populations of a Polynesian village ten years after interruption of mass chemoprophylaxis with diethylcarbamazine. *Trans R Soc Trop Med Hyg* **86**: 414-416.

Centers for Disease Control (1993). Recommendations of the International Task Force for Disease Eradication. *MMWR Morb Mortal Wkly Rep* **42**: 1-38.

Das PK, Ramaiah KD, Vanamail P, Pani SP, Yuvaraj J, Balarajan K and Bundy DA (2001). Placebo-controlled community trial of four cycles of single-dose diethylcarbamazine or ivermectin against *Wuchereria bancrofti* infection and transmission in India. *Trans R Soc Trop Med Hyg* **95**: 336-341.

Day KP, Gregory WF and Maizels RM (1991). Age-specific acquisition of immunity to infective larvae in a bancroftian filariasis endemic area of Papua New Guinea. *Parasite Immunol* **13**: 277-290.

Dreyer G, Norões J, Amaral F, Nen A, Medeiros Z, Coutinho A and Addiss D (1995). Direct assessment of the adulticidal efficacy of a single dose of ivermectin in bancroftian filariasis. *Trans R Soc Trop Med Hyg* **89**: 441-443.

Dunyo SK, Nkrumah FK and Simonsen PE (2000a). A randomized double-blind placebo-controlled field trial of ivermectin and albendazole alone and in combination for the treatment of lymphatic filariasis in Ghana. *Trans R Soc Trop Med Hyg* **94**: 205-211.

Dunyo SK, Nkrumah FK and Simonsen PE (2000b). Single-dose treatment of *Wuchereria bancrofti* infections with ivermectin and albendazole alone or in combination: evaluation of the potential for control at 12 months after treatment. *Trans R Soc Trop Med Hyg* **94**: 437-443.

Dunyo SK and Simonsen PE (2002). Ivermectin and albendazole alone and in combination for the treatment of lymphatic filariasis in Ghana: follow-up after re-treatment with the combination. *Trans R Soc Trop Med Hyg* **96**: 189-192.

Esterre P, Plichart C, Sechan Y and Nguyen NL (2001). The impact of 34 years of massive DEC chemotherapy on *Wuchereria bancrofti* infection and transmission: the Maupiti cohort. *Trop Med Int Health* **6**: 190-195.

Figueredo-Silva J, Jungmann P, Noroes J, Piessens WF, Coutinho A, Brito C, Rocha A and Dreyer G (1996). Histological evidence for adulticidal effect of low doses of diethylcarbamazine in bancroftian filariasis. *Trans R Soc Trop Med Hyg* **90**: 192-194.

Gad AM, Feinsod FM, Soliman BA, Nelson GO, Gibbs PH and Shoukry A (1994). Exposure variables in bancroftian filariasis in the Nile Delta. *J Egypt Soc Parasitol* **24**: 439-455.

Gyapong JO (2000). Impact of single-dose ivermectin on community microfilaria load in bancroftian filariasis infection: two years post treatment. *Trans R Soc Trop Med Hyg* **94**: 434-436.

Horton J, Witt C, Ottesen EA, Lazdins JK, Addiss DG, Awadzi K, Beach MJ, Belizario VY, Dunyo SK, Espinel M, Gyapong JO, Hossain M, Ismail MM, Jayakody RL, Lammie PJ, Makunde W, Richard-Lenoble D, Selve B, Shenoy RK, Simonsen PE, Wamae CN and Weerasooriya MV (2000). An analysis of the safety of the single dose, two drug regimens used in programmes to eliminate lymphatic filariasis. *Parasitology* **121**: S147-160.

Ichimori K (2001). Entomology of the filariasis control programme in Samoa, *Aedes polynesiensis* and *Ae. samoanus*. *Med. Entomol. Zool.* **52**: 11-21.

Ismail MM, Jayakody RL, Weil GJ, Nirmalan N, Jayasinghe KS, Abeyewickrema W, Rezvi Sheriff MH, Rajaratnam HN, Amarasekera N, de Silva DC, Michalski ML and Dissanaike AS (1998). Efficacy of single dose combinations of albendazole, ivermectin and diethylcarbamazine for the treatment of bancroftian filariasis. *Trans R Soc Trop Med Hyg* **92**: 94-97.

Ismail MM, Jayakody RL, Weil GJ, Fernando D, De Silva MS, De Silva GA and Balasooriya WK (2001). Long-term efficacy of single-dose combinations of albendazole, ivermectin and diethylcarbamazine for the treatment of bancroftian filariasis. *Trans R Soc Trop Med Hyg* **95**: 332-335.

Jayakody RL, De Silva CSS and Weerasinghe WMT (1993). Treatment of bancroftian filariasis with albendazole: evaluation of efficacy and adverse reactions. *Tropical Biomedicine* **10**: 19-24.

Kimura E, Spears GF, Singh KI, Samarawickrema WA, Penaia L, Sone PF, Pelenatu S, Faaiuaso ST, Self LS and Dazo BC (1992). Long-term efficacy of single-dose mass treatment with diethylcarbamazine citrate against diurnally subperiodic *Wuchereria bancrofti*: eight years' experience in Samoa. *Bull World Health Organ* **70**: 769-776.

Laigret J, Fagneaux G and Tuira E (1980). Chimiothérapie de masse par la diéthylcarbamazine en doses espacées: effets obtenus à Tahiti sur la microfilarémie à *Wuchereria bancrofti*, var. *pacifica*. *Bull World Health Organ* **58**: 779-783.

Meyrowitsch DW and Simonsen PE (1998). Long-term effect of mass diethylcarbamazine chemotherapy on bancroftian filariasis: results at four years after start of treatment. *Trans R Soc Trop Med Hyg* **92**: 98-103.

Michael E, Bundy DA and Grenfell BT (1996). Re-assessing the global prevalence and distribution of lymphatic filariasis. *Parasitology* **112**: 409-428.

Michael E and Bundy DAP (1997). Global mapping of lymphatic filariasis. *Parasitol Today* **13**: 472-476.

Michael E and Bundy DA (1998). Herd immunity to filarial infection is a function of vector biting rate. *Proc R Soc Lond B Biol Sci* **265**: 855-860.

Michael E (2000). The population dynamics and epidemiology of lymphatic filariasis. in: *Lymphatic filariasis*. (ed. TB Nutman). London, Imperial College Press. **1:** 41-81.

Newcombe RG and Altman DG (2000). Chapter 6. Proportions and their differences. in: *Statistics with confidence. Second edition*. (ed. DG Altman, D Machin, TN Bryant and MJ Gardner), BMJ Books**:** 45-56.

Norões J, Dreyer G, Santos A, Mendes VG, Medeiros Z and Addiss D (1997). Assessment of the efficacy of diethylcarbamazine on adult *Wuchereria bancrofti in vivo*. *Trans R Soc Trop Med Hyg* **91**: 78-81.

Ottesen EA (1985). Efficacy of diethylcarbamazine in eradicating infection with lymphatic-dwelling filariae in humans. *Rev Infect Dis* **7**: 341-356.

Ottesen EA, Duke BOL, Karam M and Behbehani K (1997). Strategies and tools for the control/elimination of lymphatic filariasis. *Bull World Health Organ* **75**: 491-503.

Ottesen EA, Ismail MM and Horton J (1999). The role of albendazole in programmes to eliminate lymphatic filariasis. *Parasitol Today* **15**: 382-386.

Ottesen EA (2000). The global programme to eliminate lymphatic filariasis. *Trop Med Int Health* **5**: 591-594.

Plaisier AP, Subramanian S, Das PK, Souza W, Lapa T, Furtado AF, Van der Ploeg CPB, Habbema JDF and Van Oortmarssen GJ (1998). The LYMFASIM simulation program for modeling lymphatic filariasis and its control. *Methods Inf Med* **37**: 97-108.

Plaisier AP, Cao WC, van Oortmarssen GJ and Habbema JD (1999). Efficacy of ivermectin in the treatment of *Wuchereria bancrofti* infection: a model-based analysis of trial results. *Parasitology* **119**: 385-394.

Plaisier AP, Stolk WA, van Oortmarssen GJ and Habbema JD (2000). Effectiveness of annual ivermectin treatment for *Wuchereria bancrofti* infection. *Parasitol Today* **16**: 298-302.

Rajagopalan PK, Das PK, Subramanian S, Vanamail P and Ramaiah KD (1989). Bancroftian filariasis in Pondicherry, south India: 1. Pre-control epidemiological observations. *Epidemiol Infect* **103**: 685-692.

Ramaiah KD, Das PK, Appavoo NC, Ramu K, Augustin DJ, Kumar KN and Chandrakala AV (2000). A programme to eliminate lymphatic filariasis in Tamil Nadu state, India: compliance with annual single-dose DEC mass treatment and some related operational aspects. *Trop Med Int Health* **5**: 842-847.

Ramaiah KD, Vanamail P, Pani SP, Yuvaraj J and Das PK (2002). The effect of six rounds of single dose mass treatment with diethylcarbamazine or ivermectin on *Wuchereria bancrofti* infection and its implications for lymphatic filariasis elimination. *Trop Med Int Health* **7**: 767-774.

Registrar General of India and Census Commissioner (1981). Census of India 1981, Series-30 and series-32, Pondicherry. (ed., Controller of Publications, Civil Lines, New Delhi.

Schlemper BR, Jr., Steindel M, Grisard EC, Carvalho-Pinto CJ, Bernardini OJ, de Castilho CV, Rosa G, Kilian S, Guarneri AA, Rocha A, Medeiros Z and Ferreira Neto JA (2000). Elimination of bancroftian filariasis (*Wuchereria bancrofti*) in Santa Catarina state, Brazil. *Trop Med Int Health* **5**: 848-854.

Shenoy RK, Dalia S, John A, Suma TK and Kumaraswami V (1999). Treatment of the microfilaraemia of asymptomatic brugian filariasis with single doses of ivermectin, diethylcarbamazine or albendazole, in various combinations. *Ann Trop Med Parasitol* **93**: 643-651.

Shenoy RK, John A, Babu BS, Suma TK and Kumaraswami V (2000). Two-year follow-up of the microfilaraemia of asymptomatic brugian filariasis, after treatment with two, annual, single doses of ivermectin, diethylcarbamazine and albendazole, in various combinations. *Ann Trop Med Parasitol* **94**: 607-614.

Southgate BA (1992). Intensity and efficiency of transmission and the development of microfilaraemia and disease: their relationship in lymphatic filariasis. *J Trop Med Hyg* **95**: 1-12.

Steel C, Guinea A and Ottesen EA (1996). Evidence for protective immunity to bancroftian filariasis in the Cook Islands. *J Infect Dis* **174**: 598-605.

Subramanian S, Krishnamoorthy K, Ramaiah KD, Habbema JDF, Das PK and Plaisier AP (1998). The relationship between microfilarial load in the human host and uptake and development of *Wuchereria bancrofti* microfilariae by *Culex quinquefasciatus*: a study under natural conditions. *Parasitology* **116**: 243-255.

Subramanian S, Stolk WA, Ramaiah KD, Plaisier AP, Krishnamoorthy K, Van Oortmarssen GJ, Dominic Amalraj D, Habbema JD and Das PK (2004). The dynamics of *Wuchereria bancrofti* infection: a model-based analysis of longitudinal data from Pondicherry, India. *Parasitology* **128**: 467-482.

World Health Organization (1992). Lymphatic filariasis: the disease and its control. Fifth report of the WHO Expert Committee on Filariasis. *World Health Organ Tech Rep Ser* **821**: 1-71.

World Health Organization (1997). Elimination of lymphatic filariasis as a public health problem - resolution of the executive board of the WHO (WHA50.29). Geneva, Switzerland, Fiftieth World Health Assembly.

4

Anti-*Wolbachia* treatment for lymphatic filariasis

W. A. STOLK, S. J. DE VLAS and J. D. F. HABBEMA

Department of Public Health, Erasmus MC, University Medical Center Rotterdam, the Netherlands

Lancet (2005) 365, 2067-2068

Mass treatment with antifilarial drugs is the mainstay of the Global Programme to Eliminate Lymphatic Filariasis (Molyneux & Zagaria 2002), a parasitic disease which is an important cause of chronic morbidity in tropical countries. Current drugs—diethylcarbamazine or ivermectin, usually given with albendazole—effectively kill the microfilariae (larval offspring of the parasite), but their effect on the macrofilariae (adult worms) is incomplete. The search for macrofilaricides remains a research priority (Anonymous 2004). One of the most promising leads is treatment directed at *Wolbachia*, the intracellular bacterial symbiont of filarial parasites (Taylor *et al.* 2000). In a recent study, Mark Taylor *et al.* provided convincing evidence that depletion of *Wolbachia* by doxycycline kills most adult worms, without causing severe side-effects (Taylor *et al.* 2005).

An earlier study suggested that doxycycline has no direct microfilaricidal effect, but blocks the adult worms in producing microfilariae (Hoerauf *et al.* 2003). Taylor *et al.* (2005) showed for the first time that doxycycline indirectly kills the adult worm. Their conclusion is based on the strong reduction in the number of worm nests in the scrotum and levels of filarial antigen in the blood 14 months after treatment. The complete absence of microfilariae is consistent with death of the adult worms.

The 80% difference between the doxycycline and placebo group in the mean number of worm nests observed by Taylor *et al.* (2005) with ultrasound suggests that about 80% of the adult worms were killed. (It could be higher if remaining worm nests contain fewer worms, or lower if smaller nests are missed on ultrasound.) This macrofilaricidal effect of doxycycline is high compared with that of the currently used drugs. No macrofilaricidal effect was found for ivermectin (Dreyer *et al.* 1995). Although there are indications that the fertility of worms is reduced (Plaisier *et al.* 1999), ivermectin is usually considered a pure microfilaricide, killing nearly all microfilariae (Richard-Lenoble *et al.* 2003). Some macrofilaricidal effect might occur, though, if ivermectin is combined with the broad-spectrum albendazole (Ottesen *et al.* 1999). A single dose of diethylcarbamazine has good microfilaricidal effect and is thought to kill about 50% of adult worms (Norões *et al.* 1997; Kshirsagar *et al.* 2004). Only the combination of diethylcarbamazine and albendazole had macrofilaricidal effects comparable to doxycycline (56–87%) (El Setouhy *et al.* 2004; Kshirsagar *et al.* 2004).

The availability of a new generation of drugs with a different working mechanism (killing the symbiont bacteria) is good news. New drugs are needed to anticipate the possible development of resistance in the many mass-treatment programmes that have been started worldwide for the elimination of lymphatic filariasis. For African countries, a new macrofilaricidal drug would be especially welcome: diethylcarbamazine cannot be used in this region because of severe side-effects in *Onchocerca*-infected people, and ivermectin is contraindicated where *Loa loa* is endemic. Although the 8-week treatment regimen (200 mg doxycycline daily) is not suitable for use in mass administration, as Taylor *et al.* rightly mentioned, it is interesting to contemplate how effective doxycycline would be if used in mass treatment.

Models show that mass treatment with doxycycline could well be more effective than mass treatment with ivermectin plus albendazole or only diethylcarbamazine to achieve elimination (Table 4-1). The effectiveness of doxycycline would be comparable to that of diethylcarbamazine plus albendazole, even though doxycycline was assumed to have no microfilaricidal effect. With either of these regimens, six annual rounds of mass treatment with 65% coverage would suffice in an endemic setting such as Pondicherry (India), whereas (a less realistic) 80% coverage would require only four rounds. These estimates should, however, be regarded with some care. The assumed macrofilaricidal effects are based on a few studies, and individual variation in the effects of treatment (which might even double the time to elimination if some people respond poorly) was not considered. Possible sterilisation of worms by doxycycline and ivermectin was also not considered, but the effectiveness of mass treatment would be similar if worms are sterilised rather than killed. In regions other than Pondicherry, elimination might be harder to achieve because of more favourable conditions for transmission or more problematic operational conditions. Then programmes could shift their focus to the less ambitious aim of reducing lymphatic filariasis as a public-health problem, but conclusions about the performance of doxycycline relative to other drugs will not change.

In conclusion, anti-*Wolbachia* treatment has high potential for use in lymphatic filariasis control. Research should now focus on identification of regimens, based on doxycycline or other antibiotics, that are practical for use in mass treatment and have similar strong macrofilaricidal, or equivalently sterilising, effects to doxycycline.

Table 4-1. Predicted number of annual rounds of mass drug-treatment required to achieve elimination with 99% certainty in an area such as Pondicherry.[a]

	Assumed treatment effects (proportion killed)		Predicted number of rounds for elimination, with coverage	
Drug(s)	adult worms	microfilariae	65%	80%
Ivermectin + albendazole	35%	100%	10	6
Diethylcarbamazine	50%	70%	8	5
Diethylcarbamazine + albendazole	65%	70%	6	4
Doxycycline	80%	0%	6	4

[a] Pretreatment prevalence of microfilaraemia = 8.5%. A simulation model for transmission of lymphatic filariasis, validated against longitudinal data from Pondicherry (Subramanian *et al.* 2004), was used as explained elsewhere (Stolk *et al.* 2003). Assumptions of efficacy were based on literature review, including Taylor *et al*. (2005)

References

Anonymous (2004). Towards a strategic plan for research to support the global program to eliminate lymphatic filariasis. Summary of immediate needs and opportunities for research on lymphatic filariasis. Philadelphia, Pennsylvania, USA, December 9-10, 2003. *Am J Trop Med Hyg* **71 (Suppl 5)**: iii, 1-46.

Dreyer G, Norões J, Amaral F, Nen A, Medeiros Z, Coutinho A and Addiss D (1995). Direct assessment of the adulticidal efficacy of a single dose of ivermectin in bancroftian filariasis. *Trans R Soc Trop Med Hyg* **89**: 441-443.

El Setouhy M, Ramzy RMR, Ahmed ES, Kandil AM, Hussain O, Farid HA, Helmy H and Weil GJ (2004). A randomized clinical trial comparing single- and multi-dose combination therapy with diethylcarbamazine and albendazole for treatment of bancroftian filariasis. *Am J Trop Med Hyg* **70**: 191-196.

Hoerauf A, Mand S, Fischer K, Kruppa T, Marfo-Debrekyei Y, Debrah AY, Pfarr KM, Adjei O and Buttner DW (2003). Doxycycline as a novel strategy against bancroftian filariasis--depletion of *Wolbachia* endosymbionts from *Wuchereria bancrofti* and stop of microfilaria production. *Med Microbiol Immunol (Berl)* **192**: 211-216.

Kshirsagar NA, Gogtay NJ, Garg BS, Deshmukh PR, Rajgor DD, Kadam VS, Kirodian BG, Ingole NS, Mehendale AM, Fleckenstein L, Karbwang J and Lazdins-Helds JK (2004). Safety, tolerability, efficacy and plasma concentrations of diethylcarbamazine and albendazole co-administration in a field study in an area endemic for lymphatic filariasis in India. *Trans R Soc Trop Med Hyg* **98**: 205-217.

Molyneux DH and Zagaria N (2002). Lymphatic filariasis elimination: progress in global programme development. *Ann Trop Med Parasitol* **96 (Suppl 2)**: S15-40.

Norões J, Dreyer G, Santos A, Mendes VG, Medeiros Z and Addiss D (1997). Assessment of the efficacy of diethylcarbamazine on adult *Wuchereria bancrofti in vivo*. *Trans R Soc Trop Med Hyg* **91**: 78-81.

Ottesen EA, Ismail MM and Horton J (1999). The role of albendazole in programmes to eliminate lymphatic filariasis. *Parasitol Today* **15**: 382-386.

Plaisier AP, Cao WC, van Oortmarssen GJ and Habbema JD (1999). Efficacy of ivermectin in the treatment of *Wuchereria bancrofti* infection: a model-based analysis of trial results. *Parasitology* **119**: 385-394.

Richard-Lenoble D, Chandenier J and Gaxotte P (2003). Ivermectin and filariasis. *Fundam Clin Pharmacol* **17**: 199-203.

Stolk WA, Subramanian S, Oortmarssen GJ, Das PK and Habbema JD (2003). Prospects for elimination of bancroftian filariasis by mass drug treatment in Pondicherry, India: a simulation study. *J Infect Dis* **188**: 1371-1381.

Subramanian S, Stolk WA, Ramaiah KD, Plaisier AP, Krishnamoorthy K, Van Oortmarssen GJ, Dominic Amalraj D, Habbema JD and Das PK (2004). The dynamics of *Wuchereria bancrofti* infection: a model-based analysis of longitudinal data from Pondicherry, India. *Parasitology* **128**: 467-482.

Taylor MJ, Bandi C, Hoerauf AM and Lazdins J (2000). *Wolbachia* bacteria of filarial nematodes: a target for control? *Parasitol Today* **16**: 179-180.

Taylor MJ, Makunde WH, McGarry HF, Turner JD, Mand S and Hoerauf A (2005). Macrofilaricidal activity after doxycycline treatment of *Wuchereria bancrofti*: a double-blind, randomised placebo-controlled trial. *Lancet* **365**: 2116-2121.

5

Advances and challenges in predicting the impact of lymphatic filariasis elimination programmes

W. A. STOLK, S. J. DE VLAS, J. D. F. HABBEMA

Department of Public Health, Erasmus MC, University Medical Center Rotterdam, the Netherlands

Background paper for the WHO/TDR Scientific Working Group Meeting on Lymphatic Filariasis, May 10 –12, 2005, Geneva

Abstract

Mathematical simulation models for transmission and control of lymphatic filariasis are useful to study the prospects for elimination of lymphatic filariasis. Two simulation models are currently being used. The first, EPIFIL, is a population-based, deterministic model that simulates average trends in infection intensity over time. The second, LYMFASIM, is an individual-based, stochastic model that simulates acquisition and loss of infection for each individual in the simulated population, taking account of individual characteristics. The two models, which were both quantified using data from a vector control programme in Pondicherry (India), give similar predictions of the coverage and number of treatment rounds required to bring microfilaraemia prevalence below a threshold level of 0.5%. LYMFASIM can in addition assess the risk of infection recurrence after reaching this threshold. The two main challenges for future work are: 1) quantification of the models for simulation of transmission dynamics in other regions; 2) application of the models for decision-making in ongoing elimination programmes.

Introduction

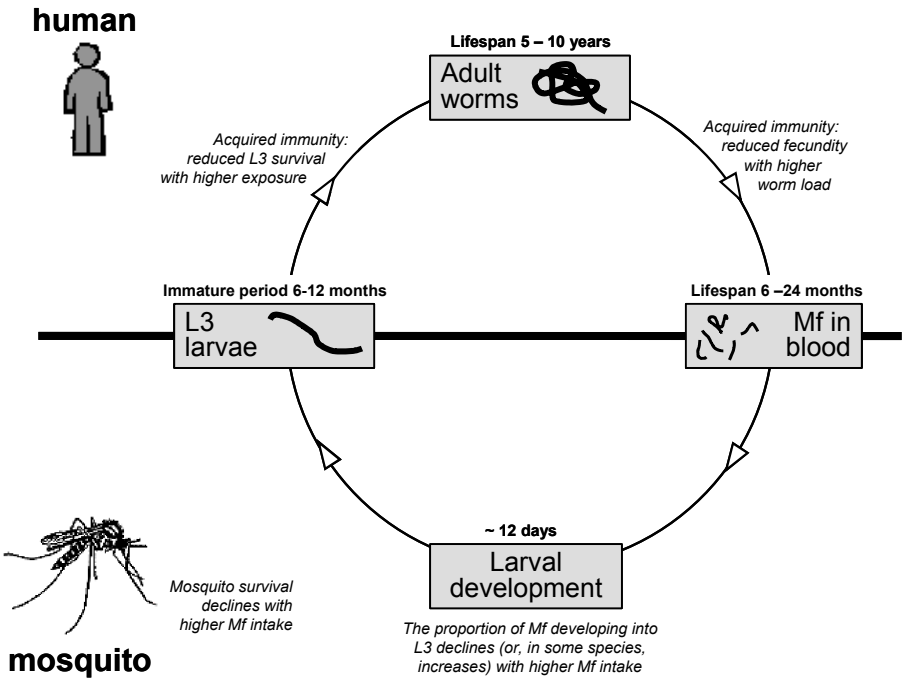
Lymphatic filariasis is a mosquito-borne parasitic disease and an important cause of chronic morbidity in tropical countries. In 1998, the Global Programme to Eliminate Lymphatic Filariasis (GPELF) was initiated, aiming at the worldwide elimination of this parasitic disease as a public health problem (Molyneux & Zagaria 2002). The main strategy in the global programme is to interrupt transmission by annual population treatment with antifilarial drugs (diethylcarbamazine or ivermectin plus albendazole). In addition, morbidity management should reduce the suffering of patients who have chronic manifestations. Thirty-two countries had started elimination programmes in 2002 (World Health Organization 2003) and this number is still growing.

The goal of elimination is ambitious. Past mass treatment programmes had varying degrees of success. In some areas transmission was apparently interrupted (Schlemper *et al.* 2000). In other areas elimination was not achieved, in spite of long-term control programmes (VCRC, Annual Report 2003; Esterre *et al.* 2001). How strategic choices, and operational or biological factors contribute to success or failure is poorly understood. It is unknown which coverage and duration of mass treatment programmes (and possible additional measures) are required to achieve elimination and how this depends on the vector and parasite strain, endemicity level, and the drugs that are used. Mathematical models can help to clarify these issues and application of such models is considered important for support of GPELF (Anonymous 2004).

Mathematical models have been used widely in parasitology. They help to understand the complex transmission dynamics of parasitic diseases and are useful tools for planning and evaluation of control programmes (Habbema *et al.* 1992; Goodman 1994). Models have also played an important role in lymphatic filariasis research (Das & Subramanian 2002; Michael *et al.* 2004). Targeted models, which consider part of the processes involved in transmission, helped for example to clarify the role of acquired immunity (Michael & Bundy 1998; Michael *et al.* 2001) and the macrofilaricidal effects of treatment (Plaisier *et al.* 1999; Stolk *et al.* in press). This paper concentrates on so-called 'full transmission models', which relate the rate of transmission to the intensity and distribution of infection in a human population and can be used to predict the impact of interventions on transmission and the probability of elimination.

To our knowledge, three full transmission models have been described in the literature. The first was specifically developed for the evaluation of a vector control programme and is not considered here (Rochet 1990). The two other models, called EPIFIL (Chan *et al.* 1998; Norman *et al.* 2000) and LYMFASIM (Plaisier *et al.* 1998), are both being used for planning and evaluation of elimination programmes. After a brief introduction of the processes involved in transmission and control of lymphatic filariasis, we describe the basic structure of these models, compare and discuss some critical model predictions, and outline future research priorities.

Figure 5-1. Transmission cycle of lymphatic filariasis with density dependent mechanisms. This figure shows the life cycle of *Wuchereria bancrofti*, the main parasitic cause of lymphatic filariasis. The adult worms (macrofilariae) are located in the lymphatic system of the human host, where they live for 5-10 years (Vanamail *et al.* 1996; Subramanian *et al.* 2004). After mating with male worms, female worms can produce millions of microfilariae (mf), which can be found in the bloodstream and have a lifespan of 6-24 months (Plaisier *et al.* 1999). A mosquito that takes a blood meal may engorge some mf. Inside the mosquito, mf develop in about 12 days into L3 stage larvae (L3), which are infectious to humans. When the mosquito takes another blood meal, the L3 can enter the human body and some will migrate to the lymphatic system and will develop into mature adult worms. The immature period lasts about 6-12 months (World Health Organization 1992). Mf cannot develop into adult worms without passing through the developmental stages in the mosquito. Larval development and mosquito survival are density dependent (Subramanian *et al.* 1998; Krishnamoorthy *et al.* 2004). Two possible mechanisms of acquired immunity are shown (Michael & Bundy 1998).



Processes in lymphatic filariasis transmission and control

Models for lymphatic filariasis control basically describe the main biological processes involved in transmission (Figure 5-1). To study the dynamics of transmission and how intervention affects transmission, it is specifically important to take account of density-dependence and heterogeneities (Anderson & May 1991; Churcher *et al.* 2005; Duerr *et al.* 2005).

Density dependence means that the outcome of a process depends on the abundance of the parasite stages involved. Several limiting mechanisms may reduce transmission when the average worm burden increases. For example, the proportion of microfilariae (mf) that develops into infectious L3 larvae saturates in *Culex quinquefasciatus* when the mf intake is higher, limiting the transmission of infection (Southgate & Bryan 1992; Subramanian *et al.* 1998). Further, the survival probability of mosquitoes is reported to reduce with their infection load (Krishnamoorthy *et al.* 2004). Acquired immunity may limit infection intensity in the human host. Different mechanisms for this have been proposed (Woolhouse 1992), but evidence for the operation of such immunity is inconclusive (Michael & Bundy 1998; Stolk *et al.* 2004). These limiting mechanisms all negatively affect the impact of interventions, because transmission becomes relatively more efficient when infection levels are lower. Density dependence, however, may also occur in the opposite direction (called facilitation). The probability that a female worm mates with a male worm increases with higher worm burdens. Further, in some anopheline mosquito species, larval development might increase with higher mf intake (Southgate & Bryan 1992). It is unknown whether density dependence, either limitation or facilitation, occurs in parasite establishment and survival in humans, their fertility, and mf survival.

The term heterogeneity points at variation between individuals. Individuals differ for example in genetic background, nutritional status and behaviour, which may cause differences in exposure to mosquitoes, susceptibility to infection, and the survival, maturation and fecundity of parasites. Therefore, individuals may be predisposed to heavy or light infection, leading to an aggregated or overdispersed distribution of parasites (with a few hosts harbouring the majority of the parasites). Individuals also differ in compliance and responsiveness to treatment, which may also contribute to aggregation of parasites (Plaisier *et al.* 1999; Stolk *et al.* in press). This aggregation enhances transmission, because it increases the probability that female and male worms mate. Heterogeneity may also occur in the parasite population, e.g. with respect to the life span and resistance to treatment.

Available models

Both available models for lymphatic filariasis transmission and control, EPIFIL and LYMFASIM, mainly differ in the amount of detail that is included. Specific variants of both models have been developed for *Wuchereria bancrofti* transmitted by *Culex quinquefasciatus*, using data from an integrated vector management control programme that was carried out in Pondicherry, India, from 1981-1985 (Norman *et al.* 2000; Subramanian *et al.* 2004). These 'Pondicherry model variants' are described below. Table 5-1 gives the quantification of several key biological parameters of the models. Figure 5-2 illustrates the good fit of both models to the precontrol (1981) data from Pondicherry.

Table 5-1. Quantification of several key biological parameters in the EPIFIL and LYMFASIM model variants for Pondicherry, where *Wuchereria bancrofti* is transmitted by *Culex quinquefasciatus*.

Parameter	EPIFIL	LYMFASIM	
		Anti-L3 immunity	Anti-fecundity immunity
Parasite lifecycle			
Average adult worm life span in years (type of distribution)	8 [a]	10.2 [b]	11.8 [b]
Average mf life span in months (type of distribution)	10 [a]	10 [a]	10 [a]
Premature period in months	-	8	8
Exposure variation by age			
Exposure at age zero as fraction of maximum exposure	0	0.26	0.40
Age in years at which maximum exposure is achieved	9	19.1	21.3
Density dependence in mosquitoes			
Maximum number of L3 larvae that can develop in mosquitoes at high mf intensities	6 [c]	6.6 [d]	6.6 [d]
Acquired immunity			
Duration of acquired immunity in years	lifelong	9.6 [e]	11.2 [e]
Other parameters			
Monthly biting rate	5760	2200	2200
Proportion of L3 larvae in mosquitoes that enters the human host when a mosquito bites	0.414*0.32 = 0.13	0.1	0.1
Proportion of inoculated L3 larvae that develops successfully into adult worms (x103)	0.113	1.03 [f]	0.42
Mf production per worm	2	0.61 [g]	4.03 [g,h]

- Not considered in the model; mf, microfilaria.
[a] Assuming a negative exponential distribution.
[b] Assuming a Weibull distribution with shape parameter α=2.
[c] Exponential saturating function with initial increase when mf intake increases from zero = 0.047.
[d] Hyperbolic saturating function with initial increase when mf intake increases from zero = 0.09.
[e] This parameter defines the period in which the strength of the immune response is halved in the absence of boosting.
[f] In the absence of anti-L3 immunity.
[g] In the presence of at least 1 male worm, scaled to the number of mf per 20 µl peripheral blood.
[h] In the absence of anti-fecundity immunity.

EPIFIL

EPIFIL simulates the average course of infection over age and time in a human population by a set of differential equations. The human population is constant in size

and age-structure. Limitation in the transmission of infection by culicine mosquitoes is taken into account, so that the number of infectious L3 larvae that can develop in mosquitoes saturates at higher mf intensities. Acquired immunity is included as a second limiting mechanism: it is triggered by incoming L3 larvae and reduces the probability that new larvae develop into adult worms. Heterogeneity is only included by age-related exposure to mosquitoes: i.e. the risk of infection increases with age, until a maximum level is reached at the age of 9 years. The mf prevalence is calculated using a negative binomial distribution, assuming a certain amount of aggregation of parasites in the human population.

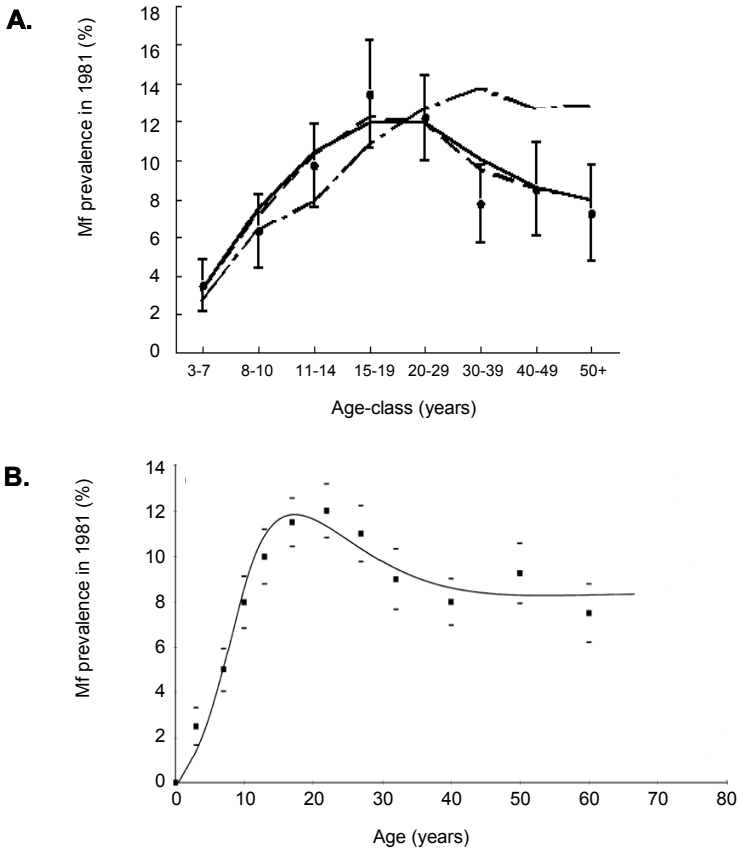
The model can be used to simulate the impact of vector control or mass treatment. Vector control is assumed to reduce the mosquito biting rate. Mass treatment leads to killing of a proportion of adult worms or mf and to temporal infertility of worms, depending on the proportion of the population that receives treatment and characteristics of the treatment regimen.

The design of this population-based, deterministic model is based on a general differential equation framework describing the dynamics of macroparasitic infections (Anderson & May 1985, 1991; Woolhouse 1992).

LYMFASIM

LYMFASIM simulates the acquisition and loss of worms over age and time in a discrete number of human individuals, using stochastic microsimulation. Individuals interact through biting mosquitoes and together they form a dynamic population of which the size and age-structure may change over time. Like EPIFIL, LYMFASIM takes account of limitation in the proportion of engorged mf that develops into L3 larvae inside the mosquito and of acquired immunity in human hosts. Two model variants were developed for Pondicherry, which differed with respect to the type of acquired immunity: 'anti-L3' immunity is triggered by incoming L3 larvae and reduces the probability of successful adult worm establishment; 'anti-fecundity' immunity is triggered by the presence of adult worms and reduces the rate of mf production by female worms. By considering individual worms in individual hosts, the model automatically takes account of the declining mating probability of female and male worms with lower average infection intensities. Age-dependent exposure is included, assuming that exposure increases until a maximum is reached at about 20 years of age. Other factors contributing to heterogeneity are variation in exposure to infection within age groups, inclination to participate in treatment programmes, the response to treatment, and the ability to develop immune responses. Parasites may vary with respect to their life span (about 10 years on average). Individual mf intensities are translated into the number of mf that would be counted in a 20 µl blood smear, taking account of random variability in these counts and reduced sensitivity of diagnostic tests at lower mf densities. The mf prevalence and (geometric or arithmetic)

Figure 5-2. Comparison of model predictions with microfilaraemia prevalence by age observed before the start of vector control in Pondicherry, India, in 1981. (A) LYMFASIM predictions for models with anti-L3 immunity (solid line), anti-fecundity immunity (dashed line), and a model variant without immunity (dot-dashed line); the latter model does not fit the data and was therefore rejected. Source: Subramanian *et al.* (2004). (B) EPIFIL predictions of a model with acquired immunity. Source: Norman *et al.* (2000). Symbols in both graphs indicate the observed prevalence levels with corresponding confidence intervals. Figures were reprinted with permission.



mean mf intensity can be directly calculated from the smear counts, using data from all simulated individuals or specific subgroups.

Similar to EPIFIL, LYMFASIM simulates the impact of vector control by reducing the mosquito biting rate. Treatment takes place at the individual level, and results in killing (part) of adult worms or mf and a temporal or permanent reduction in the fertility of female worms. Selective or mass treatment can be simulated.

This individual-based model uses the technique of stochastic microsimulation, which was earlier applied in the modelling of onchocerciasis transmission and control (Habbema *et al.* 1996).

Comparison of model predictions

Both EPIFIL and LYMFASIM have been used to predict the impact of control measures (Das & Subramanian 2002; Stolk *et al.* 2003; Michael *et al.* 2004; Stolk *et al.* 2005). In this report, we focus on model predictions of the coverage and duration of annual mass treatment programmes that will be required for elimination. All published predictions were based on the Pondicherry variants of the model, although acquired immunity was left out of the model in the EPIFIL predictions. From the predictions of both models we can conclude that it is possible to eliminate lymphatic filariasis by yearly mass treatment, but the number of treatment rounds largely depends on coverage, precontrol mf prevalence and the macrofilaricidal effects of drugs. This is illustrated in Tables 5-2 and 5-3, and Figure 5-3. Often the required number of yearly treatment rounds is predicted to be higher than the 4-6 rounds, which was hoped to be sufficient when GPELF was initiated. As an alternative to longer programmes, one might consider more frequent mass treatment (e.g. half-yearly) or applying vector control in addition to mass treatment (Figure 5-4).

The predictions of EPIFIL and LYMFASIM cannot be compared directly, because the original publications reported results for different treatment regimens, with different assumptions on efficacy of the drugs, and different precontrol mf prevalence levels. Further, different criteria for elimination were used: in EPIFIL elimination was assumed to occur if the mf prevalence after treatment was below 0.5%; in LYMFASIM elimination

Table 5-2. LYMFASIM – Predicted number of annual rounds of mass drug treatment required to achieve elimination in 99% of the simulation runs in an area like Pondicherry, for four different drugs or drug combinations and two coverage levels. Predictions are based on the anti-L3 variant of the model for Pondicherry, with a precontrol microfilaraemia prevalence of 8.5%. Elimination is defined as zero microfilaraemia prevalence 40 years after the start of treatment. Source: Stolk *et al*. (2005).

	Assumed treatment effects (proportion killed)		Predicted number of rounds for elimination, with coverage	
Drug(s)	adult worms	microfilariae	65%	80%
Ivermectin + albendazole	35%	100%	10	6
Diethylcarbamazine	50%	70%	8	5
Diethylcarbamazine + albendazole	65%	70%	6	4
Doxycycline	80%	0%	6	4

Table 5-3. Prediction of number of yearly mass treatment rounds that is required to reach a 0.5% microfilaraemia prevalence threshold, using a combination diethylcarbamazine plus albendazole in relation to endemicity and coverage. The combination treatment is assumed to kill 55% of all adult worms and 95% of the microfilariae, and to interrupt the microfilaria production for 6 months. EPIFIL simulation were published (Michael *et al.* 2004) and concerned a model without acquired immunity. LYMFASIM results from the model with anti-L3 immunity were added for comparison for an average pretreatment microfilaraemia prevalence of 10%.

	Coverage			
Pretreatment mf prevalence	60%	70%	80%	90%
EPIFIL				
2.5%	7	6	5	4
5%	9	7	6	5
10%	10	8	7	6
15%	12	9	8	7
LYMFASIM [a]				
10%	10	8	6	5

[a] Based on the average trend in microfilaraemia prevalence of 100 simulation runs.

was defined as a zero mf prevalence 40 years after the start of control in 99% of the runs. To allow better comparison of the models, we did a series of additional simulations with LYMFASIM for mass treatment with the combination of diethylcarbamazine plus albendazole, using the same assumptions on drug-efficacy and the same criterion for elimination as in published EPIFIL predictions (Table 5-3). Simulations were done with the anti-L3 variant of the LYMFASIM model.

It is reassuring that both models come to comparable conclusions regarding the number of treatment rounds required to achieve elimination, although LYMFASIM's predictions are slightly more optimistic than EPIFIL's, when population coverage is high. This finding of nearly equal predictions is not straightforward. The LYMFASIM model contains several assumptions and mechanisms, which, relative to EPIFIL, limit the impact of the intervention on transmission: 1) a longer adult worm life span (~10 vs. 8 years); 2) acquired immunity; 3) heterogeneities in exposure to mosquitoes, in compliance to mass treatment, and in adult worm life span. However, the limiting effect of these assumptions and mechanisms on the impact of mass treatment is apparently counteracted by the enhancing effect of a reduced mating probability of worms at lower average worm burdens in LYMFASIM.

Criteria for elimination

EPIFIL's predictions were based on the assumption that transmission will not continue when the mf prevalence falls below 0.5%. The choice for this threshold is somewhat

Figure 5-3. LYMFASIM – Prediction of the duration of yearly mass treatment with ivermectin required to reach elimination (zero microfilaraemia prevalence 40 years after the start of treatment) with 99% certainty, in relation to coverage. Ivermectin is assumed to sterilize 77% of female worms permanently and to kill all microfilariae. Results are shown for two variants of the LYMFASIM model for Pondicherry, that differ in the type of acquired immunity assumed, assuming a precontrol microfilaraemia prevalence of 8.5%. Source: Stolk *et al.* (2003).

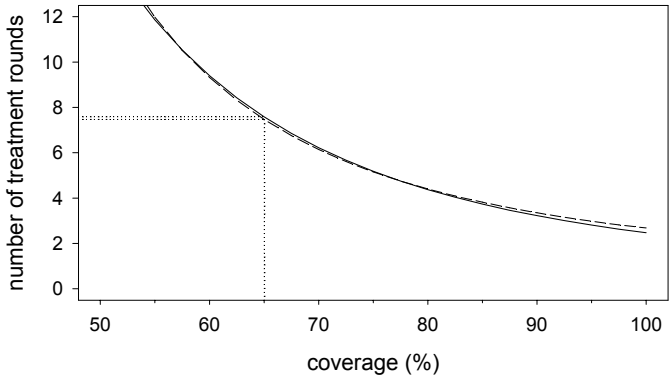
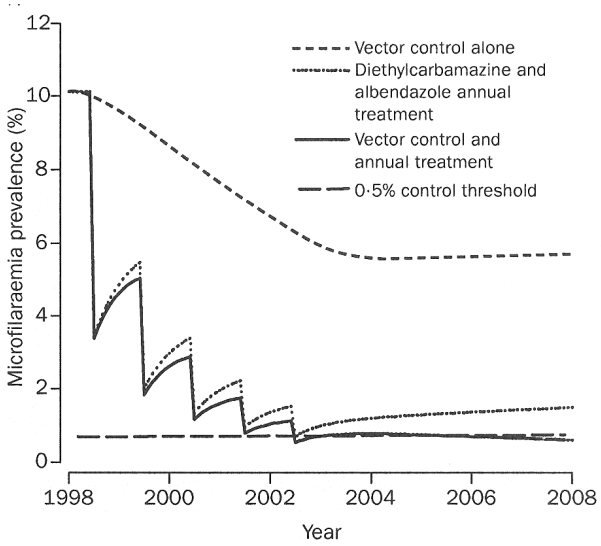


Figure 5-4. EPIFIL – The impact of different control strategies on the mean microfilaraemia prevalence in an endemic community with precontrol prevalence of 10%. The plot shows the impact of mass treatment alone (5 rounds of annual mass treatment with diethylcarbamazine + albendazole, with a coverage of 80%), vector control alone (assuming a 90% reduction in biting rate during 5 years), and the combination of the two. Reprinted from Michael *et al.* (2004), with permission.



arbitrary in the absence of evidence from the field. Given its individual-based structure, LYMFASIM is more suitable to examine in how many runs infection is 'truly' eliminated, as indicated by zero mf prevalence 40 years after the start of control. For example, in the runs with 10% precontrol prevalence, 8 rounds with 70% coverage were required to bring the average mf prevalence below 0.5% (Table 5-3). However, in only 87% of the runs this resulted in zero mf prevalence 40 years after the start of control. To be 99% certain of elimination (as was the criterion in Table 5-2), longer continuation of mass treatment would be required (1 or 2 extra rounds).

More extensive simulation studies are required to determine a more precise threshold level below which elimination would occur. This threshold level (or threshold levels) will depend on local transmission dynamics and mosquito biting rates, inmigration of parasite carriers or infected mosquitoes, but also on heterogeneities and population size in view of the stochastic processes involved.

Application of models for other regions

The existing model variants were all quantified for transmission of *W. bancrofti* by *Culex quinquefasciatus* and tested against data from Pondicherry (Norman *et al.* 2000; Subramanian *et al.* 2004). The basic structure of the models is generalisable to other areas, but various model parameters may take different values. Most importantly, this concerns the relationship between mf density in the human blood and the number of L3 larvae developing in mosquitoes. Unfortunately, few data are available to quantify this relationship for the different mosquito species involved (Snow & Michael 2002). Especially for the anopheline mosquito species that are responsible for transmission in the large parts of Africa more field research is needed. Other parameters that may need requantification relate to the composition of the human population, mosquito biting rates and heterogeneity in exposure, and operational characteristics of interventions.

Biological parameters are not expected to vary much between regions. However, our understanding of the biology of infection (in spite of in-depth model-based analysis of the Pondicherry data) is incomplete and there is uncertainty on the quantification of several key parameters, such as the parasite life span or the role of acquired immunity. Therefore, it is crucial to continue testing the validity of existing and new model variants against epidemiological data. Testing models against age-specific data may help to determine the role of acquired immunity or other processes (Duerr *et al.* 2003). Trends during vector control are especially informative on the adult worm life span (Vanamail *et al.* 1996; Subramanian *et al.* 2004). Trends during mass treatment may give information on the effects of drugs on worm survival and productivity. And trends after cessation of control may help to determine whether density-dependent mechanisms have appropriately been included in the model. Better information on all these aspects should eventually come from field research: using combinations of available diagnostic tests (mf and antigen

detection, ultrasound to visualize adult worms) it may be possible to further increase the validity of our existing models.

Some work has already been done to prepare models for use in other areas. The LYMFASIM model has been applied to age-patterns observed in an area in South-East India that has the same vector-parasite combination and presumably the same transmission dynamics as Pondicherry. This led to the development of new model variants with less strong or no immunity (Subramanian, unpublished data). Comparison of predictions from the new LYMFASIM model variant and EPIFIL with observed trends during mass treatment in this region indicated that assumptions regarding efficacy of drugs or possibly coverage and compliance patterns had to be adapted (Subramanian, unpublished data; Michael *et al.* 2004). Using published data of uptake and development of mf in *Anopheles* mosquitoes (Bryan & Southgate 1988a, b; Southgate & Bryan 1992; Boakye *et al.* 2004), LYMFASIM was adapted for transmission in Africa (Stolk, unpublished data). Model parameters were adapted so that the predicted age-prevalence reflect the observed data from this region (Stolk *et al.* 2004).

Challenges in the evaluation of current elimination programmes

The available models soon have to face new challenges in the ongoing programmes for elimination of lymphatic filariasis. Predictions of the number of treatment rounds required for elimination were only a first step. However, specific programmes also need to be monitored and evaluated. For example, the observed results can be compared with model predictions to see whether progress is as expected. If results lag behind, programmes can be adapted. Also, the models could help to determine when mass treatment can be stopped with low risk of recrudescence, taking account of the specific local conditions, local coverage and compliance levels, and the achieved reduction in mf prevalence and intensity. Analogously, models can help to determine cost-effective surveillance strategies for early detection of recrudescence of infection after cessation of control and measures to be taken to stop this recrudescence.

To address the discussed issues on monitoring and surveillance, the models must be extended to include results of antigen detection, which is widely used in the monitoring and surveillance of ongoing control programmes. Other possibly useful extensions of the model include migration of parasite carriers and infected mosquitoes and development of resistance to available drugs.

Although discussion until now focused on the elimination of transmission, this goal may be difficult to achieve in some areas. In some situations focus may shift to reducing the public health problem without explicitly eliminating infection. To address this with the models, more attention is required for the development of disease. Simple mechanisms of disease development are included in both models, but disease development has received little attention in published work until now.

Conclusions

There are currently two models for lymphatic filariasis transmission and control, LYMFASIM and EPIFIL, that have been used in the prediction of the impact of mass treatment programmes. These models give more or less similar predictions on the number of treatment rounds that will be required for elimination, at least in Pondicherry-like situations. These models differ however in defining when elimination occurs, which leads to different advices on the duration of mass treatment. In view of current elimination programmes, it is crucial to obtain better criteria on when to stop control, taking account of stochasticity in the eventual outcome of elimination. Antigen tests should be included in the model, and the disease part of the models may need more attention. Model variants that are adjusted to local situations are powerful tools to aid decision making in current control programmes.

References

Anderson RM and May RM (1985). Helminth infections of humans: mathematical models, population dynamics, and control. *Adv Parasitol* **24**: 1-101.

Anderson RM and May RM (1991). *Infectious diseases of humans: dynamics and control*. Oxford, Oxford University Press.

Anonymous (2004). Towards a strategic plan for research to support the global program to eliminate lymphatic filariasis. Summary of immediate needs and opportunities for research on lymphatic filariasis. Philadelphia, Pennsylvania, USA, December 9-10, 2003. *Am J Trop Med Hyg* **71 (Suppl 5)**: iii, 1-46.

Boakye DA, Wilson MD, Appawu MA and Gyapong J (2004). Vector competence, for *Wuchereria bancrofti*, of the *Anopheles* populations in the Bongo district of Ghana. *Ann Trop Med Parasitol* **98**: 501-508.

Bryan JH and Southgate BA (1988a). Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 1. Uptake of microfilariae. *Trans R Soc Trop Med Hyg* **82**: 128-137.

Bryan JH and Southgate BA (1988b). Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 2. Damage to ingested microfilariae by mosquito foregut armatures and development of filarial larvae in mosquitoes. *Trans R Soc Trop Med Hyg* **82**: 138-145.

Chan MS, Srividya A, Norman RA, Pani SP, Ramaiah KD, Vanamail P, Michael E, Das PK and Bundy DA (1998). Epifil: a dynamic model of infection and disease in lymphatic filariasis. *Am J Trop Med Hyg* **59**: 606-614.

Churcher TS, Ferguson NM and Basáñez M-G (2005). Density dependence and overdispersion in the transmission of helminth parasites. *Parasitology* **131**: 1-12.

Das PK and Subramanian S (2002). Modelling the epidemiology, transmission and control of lymphatic filariasis. *Ann Trop Med Parasitol* **96**: S153-S164.

Duerr HP, Dietz K and Eichner M (2003). On the interpretation of age-intensity profiles and dispersion patterns in parasitological surveys. *Parasitology* **126**: 87-101.

Duerr HP, Dietz K and Eichner M (2005). Determinants of the eradicability of filarial infections: a conceptual approach. *Trends Parasitol* **21**: 88-96.

Esterre P, Plichart C, Sechan Y and Nguyen NL (2001). The impact of 34 years of massive DEC chemotherapy on *Wuchereria bancrofti* infection and transmission: the Maupiti cohort. *Trop Med Int Health* **6**: 190-195.

Goodman B (1994). Models aid understanding, help control parasites [news]. *Science* **264**: 1862-1863.

Habbema JDF, Alley ES, Plaisier AP, van Oortmarssen GJ and Remme JHF (1992). Epidemiological modelling for onchocerciasis control. *Parasitol Today* **8**: 99-103.

Habbema JDF, De Vlas SJ, Plaisier AP and Van Oortmarssen GJ (1996). The microsimulation approach to epidemiologic modeling of helminthic infections, with special reference to schistosomiasis. *Am J Trop Med Hyg* **55**: 165-169.

Krishnamoorthy K, Subramanian S, Van Oortmarssen GJ, Habbema JD and Das PK (2004). Vector survival and parasite infection: the effect of *Wuchereria bancrofti* on its vector *Culex quinquefasciatus*. *Parasitology* **129**: 43-50.

Michael E and Bundy DA (1998). Herd immunity to filarial infection is a function of vector biting rate. *Proc R Soc Lond B Biol Sci* **265**: 855-860.

Michael E, Simonsen PE, Malecela M, Jaoko WG, Pedersen EM, Mukoko D, Rwegoshora RT and Meyrowitsch DW (2001). Transmission intensity and the immunoepidemiology of bancroftian filariasis in East Africa. *Parasite Immunol* **23**: 373-388.

Michael E, Malecela-Lazaro MN, Simonsen PE, Pedersen EM, Barker G, Kumar A and Kazura JW (2004). Mathematical modelling and the control of lymphatic filariasis. *Lancet Infect Dis* **4**: 223-234.

Molyneux DH and Zagaria N (2002). Lymphatic filariasis elimination: progress in global programme development. *Ann Trop Med Parasitol* **96 (Suppl 2)**: S15-40.

Norman RA, Chan MS, Srividya A, Pani SP, Ramaiah KD, Vanamail P, Michael E, Das PK and Bundy DA (2000). EPIFIL: the development of an age-structured model for describing the transmission dynamics and control of lymphatic filariasis. *Epidemiol Infect* **124**: 529-541.

Plaisier AP, Subramanian S, Das PK, Souza W, Lapa T, Furtado AF, Van der Ploeg CPB, Habbema JDF and Van Oortmarssen GJ (1998). The LYMFASIM simulation program for modeling lymphatic filariasis and its control. *Methods Inf Med* **37**: 97-108.

Plaisier AP, Cao WC, van Oortmarssen GJ and Habbema JD (1999). Efficacy of ivermectin in the treatment of *Wuchereria bancrofti* infection: a model-based analysis of trial results. *Parasitology* **119**: 385-394.

Rochet MJ (1990). A simple deterministic model for bancroftian filariasis transmission dynamics. *Trop Med Parasitol* **41**: 225-233.

Schlemper BR, Jr., Steindel M, Grisard EC, Carvalho-Pinto CJ, Bernardini OJ, de Castilho CV, Rosa G, Kilian S, Guarneri AA, Rocha A, Medeiros Z and Ferreira Neto JA (2000). Elimination of bancroftian filariasis (*Wuchereria bancrofti*) in Santa Catarina state, Brazil. *Trop Med Int Health* **5**: 848-854.

Snow LC and Michael E (2002). Transmission dynamics of lymphatic filariasis: density-dependence in the uptake of *Wuchereria bancrofti* microfilariae by vector mosquitoes. *Med Vet Entomol* **16**: 409-423.

Southgate BA and Bryan JH (1992). Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 4. Facilitation, limitation, proportionality and their epidemiological significance. *Trans R Soc Trop Med Hyg* **86**: 523-530.

Stolk WA, Subramanian S, Oortmarssen GJ, Das PK and Habbema JD (2003). Prospects for elimination of bancroftian filariasis by mass drug treatment in Pondicherry, India: a simulation study. *J Infect Dis* **188**: 1371-1381.

Stolk WA, Ramaiah KD, Van Oortmarssen GJ, Das PK, Habbema JD and De Vlas SJ (2004). Meta-analysis of age-prevalence patterns in lymphatic filariasis: no decline in microfilaraemia prevalence in older age groups as predicted by models with acquired immunity. *Parasitology* **129**: 605-612.

Stolk WA, de Vlas SJ and Habbema JDF (2005). Anti-*Wolbachia* treatment for lymphatic filariasis. *Lancet* **365**: 2067-2068.

Stolk WA, van Oortmarssen GJ, Pani SP, Subramanian S, Das PK and Habbema JDF (in press). Effects of ivermectin and diethylcarbamazine on microfilariae and microfilaria production in bancroftian filariasis. *Am J Trop Med Hyg.*

Subramanian S, Krishnamoorthy K, Ramaiah KD, Habbema JDF, Das PK and Plaisier AP (1998). The relationship between microfilarial load in the human host and uptake and development of *Wuchereria bancrofti* microfilariae by *Culex quinquefasciatus*: a study under natural conditions. *Parasitology* **116**: 243-255.

Subramanian S, Stolk WA, Ramaiah KD, Plaisier AP, Krishnamoorthy K, Van Oortmarssen GJ, Amalraj D, Habbema JDF and Das PK (2004). The dynamics of *Wuchereria bancrofti* infection: a model-based analysis of longitudinal data from Pondicherry, India. *Parasitology* **128**: 467-482.

Taylor MJ, Makunde WH, McGarry HF, Turner JD, Mand S and Hoerauf A (2005). Macrofilaricidal activity after doxycycline treatment of *Wuchereria bancrofti*: a double-blind, randomised placebo-controlled trial. *Lancet* **365**: 2116-2121.

Vanamail P, Ramaiah KD, Pani SP, Das PK, Grenfell BT and Bundy DA (1996). Estimation of the fecund life span of *Wuchereria bancrofti* in an endemic area. *Trans R Soc Trop Med Hyg* **90**: 119-121.

VCRC. Annual report 2003. Vector Control Research Centre, Pondicherry, India.

Woolhouse ME (1992). A theoretical framework for the immunoepidemiology of helminth infection. *Parasite Immunol* **14**: 563-578.

World Health Organization (2003). Lymphatic filariasis - Progress report on the Programme in 2002. *Wkly Epidemiol Rec* **78**: 171-179.

6

Meta-analysis of age-prevalence patterns in lymphatic filariasis: no decline in microfilaraemia prevalence in older age groups as predicted by models with acquired immunity

W. A. STOLK, K. D. RAMAIAH*, G. J. VAN OORTMARSSEN, P. K. DAS*, J. D. F. HABBEMA and S. J. DE VLAS

*Department of Public Health, Erasmus MC, University Medical Center Rotterdam, the Netherlands, and *Vector Control Research Centre, Indian Council of Medical Research, Pondicherry, India*

Parasitology (2004) 129, 605–612

Abstract

The role of acquired immunity in lymphatic filariasis is uncertain. Assuming that immunity against new infections develops gradually with accumulated experience of infection, models predict a decline in prevalence after teenage or early adulthood. A strong indication for acquired immunity was found in longitudinal data from Pondicherry, India, where microfilara (mf) prevalence was highest around the age of 20 and declined thereafter. We reviewed published studies from India and Sub-Saharan Africa to investigate whether their age-prevalence patterns support the models with acquired immunity. By comparing prevalence levels in 2 adult age groups we tested whether prevalence declined at older age. For India, comparison of age groups 20–39 and 40+ revealed a significant decline in only 6 out of 53 sites, whereas a significant increase occurred more often (10 sites). Comparison of older age groups provided no indication that a decline would start at a later age. Results from Africa were even more striking, with many more significant increases than declines, irrespective of the age groups compared. The occurrence of a decline was not related to the overall mf prevalence and seems to be a chance finding. We conclude that there is no evidence of a general age-prevalence pattern that would correspond to the acquired immunity models. The Pondicherry study is an exceptional situation that may have guided us in the wrong direction.

Introduction

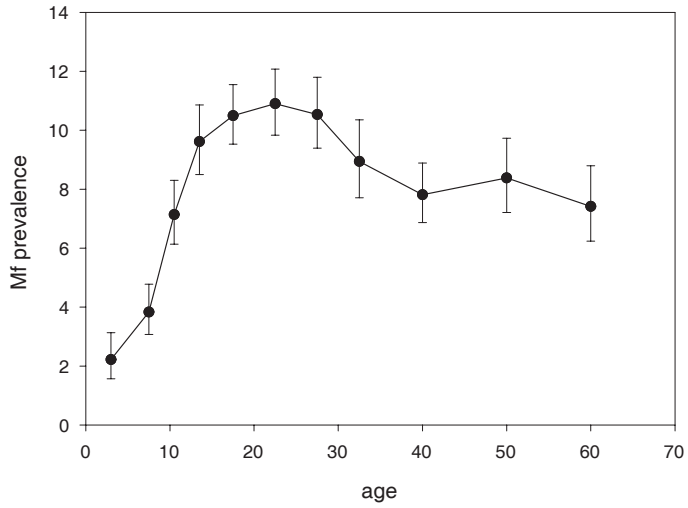
It remains unclarified whether humans, who are lifelong exposed to lymphatic filariasis infection, develop a protective immune response (Maizels *et al.* 2000). The possible operation of acquired immunity in regulating filarial infection has received special attention, because of its potential consequences for the long-term effects of control measures (Anderson & May 1985), but also because understanding immunity may help in the development of vaccines against lymphatic filariasis (Kazura 2000).

There is a large body of research on the role of acquired immunity in helminthic diseases in men, especially for schistosomiasis (Hagan 1992). In experimental animal models, protective immunity against new infections has been generated by repeated infection with infective larvae or by immunization with irradiated larvae from different filarial species (Selkirk *et al.* 1992). It is more difficult to determine whether acquired immunity also plays a role in human individuals who are naturally exposed to lymphatic filariasis, because neither an individual's exposure to infective mosquitoes nor the number of adult worms present in the human body can be quantified easily. Therefore, immunological studies in humans focussed on the correlation of various types of immune responses with infection status. Although these studies revealed many differences between infected and presumably uninfected hosts, it is unclear to which extent this is indicative of an acquired protective immune response (Kazura 2000; Ravindran *et al.* 2003).

Epidemiological studies can be helpful in investigating the role of acquired immunity in helminths. Based on pioneering epidemiological and immunological studies in Papua New Guinea, it was suggested that the acquisition of new infections may be reduced in adults due to acquired immunity against infection (Day *et al.* 1991a; Day *et al.* 1991b). Assuming that exposure is constant with age and that prolonged exposure leads to (partial) resistance against new infections, mathematical models predict an increase in infection intensity to a peak at a certain age followed by a decline in older individuals who have acquired immunity against new infections; the peak would occur at a higher level and younger age in areas with higher transmission intensity (the so-called peak-shift theorem) (Anderson & May 1985; Woolhouse 1992). If transmission intensity is stable over time, these age-patterns should be reflected in cross-sectional data on prevalence and intensity of infection.

A strong indication for the operation of acquired immunity in lymphatic filariasis was found in a study from urban Pondicherry (India) that examined the long-term effects of vector control (Rajagopalan *et al.* 1989; Subramanian *et al.* 1989). With the availability of longitudinal data on microfilaria (mf) intensity for a large number of individuals and on transmission by mosquitoes, this study is ideal for examining the dynamics of filarial infection. Mf prevalence in Pondicherry was found to decline after about 20 years of age (Figure 6-1) (Rajagopalan *et al.* 1989). Mathematical simulation models had to include strong acquired immunity to explain these data and alternative models without immunity failed (Chan *et al.* 1998; Subramanian *et al.* 2004). Additional epidemiological evidence for

Figure 6-1. Age-pattern in mf prevalence in urban Pondicherry, 1981. Figure reproduced using data from Rajagopalan *et al*. (1989). The symbols indicate the observed mf prevalence per age group with 95% confidence intervals, plotted against the mid-point of the age range.



acquired immunity in lymphatic filariasis comes from a literature review that showed a peak in prevalence in various studies. The peak appeared most pronounced in areas with high transmission intensity, and the age at which the peak occurred decreased with increasing endemicity (Michael & Bundy 1998).

However, there are also locations where mf prevalence does not decrease in the oldest age groups. Acquired immunity is not required to explain these patterns (Michael *et al.* 2001; Simonsen *et al.* 2002). This raises the question whether it is justified to attribute a decline in prevalence among older age groups, such as in the Pondicherry study, to this form of immunity. To answer this question, insight into observed patterns of lymphatic filariasis infection prevalence by age is required. We carried out a meta-analysis of all published age-specific data on prevalence of bancroftian filariasis in India and Sub-Saharan Africa, to investigate whether a decline in mf prevalence in older age groups is common in these regions and whether its occurrence is related to transmission intensity.

Material and methods

Data sources

We searched Medline (entry dates through September 2003) combining search terms Africa or India and *Wuchereria bancrofti* or filariasis to identify papers that possibly contain

age-specific data on mf prevalence. Other papers were identified by checking references from selected papers and recently published reviews. Full text copies were retrieved for all papers. Additional data were available from published books and reports from the WHO library. All publications that presented data on mf prevalence of bancroftian filariasis from India or Sub-Saharan Africa for at least 2 adult age groups were selected for inclusion in the review. Reasons for exclusion were: age-specific data on the number of individuals examined and positive were not given; the overall infection prevalence was very low (<1%); vector control or mass treatment was carried out in the 10-year period preceding the survey; the study population concerned a non-representative sample of the total population (e.g. selected on clinical or parasitological status, hospitalised patients); a large part of the population concerned migrants. Two studies reporting data from the same location were both included if the surveys took place with an interval of at least 10 years; otherwise only the study with the largest sample size was included. If a study separately presented data from different locations, these data were included as different observations in the final database and analysed separately, with the exception of 1 study that provided separate data for 17 villages with small sample size (Zielke & Chlebowsky 1979). For each observation we recorded: bibliographic information, country, and the numbers of persons examined and positive for mf in each reported age group. Differences in diagnostic tests between studies were ignored, because these were not expected to influence the patterns of mf prevalence by age. In some studies, more than one diagnostic test was used. The occasional use of different tests in children versus adults does not influence our analyses, since we compare adult age groups only. Few studies reported the use of multiple diagnostic tests in adults. If data from different diagnostic tests were provided separately, then only the data from the most sensitive diagnostic test (resulting in the highest prevalence levels) were used.

Statistical analysis

To investigate whether mf prevalence declined after the age of 20, we compared the mf prevalence in 2 adult age groups. The aim was to compare age groups 20–39 vs. 40+, but the many studies with age groups 21–40 vs. 41+ or 25–44 vs. 45+ and the few studies that only allowed comparison of age groups 15–39 vs. 40+, 16–40 vs. 41+, 15–44 vs. 45+, or 15–34 vs. 35+ were also included in this comparison. Per observation, we calculated the ratio of the prevalence rate in the older over the prevalence rate in the younger group. In order not to miss studies with a possible decline in prevalence, we assessed significance at the α=10% level. That is, we calculated 90% confidence intervals around the prevalence ratio rather than the more common, but wider, 95% confidence intervals, so that we will sooner conclude that a difference in prevalence between age groups is significant. In the few cases with zero mf prevalence in one of the age groups of interest, we calculated the relative risk and confidence limits assuming that 0.5 individual was mf positive. The number of observations that showed a significantly lower prevalence

in the oldest age group was compared to the number of observations with no change in mf prevalence or with a significantly higher prevalence in the oldest age group. Using the overall mf prevalence in the study population (children and adults) as indicator for transmission intensity, we assessed whether a possible decline in prevalence in older age groups occurred more frequently in areas with higher transmission intensity. To allow for the possibility that a decline starts in older age groups, we carried out similar analyses with 30–49 vs. 50+ and 40–59 vs. 60+.

All statistical analyses were carried out in SAS (version 6.12).

Results

We identified 79 publications that contained age-specific data on mf prevalence for either India or Sub-Saharan Africa. Together, the studies contained $n = 122$ observations, including 66 observations for Africa from 15 countries and 56 for India from 14 states. There was a large variation in the sample size, ranging from 84 to about 4000 in African studies and from 153 to 1.6 million in Indian studies. The overall community mf prevalence ranged from 2.7% to 48.1% in the African data and from 1.2% to 18.8% in the Indian data. A complete list of the articles that provide data for the current analysis is given in the Appendix to this chapter. For each study it is indicated whether comparisons of age groups 20–39 vs. 40+, 30–49 vs. 50+ and 40–59 vs. 60+ were included.

Figure 6-2A plots the relative risks of infection in the 40+ groups compared with 20–39 year olds with 90% confidence limits for India. Values <1 indicate a lower mf prevalence in the older group. A significant decline with age was found in only 6 out of 53 Indian observations. A significant increase occurred more frequently (10 observations), but most often the difference between the two age groups was not significant. The data in Figure 6-2A were sorted by overall mf prevalence in the community. An association with endemicity level is not apparent. When age groups 30–49 and 50+ were compared, only 6 out of 52 observations showed a significant difference: 4 with lower and 2 with higher prevalence in the oldest age groups. Out of 17 observations that allowed comparison between age groups 40–59 and 60+, there was none with a significant decline and 1 with a significant increase.

In Africa, the comparison between age groups 20–39 and 40+ revealed only 1 out of 65 observations with a significantly lower prevalence in the oldest group and 18 with a significantly higher prevalence. Taking non-significant increases into account, 80% of observations had higher mf prevalence in the oldest group. This indicates that any decline in prevalence would occur at a later age than in India. However, in the comparison of age groups 30–49 and 50+ respectively 1 and 9 out of 48 observations showed significantly lower and higher prevalence among 50+ (Figure 6-2B). In the comparison between 40–59 vs. 60+ these numbers were 0 and 4 ($n = 41$). As in India, a decline was not more common in areas with higher prevalence.

Figure 6-2 (A). Relative risk of infection with mf in two adult age groups. Results for India: ratio of mf prevalence in age group 40+ vs. 20–39. On the Y-axis, overall mf prevalence in the entire study population and the study number are given for each observation; study numbers refer to the list in the Appendix. Symbols indicate the point-estimate for the relative risk of infection in the older vs. the younger group; horizontal bars give the 90% confidence intervals around the point-estimate. Plus and minus signs on the right side of the figure indicate observations with a significantly higher (+) or lower (–) prevalence in the older group.

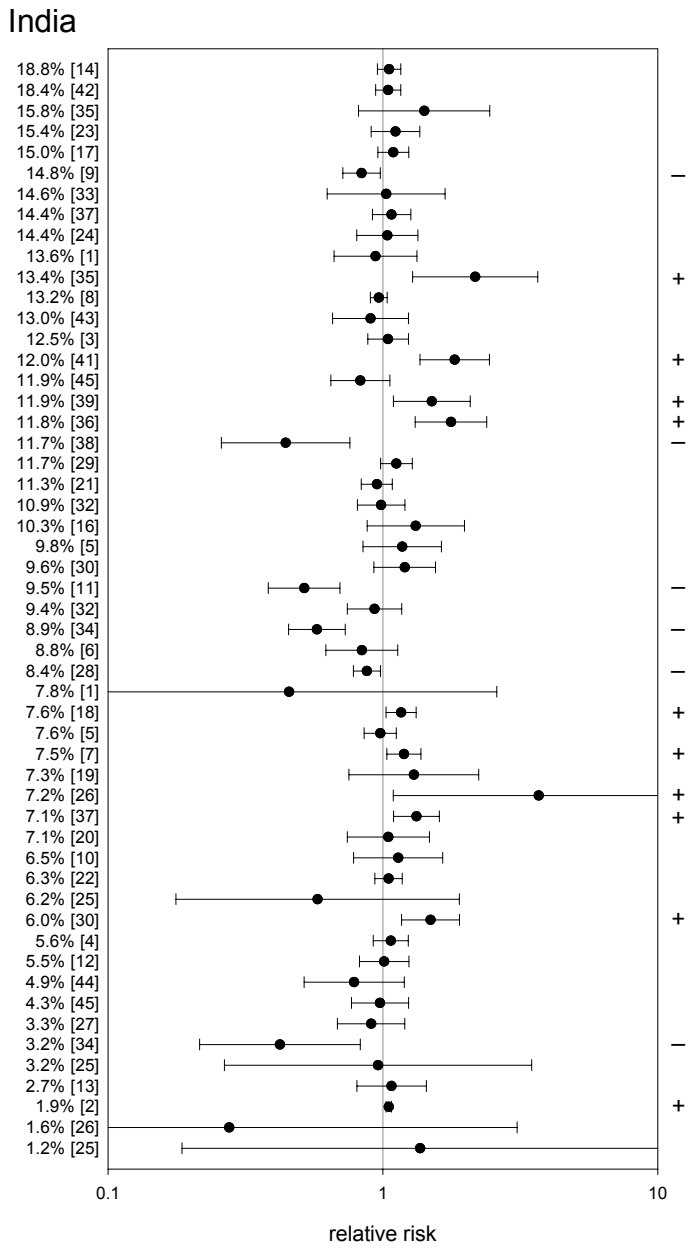
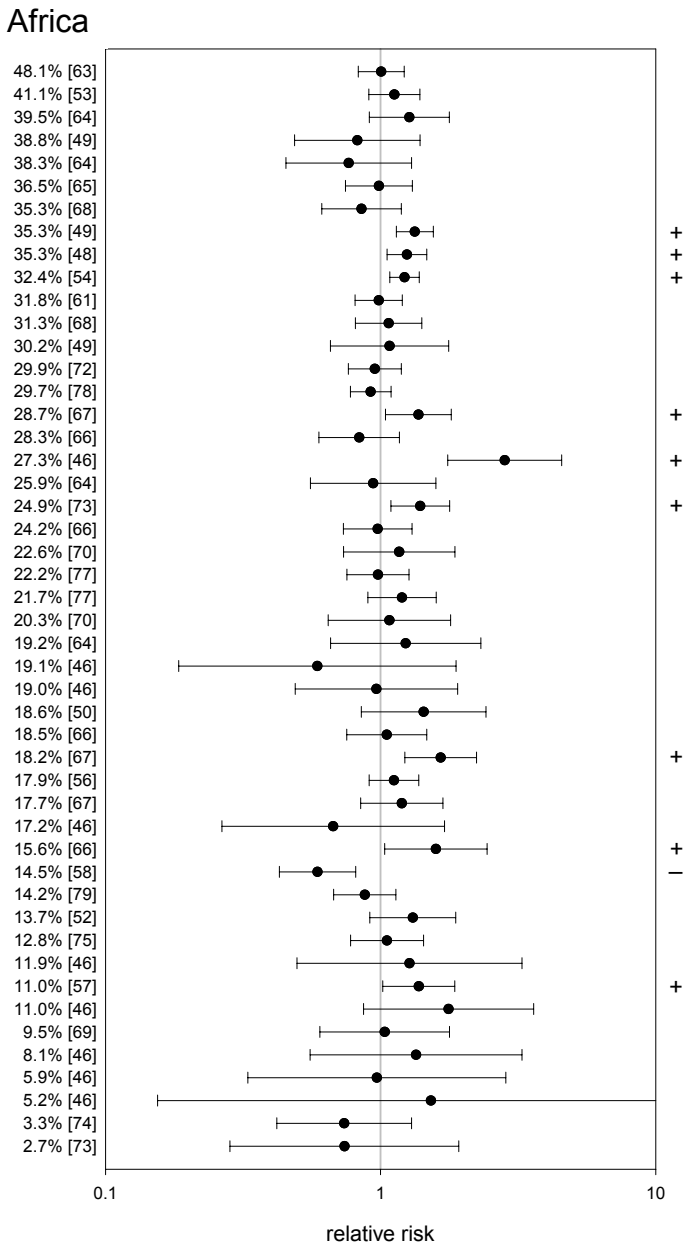


Figure 6-2 (B). Relative risk of infection with mf in two adult age groups. Results for Africa: ratio of mf prevalence in age group 50+ vs. 30–49. On the Y-axis, overall mf prevalence in the entire study population and the study number are given for each observation; study numbers refer to the list in the Appendix. Symbols indicate the point-estimate for the relative risk of infection in the older vs. the younger group; horizontal bars give the 90% confidence intervals around the point-estimate. Plus and minus signs on the right side of the figure indicate observations with a significantly higher (+) or lower (–) prevalence in the older group.



Discussion

This meta-analysis shows that patterns with declining prevalence in the oldest age groups, which would be expected if acquired immunity plays an important role in preventing infection, are not common in areas endemic for bancroftian filariasis. In India, comparison of age groups 20–39 vs. 40+ showed that the number of sites with a significant decrease in prevalence with age was low and comparable to the number of sites with a significant increase. In Africa, comparison of age groups 30–49 vs. 50+ even showed that an increase in prevalence with age occurred much more frequently than a decrease. Assessing significance at the α=5% level resulted in a somewhat lower number of studies with significant differences between the age groups of interest, but did not lead to different proportions of significant decreases and increases.

Based on a recent study of age-infection patterns of lymphatic filariasis in East Africa, it was suggested that the impact of acquired immunity in moderating infection levels, may only be apparent in areas with high transmission intensity and especially in the oldest age groups (Michael *et al.* 2001). This hypothesis is not supported by our results: using overall mf prevalence in the study population as an indicator for transmission intensity, we found no indication that a decline in prevalence occurred more frequently in areas with higher transmission intensity. This pattern did not change when we compared older age groups. A peak in mf prevalence and subsequent decline seems to be a chance finding, which has no relation to endemicity level.

Our results do not confirm the results of the earlier study by Michael & Bundy (1998), who also analysed age-prevalence patterns to investigate the role of acquired immunity in lymphatic filariasis transmission. Their analysis was restricted to locations for which combined data were available on annual infective biting rate (as the indicator for transmission intensity) and age-specific mf prevalence. The authors showed that a peak in mf prevalence occurred at younger ages and higher levels in areas with higher transmission intensity; this 'peak shift' has been interpreted as a strong indication for the operation of acquired immunity. However, the authors *a priori* assumed a peak in mf prevalence in all studies and estimated the peak level and age at which the peak occurred by fitting a quadratic curve to the data from each study. This curve, though, does not accurately describe patterns with stabilizing prevalence above a certain age. In fact, the estimated peak level was sometimes considerably higher than the prevalence level observed in any age group. Based on the results of our meta-analysis, the earlier conclusion that prevalence patterns are shaped by acquired immunity may have to be reconsidered.

The quality of data in our study may to some extent be compromised by the variation in sample sizes. Several Indian studies provided highly aggregated data, e.g. for an entire district, with very low overall mf prevalence levels. Age-patterns from these studies could be biased if endemicity levels vary within the region and if there was imbalance in sampling of different age groups from different locations. Also, details on past control activities in Indian sites were often not provided. For example, in many

urban areas, vector control and selective treatment may have taken place as part of the National Filariasis Control Program (NFCP). Nevertheless, there is no reason to assume that these factors introduce such strong bias that patterns with declining prevalence were masked completely. African studies were usually confined to well-defined, small geographical areas and, in most areas, there were no previous control activities.

Overall, our results do not suggest that prevalence is systematically reduced in older age groups, which would be expected as a consequence of acquired immunity. This has implications for the modelling of lymphatic filariasis transmission. Two currently available simulation models, which were both quantified based on data from Pondicherry, included strong acquired immunity to explain the data from this area (Chan *et al.* 1998; Subramanian *et al.* 2004). Our study revealed that Pondicherry is one of only few locations with declining prevalence at higher ages (study number 28 in Figure 6-2A). Nevertheless, this exceptional pattern was found in data from both the integrated vector management arm and the control arm (Rajagopalan *et al.* 1989). Also, it was visible in subsequent cross-sectional surveys from the area (Das *et al.* 1992; Manoharan *et al.* 1997) and in individual-level longitudinal data (Vanamail *et al.* 1989). Other factors than immunity may have to be considered to explain these data, such as trends in transmission intensity over time, immigration from areas with low endemicity levels or emigration of infected cases from urban Pondicherry, differences in treatment history between age groups, or a site-specific decline in exposure to mosquito bites with age. Changing assumptions on acquired immunity may influence model predictions of the long-term effects of mass treatment and of the probability of elimination (Stolk *et al.* 2003).

The absence of a decline in mf prevalence in older ages does not necessarily preclude the operation of acquired immunity. Theoretically, it is possible that exposure increases until the oldest age groups but that prevalence stabilizes at a certain level due to acquired immunity. However, there is no reason to assume that exposure would increase with age among adults. It is also possible that the immune response regulates the density of mf rather than presence or absence. However, the number of studies reporting age-specific data on mf intensity is much smaller than the number of studies that report prevalence data and information on variance to be used for statistical comparison is usually lacking. Scanning through the available articles for patterns on mf density, though, we also found no indication of a regularly occurring decline in mf intensity in older age groups (unpublished data). It may also be useful to analyse data on prevalence and intensity of antigenaemia by age in a similar way (Simonsen *et al.* 1996; Onapa *et al.* 2001; Steel *et al.* 2001; Tisch *et al.* 2001; Simonsen *et al.* 2002). Nevertheless, the age-patterns of mf prevalence in published studies were not consistent with existing models of acquired immunity. Possibly, models for acquired immunity can be adapted so that the predicted patterns are more consistent with the aggregated data from literature (e.g. with different assumptions on parasite mortality, the parasite stages that trigger immunity, the rates of acquisition or decay of immunity, the effects of immune responses, or the strength of immunity). In this respect, it is interesting to note that Day *et al.* (1991b), who also did not find a decline in infection intensity in older age groups, suggested that acquired immunity

may only affect the rate of parasite establishment and the plateau worm burden. Further, even if acquired immunity does not protect against new infections, it may for example protect against development of disease.

This meta-analysis has shown that a decline in prevalence in older age groups is not found more frequently than an increase in *W. bancrofti*-endemic areas, and that the occurrence of such patterns is not related to transmission intensity. The aggregated data thus provide no indication that mf prevalence among adults is moderated by a form of acquired immunity. More detailed analysis of age-patterns in lymphatic filariasis infection may enhance our understanding of the factors that shape age-prevalence curves. For vaccine development, for predicting the long-term effects of mass treatment and for assessing the prospects of achieving elimination, better understanding of the dynamics of infection in the human host and the role of acquired immunity is crucial.

Acknowledgements

This investigation received financial support from the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

Appendix

Bibliographic information of articles with source data for the analysis presented in this chapter. Letters between brackets indicate that the study was included in the comparison of the following age groups: [a] 20-39 vs. 40+, [b] 30-49 vs. 50+, [c] 40-59 vs. 60+.

India

1. Bhattacharya NC, Gubler DJ, *Indian J Med Res* **61**, 8 (1973) [abc]
2. Biswas H *et al*, *J Commun Dis* **21**, 272 (1989) [a]
3. Chand D, Singh MV, Bhaskar VK, *Indian J Malariol* **15**, 149 (1961) [ab]
4. Chand D, Singh MV, Srivastava RN, *Indian J Malariol* **15**, 175 (1961) [ab]
5. Chand D, Singh MV, Pathak VK, *Indian J Malariol* **15**, 21 (1961) [ab]
6. Chand D *et al*, *Indian J Malariol* **15**, 39 (1961) [ab]
7. Chand D, Singh MV, Pathak VK, *Indian J Malariol* **15**, 31 (1961) [ab]
8. Chand D, Singh MV, Vyas LC, *Indian J Malariol* **16**, 269 (1962) [ab]
9. Chhotray GP *et al*, *Indian J Med Res* **114**, 65 (2001) [abc]
10. Dutta SN, Diesfeld HJ, *J Commun Dis* **26**, 43 (1994) [abc]
11. Jain DC *et al*, *J Commun Dis* **19**, 317 (1987) [ac]
12. Jain DC *et al*, *J Commun Dis* **21**, 265 (1989) [ab]
13. Joseph C, Peethambaran P, *Indian J Malariol* **17**, 33 (1963) [ab]
14. Kant L, Ken SK, Puri BS, *Indian J Malariol* **10**, 199 (1956) [ab]
15. Kar SK, Mania J, Kar PK, *Acta Trop* **55**, 53 (1993) [c]
16. Khan AM *et al*, *J Commun Dis* **31**, 101 (1999) [ab]
17. Krishnaswami AK, *Indian J Malariol* **9**, 1 (1955) [ab]
18. Kumar A, Chand SK, *J Commun Dis* **22**, 209 (1990) [abc]
19. Kumar A, Dash AP, Mansing GD, *J Commun Dis* **26**, 215 (1994) [ab]
20. Mishra SS, Dwivedi MP, *Indian J Public Health* **23**, 7 (1979) [abc]
21. Nair CP, Roy R, Joseph C, *Indian J Malariol* **14**, 223 (1960) [ab]
22. Nair CP, *Indian J Malariol* **14**, 233 (1960) [ab]

23. Nair CP, *Indian J Malariol* **15**, 263 (1961) [ab]
24. Nair CP, *Indian J Malariol* **16**, 47 (1962) [ab]
25. Pawar RG, Mittal MC, *Indian J Med Res* **56**, 370 (1968) [ab]
26. Rahman NM, Bhattacharyya MN, *J Indian Med Assoc* **56**, 363 (1971) [abc]
27. Rajagopalan PK, Shetty PS, Arunachalam N, *Indian J Med Res* **73**, 73 (1981) [abc]
28. Rajagopalan PK *et al*, *Epidemiol Infect* **103**, 685 (1989) [abc]
29. Ramaiah KD *et al*, *Indian J Med Res* **89**, 184 (1989) [abc]
30. Rao NS, Murthy NS, Marwah SM, *Indian J Med Res* **61**, 943 (1973) [ab]
31. Rao CK *et al*, *Indian J Med Res* **71**, 712 (1980) [b]
32. Rath RN *et al*, *J Commun Dis* **16**, 104 (1984) [abc]
33. Ravindran B *et al*, *Parasite Immunol* **22**, 633 (2000) [ab]
34. Rudra SK, Chandra G, *Ann Trop Med Parasitol* **94**, 365 (2000) [abc]
35. Russel S, Das M, Rao CK, *J Commun Dis* **7**, 15 (1975) [ab]
36. Shriram AN *et al*, *Trop Med Int Health* **7**, 949 (2002) [ab]
37. Singh J, Raghavan NGS, Krishnaswami AK, *Indian J Malariol* **10**, 219 (1956) [ab]
38. Sinha AP *et al*, *Indian J Malariol* **13**, 159 (1959) [ab]
39. Srivastava RN, Prasad BG, *Indian J Med Res* **57**, 528 (1969) [ab]
40. Sunish IP *et al*, *Trop Med Int Health* **8**, 316 (2003).
41. Tewari SC, Hiriyan J, Reuben R, *Trans R Soc Trop Med Hyg* **89**, 163 (1995) [ab]
42. Varma BK, Dass NL, Sinha VP, *Indian J Malariol* **15**, 185 (1961) [ab]
43. Varma BK, Dass NL, Sinha VP, *Indian J Malariol* **15**, 285 (1961) [ab]
44. Varma BK, Dass NL, Sinha VP, *Indian J Malariol* **15**, 293 (1961) [ab]
45. Varma BK *et al*, *Indian J Malariol* **16**, 17 (1962) [ab]

Africa

46. Akogun OB, *J Hyg Epidemiol Microbiol Immunol* **35**, 383 (1991) [abc]
47. Brengues J, Subra R, Bouchite B, *Cah ORSTOM sér Ent méd et Parasitol* **7**, 279 (1969) [a]
48. Brengues J, *Mémoires ORSTOM*, vol. **79**, 299p (1975) [ab]
49. Brunhes J, *Mémoires ORSTOM,* vol. **81**, 212p (1975) [abc]
50. Charafoudine H, Pesson B, *Bull Soc Path Ex* **79**, 229 (1986) [ab]
51. Dzodzomenyo M *et al*, *Trop Med Int Health* **4**, 13 (1999) [ac]
52. Estambale BB *et al*, *Ann Trop Med Parasitol* **88**, 145 (1994) [ab]
53. Gyapong JO *et al*, *J Trop Med Hyg* **96**, 317 (1993) [abc]
54. Gyapong JO, Magnussen P, Binka FN, *Trans R Soc Trop Med Hyg* **88**, 555 (1994) [abc]
55. Gyapong JO, Adjei S, Sackey SO, *Trans R Soc Trop Med Hyg* **90**, 26 (1996) [ac]
56. Gyapong JO, Omane-Badu K, Webber RH, *Trans R Soc Trop Med Hyg* **92**, 407 (1998) [abc]
57. Huehns ER, *Trans R Soc Trop Med Hyg* **47**, 549 (1953) [abc]
58. Ivoke N, *J Commun Dis* **32**, 254 (2000) [abc]
59. Jemaneh L, Kebede D, *Ethiop Med J* **33**, 143 (1995).
60. Juminer B, Diallo S, Diagne S, *Arch Inst Pasteur Tunis* **48**, 231 (1971) [a]
61. Massaga JJ, Salum FM, Savael ZX, *Cent Afr J Med* **46**, 237 (2000) [abc]
62. Matola YG, *Trop Geogr Med* **37**, 108 (1985) [a]
63. Maxwell CA *et al*, *Trans R Soc Trop Med Hyg* **84**, 709 (1990) [ab]
64. McFadzean JA, *Trans R Soc Trop Med Hyg* **48**, 267 (1954) [abc]
65. McGregor IA, Hawking F, Smith DA, *Br Med J* **ii**, 908 (1952) [ab]
66. McMahon JE *et al*, *Ann Trop Med Parasitol* **75**, 415 (1981) [abc]
67. Meyrowitsch DW, Simonsen PE, Makunde WH, *Ann Trop Med Parasitol* **89**, 665 (1995) [abc]
68. Meyrowitsch DW, Simonsen PE, Makunde WH, *Ann Trop Med Parasitol* **89**, 653 (1995) [abc]
69. Myung K *et al*, *Am J Trop Med Hyg* **59**, 222 (1998) [abc]
70. Nielsen NO *et al*, *Trans R Soc Trop Med Hyg* **96**, 133 (2002) [ab]
71. Simonsen PE *et al*, *Acta Trop* **60**, 179 (1995) [a]
72. Simonsen PE *et al*, *Am J Trop Med Hyg* **55**, 69 (1996) [abc]
73. Simonsen PE *et al*, *Am J Trop Med Hyg* **66**, 550 (2002) [abc]
74. Udonsi JK, *Ann Trop Med Parasitol* **80**, 425 (1986) [abc]
75. Udonsi JK, *Folia Parasitol* **35**, 147 (1988) [abc]
76. Wamae CN *et al*, *Parasitology* **116**, 173 (1998) [a]
77. Wijers DJ, Kinyanjui H, *Ann Trop Med Parasitol* **71**, 333 (1977) [abc]
78. Wijeyaratne PM *et al*, *Indian J Med Res* **76**, 534 (1982) [abc]
79. Zielke E, Chlebowsky HO, *Tropenmed Parasitol* **30**, 91 (1979) [ab]

References

Anderson RM and May RM (1985). Herd immunity to helminth infection and implications for parasite control. *Nature* **315**: 493-496.

Chan MS, Srividya A, Norman RA, Pani SP, Ramaiah KD, Vanamail P, Michael E, Das PK and Bundy DA (1998). Epifil: a dynamic model of infection and disease in lymphatic filariasis. *Am J Trop Med Hyg* **59**: 606-614.

Das PK, Manoharan A, Subramanian S, Ramaiah KD, Pani SP, Rajavel AR and Rajagopalan PK (1992). Bancroftian filariasis in Pondicherry, south India--epidemiological impact of recovery of the vector population. *Epidemiol Infect* **108**: 483-493.

Day KP, Gregory WF and Maizels RM (1991a). Age-specific acquisition of immunity to infective larvae in a bancroftian filariasis endemic area of Papua New Guinea. *Parasite Immunol* **13**: 277-290.

Day KP, Grenfell B, Spark R, Kazura JW and Alpers MP (1991b). Age specific patterns of change in the dynamics of *Wuchereria bancrofti* infection in Papua New Guinea. *Am J Trop Med Hyg* **44**: 518-527.

Hagan P (1992). Reinfection, exposure and immunity in human schistosomiasis. *Parasitol Today* **8**: 12-16.

Kazura JW (2000). Resistance to infection with lymphatic-dwelling filarial parasites. in: *Lymphatic filariasis*. (ed. TB Nutman). London, Imperial College press. **1:** 83-102.

Maizels RM, Allen JE and Yazdanbakhsh M (2000). Immunology of lymphatic filariasis: current controversies. in: *Lymphatic filariasis*. (ed. TB Nutman). London, Imperial College Press. **1:** 217-264.

Manoharan A, Das PK, Keerthiseelan VB and Ramaiah KD (1997). Trend of *Wuchereria bancrofti* infection in Pondicherry urban agglomeration after the withdrawal of a five year vector control programme. *J Commun Dis* **29**: 255-261.

Michael E and Bundy DA (1998). Herd immunity to filarial infection is a function of vector biting rate. *Proc R Soc Lond B Biol Sci* **265**: 855-860.

Michael E, Simonsen PE, Malecela M, Jaoko WG, Pedersen EM, Mukoko D, Rwegoshora RT and Meyrowitsch DW (2001). Transmission intensity and the immunoepidemiology of bancroftian filariasis in East Africa. *Parasite Immunol* **23**: 373-388.

Onapa AW, Simonsen PE, Pedersen EM and Okello DO (2001). Lymphatic filariasis in Uganda: baseline investigations in Lira, Soroti and Katakwi districts. *Trans R Soc Trop Med Hyg* **95**: 161-167.

Rajagopalan PK, Das PK, Subramanian S, Vanamail P and Ramaiah KD (1989). Bancroftian filariasis in Pondicherry, south India: 1. Pre-control epidemiological observations. *Epidemiol Infect* **103**: 685-692.

Ravindran B, Satapathy AK, Sahoo PK and Mohanty MC (2003). Protective immunity in human lymphatic filariasis: problems and prospects. *Med Microbiol Immunol (Berl)* **192**: 41-46.

Selkirk ME, Maizels RM and Yazdanbakhsh M (1992). Immunity and the prospects for vaccination against filariasis. *Immunobiology* **184**: 263-281.

Simonsen PE, Lemnge MM, Msangeni HA, Jakobsen PH and Bygbjerg IC (1996). Bancroftian filariasis: the patterns of filarial-specific immunoglobulin G1 (IgG1), IgG4, and circulating antigens in an endemic community of northeastern Tanzania. *Am J Trop Med Hyg* **55**: 69-75.

Simonsen PE, Meyrowitsch DW, Jaoko WG, Malecela MN, Mukoko D, Pedersen EM, Ouma JH, Rwegoshora RT, Masese N, Magnussen P, Estambale BB and Michael E (2002). Bancroftian filariasis infection, disease, and specific antibody response patterns in a high and a low endemicity community in East Africa. *Am J Trop Med Hyg* **66**: 550-559.

Steel C, Ottesen EA, Weller PF and Nutman TB (2001). Worm burden and host responsiveness in *Wuchereria bancrofti* infection: use of antigen detection to refine earlier assessments from the South Pacific. *Am J Trop Med Hyg* **65**: 498-503.

Stolk WA, Subramanian S, Oortmarssen GJ, Das PK and Habbema JDF (2003). Prospects for elimination of bancroftian filariasis by mass drug treatment in Pondicherry, India: a simulation study. *J Infect Dis* **188**: 1371-1381.

Subramanian S, Pani SP, Das PK and Rajagopalan PK (1989). Bancroftian filariasis in Pondicherry, south India: 2. Epidemiological evaluation of the effect of vector control. *Epidemiol Infect* **103**: 693-702.

Subramanian S, Stolk WA, Ramaiah KD, Plaisier AP, Krishnamoorthy K, Van Oortmarssen GJ, Amalraj D, Habbema JDF and Das PK (2004). The dynamics of *Wuchereria bancrofti* infection: a model-based analysis of longitudinal data from Pondicherry, India. *Parasitology* **128**: 467-482.

Tisch DJ, Hazlett FE, Kastens W, Alpers MP, Bockarie MJ and Kazura JW (2001). Ecologic and biologic determinants of filarial antigenemia in bancroftian filariasis in Papua New Guinea. *J Infect Dis* **184**: 898-904.

Vanamail P, Subramanian S, Das PK, Pani SP, Rajagopalan PK, Bundy DA and Grenfell BT (1989). Estimation of age-specific rates of acquisition and loss of *Wuchereria bancrofti* infection. *Trans R Soc Trop Med Hyg* **83**: 689-693.

Woolhouse ME (1992). A theoretical framework for the immunoepidemiology of helminth infection. *Parasite Immunol* **14**: 563-578.

Zielke E and Chlebowsky HO (1979). Studies on Bancroftian filariasis in Liberia, West Africa. I. Distribution and prevalence in the north-western savanna area. *Tropenmed Parasitol* **30**: 91-96.

7

Assessing density dependence in the transmission of lymphatic filariasis: uptake and development of *Wuchereria bancrofti* microfilariae in the vector mosquitoes

W. A. STOLK, G. J. VAN OORTMARSSEN, S. SUBRAMANIAN*, P. K. DAS*, G. J. J. M. BORSBOOM, J. D. F. HABBEMA and S. J. DE VLAS

*Department of Public Health, Erasmus MC, University Medical Center Rotterdam, the Netherlands, and *Vector Control Research Centre, Indian Council of Medical Research, Pondicherry, India*

Medical and Veterinary Entomology (2004) 18, 57–60

Abstract

Understanding density dependence in the transmission of lymphatic filariasis is essential for assessing the prospects of elimination. This study seeks to quantify the relationship between microfilaria (mf) density in human blood and the number of third stage (L3) larvae developing in the mosquito vectors *Aedes polynesiensis* Marks and *Culex quinquefasciatus* Say (Diptera: Culicidae) after blood-feeding. Two types of curves are fitted to previously published data. Fitting a linearized power curve through the data allows for correction for measurement error in human mf counts. Ignoring measurement error leads to overestimation of the strength of density dependence; the degree of overestimation depends on the accuracy of measurement of mf density. For use in mathematical models of transmission of lymphatic filariasis, a hyperbolic saturating function is preferable. This curve explicitly estimates the mf uptake and development at lowest mf densities and the average maximum number of L3 that can develop in mosquitoes. This maximum was estimated at 23 and 4 for *Ae. polynesiensis* and *Cx. quinquefasciatus*, respectively.

Better understanding of the transmission of lymphatic filariasis is crucial for predicting the impact of control programmes and assessing the prospects of elimination. The occurrence of density dependence in the vector part of the transmission cycle has been addressed in several studies. In a recently published meta-analysis in this journal, Snow & Michael (2002) examined density dependence in the uptake of microfilariae (mf) in relation to mf density in the human blood for the three major vectors of *Wuchereria bancrofti*, the predominant cause of lymphatic filariasis: *Culex*, *Aedes* and *Anopheles*. Their study showed 'limitation' in mf uptake by all three species: the mf uptake relative to mf density in the human blood decreases when human mf densities are higher. This effect appeared to be strongest for *Anopheles* and weakest for *Culex* species.

Density dependence, however, also occurs in the subsequent development of mf into L3 larvae: the proportion of ingested mf that develops successfully into L3 larvae may decrease (limitation) or increase (facilitation) with higher mf uptake (Southgate & Bryan 1992; Pichon 2002). The combined impact of density dependence in both uptake and development of mf determines the relationship between mf density in the human blood and the number of L3 larvae eventually developing in mosquitoes after feeding. For modelling the transmission of lymphatic filariasis, we aimed to describe this relationship quantitatively. In this short communication, we discuss several issues that play a role in choosing a mathematical function and estimating its parameters.

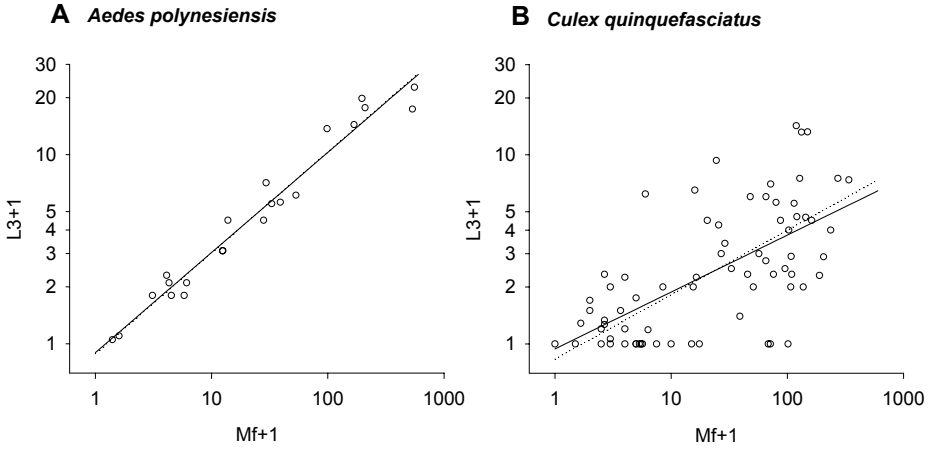
We re-analysed paired data on human mf density and the average number of L3 larvae per mosquito for *Ae. polynesiensis* and *Cx. quinquefasciatus*. Data for *Ae. polynesiensis* were available from Tahiti, French Polynesia (Rosen 1955). This dataset included 22 paired observations from 17 individuals. Mf density in the human blood was determined as the average count in eight 20-μL blood smears; 13–138 (median 46) mosquitoes were dissected for L3 larvae 13 days after feeding. For *Cx. quinquefasciatus* we used data from Pondicherry, India (Subramanian *et al.* 1998). This dataset included 72 paired observations from 12 individuals. Mf density in the human blood was determined as the average count of two or three 20 μL blood smears; 1–22 (median 5) mosquitoes were dissected for L3 larvae 13 days after feeding.

Analogously to Snow & Michael (2002), we quantified the association between mf density in human blood and L3 in vector mosquitoes by fitting the power curve $L3 = a\,mf^{\,b}$ through the data; the curve is linearized by $\log_{10}$ transformation of both sides of the equation after adding +1 to mf and L3 counts:

$$\log(L3+1) = \log(a) + b\log(mf+1) \qquad (7\text{-}1)$$

The parameters of this equation were estimated by standard linear regression and the resulting equations are plotted in Figure 7-1 (solid lines). The steeper slope for *Ae. polynesiensis* (b=0.53, 95% CI 0.48–0.58) compared to *Cx. quinquefasciatus* (b=0.30, 95% CI 0.22–0.39) indicates that the combined impact of limitation in uptake and development is much stronger for *Culex*. The estimate of b for *Cx. quinquefasciatus* was much lower than that estimated by Snow & Michael (2002) for the association between mf in the blood and mf uptake by this mosquito (b=0.73). This suggests that limitation occurs not only in mf

Figure 7-1. Average number of L3 developing in mosquitoes in relation to mf density in the human blood (mf / 20 μL) for *Ae. polynesiensis* (A) and *Cx. quinquefasciatus* (B). The open circles indicate the observations, plotted on a log-scale. The lines show the best fitting linearized power curve of Equation 7-1 without (solid) or with (dotted) taking account of measurement error in the human mf density. The parameters of the regression equation were the same in both analyses for *Ae. polynesiensis*: $\log_{10}(a) = -0.05$, $b = 0.53$. For *Cx. quinquefasciatus* the parameter estimates were $\log_{10}(a) = -0.03$, $b = 0.30$ when measurement error was not taken into account, and $\log_{10}(a) = -0.08$, $b = 0.34$ after correction.



uptake but also in the subsequent development of engorged mf. For *Ae. polynesiensis* the slopes from the current study (b=0.53) and Snow & Michael (2002) (b=0.57) were comparable, suggesting that density dependence in the development of mf into L3 is limited. Thus, whereas Snow & Michael (2002) found that limitation in mf uptake was stronger for *Aedes* than for *Culex*, the current analysis shows that limitation may be stronger in the latter species when density dependence in the development of mf in L3 larvae is also taken into account.

The parameter estimates presented above did not take account of measurement error in the mf density in human blood. There is therefore a risk of underestimating the slopes of the regression equations (Armitage & Berry 1987). The degree of measurement error depends on the diagnostic test, the volume of blood that is examined and the amount of 'true' variation that may occur between mf counts in the same individual due to periodicity or day-to-day variation. 'Deming regression' takes account of measurement error, assuming that variance in the independent variable is proportional to the variance in the dependent variable (Polman *et al.* 2001). We used this method to explore the impact of measurement error on the accuracy of the estimated regression equations, assuming that variances in the log-transformed mf + 1 and L3 + 1 are equal (ratio $\lambda = 1$). The difference between corrected and uncorrected slopes for *Ae. polynesiensis* was negligible (see Figure 7-1). The data on *Cx. quinquefasciatus* showed a much weaker association,

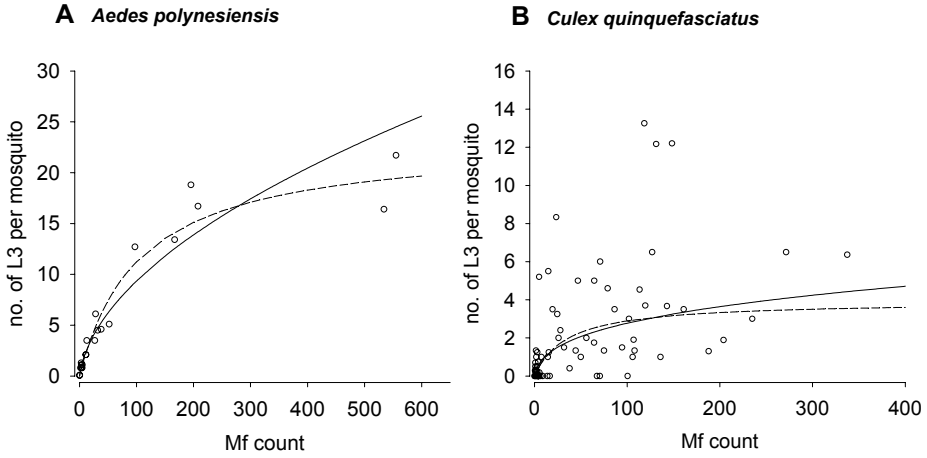
which can be explained by less accurate estimation of both mf density in human blood (based on two or three mf counts instead of eight) and the average number of L3 developing in mosquitoes (based on dissection of a median of five mosquitoes rather than 46). Using Deming regression we found a somewhat higher slope, implying less strong limitation ($b = 0.34$, CI 0.25–0.42). If the variance in the human mf density was larger than the variance in the number of L3 per mosquito ($\lambda > 1$), the underestimation of the slope – and thereby overestimation of the limitation effect would be stronger than shown in Figure 7-1. For a more accurate estimation of the slopes of the regression equation, the parameter λ should be estimated from ideally independent but otherwise similar data.

Fitting a linearized power curve is very convenient for assessing the strength of density dependence in the relationship between mf density in the blood and intake and development of mf in mosquitoes, and standard methods – such as Deming regression – can be used to correct for intra-individual variation in mf counts. However, this curve is not ideal to give a realistic description of this relationship in transmission models such as LYMFASIM or EPIFIL (Plaisier *et al.* 1998; Norman *et al.* 2000). The relationship between mf density in the blood and the number of L3 developing in the mosquito is distorted at the lowest mf densities due to the log(+1) transformation of mf counts and (unless $a = 1$) the curve of Equation 7-1 does not go through the origin. Using the original power function $L3 = a\,mf^{\,b}$ would lead to an infinite slope at the start of the curve, where a proportional association would be more realistic. Furthermore, biologists would argue that there is a maximum to the number of L3 that can develop successfully, which is not properly accounted for by the continuously increasing curve of Equation 7-1. For mathematical modelling, the correct estimation of the number of L3 developing in mosquitoes after biting on a carrier with very low mf density is crucial for predicting the probability of interrupting transmission after reducing mf density by mass treatment, whereas the saturation level is an important determinant of the endemicity level in the absence of control efforts. The hyperbolic saturating curve is a better alternative for relating L3 to mf in a transmission model (Pichon *et al.* 1974; Subramanian *et al.* 1998):

$$L3 = \frac{\alpha Mf}{1 + \alpha/\beta\, Mf} \qquad (7\text{-}2)$$

Parameter α quantifies the initial slope of the relationship, whereas β indicates the average maximum number of L3 that can develop in mosquitoes. We fitted this curve to the data by the ordinary least squares method after log(+1) transformation of both sides of the equation to stabilize the variance in the dependent variable. In the absence of standard methods to correct for measurement error in the independent variable in non-linear regression, measurement error in mf counts was not taken into account. Therefore, the results for *Cx. quinquefasciatus* especially should be interpreted with caution. The results are plotted in Figure 7-2 and compared with the (uncorrected) linearized power curve. The average number of L3 larvae per mosquito was found to saturate at 23.1 (95% CI 16.9–29.3) for *Ae. polynesiensis*; especially at higher mf densities (>100 mf/20 µL) the hyperbolic

Figure 7-2. The relationship between the number of L3 developing in mosquitoes and the mf density in the human blood for *Ae. polynesiensis* and *Cx. quinquefasciatus*. The dots indicate the observations. The lines show the linearized power curve of Equation 7-1 (solid) and the best fitting hyperbolic saturating curve of Equation 7-2 (dashed). Parameter estimates for the hyperbolic saturating function were α=0.22, β=23.15 for *Ae. polynesiensis* and α=0.11, β=3.92 for *Cx. quinquefasciatus*. Parameters for the linearized power curve are given in the legend of Figure 7-1.



saturating function performs better than the power curve. The saturation level was much lower for *Cx. quinquefasciatus* (3.9, 95% CI 2.0–5.8). Based on the same dataset, the saturation level was previously estimated at 6.6 (95% CI 4.3–17.0) (Subramanian *et al.* 1998). In this earlier publication, individual level data were used, whereas the current methods are based on analysis of aggregated data, which are more widely available in literature.

Our study showed that there is limitation in the relationship between mf density in the human blood and the number of L3 larvae developing in mosquitoes, which is stronger for *Cx. quinquefasciatus* than for *Ae. polynesiensis*. Measurement error in the mf density in the human blood can lead to overestimation of the strength of limitation when this is not accounted for in the analysis. A hyperbolic saturating curve may be more appropriate to describe the association in mathematical models, but its use is limited to datasets with minimal error in the measurement of mf density. To understand fully how transmission intensity depends on the mf density in the blood of human individuals, excess mortality among (heavily) infected mosquitoes should be considered as well (Das *et al.* 1995; Failloux *et al.* 1995).

Acknowledgements

This investigation received financial support from the World Health Organization.

References

Armitage P and Berry G (1987). *Statistical methods in medical research*. Oxford, Blackwell Scientific Publications.

Das PK, Subramanian S, Manoharan A, Ramaiah KD, Vanamail P, Grenfell BT, Bundy DA and Michael E (1995). Frequency distribution of *Wuchereria bancrofti* infection in the vector host in relation to human host: evidence for density dependence. *Acta Trop* **60**: 159-165.

Failloux AB, Raymond M, Ung A, Glaziou P, Martin PM and Pasteur N (1995). Variation in the vector competence of Aedes polynesiensis for *Wuchereria bancrofti*. *Parasitology* **111 (Pt 1)**: 19-29.

Norman RA, Chan MS, Srividya A, Pani SP, Ramaiah KD, Vanamail P, Michael E, Das PK and Bundy DA (2000). EPIFIL: the development of an age-structured model for describing the transmission dynamics and control of lymphatic filariasis. *Epidemiol Infect* **124**: 529-541.

Pichon G, Perrault G and Laigret J (1974). Rendement parasitaire chez les vecteurs de filarioses. *Bull World Health Organ* **51**: 517-524.

Pichon G (2002). Limitation and facilitation in the vectors and other aspects of the dynamics of filarial transmission: the need for vector control against *Anopheles*-transmitted filariasis. *Ann Trop Med Parasitol* **96**: S143-S152.

Plaisier AP, Subramanian S, Das PK, Souza W, Lapa T, Furtado AF, Van der Ploeg CPB, Habbema JDF and Van Oortmarssen GJ (1998). The LYMFASIM simulation program for modeling lymphatic filariasis and its control. *Methods Inf Med* **37**: 97-108.

Polman K, De Vlas SJ, Van Lieshout L, Deelder AM and Gryseels B (2001). Evaluation of density-dependent fecundity in human *Schistosoma mansoni* infections by relating egg counts to circulating antigens through Deming regression. *Parasitology* **122**: 161-167.

Rosen L (1955). Observations on the epidemiology of human filariasis in French Oceania. *Am J Hyg* **61**: 219-248.

Snow LC and Michael E (2002). Transmission dynamics of lymphatic filariasis: density-dependence in the uptake of *Wuchereria bancrofti* microfilariae by vector mosquitoes. *Med Vet Entomol* **16**: 409-423.

Southgate BA and Bryan JH (1992). Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 4. Facilitation, limitation, proportionality and their epidemiological significance. *Trans R Soc Trop Med Hyg* **86**: 523-530.

Subramanian S, Krishnamoorthy K, Ramaiah KD, Habbema JDF, Das PK and Plaisier AP (1998). The relationship between microfilarial load in the human host and uptake and development of *Wuchereria bancrofti* microfilariae by *Culex quinquefasciatus*: a study under natural conditions. *Parasitology* **116**: 243-255.

8

Effects of ivermectin and diethylcarbamazine on microfilariae and overall microfilaria production in bancroftian filariasis

W. A. STOLK, G. J. VAN OORTMARSSEN, S. P. PANI*, S. J. DE VLAS, S. SUBRAMANIAN*, P. K. DAS*, and J. D. F. HABBEMA

*Department of Public Health, Erasmus MC, University Medical Center Rotterdam, the Netherlands, and *Vector Control Research Centre, Indian Council of Medical Research, Pondicherry, India*

American Journal of Tropical Medicine and Hygiene (in press)

Abstract

Ivermectin and diethylcarbamazine (DEC) are used in mass treatment programmes for the elimination of lymphatic filariasis because of their strong effects on microfilaraemia. However, the effects of treatment on adult worms and the degree of individual variation in efficacy are unclear. We analyzed series of microfilaria (mf) counts from individuals treated with a single dose of 400 μg/kg ivermectin or 6 mg/kg DEC (n=23 in each group; 1 year follow-up). For each individual, we estimated the microfilaricidal effect and the reduction in overall mf production (e.g., caused by death or sterilization of worms, or inhibited mf release from the female worm uterus). Ivermectin on average killed 96% of mf and reduced mf production by 82%. DEC killed 57% of mf and reduced mf production by 67%, with some individuals responding very poorly. The strong reduction in overall mf production is good news for control of lymphatic filariasis, but the prospects of elimination will be diminished if part of the population systematically responds poorly to treatment.

Introduction

Programmes are being initiated worldwide to eliminate lymphatic filariasis by yearly mass treatment with ivermectin or diethylcarbamazine (DEC), given alone or in combination with albendazole. It is unclear, though, how many treatment rounds will be required to achieve the goal of elimination. A major problem is our incomplete understanding of the effects of treatment on the adult worm. Control needs to be continued for many years if overall microfilaria (mf) production by adult worms is largely unaffected by treatment. Antigen tests have been used to demonstrate macrofilaricidal effects (Weil *et al.* 1991; McCarthy *et al.* 1995), but it is unclear how the reduction in antigen level relates to the proportion of worms killed. Ultrasound has been used to assess the macrofilaricidal effects of treatment directly (Dreyer *et al.* 1995a; Norões *et al.* 1997); however, its application is limited to the scrotal area and superficial lymphatics. Neither of these tools can assess an effect on fecundity.

Most commonly, the effects of treatment have been assessed by measuring the change in mf density over time. Many clinical trials and community-based interventions showed that treatment with ivermectin or DEC, given alone or in combination with albendazole, leads to a strong and sustained reduction in mf density (reviewed in Ottesen *et al.* 1999; Brown *et al.* 2000; Melrose 2002; International Filariasis Review Group 2004). Mathematical models that describe the development of parasites in the human body can be used to analyze such trends in mf density for indirect quantification of the effects of treatment. In this way, it was estimated from published data that a single dose treatment with 200 or 400 μg /kg ivermectin not only results in immediate killing of all mf, but also in a reduction in the overall mf production in the follow-up period of respectively 35% or 65% at least (Plaisier *et al.* 1999). The reduction in mf production indicates that adult worms are affected, but the nature of this effect (e.g., death or sterilization of worms, reduced mf release from female worm uterus) cannot be determined. However, there was no indication that the reduction in mf production was only temporary. Similar estimates for the efficacy of a single dose of DEC are not available yet.

Another aspect of interest is the variation in treatment efficacy that occurs between individuals. This has received little attention in literature. However, the impact of mass treatment may be undermined when there is a number of individuals who respond poorly to treatment and who continue to transmit infection in the population (Stolk *et al.* 2003).

Here, we present the results of a double-blind, randomized, hospital-based trial that was carried out to investigate the efficacy of a single dose of ivermectin (400 μg/kg body weight) or DEC (6 mg/kg body weight) for treatment of bancroftian filariasis (Subramanyam Reddy *et al.* 2000). We analyzed the one-year follow-up trends in mf density at the individual level to quantify the effects of treatment and the individual variation in these effects.

Material and methods

Data

A double-blind, randomized, hospital-based trial was carried out in Pondicherry, India, to compare the safety and efficacy of a single dose of ivermectin (400 μg/kg body weight) or DEC (6 mg/kg body weight) for treatment of bancroftian filariasis (Subramanyam Reddy *et al.* 2000). In each treatment group, 30 mf carriers with pretreatment mf counts ≥100 mf/mL were included. Mf density in the blood was determined by membrane filtration of 1 mL venous night blood, and all blood samples were taken between 8.30 PM and 9.30 PM (not always on the exact same time for an individual). Mf counts were taken with monthly intervals during the first year after treatment. Available observations for part of the individuals made 24 months after treatment were not included in our analysis. This was because these observations are not only determined by the effects of treatment, but to a large extent also by trends in transmission intensity or other external factors that are not accounted for in our model. One year follow-up is long enough to measure the effects of treatment, but distortion of the trends due to reinfection will be minimal because of the long immature period of the worms. Only individuals with complete follow-up were included in the analysis (23 individuals in each group).

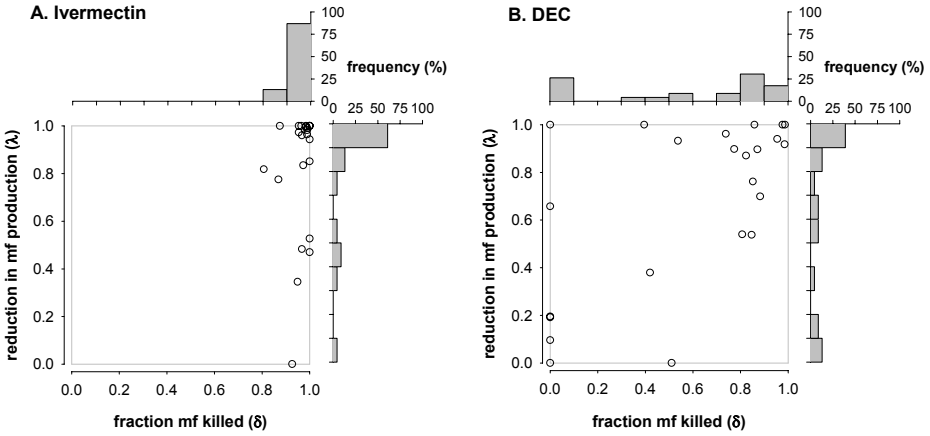
The two treatment groups were comparable with respect to age and gender: the mean age was 20 years in the ivermectin group and 22 years in the DEC group, and the male:female ratio was 14:9 and 12:11, respectively. The mean pretreatment mf load was higher in the ivermectin group than in the DEC group (538 mf /mL vs. 338 mf /mL), but this difference was not significant (t-test on log-transformed values, p=0.118).

Statistical analysis

We used a mathematical model, which describes the course of *Wuchereria bancrofti* infection in individuals over time and the impact of treatment on the different parasite stages (see the Appendix to this chapter). We assumed that the pretreatment mf density represents an equilibrium situation where the acquisition of worms and mf is balanced by the loss. This equilibrium is disturbed by two immediate and irreversible effects of treatment: a fraction of mf is killed (resulting in an immediate drop in mf density) and the overall mf production is reduced by a certain fraction (resulting in a lower rate of mf recurrence in the blood than expected if mf production had not been affected). The cause of the reduced mf production (e.g., death or sterilization of adult worms or any other mechanism that inhibits the release of mf from the female worm uterus) cannot be determined from the data on mf density.

The rate of recurrence of mf after treatment (relative to an individual's pretreatment level) depends not only on the effects of treatment, but also on assumptions on the duration of the immature period of worms and the adult worm and mf life span. Based on literature, we assumed these durations to be, respectively, 8 months (World Health Organization 1992), 8 years (Vanamail *et al.* 1996; Subramanian *et al.* 2004), and 12

Figure 8-1. Individual estimates of the fraction of mf killed and the reduction in overall mf production due to treatment with ivermectin (A) or DEC (B). The histograms along the upper and right axes of the graphs show the corresponding frequency distributions of the efficacy estimates.



months (Plaisier *et al.* 1999) on average. As argued above, new infections acquired during the first year after treatment will have little impact on trends in mf density and were ignored in this analysis. Under these assumptions, mf density one year after treatment is 61% of the pretreatment level if treatment kills all mf but has no effect on adult worms. The behavior of the model is further explained elsewhere (Plaisier *et al.* 1999).

Individual trends in mf density are described by the pretreatment force-of-infection (β), the fraction mf killed due to treatment (δ), and the effect of treatment on overall mf production (λ). The values of these parameters are estimated by fitting the model to the individual data using non-linear regression and assuming extra-Poisson variation. A more detailed description of the model and the estimation procedure is given in the Appendix.

In a sensitivity analysis, we assessed how the estimates of the efficacy parameters depend on assumptions on the immature period and the worm and mf life span by halving and doubling their values. We also checked how the results change if we take account of new infections acquired during follow-up with the rate of acquisition being equal to the pretreatment rate. Spearman's rank correlation was used to test for correlations between efficacy estimates (δ or λ) and the predicted pretreatment mf intensities (reflected by β).

Results

The results of the analysis are summarized in Figure 8-1 and Table 8-1. On average, the efficacy of ivermectin was higher than that of DEC. The fraction of mf killed (δ) was high

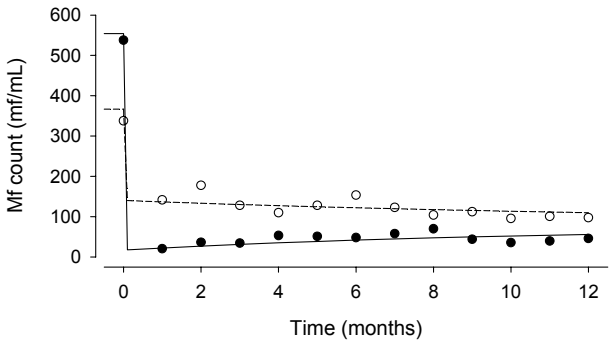
Table 8-1. Variation in the estimated fraction of microfilariae (mf) killed and the reduction in overall mf production between individuals who were treated with ivermectin or diethylcarbamazine (DEC).

Parameter and description	Ivermectin (n=23)	DEC (n=23)
Impact on mf		
Fraction of mf killed (δ)		
Average (sd)	0.96 (0.05)	0.57 (0.39)
Median (25^{th} – 75^{th} percentile)	0.98 (0.95 – 1.00)	0.77 (0.00 – 0.87)
Number (%) of individuals with all mf killed ($\delta > 0.999$)	7 (30%)	0 (0%)
Number (%) of individuals with no mf killed ($\delta < 0.001$)	0 (0%)	6 (26%)
Impact on mf production		
Reduction in overall mf production (λ)		
Average (sd)	0.82 (0.27)	0.67 (0.36)
Median (25^{th} – 75^{th} percentile)	0.96 (0.78 – 1.00)	0.87 (0.38 – 0.96)
Number (%) of individuals with complete cessation of mf production ($\lambda > 0.999$)	7 (30%)	5 (22%)
Number (%) of individuals with no change in mf production ($\lambda < 0.001$)	1 (4%)	2 (9%)

in all ivermectin-treated individuals; in 87% of the individuals even more than 90% of the mf was killed. Usually there was also a strong reduction in overall mf production (λ). The effects of DEC treatment were somewhat lower on average and varied strongly between individuals. In both groups, there was no significant correlation between the individual estimates of δ or λ and the pretreatment mf intensity β, indicating that the effects of treatment do not depend on the pretreatment level of infection.

Figure 8-2 shows the average trend in observed and predicted mf intensities. Figure 8-3 gives some typical examples of individual trends in mf density after treatment. Ivermectin led to a strong initial reduction of mf density in all individuals and usually the density remained low during follow-up (Figure 8-3A and B), indicating that the treatment killed nearly all mf and almost completely interrupted mf production. Three individuals even had zero mf counts at all measurements post-treatment, suggesting complete effectiveness of treatment. In several individuals, the strong immediate reduction was followed by a gradual increase, which indicates that mf production was not completely interrupted (Figure 8-3C). In the DEC-group, only few individuals showed the nearly ideal pattern of Figure 8-3A or B and there were no individuals who had zero mf counts during the entire follow-up period. Often, a limited immediate reduction in mf density after treatment was followed by gradual decline during the follow-up period (Figure 8-3D). This pattern reflects little direct effect on mf and a strong effect on mf production.

Figure 8-2. Observed and predicted trends in arithmetic mean mf density. Symbols indicate the mean of the observed individual mf counts; the lines show the average of the individual predicted mf densities (○ and -- for DEC; ● and — for ivermectin).



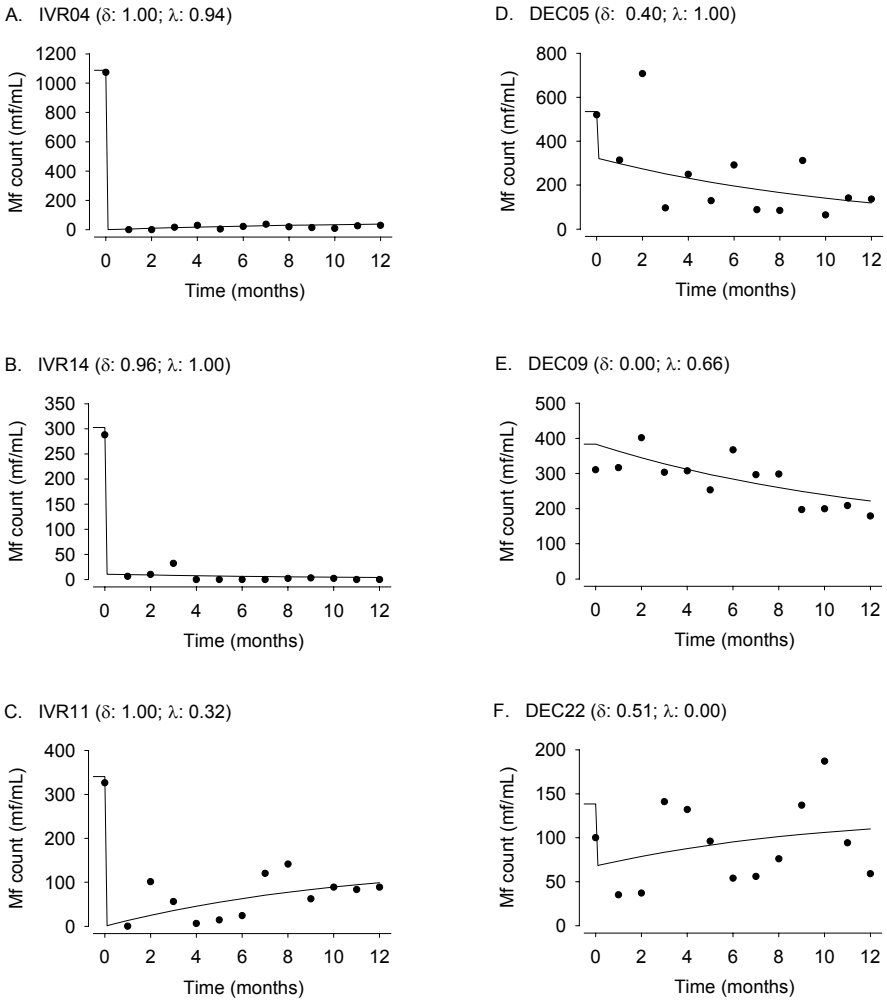
Sometimes there was no immediate effect on microfilaraemia (Figure 8-3E). In several DEC-treated individuals, mf counts during follow-up remained high. Due to the high variability in mf counts, such trends were difficult to interpret, but anyway the effects are poor (Figure 8-3F). In one individual treated with DEC, treatment did not have any effect on mf or mf production.

The sensitivity analysis showed that our results did not depend on the duration of the immature period and worm life span. Only the assumptions on mf life span influenced the individual efficacy estimates, although the change was not always in the same direction. Assuming a mf life span of 6 or 24 months, the average reduction in overall mf production was 0.63 or 0.69, respectively, in the DEC group and 0.86 or 0.75, respectively, in the ivermectin group. Allowing for acquisition of new infections during the post-treatment period, we found slightly (around 0-3%) higher estimates for the reduction in overall mf production; the estimated fraction of mf killed hardly changed at all.

Discussion

Our analysis of individual-level trends in mf density after treatment showed that a single dose of ivermectin (400 μg/kg) in all treated individuals resulted in death of a large fraction of mf and in most instances also in a strong reduction in overall mf production. The effects of DEC were somewhat lower on average and more variable. In some individuals treated with DEC, almost all mf were killed and mf production was nearly completely interrupted; in others, the drug had little effect. The data provide no information about the cause of the reduction in overall mf production. For DEC it is

Figure 8-3. Observed and predicted trends in mf density for 6 individuals with typical patterns for ivermectin (A-C) or DEC (D-F). Corresponding values of both efficacy parameters are given above each graph.



probably explained by a macrofilaricidal effect (Weil *et al.* 1991; Figueredo-Silva *et al.* 1996; Norões *et al.* 1997). Ivermectin probably does not kill adult worms (Dreyer *et al.* 1995c; Dreyer *et al.* 1996). Possibly, ivermectin causes damage to the reproductive system of female worms, so that embryogenesis, maturation or release of mf from the uterus is inhibited.

Based on ultrasound examination of the male scrotum, it was previously estimated that a single dose of DEC kills about half of the adult worms (Norões *et al.* 1997). The estimated reduction in overall mf production in our study was only slightly higher. Care is required in this comparison: the reduction in mf production may be higher than the proportion of adult worms affected, because unmated worms may have survived and retained their ability to produce mf. Any effect of ivermectin on the fertility of adult worms cannot directly be measured. However, the current estimates are in agreement with the results of a previous model-based analysis (Plaisier *et al.* 1999). This analysis of 2-year follow-up data provided no indication that the effect on mf production was only temporal, but studies with longer follow-up are required to be more certain on this aspect. Analysis of combined data on mf and antigen density and on the presence of motile worms (Freedman *et al.* 2001; Ismail *et al.* 2001; El Setouhy *et al.* 2004; Kshirsagar *et al.* 2004) may enhance our qualitative and quantitative understanding of the effects of treatment on adult worms.

The validity of our efficacy estimates depends on the validity of the model that was used to describe the average trends. We do not know exactly how the filarial worm develops in the human body. However, assumptions about the immature period or worm life span proved to have little impact on our efficacy estimates and did not change the main conclusions. The results were more sensitive to assumptions about the mf life span. The effect of changing the assumed mf life span depends on the observed trend. Assuming a shorter mf life span results in higher estimates of the reduction in overall mf production, if a strong initial decline in mf density is followed by a gradual increase. However, it results in lower estimates, if a gradual decline in mf density is observed over time. Assuming a longer mf life span results in changes in the other direction. Although individual estimates were influenced by assumptions on the mf life span, the impact on the average efficacy estimate was rather limited and strong variability remained.

Assumptions on the acquisition of new infections during follow-up had little impact on the outcomes. Because of the long immature period of the worm (8 months), the contribution of newly acquired infection on the mf density one year after treatment is very limited. Indeed, when we allowed for the acquisition of new infections, assuming that transmission in the post-treatment period continues at the same rate as before treatment, we found only slightly higher estimates for the reduction in overall mf production and the estimated fraction mf killed hardly changed.

To assess the generalizability of our efficacy estimates, we compared our data with that from other trials. Higher effectiveness of ivermectin (400 μg/kg) compared to DEC (6 mg/kg) was reported in several studies (Addiss *et al.* 1993; Kazura *et al.* 1993; Moulia-Pelat *et al.* 1993), but other studies revealed only small differences between both treatment regimens (Moulia-Pelat *et al.* 1996; Nicolas *et al.* 1997) and one study found that DEC was even more effective than ivermectin (Dreyer *et al.* 1995b). For DEC, the geometric mean mf density one year after treatment varied widely in published studies from 4.5% to 33.4% (average 12%) of the pretreatment level (Kimura *et al.* 1985; Addiss *et al.* 1993; Moulia-Pelat *et al.* 1993; Andrade *et al.* 1995; Dreyer *et al.* 1995b; Moulia-Pelat *et al.* 1996;

Nicolas *et al.* 1997; Pani *et al.* 2002). In our data, it was reduced to about 17% of the pretreatment level, which is within the range of other studies. For ivermectin, too, trends in mf density varied between studies (Cao *et al.* 1997). Analysis of data from other studies may therefore yield somewhat different efficacy estimates.

For part of the individuals in our study one additional observation made two years after treatment was available, but these observations were not used. These observations were usually low relative to the observed trend during the first year after treatment (Cao *et al.* 1997). Explorative attempts to fit the model to all data (including the 2-year follow-up data) resulted in somewhat higher estimates of the reduction in overall mf production, but a poorer fit. This suggests that these observations were probably influenced by (external) factors that are not accounted for by our model.

A problem in the analysis of individual level data is the large variability in mf counts, so that sometimes trends were difficult to interpret. The pretreatment mf density was based on only one measurement. In some individuals the pretreatment mf count by chance will have been lower than the true density. This was probably seen in some DEC-treated individuals, who had higher mf counts during follow-up than before treatment (e.g. Figure 8-3E and F). In other individuals, the observed mf count will by chance have been higher than the true mf density. With our approach, however, we cannot identify when this occurs. This might have led to a small overestimation of the average effects of treatment. The selection of mf positives for our study population may have added to the overestimation. In the whole population, therefore, the average efficacy may be somewhat lower than we estimated.

Our study provides important information for the ongoing elimination programmes for lymphatic filariasis, which are based on mass treatment with DEC and ivermectin in combination with albendazole. The average effects of DEC and ivermectin treatment are high, which triggers optimism about the potential impact of mass treatment. However, ivermectin is usually given in lower dosages (150-200 μg/kg instead of the 400 μg/kg given in this study), which is less effective in reducing the overall mf production (Plaisier *et al.* 1999). It is unknown to what extent the impact of treatment is improved by giving the drugs in combination with albendazole (International Filariasis Review Group 2004).

Especially in the DEC group, there was much variation in treatment efficacy and in several individuals the effects were poor. A remaining question is whether the observed variation is random or systematic. More information is needed about the impact of a second treatment in individuals who had a poor response. The presence of systematic non-responders in a population will considerably reduce the probability of elimination, or at least necessitate a longer duration of treatment programmes (until most adult worms have died naturally). It would be interesting to study whether the average efficacy of treatment increases and whether the number of people with poor response to treatment is reduced when ivermectin or DEC are given in combination with albendazole, as is recommended for the ongoing elimination programmes (Ottesen *et al.* 1997).

Acknowledgements

We thank Paul Simonsen and Dan Meyrowitsch of the Danish Bilharziasis Laboratory (DBL) and Anton Plaisier for their contribution to this work in earlier stages of the project. We thank Theo Stijnen for his statistical advice. This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

Appendix

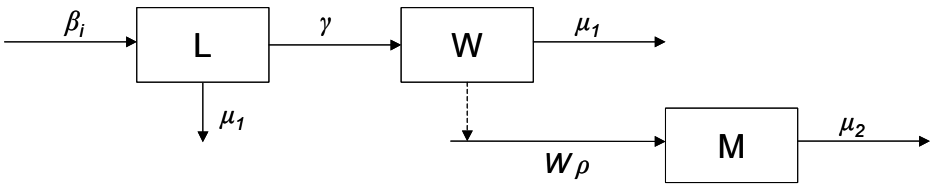
Mathematical description of the model

The structure of the model is schematically presented in Figure 8A-1. The dynamics of parasite development and mf production are described by the following set of differential equations:

$$\left.\begin{aligned} \frac{dL(t)}{dt} &= \beta_i - (\gamma + \mu_1)L(t) \\ \frac{dW(t)}{dt} &= \gamma L(t) - \mu_1 W(t) \\ \frac{dM(t)}{dt} &= \rho W(t) - \mu_2 M(t) \end{aligned}\right\} \qquad \text{(A1)}$$

Let W be the number of adult and productive worms in a person, L the number of immature worms, and M the number of mf. The rate of acquisition of new worms depends on the force-of-infection β_i, which is defined as the average number of successfully inoculated new parasites per year. The rate of maturation, γ, is defined by the duration of the immature period (immature period = $1/\gamma$). Similarly, the death rate of larvae and worms, μ_1, is defined by the average life span of the parasites (parasite life span = $1/\mu_1$). Mature adult worms start producing mf (M) at a constant per capita rate ρ. Parameter ρ is defined as the rate of mf production per mature worm per unit of blood

Figure 8A-1. Flow-chart of the model, showing the dynamics of immature worms (L), adult worms (W), and microfilariae (M) in the human host.



taken for diagnosis. The death rate of larvae and worms, μ_2, is defined by the average mf life span (mf life span = $1/\mu_2$).

We assume that the force-of-infection has been constant over time, so that the worm and mf density are in equilibrium prior to treatment, meaning that death of worms is balanced by new infections. The force-of-infection varies between individuals, reflected in different pretreatment counts. Because of the long immature period of worms, new infections acquired during the first year after treatment will have little impact on trends in mf density, and we ignore these in our analysis. In other words, $\beta_i = 0$ in the post-treatment period for all individuals.

At the moment of treatment ($t = 0$), a fraction δ_i of the mf (M) is being killed instantaneously and a fraction λ_i of all worms present in the body (L and W) stop producing mf or, in the case of immature worms, lose their ability to produce mf.

Solution of the differential equations

For estimating the effects of treatment, we are interested in the relationship between the mf density M and time t. By solving the set of differential equations A1 for $dL(t)/dt = dW(t)/dt = dM(t)/dt = 0$, we derived the following relationship for the equilibrium mf density pretreatment M^*:

$$M^* = \frac{\rho\beta_i\gamma}{\mu_1\mu_2(\gamma+\mu_1)} \qquad \text{(A2)}$$

From the moment of treatment onwards, the relationship is given by a non-linear function:

$$M(t) = \frac{\rho\beta_i}{\mu_1\mu_2(\gamma+\mu_1)}\left[\gamma(1-\delta_i)e^{-\mu_2 t} - \frac{\mu_2(\gamma+\mu_1)}{\mu_2-\mu_1}(\lambda_i-1)\left(e^{-\mu_1 t}-e^{-\mu_2 t}\right) + \frac{\mu_1\mu_2}{\mu_2-\gamma-\mu_1}(\lambda_i-1)\left(e^{-(\gamma+\mu_1)t}-e^{-\mu_2 t}\right)\right] \qquad \text{(A3)}$$

with β_i reflecting the pretreatment individual force-of-infection. For $t = 0$ (i.e. directly after treatment), this becomes:

$$M(0) = \frac{\rho\beta_i\gamma(1-\delta_i)}{\mu_1\mu_2(\gamma+\mu_1)} \qquad \text{(A4)}$$

Estimation of model parameters

Equations A2 and A3 were fitted to the data. Since we have no sound knowledge of the worm load of a person or the mf production per worm, and since mathematically one of

the parameters β_i and ρ is redundant, we put $\rho=1$ and only estimated β_i. Further, we estimated the individual values of δ_i and λ_i. These parameters were estimated by non-linear regression, using SAS (v. 8.2). In doing so, we assumed that mf counts follow a Poisson distribution with overdispersion (i.e. extra-Poisson variation, the variance being a factor θ larger than the mean mf density). The value of θ was estimated at 30.9, indicating a high variation in mf counts. Assuming a negative binomial distribution of mf counts resulted in a worse fit to the data.

Explorative analyses showed that the individual level parameters did not follow a normal distribution and that efficacy estimates were frequently on the boundaries of the possible range of values (implying full or no effect on mf or mf production). Including these parameters as random effects in the model was not useful, and we therefore estimated all parameters as fixed effects.

References

Addiss DG, Eberhard ML, Lammie PJ, McNeeley MB, Lee SH, McNeeley DF and Spencer HC (1993). Comparative efficacy of clearing-dose and single high-dose ivermectin and diethylcarbamazine against *Wuchereria bancrofti* microfilaremia. *Am J Trop Med Hyg* **48**: 178-185.

Andrade LD, Medeiros Z, Pires ML, Pimentel A, Rocha A, Figueredo-Silva J, Coutinho A and Dreyer G (1995). Comparative efficacy of three different diethylcarbamazine regimens in lymphatic filariasis. *Trans R Soc Trop Med Hyg* **89**: 319-321.

Brown KR, Ricci FM and Ottesen EA (2000). Ivermectin: effectiveness in lymphatic filariasis. *Parasitology* **121**: S133-146.

Cao WC, Van der Ploeg CPB, Plaisier AP, van der Sluijs IJS and Habbema JDF (1997). Ivermectin for the chemotherapy of bancroftian filariasis: a meta-analysis of the effect of single treatment. *Trop Med Int Health* **2**: 393-403.

Dreyer G, Amaral F, Norões J, Medeiros Z and Addiss D (1995a). A new tool to assess the adulticidal efficacy *in vivo* of antifilarial drugs for bancroftian filariasis. *Trans R Soc Trop Med Hyg* **89**: 225-226.

Dreyer G, Coutinho A, Miranda D, Norões J, Rizzo JA, Galdino E, Rocha A, Medeiros Z, Andrade LD, Santos A, Figueredo-Silva J and Ottesen EA (1995b). Treatment of bancroftian filariasis in Recife, Brazil: a two-year comparative study of the efficacy of single treatments with ivermectin or diethylcarbamazine. *Trans R Soc Trop Med Hyg* **89**: 98-102.

Dreyer G, Norões J, Amaral F, Nen A, Medeiros Z, Coutinho A and Addiss D (1995c). Direct assessment of the adulticidal efficacy of a single dose of ivermectin in bancroftian filariasis. *Trans R Soc Trop Med Hyg* **89**: 441-443.

Dreyer G, Addiss D, Noroes J, Amaral F, Rocha A and Coutinho A (1996). Ultrasonographic assessment of the adulticidal efficacy of repeat high-dose ivermectin in bancroftian filariasis. *Trop Med Int Health* **1**: 427-432.

El Setouhy M, Ramzy RMR, Ahmed ES, Kandil AM, Hussain O, Farid HA, Helmy H and Weil GJ (2004). A randomized clinical trial comparing single- and multi-dose combination therapy with diethylcarbamazine and albendazole for treatment of bancroftian filariasis. *Am J Trop Med Hyg* **70**: 191-196.

Figueredo-Silva J, Jungmann P, Noroes J, Piessens WF, Coutinho A, Brito C, Rocha A and Dreyer G (1996). Histological evidence for adulticidal effect of low doses of diethylcarbamazine in bancroftian filariasis. *Trans R Soc Trop Med Hyg* **90**: 192-194.

Freedman DO, Plier DA, De Almeida AB, De Oliveira AL, Miranda J and Braga C (2001). Effect of aggressive prolonged diethylcarbamazine therapy on circulating antigen levels in bancroftian filariasis. *Trop Med Int Health* **6**: 37-41.

International Filariasis Review Group, (David Addiss, Julia Critchley, Henry Ejere, Paul Garner, Hellen Gelband, Carroll Gamble) (2004). Albendazole for lymphatic filariasis (Cochrane Review). Chichester, UK, John Wiley & Sons, Ltd.

Ismail MM, Jayakody RL, Weil GJ, Fernando D, De Silva MS, De Silva GA and Balasooriya WK (2001). Long-term efficacy of single-dose combinations of albendazole, ivermectin and diethylcarbamazine for the treatment of bancroftian filariasis. *Trans R Soc Trop Med Hyg* **95**: 332-335.

Kazura J, Greenberg J, Perry R, Weil G, Day K and Alpers M (1993). Comparison of single-dose diethylcarbamazine and ivermectin for treatment of bancroftian filariasis in Papua New Guinea. *Am J Trop Med Hyg* **49**: 804-811.

Kimura E, Penaia L and Spears GF (1985). The efficacy of annual single-dose treatment with diethylcarbamazine citrate against diurnally subperiodic bancroftian filariasis in Samoa. *Bull World Health Organ* **63**: 1097-1106.

Kshirsagar NA, Gogtay NJ, Garg BS, Deshmukh PR, Rajgor DD, Kadam VS, Kirodian BG, Ingole NS, Mehendale AM, Fleckenstein L, Karbwang J and Lazdins-Helds JK (2004). Safety, tolerability, efficacy and plasma concentrations of diethylcarbamazine and albendazole co-administration in a field study in an area endemic for lymphatic filariasis in India. *Trans R Soc Trop Med Hyg* **98**: 205-217.

McCarthy JS, Guinea A, Weil GJ and Ottesen EA (1995). Clearance of circulating filarial antigen as a measure of the macrofilaricidal activity of diethylcarbamazine in *Wuchereria bancrofti* infection. *J Infect Dis* **172**: 521-526.

Melrose WD (2002). Lymphatic filariasis: new insights into an old disease. *Int J Parasitol* **32**: 947-960.

Moulia-Pelat JP, Glaziou P, Nguyen LN, Chanteau S, Martin PM and Cartel JL (1993). Long-term efficacy of single-dose treatment with 400 micrograms.kg-1 of ivermectin in bancroftian filariasis: results at one year. *Trop Med Parasitol* **44**: 333-334.

Moulia-Pelat JP, Nguyen LN, Hascoet H and Nicolas L (1996). Associations de l'ivermectine et de la diéthylcarbamazine pour obtenir un meilleur contrôle de l'infection en filariose lymphatique. *Parasite* **3**: 45-48.

Nicolas L, Plichart C, Nguyen LN and Moulia-Pelat JP (1997). Reduction of *Wuchereria bancrofti* adult worm circulating antigen after annual treatments of diethylcarbamazine combined with ivermectin in French Polynesia. *J Infect Dis* **175**: 489-492.

Norões J, Dreyer G, Santos A, Mendes VG, Medeiros Z and Addiss D (1997). Assessment of the efficacy of diethylcarbamazine on adult *Wuchereria bancrofti in vivo*. *Trans R Soc Trop Med Hyg* **91**: 78-81.

Ottesen EA, Duke BOL, Karam M and Behbehani K (1997). Strategies and tools for the control/elimination of lymphatic filariasis. *Bull World Health Organ* **75**: 491-503.

Ottesen EA, Ismail MM and Horton J (1999). The role of albendazole in programmes to eliminate lymphatic filariasis. *Parasitol Today* **15**: 382-386.

Pani S, Subramanyam Reddy G, Das L, Vanamail P, Hoti S, Ramesh J and Das P (2002). Tolerability and efficacy of single dose albendazole, diethylcarbamazine citrate (DEC) or co-administration of albendazole with DEC in the clearance of *Wuchereria bancrofti* in asymptomatic microfilaraemic volunteers in Pondicherry, South India: a hospital-based study. *Filaria J* **1**: 1.

Plaisier AP, Cao WC, van Oortmarssen GJ and Habbema JD (1999). Efficacy of ivermectin in the treatment of *Wuchereria bancrofti* infection: a model-based analysis of trial results. *Parasitology* **119**: 385-394.

Stolk WA, Subramanian S, Oortmarssen GJ, Das PK and Habbema JDF (2003). Prospects for elimination of bancroftian filariasis by mass drug treatment in Pondicherry, India: a simulation study. *J Infect Dis* **188**: 1371-1381.

Subramanian S, Stolk WA, Ramaiah KD, Plaisier AP, Krishnamoorthy K, Van Oortmarssen GJ, Amalraj D, Habbema JDF and Das PK (2004). The dynamics of *Wuchereria bancrofti* infection: a model-based analysis of longitudinal data from Pondicherry, India. *Parasitology* **128**: 467-482.

Subramanyam Reddy G, Vengatesvarlou N, Das PK, Vanamail P, Vijayan AP, Kala S and Pani SP (2000). Tolerability and efficacy of single-dose diethyl carbamazine (DEC) or ivermectin in the clearance of *Wuchereria bancrofti* microfilaraemia in Pondicherry, south India. *Trop Med Int Health* **5**: 779-785.

Vanamail P, Ramaiah KD, Pani SP, Das PK, Grenfell BT and Bundy DA (1996). Estimation of the fecund life span of *Wuchereria bancrofti* in an endemic area. *Trans R Soc Trop Med Hyg* **90**: 119-121.

Weil GJ, Lammie PJ, Richards FO, Jr. and Eberhard ML (1991). Changes in circulating parasite antigen levels after treatment of bancroftian filariasis with diethylcarbamazine and ivermectin. *J Infect Dis* **164**: 814-816.

World Health Organization (1992). Lymphatic filariasis: the disease and its control. Fifth report of the WHO Expert Committee on Filariasis. *World Health Organ Tech Rep Ser* **821**: 1-71.

9

Model-based analysis of trial data: microfilaria and worm-productivity loss after diethylcarbamazine-albendazole or ivermectin-albendazole combination therapy against *Wuchereria bancrofti*

M. E. A. DE KRAKER, W. A. STOLK, G. J. VAN OORTMARSSEN, and J. D. F. HABBEMA

Department of Public Health, Erasmus MC, University Medical Center Rotterdam, the Netherlands

(submitted)

Abstract

Combinations of ivermectin (IVM) or diethylcarbamazine (DEC) and albendazole (ALB) are recommended for use in mass treatment programmes against lymphatic filariasis. We reviewed published trends in microfilaria (mf) intensities after treatment with these combination therapies. By fitting a mathematical model of treatment effects to the trial data, we quantified the efficacy of treatment, distinguishing between the killing of mf (mf loss) and a reduction in mf production by adult worms (worm-productivity loss). The mf density after DEC-ALB treatment showed an immediate drop, followed by a slow but steady further decrease (n=4 trials). After IVM-ALB treatment, mf densities immediately dropped to near-zero levels, followed by a small increase (n=5). For DEC-ALB, the average mf loss and worm-productivity loss were estimated, respectively, at 83% (ranging from 54-100% in the different studies) and 100% (for all studies). For IVM-ALB, the respective estimates were 100% (ranging from 98-100%) and 96% (ranging from 83-100%). Stronger effects were found for treatment with higher doses. Sensitivity analysis showed that the estimates did not depend on assumptions on worm life span or premature period and little on assumptions on mf life span. It can be concluded that the two therapies differ with respect to their direct effect on mf, but both are highly effective against adult worms. With high coverage, mass treatment with these combination therapies can have a large impact on lymphatic filariasis transmission.

Introduction

Lymphatic filariasis is endemic in 80 countries, with the largest population at risk in Africa and the Indian region. An estimated 119 million people are affected by lymphatic filariasis worldwide, with 43 million people suffering from elephantiasis or hydrocele; *Wuchereria bancrofti* accounts for 89% of the cases (Michael & Bundy 1997). These chronic manifestations can be severely disabling and have a large social impact due to stigma, embarrassment, depression and sexual dysfunction; economic losses occur due to costs of medical care, but also due to temporary or permanent productivity loss (Evans *et al.* 1993).

Lymphatic filariasis is considered a potentially eradicable disease, due to the absence of a non-human reservoir for *W. bancrofti,* the availability of cheap and highly effective drugs (DEC, ivermectin and albendazole) and of easy-to-use, highly specific and sensitive antigen tests (Centers for Disease Control 1993). In 1997, WHO therefore adopted a resolution to advocate the global elimination of lymphatic filariasis as a public health problem by interrupting its transmission (World Health Organization 1997). Yearly mass drug administration (MDA) has become the primary strategy (Ottesen *et al.* 1997).

Nowadays, the recommended treatment regimens for use in elimination programmes are combinations of diethylcarbamazine (DEC, 6 mg/kg) and albendazole (ALB, 400 mg) or of ivermectin (IVM, 200 μg/kg) and ALB (400 mg), administered yearly in a single dose. These treatment regimens have shown to be very effective in reducing microfilaria (mf) intensity in several trials (Ottesen *et al.* 1997).

The success of MDA greatly depends on drug effects on mf and adult worms. Especially the effects on adult worms will greatly determine the long-term impact of MDA. Measuring the effect of therapy on the adult worms is difficult. Ultrasound detection is a powerful tool to visualize living adult worms in an individual and to investigate the macrofilaricidal effect of treatment (Dreyer *et al.* 1996; Norões *et al.* 1997). However, it has some limitations: living worms cannot be visualized in all parts of the body and sterilization of worms cannot be measured. Other tools to detect the amount of living adult worms in an individual are not yet available. Mathematical models provide a means for indirect estimation of the effects of treatment. Plaisier *et al.* (1999) developed a mathematical model that considers the life cycle of the worm, mf production and survival, and the effects of treatment on mf and on mf production by adult worms. The latter can be reduced by death or sterilization of the worm. This model was used to estimate the efficacy of ivermectin treatment from published trends in mf intensity. The authors concluded that IVM (400 μg/kg) reduced mf production by adult worms by at least 68%; for a lower dosage of 200 μg/kg (the one used in MDA) they found a reduction in mf production of the adult worms of at least 36%.

In this study, we review the published trends in mf intensities after treatment with a combination of ALB with DEC or IVM and quantify the efficacy of treatment, distinguishing between the killing of mf and the reduction in mf production by the adult worm using an adapted version of the mathematical model of Plaisier *et al.* (1999).

Materials and methods

Literature search

The MEDLINE database was searched up to May 2004 to identify all published studies about treatment of lymphatic filariasis with combinations of ALB with IVM or DEC. The search terms used were: 'albendazole', 'filariasis', '*Wuchereria bancrofti*', or 'clinical trial', combined with 'ivermectin', 'DEC' or 'diethylcarbamazine'. The hits were screened on basis of title and abstract, the relevant full text articles were retrieved and references were screened for other potentially relevant articles.

Study selection and quality assessment

The studies were selected using the following inclusion criteria: *W. bancrofti* infection was treated with combination therapy consisting of DEC and ALB or IVM and ALB; results were reported for a group of individuals who were all mf positive at the start of the treatment; follow-up was at least one year and mf density was measured at least at three time-points post-treatment. If results from one study were reported in several articles, the article that reported most post-treatment measurements of mf density was included. The methodological quality of the included studies was assessed using the criteria of the Cochrane Infectious Diseases Group (Garner *et al.* 2004).

Data

Our analysis concerns trends in mf density after treatment in groups of individuals who were all mf positive before the start of treatment. Most studies included several subgroups, that were treated with different treatment regimens or concerned different groups of patients. We use the term 'study arm' to refer to subgroups in studies. Mf intensities as a percentage of pre-treatment level were extracted and entered in an Excel database for each relevant study arm. When a second treatment was given, observations after this second treatment were ignored. If relative mf intensities were not provided, these values were calculated by dividing geometric mean absolute mf intensities at different follow-up moments by pre-treatment geometric mean mf intensity. Several studies have already shown that higher doses of IVM or DEC induce greater and more sustained mf reduction (Ottesen 1985; Cao *et al.* 1997). Therefore four treatment regimen groups were distinguished: low dose IVM-ALB (IVM ≤200 μg/kg, ALB 400 mg), high dose IVM-ALB (IVM >200 μg/kg, ALB ≥400 mg), low dose DEC-ALB (DEC ≤6 mg/kg, ALB 400 mg) and high dose DEC-ALB (DEC >6 mg/kg, ALB ≥400 mg). In the remainder of the paper we will refer to these groups by 'treatment regimen group'. The lower dosages are currently recommended for use in MDA programmes.

Model

Description. A description of the model is given in the appendix. Because study participants are from known endemic areas and probably were not treated previously for lymphatic filariasis (4 studies stated this explicitly; only Ismail 1998/2001 gave no information on this), we can assume that the pre-treatment infection intensity represents an equilibrium situation: mortality of worms and mf is balanced by new infections and newly produced mf. Freshly acquired parasites are unproductive during the premature period. Thereafter, the mature adult parasites produce mf at a per capita constant rate (ρ). The effect of treatment is assumed to be two-fold: a fraction δ of mf is killed and a fraction λ of the worms stops producing mf (mature worms) or lose their ability to do so (premature worms). In the remainder of the paper we will refer to these effects as 'mf loss' or 'worm-productivity loss'. The latter can be due to death (as assumed for DEC and ALB), sterilization (as assumed for IVM), or another mechanism that prevents release of mf into the blood. We assume that worm-productivity loss is permanent, or at least not reversed within the 2-year follow-up period. New infections could be acquired during follow-up, if transmission continues. The key outcome of the model is the calculated relative trend in mf density over time after treatment.

Parameter quantification. Based on estimates from literature and earlier analyses, the premature period was assumed to be eight months (World Health Organization 1992), the life span of the adult female worm eight years (Vanamail *et al.* 1996; Subramanian *et al.* 2004) and the life span of mf one year (Thooris 1956; Plaisier *et al.* 1999). We ignored the possible acquisition of new infections during the post-treatment period. In the community-based studies, the reinfection rate will be low due to reduced transmission. But even if new infections occur, they are expected to have little influence on post-treatment trends of lymphatic filariasis, because of the long premature period of the worm. Alternative values for these parameters were considered in a univariate sensitivity analysis. By fitting the model outcomes to the observed trends in mf intensity, we estimated the following three parameters: the fraction of mf killed (δ), the fraction of worm-productivity loss (λ) and the linear factor 'reinfection rate pre-treatment × mf production' ($\beta_0 \times \rho$). This is further explained below.

Sensitivity analysis. We examined how the results would change if we assumed reinfection to occur post-treatment (assuming that transmission intensity was not affected by treatment). We further tested how halving and doubling the assumed durations of the premature period, worm life span and mf life span would affect the efficacy estimates and the goodness of fit in the situation with and without reinfection. Univariate and multivariate sensitivity analysis was done.

Estimation procedure. Parameters were estimated by fitting the model outcomes to the observed data. Assuming that the relative mean mf intensities follow a normal distribution, the least squares method was used for testing the goodness of fit. In a first analysis, we estimated mf and worm-productivity loss for each treatment regimen group, assuming that there was no difference between study arms within each group. Because

sample sizes varied between studies, we used weighted least squares, weighing for the number of patients at inclusion. The following expression was minimized:

$$SS = \sum_{i=1}^{d} \sum_{j=1}^{s_i} n_i \left[m_{p,i}\left(t_j, \delta, \lambda, \beta \cdot \rho\right) - m_{o,i}\left(t_j\right) \right]^2 \qquad (9\text{-}1)$$

We subsequently tested whether the goodness-of-fit improved in a second analysis, in which we estimated the efficacy for each study arm separately, thus allowing variation in efficacy between study arms within a treatment regimen group. Since loss-to-follow-up was limited and data on the number of patients at every follow-up time point were lacking, we used ordinary least squares for parameter estimation, minimizing the following expression:

$$SS = \sum_{j=1}^{s_i} \left[m_{p,i}\left(t_j, \delta, \lambda, \beta \cdot \rho\right) - m_{o,i}\left(t_j\right) \right]^2 \qquad (9\text{-}2)$$

With:

SS sum of squared errors;
i index for study arm;
d total number of study arms within a treatment regimen;
j post-treatment follow-up time-point of study arm *i;*
s_i total number of follow-up time-points of study arm *i;*
n_i number of patients in study arm i at inclusion;
$m_{p,i}(t_j, \delta, \lambda, \beta.\rho)$ model-predicted relative mf density of study arm *i*, at follow-up time-point *j* as a function of time and the parameters $\delta, \lambda, \beta.\rho$;
$m_{0,i}(t_j)$ observed relative mf density of study arm *i*, at follow-up moment *j* ;

Likelihood-based confidence intervals were calculated for δ and λ (Kalbfleish 1979). Briefly, we calculated the *scale* as SS_{opt} / df, with SS_{opt} = the sum of squared errors of the optimised model (corresponding to the point-estimates for δ and λ) and *df* = the degrees of freedom. The maximum acceptable *SS* (corresponding to the boundaries of the 95% confidence interval for the parameters) are then given by $SS_{opt} + 3.84 \times scale$. The corresponding confidence intervals for δ and λ were derived.

Results

Nineteen potentially relevant articles were identified. Six articles met the inclusion criteria, with 11 relevant study arms in total (Ismail *et al.* 1998; Dunyo *et al.* 2000; Ismail *et al.* 2001; Pani *et al.* 2002; Makunde *et al.* 2003; El Setouhy *et al.* 2004). In Table 9-1, characteristics of the included studies can be found per study arm. The study arms will further be referred to by name of first author and year and, in two cases, an additional two-letter code to denote special features of the study arm.

Table 9-1. Details of included study arms, grouped into IVM-ALB and DEC-ALB treatment and low and high dose.

Study arm by treatment regimen	Combination of drugs and dosage within study arms[a]	Study area (setting of study)	Diagnostic tool	n	Age range in years	GM mf density pre-treatment in mf/ml (range)	Follow-up in days (nr. of measurements)	Loss-to-follow-up at end of study
IVM-ALB low dose								
Dunyo 2000	IVM150-200 & ALB400	Ghana (C)	Cnt. ch., fp	62	7-72	1585 (1069-2350)	360 (4)	0%
Makunde 2003	IVM 150 & ALB 400	Tanzania (C)	Filtration, vb	12	15-55 [d]	508 (108-2232) [d]	360 (7)	0%
Makunde 2003 CI [b]	IVM 150 & ALB 400	Tanzania (C)	Filtration, vb	15	15-55 [d]	422 (108-2232) [d]	360 (7)	0%
Ismail 2001	IVM 200 & ALB 400	Sri Lanka (H)	Filtration, vb	16	18-58 [d]	1222 (270-2806)	720 (10)	6%
IVM-ALB high dose								
Ismail 1998	IVM 400 & ALB 600	Sri Lanka (H)	Filtration, vb	13	18-58 [d]	858 (67-8280)	450 (7)	0%
Ismail 2001	IVM 400 & ALB 600	Sri Lanka (H)	Filtration, vb	15	18-58 [d]	923 (116-6524)	720 (10)	7%
DEC-ALB low dose								
El Setouhy 2004	DEC 6 & ALB 400	Egypt (C)	Filtration, vb	28	na	359 (90-3720)	360 (6)	7%
Pani 2002	DEC 6 & ALB 400	India (H)	vb	18	10-57 [d]	79 (24-223)	360 (8)	na
Ismail 1998	DEC 6 & ALB 400	Sri Lanka (H)	Filtration, vb	13	18-58 [d]	956 (254-4244)	450 (7)	15%
Ismail 2001	DEC 6 & ALB 400	Sri Lanka (H)	Filtration, vb	16	18-58 [d]	1013 (164-5426)	720 (10)	19%
DEC-ALB high dose								
El Setouhy 2004 MD [c]	DEC 6 & ALB 400 x7	Egypt (C)	Filtration, vb	30		400 (100-4531)	360 (6)	7%

Abbreviations: *n*, number of patients; GM, geometric mean; mf, microfilaria; H, hospital-based; C, community-based; Cnt. ch., counting chamber; fp, fingerprick blood; vb, venous blood; na, not available.

[a] ALB, albendazole in mg; IVM, ivermectin in μg/kg; DEC, diethylcarbamazine citrate in mg/kg.

[b] CI, co-infection: persons in this study arm were co-infected with *O. volvulus.*

[c] MD, multi-dose therapy: persons in this study arm were treated with a single dose of 6 mg/kg DEC + 400 mg ALB on 7 subsequent days.

[d] These ranges apply to the whole study population; ranges per study arm were not available.

Table 9-2. Point estimates (confidence intervals between parentheses) of fraction of microfilariae killed (mf loss) and fraction of worms with productivity loss (worm-productivity loss) per study arm after low or high dose IVM-ALB or DEC-ALB treatment. Model assumptions: mf life span 1 year, worm life span 8 years, premature period 8 months and reinfection rate post-treatment 0.

Study arm by treatment regimen [a]	Mf loss (δ)	Worm-productivity loss (λ)
IVM-ALB low dose		
Dunyo 2000	1.00 (0.94-1.00)	0.83 (0.76-0.92)
Makunde 2003	1.00 (1.00-1.00)	1.00 (1.00-1.00)
Makunde 2003 CI	0.98 (0.96-1.00)	0.96 (0.92-1.00)
Ismail 2001	1.00 (1.00-1.00)	0.97 (0.97-0.97)
Average	*1.00*	*0.94*
IVM-ALB high dose		
Ismail 1998	1.00 (1.00-1.00)	0.99 (0.98-0.99)
Ismail 2001	1.00 (1.00-1.00)	0.98 (0.98-0.98)
Average	*1.00*	*0.99*
DEC-ALB low dose		
El Setouhy 2004	0.85 (0.82-0.89)	1.00 (0.94-1.00)
Pani 2002	0.54 (0.31-0.69)	1.00 (0.84-1.00)
Ismail 1998	0.83 (0.80-0.86)	1.00 (0.96-1.00)
Ismail 2001	0.91 (0.88-0.95)	1.00 (0.97-1.00)
Average	*0.78*	*1.00*
DEC-ALB high dose		
El Setouhy 2004 MD	1.00 (1.00-1.00)	1.00 (1.00-1.00)

Note: These fractions are rounded; therefore 1.00 can be any value ≥0.995. This implies that mf density does not have to be reduced to zero post-treatment, even when worm-productivity loss (λ) and mf loss (δ) both have the value of 1.00.

[a] See Table 9-1 for explanation.

For DEC-ALB treatment, four study arms used the low dose; one study arm, El Setouhy 2004 MD, used multidosing and was included in the high dose group. For IVM-ALB, four and two study arms, respectively, were included in the low dose and high dose group. Drug allocation was always randomised. Details about allocation concealment were usually not mentioned, but Pani 2002 and Dunyo 2000 used look-alike drugs coded by a third party. Double blinding was applied in most studies, but in Makunde 2003, El Setouhy 2004 and El Setouhy 2004 MD no blinding was used. Loss-to-follow-up at the end of the study was usually smaller than 10%. Only the DEC-ALB study arms of Ismail 1998 and Ismail 2001 had a higher loss-to-follow-up of 15% and 19%, respectively.

Most studies reported geometric mean mf density, calculated as antilog $[\Sigma(\log(x+1))/n] - 1$, where x was mf intensity in mf/ml and n the number of individuals in the

study arm. Ismail 1998 (DEC-ALB and IVM-ALB) calculated the relative mf intensity per individual, as percentage of pre-treatment level, and then calculated the geometric mean of these percentages. For three study arms (Dunyo 2000, El Setouhy 2004, El Setouhy 2004 MD) relative geometric mean mf intensities were presented in a table, for the others these data had to be read from graphs.

Review of published trends

The observed relative mf densities are plotted in Figure 9-1 (symbols). In all four treatment regimen groups a decrease in mf intensity can be found. The initial decrease is, however, more pronounced and immediate after IVM-ALB than after DEC-ALB treatment. The more gradual decrease caused by DEC-ALB treatment did not show a tendency to bounce back during the whole of the 720 days of post-treatment follow up, in contrast to the relative mf density after IVM-ALB treatment. For low dose IVM-ALB or DEC-ALB, treatment reductions in relative mf density were variable and smaller than for the high dose treatment regimens groups.

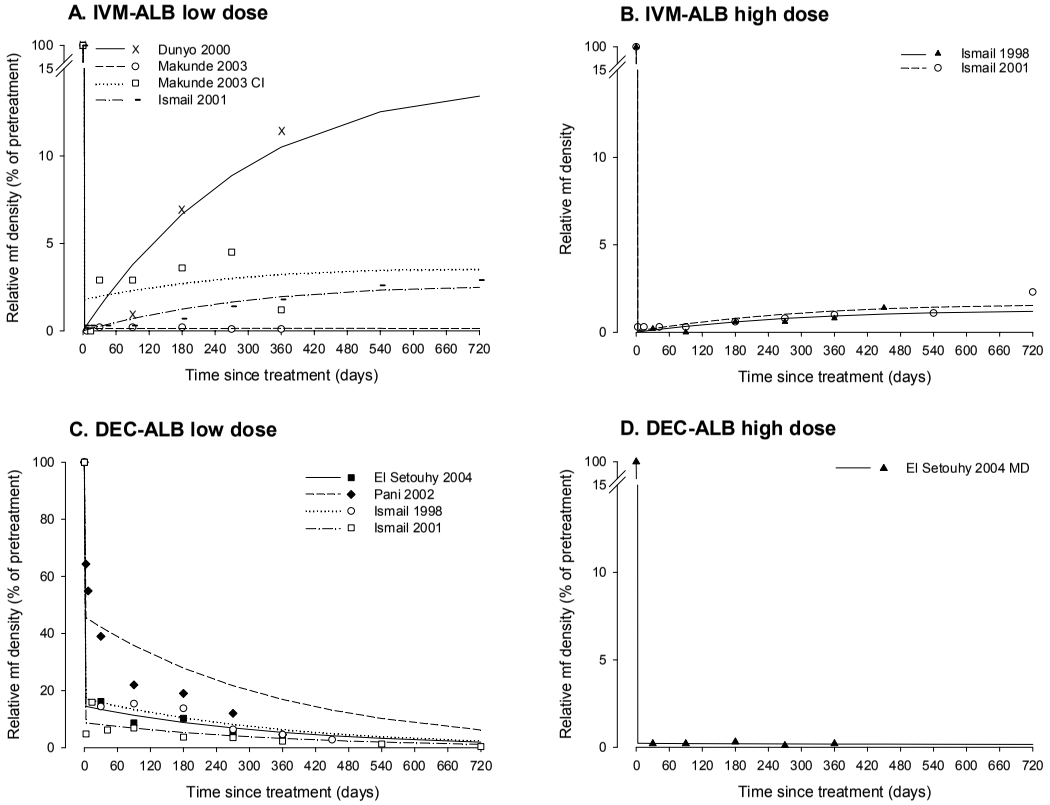
Efficacy estimates

Assuming that the effects of treatment on mf and on worm-productivity differed between study arms resulted in a significantly better sum of squared errors than assuming an equal effect within each of the four treatment regimen groups, especially in the low dose groups.

The results of the analysis per study arm are summarised in Figure 9-1 and Table 9-2. In general, predicted trends fitted the observations closely. For low-dose IVM-ALB, estimated mf loss was near 100% in all study arms; worm-productivity loss was more variable. Relative mf density increased much faster in Dunyo 2000 than in the other study arms (Figure 9-1A), resulting in a lower estimate of worm-productivity loss. For high dose IVM-ALB, estimated mf and worm-productivity losses were very high, approximating 100% (Figure 9-1B). In the low dose DEC-ALB group, estimated worm-productivity loss was 100% for all study arms, but the mf loss was variable. Mf loss was lowest for Pani 2002, which showed a smaller initial decline in mf intensity than the other study arms (Figure 9-1C). For this study arm, the model predicted higher mf intensities on the long term than observed. For the one study arm that used high dose DEC-ALB, both mf and worm-productivity losses were estimated at 100% (Figure 9-1D).

Allowing for acquisition of new infections during follow-up (with the rate equalling that of the pre-treatment situation) resulted in a better model-fit, but did not influence the efficacy estimates: we only found very minor increases in estimated mf loss for DEC-ALB and worm-productivity loss for IVM. Assumptions on mf life span had more impact on goodness of fit and efficacy estimates. A shorter mf life span usually gave a better fit for the DEC-ALB study arms, whereas a longer mf life span gave a better fit for

Figure 9-1. Observations (symbols) and model predictions (lines) of the relative mf density after low or high dose IVM-ALB or DEC-ALB treatment. Model assumptions: mf lifespan 1 year, worm lifespan 8 years, premature period 8 months, reinfection rate post-treatment 0. Note the difference in Y-scale between graph C and graphs A, B and D.



the IVM-ALB study arms. Nevertheless, efficacy estimates for the different study arms usually did not change much, except for Pani 2002 and Dunyo 2000. For Pani 2002 (DEC-ALB low dose), the estimated mf loss was considerably lower (0.46) or higher (0.61) when we assumed the mf life span to be six or 24 months, respectively; the estimated worm-productivity did not depend on mf life span. For Dunyo 2000 (IVM-ALB low dose), the estimated worm-productivity loss was higher (0.89) or lower (0.71) for the shorter and longer mf life span, respectively; the estimated mf loss did not depend on mf life span. Halving and doubling the premature period or the worm life span did not have an effect on the estimates for any study arm. The effect of changes in the various model-parameters remained the same, when they were varied at the same time in a multivariate sensitivity analysis.

Discussion

Nowadays DEC-ALB and IVM-ALB are the recommended combination therapies in mass drug administration programmes for lymphatic filariasis. In the studies analysed here, both therapies proved to be very effective. IVM-ALB immediately reduced mf density to extremely low levels, and although the density slightly increased during follow-up, it remained below 5% of pre-treatment level in most studies. In spite of a lower immediate decline, on the long term DEC-ALB also reduced mf density to less than 5% of pre-treatment level at one year post-treatment. Using a mathematical model, we estimated that DEC-ALB treatment reduced worm-productivity to zero in all study arms, whereas the immediate mf loss was variable (range 54%-100%). IVM-ALB had a very strong effect on both mf and worms (estimated mf loss 98-100%; estimated worm-productivity loss 83%-100%). For both drug-combinations, efficacy estimates were higher in the high-dose group. Sensitivity analysis showed that these estimates did not depend much on assumptions on worm life span, premature period, or changes in parasite reinfection rates, and only slightly on assumptions on mf life span (see below).

Explanations in literature for worm-productivity loss include death of the adult worms (as assumed for DEC and ALB) (Ottesen 1985; Ottesen *et al.* 1999) and irreversible sterilization (as assumed for IVM) (Dunyo *et al.* 2000b). Based on the available data we cannot determine with certainty whether the (nearly) complete worm-productivity loss is irreversible; this would require longer follow-up. Plaisier *et al.* (1999) assumed a 'recovery period' for the adult worms in their mathematical model during which mf production is temporarily interrupted, but found no evidence for such a transient effect in addition to an irreversible productivity loss.

Overall, the methodological quality of the studies included in our review was good, although loss-to-follow-up in Ismail 1998 and Ismail 2001 was high. One drawback of our study was the data extraction from graphs. Graphs may be inaccurate and reading data from graphs may introduce an error. However, a small error in reading the mf

density will not have a large effect on the analysis of the relative trends and the resulting efficacy estimates.

The trend in mf density in Pani 2002 showed a much smaller initial decline in mf density than the other DEC-ALB studies. The observed trend in this study, which had a very low mean mf count before treatment in comparison to other studies (Table 9-1), may have been influenced by reduced sensitivity of the mf diagnostic test at lower densities and relatively large fluctuations in mf counts. However, other differences between the studies (done under different circumstances in different areas) might also have played a role. Dunyo 2000 showed high resurgence of the relative mf density post-treatment compared to the other IVM-ALB studies. There is no reason to assume that the different diagnostic tool used in this study (see Table 9-1) could explain the different pattern. It might be due to the relatively high mf load pre-treatment, which would indicate a larger worm load and possibly a greater chance of worm pairs surviving after treatment and producing mf. The number of included studies is too small to come to a profound understanding of the causes underlying these different patterns.

Uncertainty about the dynamics of parasite development in the human host complicates our analysis. For example, the mf life span determines the death rate of mf that survived treatment and the rate of mf recurrence of mf due to mf producing worms. Uncertainty on the mf life span therefore influences the estimated effect of treatment. Assumptions in this life span had strongest impact in Pani 2002 and Dunyo 2000, but had less influence in other studies where the effects of treatment were more complete.

Another uncertainty in the model was the change in the rate of parasite acquisition after treatment. It could be expected that in hospital-based studies transmission intensity would not change much due to the limited number of individuals treated within a community, whereas it could decrease in community-based trials. The model, however, gave a better fit when post-treatment reinfection rates were assumed to be zero for all studies, hospital-based and community-based. There may be other explanations for the long-term reduction in mf density, though, that were not considered in our model. Treatment could not only have a direct effect on present infection (different parasite stages), but might also have a long-term prophylactic effect against new incoming infections, which is not included in the model. It is also possible that the impact of new infections is not visible in the mf density in the blood in the first two years after treatment. Furthermore monitoring effects may have had an effect: trial participants may have been more careful in preventing mosquito bites.

The relative trends analysed in this study were based on geometric mean mf counts (obtained from log-transformed data to which 1 had been added). Smaller mf counts receive more weight in this measure; therefore reductions in mf intensities will be stronger than when considering the individual mf intensities (Fulford 1994). Together with a diagnostic test that is less sensitive with lower mf counts this probably has led to a systematic overestimation of the effect. In addition, only mf-positives were included; mf-negatives becoming positive despite treatment were disregarded, which could lead to further overestimation of the effects of treatment.

Model-based analysis of trial data concerning IVM (≥ 200 μg/kg) treatment estimated 100% microfilaricidal effect and a loss of mf production of ≥ 77%, while using the same parasite demographic parameter values as in this study (Plaisier *et al.* 1999). Our estimates for IVM-ALB were higher, which could be explained by the added effect of ALB (Addiss *et al.* 1997; Dunyo *et al.* 2000; Makunde *et al.* 2003). Using this model, we cannot determine whether the worm-productivity loss results from killing of adult worms, sterilization or another mechanisms that inhibits the appearance of mf in the blood. Using ultrasound, the macrofilaricidal effect of treatment can be estimated directly. In this way, it was estimated that DEC in doses of 6 mg/kg or higher killed 51% of the worm nests (Norões *et al.* 1997). Ultrasound investigations after IVM treatment indicated no killing of worms (Dreyer *et al.* 1996). Our estimates for the worm-productivity loss caused by DEC-ALB and IVM-ALB were much higher than those indicated by the ultrasound studies. The added effect of ALB may not be the only explanation for this finding. Sterilization of (female) worms could also explain this difference: worms stop producing mf, but remain visible on ultrasound. Similarly, single-sex or single-worm infections may remain visible after treatment, although these infections do not contribute to mf density. In addition, ultrasound detection can only evaluate the effect on whole nests in the scrotum and the superficial lymphatics (Dreyer *et al.* 1996; Norões *et al.* 1997).

In four studies included in our review, circulating filarial antigen was measured post-treatment (Ismail *et al.* 1998; Dunyo *et al.* 2000; Ismail *et al.* 2001; Pani *et al.* 2002). It is still not clear how circulating filarial antigen is associated with death or sterilization of worms (Eberhard *et al.* 1997). We did not analyse the antigen data. It was striking, however, that our model predicted a very high worm-productivity loss, whereas few of the subjects totally cleared circulating filarial antigen.

In conclusion, the observed data showed that treatment with combinations of IVM-ALB or DEC-ALB results in a strong reduction in mf density for long periods. The estimated mf loss and worm-productivity loss after treatment with either of the combinations were very high, even if uncertainties and possible overestimation of the effect due to the use of geometric means are taken into account. Applied in yearly MDA, these drug-combinations can have strong impact on lymphatic filariasis transmission, provided that coverage and compliance are sufficiently high. Although high-dose regimens may be more effective, the lower (standard) dosages may be preferred for use in MDA because of practical reasons. Widespread use of drugs in MDA entails a risk that resistance develops. This has not been observed yet, and is not expected to develop fast because the transmission cycle from one generation of *W. bancrofti* to the next is very long compared to other nematodes in which drug resistance has occurred (Eberhard *et al.* 1991) and drug combinations are used instead of single drugs. Since ALB is also highly effective for the treatment of common species of intestinal helminths of humans (Horton 2000), the impact of MDA has a broader public health impact, which goes beyond lymphatic filariasis.

Acknowledgements

This investigation received financial support from the UNICEF / UNDP / WORLD BANK / WHO Special Programme for Research and Training in Tropical Diseases (TDR). The authors thank Caspar Looman for his statistical support.

Appendix

Formal description of the model

NB. This model is the same as the model used in Chapter 8 of this thesis.

The dynamics of parasite development and mf production are described by a set of differential equations. Let W be the number of adult and productive worms in a person, L the number of premature worms, and M the number of mf. T_p, T_l and T_m are the premature period, the life span of the worm and the life span of the mf respectively. T_l - T_p is the productive life span of worms. Then:

$$\left.\begin{aligned} \frac{dL(t)}{dt} &= \beta_{0,i} - (\gamma + \mu_1)L(t) \\ \frac{dW(t)}{dt} &= \gamma L(t) - \mu_1 W(t) \\ \frac{dM(t)}{dt} &= \rho W(t) - \mu_2 M(t) \end{aligned}\right\} \qquad \text{(A1)}$$

with $\beta_{0,i}$ = the pre-treatment force of infection (no. of new worms/person/year), γ = the per capita rate of maturation to adult and productive parasite ($\gamma = 1/T_p$), μ_1 = the per capita death rate of premature and adult worms ($=1/T_l$), μ_2 = per capita death rate of mf ($=1/T_m$), ρ = the rate of mf production of an adult worm per unit of blood taken for diagnosis, and i an index for study arm: persons treated with a certain therapy and a certain dose in a certain study.

Assuming that the force-of-infection, $\beta_{0,i}$, in the population has been constant for a long time, the pre-treatment numbers of premature and mature worms and mf are equal to the equilibrium values L, W, and M (denoted with *), which can be derived by solving the equations for $dL(t)/dt = dW(t)/dt = dM(t)/dt = 0$:

$$\left.\begin{aligned} L^* &= \frac{\beta_{0,i}}{\gamma + \mu_1} \\ W^* &= \frac{\beta_{0,i}\gamma}{\mu_1(\gamma + \mu_1)} \\ M^* &= \frac{\beta_{0,i}\rho\gamma}{\mu_1\mu_2(\gamma + \mu_1)} \end{aligned}\right\} \qquad \text{(A2)}$$

Equations A1 and A2 are the same as in Plaisier *et al.* (1999). However, the effects of treatment in the current paper are slightly different: we do not consider a temporal effect of treatment so that 'recovering' worms are not considered in the present model; furthermore, we assume that both premature and mature worms are affected by the treatment. At the moment of treatment, a fraction δ_i of the mf (M) is killed instantaneously and a fraction λ_i of all worms present in the body (L and W) stops producing mf (W) or loses its potential ability to produce mf (L) in the case of premature worms. Hence, at treatment time-point t, the following immediate changes occur:

$$\left.\begin{aligned} L(t) &\Leftarrow (1-\lambda_i)L(t) \\ W(t) &\Leftarrow (1-\lambda_i)W(t) \\ M(t) &\Leftarrow (1-\delta_i)M(t) \end{aligned}\right\} \qquad \text{(A3)}$$

(the symbol $\Leftarrow$ means 'becomes')

After treatment, individuals are again exposed to infection. The post-treatment force-of-infection ($\beta_{t,i}$) is defined as a fraction s of the pre-treatment force, so that $\beta_{t,i} = s\,\beta_{0,i}$.

References

Addiss DG, Beach MJ, Streit TG, Lutwick S, LeConte FH, Lafontant JG, Hightower AW and Lammie PJ (1997). Randomised placebo-controlled comparison of ivermectin and albendazole alone and in combination for *Wuchereria bancrofti* microfilaraemia in Haitian children. *Lancet* **350**: 480-484.

Cao WC, Van der Ploeg CPB, Plaisier AP, van der Sluijs IJS and Habbema JDF (1997). Ivermectin for the chemotherapy of bancroftian filariasis: a meta-analysis of the effect of single treatment. *Trop Med Int Health* **2**: 393-403.

Centers for Disease Control (1993). Recommendations of the International Task Force for Disease Eradication. *MMWR Morb Mortal Wkly Rep* **42**: 1-38.

Dreyer G, Addiss D, Noroes J, Amaral F, Rocha A and Coutinho A (1996). Ultrasonographic assessment of the adulticidal efficacy of repeat high-dose ivermectin in bancroftian filariasis. *Trop Med Int Health* **1**: 427-432.

Dunyo SK, Nkrumah FK and Simonsen PE (2000). A randomized double-blind placebo-controlled field trial of ivermectin and albendazole alone and in combination for the treatment of lymphatic filariasis in Ghana. *Trans R Soc Trop Med Hyg* **94**: 205-211.

Eberhard ML, Hightower AW, Addiss DG and Lammie PJ (1997). Clearance of *Wuchereria bancrofti* antigen after treatment with diethylcarbamazine or ivermectin. *Am J Trop Med Hyg* **57**: 483-486.

El Setouhy M, Ramzy RMR, Ahmed ES, Kandil AM, Hussain O, Farid HA, Helmy H and Weil GJ (2004). A randomized clinical trial comparing single- and multi-dose combination therapy with diethylcarbamazine and albendazole for treatment of bancroftian filariasis. *Am J Trop Med Hyg* **70**: 191-196.

Evans DB, Gelband H and Vlassoff C (1993). Social and economic factors and the control of lymphatic filariasis: a review. *Acta Trop* **53**: 1-26.

Garner P, Robb R and Group ID (2004). November 2001 to present: Assessment of methodological quality of randomized controlled trials. *The Cochrane Library*.

Horton J (2000). Albendazole: a review of anthelmintic efficacy and safety in humans. *Parasitology* **121**: S113-132.

Ismail MM, Jayakody RL, Weil GJ, Nirmalan N, Jayasinghe KS, Abeyewickrema W, Rezvi Sheriff MH, Rajaratnam HN, Amarasekera N, de Silva DC, Michalski ML and Dissanaike AS (1998). Efficacy of single dose combinations of albendazole, ivermectin and diethylcarbamazine for the treatment of bancroftian filariasis. *Trans R Soc Trop Med Hyg* **92**: 94-97.

Ismail MM, Jayakody RL, Weil GJ, Fernando D, De Silva MS, De Silva GA and Balasooriya WK (2001). Long-term efficacy of single-dose combinations of albendazole, ivermectin and diethylcarbamazine for the treatment of bancroftian filariasis. *Trans R Soc Trop Med Hyg* **95**: 332-335.

Kalbfleish JG (1979). *Probability and statistical inference II*. New York, Springer-Verlag.

Makunde WH, Kamugisha LM, Massaga JJ, Makunde RW, Savael ZX, Akida J, Salum FM and Taylor MJ (2003). Treatment of co-infection with bancroftian filariasis and onchocerciasis: a safety and efficacy study of albendazole with ivermectin compared to treatment of single infection with bancroftian filariasis. *Filaria J* **2**: 15.

Michael E and Bundy DAP (1997). Global mapping of lymphatic filariasis. *Parasitol Today* **13**: 472-476.

Norões J, Dreyer G, Santos A, Mendes VG, Medeiros Z and Addiss D (1997). Assessment of the efficacy of diethylcarbamazine on adult *Wuchereria bancrofti in vivo*. *Trans R Soc Trop Med Hyg* **91**: 78-81.

Ottesen EA (1985). Efficacy of diethylcarbamazine in eradicating infection with lymphatic-dwelling filariae in humans. *Rev Infect Dis* **7**: 341-356.

Ottesen EA, Duke BOL, Karam M and Behbehani K (1997). Strategies and tools for the control/elimination of lymphatic filariasis. *Bull World Health Organ* **75**: 491-503.

Pani S, Subramanyam Reddy G, Das L, Vanamail P, Hoti S, Ramesh J and Das P (2002). Tolerability and efficacy of single dose albendazole, diethylcarbamazine citrate (DEC) or co-administration of albendazole with DEC in the clearance of *Wuchereria bancrofti* in asymptomatic microfilaraemic volunteers in Pondicherry, South India: a hospital-based study. *Filaria J* **1**: 1.

Plaisier AP, Cao WC, van Oortmarssen GJ and Habbema JD (1999). Efficacy of ivermectin in the treatment of *Wuchereria bancrofti* infection: a model-based analysis of trial results. *Parasitology* **119**: 385-394.

Subramanian S, Stolk WA, Ramaiah KD, Plaisier AP, Krishnamoorthy K, Van Oortmarssen GJ, Amalraj D, Habbema JDF and Das PK (2004). The dynamics of *Wuchereria bancrofti* infection: a model-based analysis of longitudinal data from Pondicherry, India. *Parasitology* **128**: 467-482.

Thooris GC (1956). Le traitement experimental de la filariose a *Wuchereria bancrofti* en Océanie Francaise par la suramine. *Bull Soc Pathol Exot Filiales* **49**: 311-317.

Vanamail P, Ramaiah KD, Pani SP, Das PK, Grenfell BT and Bundy DA (1996). Estimation of the fecund life span of *Wuchereria bancrofti* in an endemic area. *Trans R Soc Trop Med Hyg* **90**: 119-121.

World Health Organization (1992). Lymphatic filariasis: the disease and its control. Fifth report of the WHO Expert Committee on Filariasis. *World Health Organ Tech Rep Ser* **821**: 1-71.

World Health Organization (1997). Elimination of lymphatic filariasis as a public health problem - resolution of the executive board of the WHO (WHA50.29). Geneva, Switzerland, Fiftieth World Health Assembly.

10

General discussion

This thesis aims to support decision making in lymphatic filariasis control, through the development and application of a transmission model for this infection. This final chapter provides concise answers to the questions posed in the introduction (section 10.1), discusses remaining challenges for model-based support of lymphatic filariasis control (section 10.2), and lists the main conclusions and recommendations (section 10.3).

10.1 Answering the research questions

10.1.1 What are the prospects for elimination of lymphatic filariasis by mass treatment?

Our simulation studies on the impact of mass treatment (chapters 3 and 4) showed that prospects of elimination are good if coverage levels are sufficiently high – at least in areas like Pondicherry (India), where infection is transmitted by *Culex quinquefasciatus* and the pretreatment microfilariae (mf) prevalence is about 8.5%. Six annual rounds of mass treatment with the recommended combination of diethylcarbamazine (DEC) and albendazole are needed for elimination if population coverage is 65% per round. Only four rounds are sufficient if coverage is 80%. More treatment rounds are required if DEC is used without albendazole or if the pretreatment mf prevalence level is higher.

There is uncertainty in our model-prediction, because the processes involved in transmission and mass treatment are not completely understood and quantified. Important uncertain factors are the parasite life span, the effects of existing antifilarial drugs on adult worm viability or mf productivity, and the role of human immune responses (section 10.1.2.). Although the accuracy of our predictions can only be determined in retrospect, important conclusions can nevertheless be drawn.

Our studies clearly showed the overwhelming importance of achieving high coverage levels for the success of elimination programmes. Coverage levels vary widely in ongoing programmes. Countries like Egypt and French Polynesia report very high coverage of >90% (World Health Organization 2005), but in a number of Indian studies much lower coverage levels were achieved (Ramaiah *et al.* 2000; Vanamail *et al.* 2005). Programmes should make strong efforts to reach and maintain high coverage levels. The determinants of population coverage and compliance are still incompletely understood and it is not clear how social mobilization should be organized in different resource-poor settings. For strengthening the Global Programme to Eliminate Lymphatic Filariasis (GPELF) more research is urgently needed on these issues (Anonymous 2004).

We also showed that the duration of mass treatment required for elimination strongly depends on the efficacy of the treatment regimen, especially on the effect on adult worms. Although existing drugs are quite effective, the prospects for elimination would improve if drugs with better macrofilaricidal efficacy were available. For this reason, but also to anticipate the possible development of resistance against existing drugs, the search for new drugs or drug-combinations remains an important priority for

further research (Anonymous 2004). Anti-*Wolbachia* treatment has shown promising results in this respect, but the investigated 6- or 8-week treatment regimens (Hoerauf *et al.* 2003; Taylor *et al.* 2005) are not suitable for use in mass treatment.

Our simulations further indicated that the required duration of mass treatment increases with pre-treatment endemicity level. This has two reasons. Firstly, the average worm burden is higher and more treatment rounds are needed to clear the infection with the available drugs. Secondly, the higher pre-treatment prevalence levels are presumably caused by higher mosquito biting rates, implying a higher risk of infection recurrence after stopping control. Prevalence levels therefore will have to be reduced to lower absolute levels.

These findings are important for GPELF. Based on an assumed adult worm life span of about 5 years, it was hoped that 4-6 years of annual mass treatment would be sufficient for elimination in most situations (Gyapong *et al.* 2005). Programme managers and policy makers should be aware that the duration can be considerably longer if coverage levels are low or endemicity levels are high. For reducing the total duration of elimination programmes in highly endemic areas, one might consider to increase the frequency of mass treatment (e.g. from yearly to 6-monthly) or to implement vector control in addition (Michael *et al.* 2004). Distribution of DEC-medicated cooking salt provides an interesting alternative approach to mass treatment (Houston 2000). If circumstances make elimination of the parasite very difficult, focus may be shifted to elimination of the public health problem rather than the infection. To achieve this goal, transmission does not necessarily have to be interrupted completely, but sustained control measures are required to keep transmission at such low levels that serious disease will be infrequent.

Some important aspects remain to be investigated, such as the potential impact of parasite resistance against the used drugs or the risk of recurrence of infection due to migration. But first and foremost, we need to study the prospects of elimination for regions with other vector-parasite combinations than in Pondicherry, in particular for Sub-Sahara Africa where mf prevalence levels can be considerably higher than in India (Stolk *et al.* 2004). Recent advances in this respect are reported in section 10.2.1.

10.1.2 Does protective immunity develop after prolonged exposure to lymphatic filariasis infection?

In our model-based analysis of longitudinal data from Pondicherry, India, we attributed the observed pronounced decline in prevalence in the older age groups to acquired immunity (chapter 2). If this acquired immunity assumption is correct, a similar decline in prevalence would be expected in other areas. However, our subsequent analysis of published age-patterns from India and Africa showed that such a decline is an exception rather than the rule (chapter 6). The acquired immunity assumptions in the Pondicherry model should be reconsidered.

We cannot definitely exclude a role for acquired immunity, but, if it exists, it will work in a different way than assumed for Pondicherry. Animal studies found evidence that past exposure to infection can indeed reduce the acquisition of new infections (Selkirk *et al.* 1992). Theoretical work further showed that acquired immunity does not necessarily lead to down regulation of infection in the oldest age groups (Woolhouse 1992). For example, a decline is not expected if immunity rapidly decays when the host is not longer exposed to the infection. Better understanding of the role of human immune responses is required for accurate assessment of the elimination prospects for lymphatic filariasis.

It was initially hoped that the Pondicherry model with minimal changes could be used for other Indian areas as well, since the vector species is the same (*Cx. quinquefasciatus*) and transmission dynamics are thought to be similar. However, the Pondicherry model does not correctly simulate the generally observed age-patterns of infection. This underscores the importance of validating models against several independent datasets. A model without acquired immunity may better explain normal age-patterns in India, but in chapter 2 we found that such a model has difficulty to explain the low mf prevalence level in Pondicherry (8.5%) in the presence of a ubiquitous vector. The challenge to develop a more widely applicable model for *Culex*-transmitted infection in India has been taken up by the Vector Control Research Centre in Pondicherry (Subramanian *et al.*, unpublished work).

10.1.3 How do mosquito species differ with respect to their efficiency in transmitting lymphatic filariasis infection?

A large number of mosquito species can transmit lymphatic filariasis infection and we considered only *Cx. quinquefasciatus* and *Aedes polynesiensis* (chapter 7). For both species, we found saturation in the number of mf that on average develops successfully into L3 larvae (i.e. limitation), but the maximum was much higher for *Ae. polynesiensis* than for *Cx. quinquefasciatus* (23 vs. 4).

The relationship between infection intensity in humans and L3 larvae in mosquitoes (here referred to as 'vector uptake curve') has received much attention, because density dependence in this relationship influences the impact of control (Southgate & Bryan 1992; Duerr *et al.* 2005). In the absence of density dependence in the vector uptake curve, the number of L3 larvae in mosquitoes would increase linearly with mf density in the human blood. In case of 'limitation', the number of L3 larvae increases less than proportional with mf density in the blood and approximates a maximum at higher densities. The transmission is most efficient at lowest mf densities: few mf may be engorged, but a large proportion will survive to become L3. In case of 'facilitation', the number of L3 increases more than proportional with mf density (until at higher densities limiting mechanisms get the upper hand). Transmission efficiency is least efficient at the lowest densities: only a small proportion of few engorged mf will survive. For elimination,

a situation with facilitation is favourable, because of the low transmission efficiency at lowest mf densities.

An earlier analysis already showed that there is limitation in the vector uptake curve for *Cx. quinquefasciatus* (Subramanian *et al.* 1998), the main vector in India. We found the same for *Ae. polynesiensis*, the vector in French Polynesia. The two curves were very different, though, with many more L3 per mosquito in *Aedes* than in *Culex*. Implications of this difference for elimination are not straightforward, since transmission also depends on other factors such as vector density, feeding behaviour, density dependence in mosquito survival, etc. The uptake curve for *Ae. polynesiensis* can be used to develop a simulation model for transmission in French Polynesia.

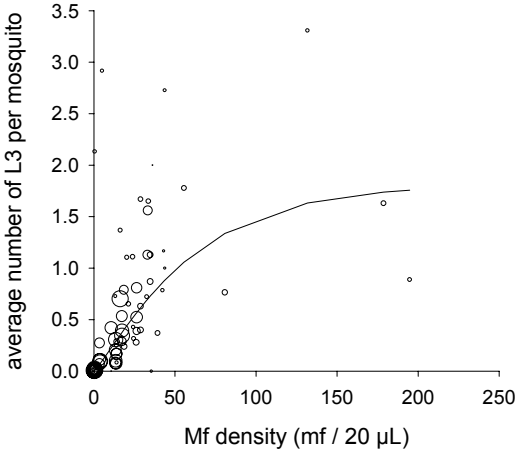
An important gap in knowledge concerns the quantification of the vector uptake curve for *Anopheles*. Mosquitoes of the genus *Anopheles* are responsible for transmission in Africa and are the second most important for filariasis transmission after *Culex quinquefasciatus* (Zagaria & Savioli 2002). There is evidence of facilitation in *Anopheles* mosquitoes (Southgate & Bryan 1992; Pichon 2002). However, accurate quantification of the uptake curve for *Anopheles* is currently difficult, because only few studies investigated the relationship. These studies are not directly comparable, because they considered different *Anopheles* species, used natural or laboratory-reared mosquito populations, used different methods for mosquito feeding, and used different diagnostic tests for measuring mf density in the human blood. In an explorative analysis, we nevertheless aggregated the data from these studies for quantification of the vector uptake curve (Appendix A). Results are shown in Figure 10-1. The number of L3 larvae developing in anopheline mosquitoes seems to be lower than in *Cx. quinquefasciatus*, but the data do not allow accurate estimation of the maximum. There was no indication of strong facilitation. The resulting curve was implemented in LYMFASIM as part of a simulation model for the African situation (see Section 10.2.1).

In our studies, we only considered density dependence in the relationship between mf density in human blood and the number of L3 developing in mosquitoes. However, density dependence may also occur in mosquito survival (Pichon 2002; Krishnamoorthy *et al.* 2004). If this increases the total degree of limitation in the transmission cycle, it would further limit the eradicability of the infection. Possible density dependence in mosquito survival is currently not considered in the LYMFASIM model and remains to be quantified for many species.

10.1.4 What are the effects of DEC, ivermectin, and their combinations with albendazole, on adult worms and microfilariae?

By analyzing trends in mf density after treatment, we estimated that a 6-mg/kg dose of DEC kills on average 57% of mf and reduces mf production by 67% (chapter 8). The reduction in mf production is presumably caused by worm death. A 400-µg/kg dose of ivermectin was found to be more effective, killing almost all mf (96%) and reducing mf

Figure 10-1. The relationship between mf density in the human blood and the number of L3 developing in *Anopheles* mosquitoes. Dots give the observed data, sized according to their weight in the analysis; the line gives the fitted curve (fit based on log-transformed values for the average number of L3 per mosquito; presented curve gives values after back-transformation).



production by 82%. The latter effect is probably caused by reduced fertility or inhibition of mf release from the female uterus. Especially the DEC effects varied strongly between individuals. Treatment efficacy estimates were higher for these drugs in combination with albendazole (chapter 9).

Effects of treatment cannot directly be measured and we therefore used the indirect approach of analyzing post-treatment trends in mf density. Although information from other studies (histology, ultrasound) is required to determine the nature of the effect on adult worms, our approach is powerful for its quantification and as yet the best way to quantify an effect on fertility. Our efficacy estimates could be too optimistic, because observed trends may have been biased by the reduced sensitivity of mf diagnostic tests at very low mf densities (Dreyer *et al.* 1996) and the use of geometric means (Fulford 1994). Further, we should be aware that the reduction in mf production can be larger than the proportion of adult worms affected, because surviving worms may have been left unmated and therefore also have stopped producing mf.

The estimated effects of DEC treatment on adult worms can be compared with results from ultrasound studies: the proportion of worms killed can be estimated from the disappearance of the so-called filarial dance sign after treatment (i.e. random movement of the adult worms, visible on ultrasound). The few available ultrasound studies suggest that the proportion of worms killed by DEC treatment is slightly lower (~50%) than our estimate of the reduction in mf production (Norões *et al.* 1997; Kshirsagar *et al.* 2004). The estimated effects on adult worms of ivermectin treatment cannot be compared with other studies: the drug does not kill worms and there are no methods available to measure an effect on adult worm fertility or mf-productivity.

Table 10-1. The probability of elimination after 5 rounds of mass treatment with 65% coverage, in relation to assumptions on variation in treatment efficacy. Exact, asymmetric 95%-confidence intervals (CI) are given. Elimination was said to occur if the prevalence had been reduced to zero, 35 years after the last treatment. Mean and variability in treatment effects were quantified based on results from chapter 8. On average, the effects of treatment are always the same: DEC is assumed to kill 57% of mf and 67% of worms. Ivermectin is assumed to kill 96% of mf and to reduce the female worm fertility by 82%.

	Probability of elimination after 5 rounds of MDA with 65% coverage (95% CI)	
Assumptions on variation in treatment efficacy	DEC	Ivermectin
No variation	98% (93% – 100%)	81% (72% – 88%)
Random variation	92% (85% – 96%)	80% (71% – 87%)
Systematic between-person variation	43% (33% – 53%)	72% (62% – 81%)

Our individual-level analysis provided important new information on variability in treatment effects. Variability limits the impact of mass treatment, especially if some individuals systematically have a poor response to treatment. To investigate how strong this effect can be, we did some additional simulations with the Pondicherry model of chapter 2. We simulated the probability of elimination after 5 rounds of mass treatment with 65% coverage. Estimates of treatment effects (mean and variability) were directly taken from chapter 8, assuming that the reduction in mf production is caused by killing of worms for DEC and by permanent sterilization of female worms for ivermectin. Results are shown in Table 10-1. For DEC, our model predicted elimination in 98% of runs (n=100) when we assumed no variation in treatment efficacy. Elimination still occurred in 92% of the runs, when we assumed treatment efficacy to vary randomly without any relation to personal characteristics. However, when we assumed that individuals always have the same (sometimes poor) response to treatment, elimination occurred in only 43% of the runs. The differences were smaller for ivermectin, because this drug had less variable effects and never had no effect at all.

10.2 Remaining challenges for model-based support of lymphatic filariasis control

We have worked on the development and application of a lymphatic filariasis transmission model, aiming to support decision making in lymphatic filariasis control. Chapter 5 described the advances in this respect and identified remaining challenges in view of the rapidly expanding GPELF. The two main challenges are: 1) quantification of models for regions with different vector-parasite combinations, and 2) application of models to monitoring and evaluation issues of relevance for current elimination programmes. These issues are further discussed in this section.

10.2.1 Application of models for regions with different vector-parasite combinations

The limited field evidence available and our model analyses for Pondicherry have provided an indication of the duration of mass treatment required for elimination, but it is unclear whether the same outcomes can be expected in regions with other vector species or parasite strains and higher endemicity levels. Models can be used to explore how the prospects of elimination depend on the vector-parasite combination and local transmission dynamics.

Since almost 40% of the global burden of lymphatic filariasis is in Africa, where *Anopheles* species are the main vectors of lymphatic filariasis, it is of particular relevance to develop a model for this vector species. Building on the work presented in this thesis, we developed a first, preliminary version of an Africa-model ("Africa-model v0.1"). In line with conclusions of chapter 6, acquired immunity was not considered. We further used the uptake curve of Figure 10-1 and quantified the other model-parameters as described in Appendix B. The resulting model could explain the whole range of prevalence levels occurring in Africa (from <5% to >40%), the observed age-patterns, and the observed relationship between mf prevalence and mf intensity, but has some difficulty in explaining the relationship between overall mf prevalence with the mosquito biting rates (Figure 10-2). However, the observations of Figure 10-2-F are somewhat difficult to interpret for several reasons. Large measurement errors in average biting rate estimates introduce a bias in the observations, leading to a lower slope in the observations than expected. This effect is strengthened because biting rates are not constant over time. The relationship is further blurred because the observations come from different studies, which employed various different mf diagnostic tests (not necessarily the same as in our model). We therefore accepted the relatively poor fit in Figure 10-2-F for now.

We did some explorative simulation runs with the Africa-model, to investigate the prospects of elimination by yearly mass treatment (Table 10-2). These prospects seem to be greatly determined by the pre-treatment mf prevalence level (which varies with the monthly biting rate according to the line in Figure 10-2-F). In areas with relatively low mf prevalence levels of 10%, elimination can be achieved in a limited number of treatment rounds, even if coverage levels are low. The higher the prevalence, the more difficult it becomes to achieve elimination. In areas with prevalence of 30% or 40%, very high coverage levels and many yearly treatment rounds would be needed to reach this goal.

These preliminary results are worrying for current elimination efforts in Africa: high prevalence levels are not uncommon (see Figure 6-2) and experience learns that often it is difficult to achieve high coverage levels (World Health Organization 2005). Especially in high-endemic areas, therefore, we may want to consider alternative interventions or to shift focus to bringing down infection to such low levels that disease is prevented without completely interrupting transmission.

Clearly, these model-predictions must be interpreted with care. Some further work should be done to investigate whether the goodness-of-fit of the Africa-model can be

Figure 10-2. Comparison of predicted (lines) and observed (dots) microfilaria (mf) prevalence and intensities, for the Africa-model v0.1. (A) Mf prevalence by age; (B) Geometric mean mf intensity (GMI) among mf-positives by age; (C) Relative mf prevalence by age, calculated as age-specific prevalence / overall prevalence in the study population; (D) Relative mf intensity by age, calculated as age-specific GMI among postives / overall GMI among positives in the study population; (E) GMI among mf-positives by mf prevalence; (F) Overall mf prevalence by monthly biting rate (mbr, average number of bites per person per month). GMI is always in mf per 20 μl night blood. Lines in figures A-E show the simulation results for different biting rate levels (solid: mbr = 500, long-dashed: mbr = 752; short-dashed: mbr = 2015). In figure F, closed dots were used for studies that used repeated landing catches to determine biting rates, open dots for all other methods (the former are considered most reliable).

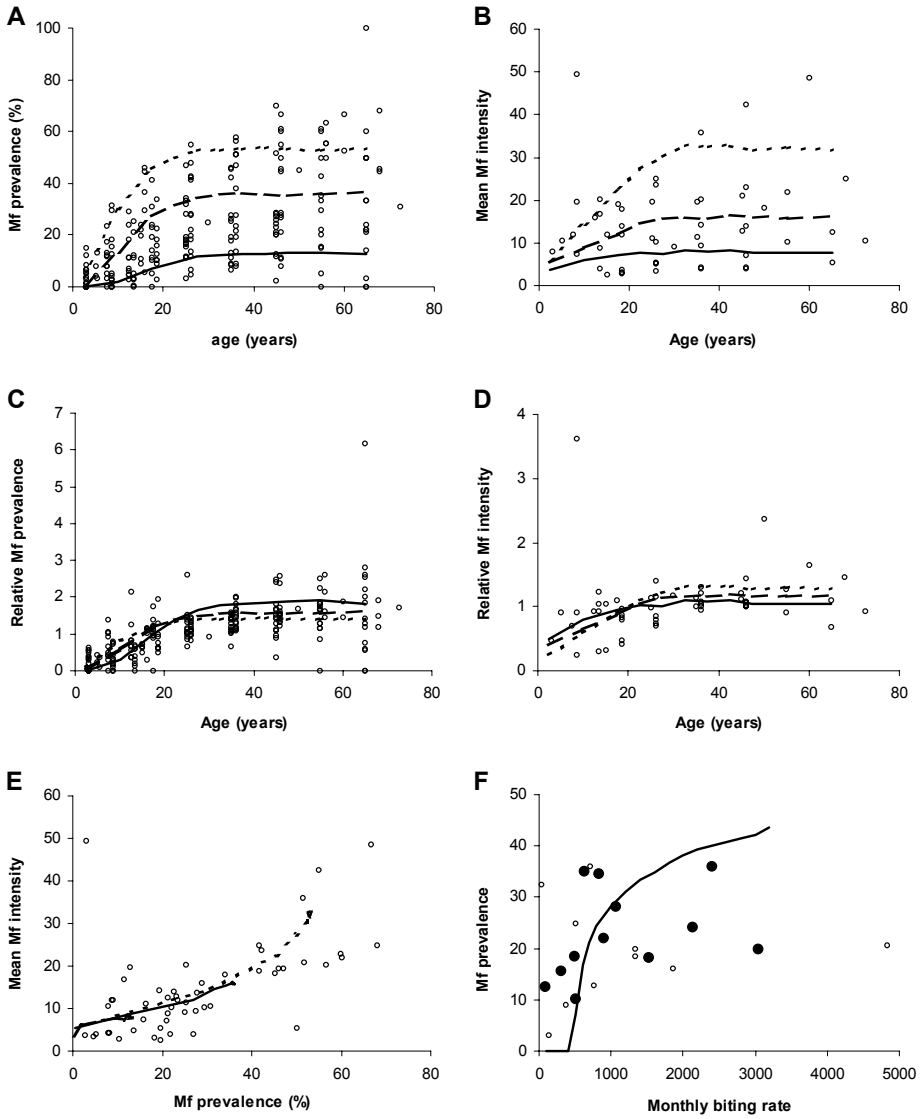


Table 10-2. Predicted number of treatment rounds that is required to be 99% certain of elimination in African communities with varying pretreatment mf prevalence levels. Predictions for Pondicherry, India, are shown for comparison. Methods are as described in chapter 3, using the models that were developed for Africa (section 10.2.1) and Pondicherry, India (chapter 2). Treatment is assumed to kill 65% of adult worms and 70% of mf per treatment, without variability.

	Pretreatment	Population coverage			
Model	mf prevalence	60%	70%	80%	90%
Africa	10%	6	4	3	3
	20%	14	9	7	5
	30%	*	*	11	8
	40%	**	*	*	13
Pondicherry (India)	8.5%	7	5	4	3

* / ** Estimated number of treatment rounds was 16-29 (*) or 30+ (**); in both cases, estimates were not exact, because they were based on logistic regression extrapolations beyond 15 treatment rounds.

improved, e.g. by including another density dependent mechanism besides limitation in the vector uptake curve. Further, the model needs to be validated by comparison of predicted trends during and after mass treatment with already available data. An interesting dataset in this respect is available from Tanzania, which has 10 year follow-up data after three different strategies of mass treatment with diethylcarbamazine (Meyrowitsch *et al.* 2004).

Similar approaches can be used to quantify the model for other regions. This is a time-consuming process, because data on different aspects of transmission must be brought together. Excellent understanding of the data and of the processes involved in transmission and control is required. This basic model quantification can therefore best be done by experienced modellers, hand in hand with researchers who are familiar with the local situation considered. Ideally, this yields a vector-parasite specific model that can easily be calibrated to local endemicity levels by adjustment of just 1 or 2 parameters.

10.2.2 Application of models to monitoring and evaluation issues of current elimination programmes

Many countries have initiated mass treatment and others will follow. All these elimination initiatives face the same questions: Is the program making enough progress to achieve elimination within the expected timeframe or do we need to intensify / adapt our control efforts? When can mass treatment be stopped?

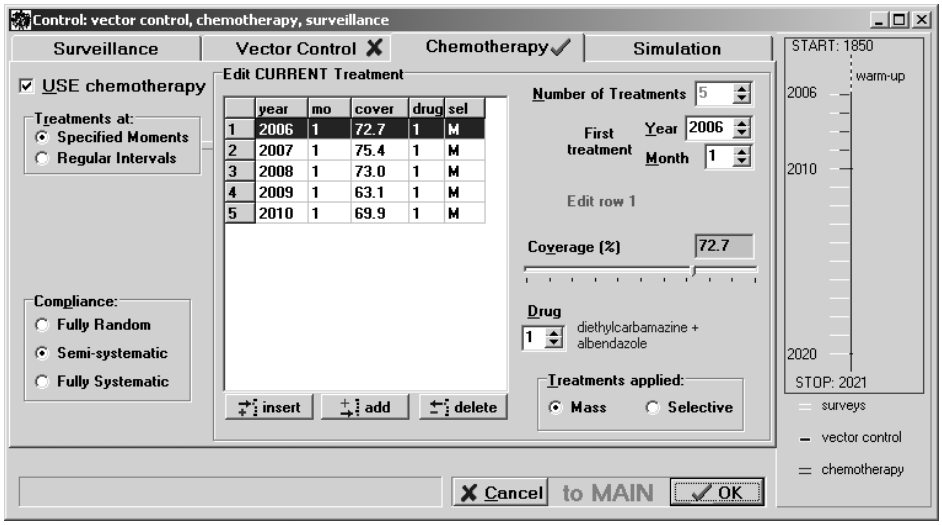
To help address these issues, extensions of the available models (LYMFASIM and EPIFIL) could be useful. For example, current elimination programmes use different diagnostic tests to monitor their progress. Besides mf detection, this includes antigen detection and xenomonitoring (determining infection prevalence and intensity in

mosquitoes). These tests should be included in the model output for comparison of observed and predicted trends. This would allow the determination of stop-criteria based on the different tests or their combinations. Simulation models should also consider disease development (hydrocele, lymphoedema, acute attacks), to predict the impact of intervention on disease prevalence and severity. This might be especially relevant in areas where elimination of the parasite is difficult.

Quantified and validated models can be used in simulation experiments to address issues that are of relevance for all ongoing programmes. For example, simulation experiments can be done to estimate the threshold level of mf or antigen prevalence, below which transmission will usually extinguish without further intervention. They can also help to identify cost-effective approaches for enhancing the effectiveness of the programme or for monitoring trends during and after mass treatment. Model-predicted trends in infection and disease are useful to check whether a programme is on track and will achieve its goal of elimination in the expected timeframe.

From the field, there is a strong demand to use the model-tool for evaluation of specific ongoing elimination programmes. This demand follows the positive experience of using the ONCHOSIM simulation model (Plaisier *et al.* 1990) in planning and evaluation of the large-scale Onchocerciasis Control Programme in West-Africa that ran from 1975-2002 (Habbema *et al.* 1992; Plaisier *et al.* 1997). The situation in lymphatic filariasis is a bit more complex, because transmission dynamics can differ markedly between regions due to different vector-parasite combinations and other factors. Before models can be used

Figure 10-3. Input screen of the Windows-interface for LYMFASIM, which contains specifications for predicting the trends after 5 rounds of mass treatment with varying levels of coverage (hypothetical situation).



for evaluation of specific programmes, they should first be quantified and validated for the local situation as illustrated above for Africa. When this is completed, models could be transferred to programme managers and others involved in planning and evaluation of lymphatic filariasis control programmes.

Indeed, eventual transfer of the model to ongoing elimination programmes has received due attention in our project. Successful use of the model by others requires a more user-friendly interface and some training. A simple Windows-interface is in development: the user will be able to adapt a (vector-specific) model to the local situation in a simple way and to simulate the impact of interventions (Figure 10-3). Several people from the Vector Control Research Centre (Pondicherry, India) and the World Health Organization-secretariat of the GPELF have already been trained in the use of LYMFASIM. Representatives of control programmes of specific countries will follow in the framework of ongoing and new collaborations.

10.3 Conclusions and recommendations

Conclusions:

- The prospects for elimination of lymphatic filariasis by mass treatment vary between regions with different vector-parasite strains and depend strongly on the pre-treatment endemicity level, the applied treatment regimen, and the proportion of the population treated per round.
- Prospects for elimination of bancroftian filariasis in a Pondicherry-like situation (an Indian area with about 8.5% pretreatment mf prevalence) are good if the highly effective combination of DEC and albendazole is used in mass treatment and coverage is sufficiently high: predictions suggests that six annual rounds of mass treatment with population coverage of 65% are sufficient for elimination.
- It is too optimistic to assume that elimination of lymphatic filariasis can be achieved by 4 to 6 rounds of mass treatment in any area: many more rounds may be required when coverage is low or pretreatment endemicity levels are high. The goal of eliminating the disease as a public health problem without necessarily interrupting transmission may sometimes be more realistic.
- The strength and direction of density dependence in the relationship between mf density in the human blood and the average number of L3 developing in mosquitoes vary between mosquito species. Therefore, vector-specific models should be used for prediction and results should not be generalized across areas with different vectors.
- Summarized epidemiological data of mf prevalence by age from India and Africa provide no indication that the prevalence of infection is down regulated in older age groups as a consequence of acquired immunity. The acquired immunity assumptions of the Pondicherry model should therefore be reconsidered.

- DEC and ivermectin, given alone or in combination with albendazole, are highly effective, killing a large proportion of mf and affecting viability or reproductive capacity of adult worms

Recommendations:

- Elimination programmes should be monitored carefully, using operational indicators (population coverage, systematic non-participation) and epidemiological indicators (infection prevalence and intensity).
- Simulation models should be quantified for different vector-parasite combinations and their validity should be tested against data from areas with varying endemicity levels.
- Validated simulation models should be used to address the following issues, which are of crucial importance for the Global Programme to Eliminate Lymphatic Filariasis:
  - Define criteria for stopping mass treatment;
  - Identify cost-effective approaches to enhance programme effectiveness;
  - Determine the short- and long-term impact of mass treatment on disease prevalence and severity;
  - Determine the circumstances under which elimination of infection is so difficult, that programmes should better focus on elimination of the disease as a public health problem.
- Validated models should be transferred to policy makers and programme managers for use in planning and evaluation of ongoing programmes.
- Research for drugs with better macrofilaricidal efficacy than the existing ones should continue to further improve elimination prospects and anticipate the potential development of resistance.
- Potential density dependence in parasite establishment or mf production in the human host should be examined, using the modern diagnostic methods that allow quantification of the adult worm burden.

10.4 References

Anonymous (2004). Towards a strategic plan for research to support the global program to eliminate lymphatic filariasis. Summary of immediate needs and opportunities for research on lymphatic filariasis. Philadelphia, Pennsylvania, USA, December 9-10, 2003. *Am J Trop Med Hyg* **71 (Suppl 5)**: iii, 1-46.

Dreyer G, Santos A, Norões J, Rocha A and Addiss D (1996). Amicrofilaraemic carriers of adult *Wuchereria bancrofti*. *Trans R Soc Trop Med Hyg* **90**: 288-289.

Duerr HP, Dietz K and Eichner M (2005). Determinants of the eradicability of filarial infections: a conceptual approach. *Trends Parasitol* **21**: 88-96.

Fulford AJ (1994). Dispersion and bias: can we trust geometric means? *Parasitol Today* **10**: 446-448.

Gyapong JO, Kumaraswami V, Biswas G and Ottesen EA (2005). Treatment strategies underpinning the global programme to eliminate lymphatic filariasis. *Expert Opin Pharmacother* **6**: 179-200.

Habbema JDF, Alley ES, Plaisier AP, van Oortmarssen GJ and Remme JHF (1992). Epidemiological modelling for onchocerciasis control. *Parasitol Today* **8**: 99-103.

Hoerauf A, Mand S, Fischer K, Kruppa T, Marfo-Debrekyei Y, Debrah AY, Pfarr KM, Adjei O and Buttner DW (2003). Doxycycline as a novel strategy against bancroftian filariasis--depletion of *Wolbachia* endosymbionts from *Wuchereria bancrofti* and stop of microfilaria production. *Med Microbiol Immunol (Berl)* **192**: 211-216.

Houston R (2000). Salt fortified with diethylcarbamazine (DEC) as an effective intervention for lymphatic filariasis, with lessons learned from salt iodization programmes. *Parasitology* **121**: S161-173.

Krishnamoorthy K, Subramanian S, Van Oortmarssen GJ, Habbema JDF and Das PK (2004). Vector survival and parasite infection: the effect of *Wuchereria bancrofti* on its vector *Culex quinquefasciatus*. *Parasitology* **129**: 43-50.

Kshirsagar NA, Gogtay NJ, Garg BS, Deshmukh PR, Rajgor DD, Kadam VS, Kirodian BG, Ingole NS, Mehendale AM, Fleckenstein L, Karbwang J and Lazdins-Helds JK (2004). Safety, tolerability, efficacy and plasma concentrations of diethylcarbamazine and albendazole co-administration in a field study in an area endemic for lymphatic filariasis in India. *Trans R Soc Trop Med Hyg* **98**: 205-217.

Meyrowitsch DW, Simonsen PE and Magesa SM (2004). Long-term effect of three different strategies for mass diethylcarbamazine administration in bancroftian filariasis: follow-up at 10 years after treatment. *Trans R Soc Trop Med Hyg* **98**: 627-634.

Michael E, Malecela-Lazaro MN, Simonsen PE, Pedersen EM, Barker G, Kumar A and Kazura JW (2004). Mathematical modelling and the control of lymphatic filariasis. *Lancet Infect Dis* **4**: 223-234.

Norões J, Dreyer G, Santos A, Mendes VG, Medeiros Z and Addiss D (1997). Assessment of the efficacy of diethylcarbamazine on adult *Wuchereria bancrofti in vivo*. *Trans R Soc Trop Med Hyg* **91**: 78-81.

Pichon G (2002). Limitation and facilitation in the vectors and other aspects of the dynamics of filarial transmission: the need for vector control against *Anopheles*-transmitted filariasis. *Ann Trop Med Parasitol* **96**: S143-S152.

Plaisier AP, van Oortmarssen GJ, Habbema JDF, Remme J and Alley ES (1990). ONCHOSIM: a model and computer simulation program for the transmission and control of onchocerciasis. *Comput Methods Programs Biomed* **31**: 43-56.

Plaisier AP, Alley ES, van Oortmarssen GJ, Boatin BA and Habbema JDF (1997). Required duration of combined annual ivermectin treatment and vector control in the Onchocerciasis Control Programme in west Africa. *Bull World Health Organ* **75**: 237-245.

Ramaiah KD, Das PK, Appavoo NC, Ramu K, Augustin DJ, Kumar KN and Chandrakala AV (2000). A programme to eliminate lymphatic filariasis in Tamil Nadu state, India: compliance with annual single-dose DEC mass treatment and some related operational aspects. *Trop Med Int Health* **5**: 842-847.

Selkirk ME, Maizels RM and Yazdanbakhsh M (1992). Immunity and the prospects for vaccination against filariasis. *Immunobiology* **184**: 263-281.

Southgate BA and Bryan JH (1992). Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 4. Facilitation, limitation, proportionality and their epidemiological significance. *Trans R Soc Trop Med Hyg* **86**: 523-530.

Stolk WA, Ramaiah KD, Van Oortmarssen GJ, Das PK, Habbema JDF and De Vlas SJ (2004). Meta-analysis of age-prevalence patterns in lymphatic filariasis: no decline in microfilaraemia prevalence in older age groups as predicted by models with acquired immunity. *Parasitology* **129**: 605-612.

Subramanian S, Krishnamoorthy K, Ramaiah KD, Habbema JDF, Das PK and Plaisier AP (1998). The relationship between microfilarial load in the human host and uptake and development of *Wuchereria bancrofti* microfilariae by *Culex quinquefasciatus*: a study under natural conditions. *Parasitology* **116**: 243-255.

Taylor MJ, Makunde WH, McGarry HF, Turner JD, Mand S and Hoerauf A (2005). Macrofilaricidal activity after doxycycline treatment of *Wuchereria bancrofti*: a double-blind, randomised placebo-controlled trial. *Lancet* **365**: 2116-2121.

Vanamail P, Ramaiah KD, Subramanian S, Pani SP, Yuvaraj J and Das PK (2005). Pattern of community compliance with spaced, single-dose, mass administrations of diethylcarbamazine or ivermectin, for the elimination of lymphatic filariasis from rural areas of southern India. *Ann Trop Med Parasitol* **99**: 237-242.

Woolhouse ME (1992). A theoretical framework for the immunoepidemiology of helminth infection. *Parasite Immunol* **14**: 563-578.

World Health Organization (2005). Global Programme to Eliminate Lymphatic Filariasis - Annual Report on Lymphatic Filariasis 2003. Geneva, Switzerland, World Health Organization**:** 150.

Zagaria N and Savioli L (2002). Elimination of lymphatic filariasis: a public-health challenge. *Ann Trop Med Parasitol* **96 Suppl 2**: S3-13.

Appendix A. The uptake curve for *Anopheles*

Data

To quantify the uptake curve for *Anopheles* (describing the relationship between mf density in the human blood and the number of L3 larvae developing in mosquitoes) we analyzed paired data on human mf density and the average number of L3 developing in mosquitoes after taking a blood meal. Detailed data were available from a study in Ghana that was carried out to investigate this relationship for anopheline mosquitoes (Boakye *et al.* 2004). The few data that were available from literature were also used (Bryan & Southgate 1988a, b; Southgate & Bryan 1992). To come to a crude quantification of the vector uptake curve, we aggregated the data from different studies, even though these studies differed in the type of mosquitoes considered (natural populations or laboratory-reared mosquitoes of different *Anopheles*-species), used different methods for mosquito feeding, and used different methods to determine mf density.

All mf densities were first scaled to mf counts in 20 µl night blood smears. If mf counts were measured in fingerprick blood taken at night, this only concerned a correction for the volume of blood considered. If mf counts were measured by filtration of 1 ml venous blood taken at night, we used the following relationship to calculate mf density in 20 µl fingerprick blood, which was derived by Snow & Michael (2002):

$$y = 0.037x + 0.1449x^2 - 0.0309 \qquad \text{(A-1)}$$

with y = $\log_{10}$ (1+mf count in 20 µL finger prick blood); and x = $\log_{10}$ (1+mf count in 1 mL venous blood). At the lowest mf counts (<1.246 per 1 mL venous blood), this function yields negative values for mf in 20 µl fingerprick blood, which are replaced by zero's. This reflects the higher detection limit of mf diagnosis in the smaller 20 µl blood sample. Correcting for bloodvolume, the estimated mf densities in finger prick blood are higher than in venous blood (except for the lowest mf densities), as has been observed in field data.

Fitted curve

It has been suggested that there is facilitation in the mf uptake and development, meaning that the proportion of mf developing into L3 larvae initially increases with mf density in the human blood and saturates only at higher mf intensities (Southgate & Bryan 1992; Duerr *et al.* 2005). Such a pattern can be described by the sigmoid curve of equation A-2, which was fitted to the aggregated data.

$$L3 = a\left(1 - e^{-(bM)^c}\right) \qquad \text{(A-2)}$$

with *L3* = the number of L3 larvae developing in mosquitoes; and *M* = the mf density in human blood (mf / 20 µl night blood).

To quantify the vector uptake curve, we fitted Equation A-2 to the pooled data from the three studies. Both sides of the equation were $\log_{10}$-transformed to normalize the residuals. To deal with zero's, it is common practice to add 1 to the observed and predicted number of L3. However, since adding 1 introduces a major distortion to the data, we rather added a number equal to the half detection limit (*D*) to the average number of L3 per mosquito (*D* is calculated as 0.5 / total number of mosquitoes examined). Thus, we fitted the curve of Equation A-3:

$$\log_{10}(L3 + D) = \log_{10}\left(a\left(1 - e^{-(bM)^c}\right) + D\right) \qquad \text{(A-3)}$$

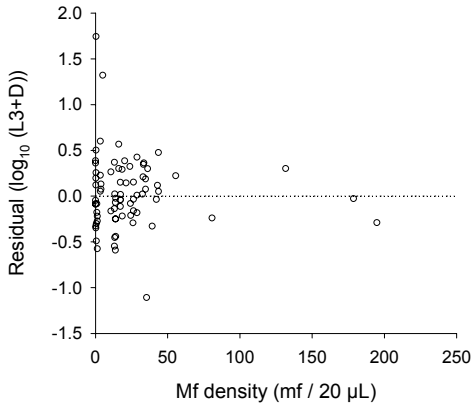
Parameter estimation

Using the non-linear regression procedure (PROC NLIN) in SAS (v8.2), we estimated the values of parameters *a*, *b* and *c* in Equations A-2 and A-3 with the least squares method. Observations were weighed for the number of mosquitoes examined, weights (W_i) being calculated as:

$$W_i = \sqrt{x_i} \Big/ \sum_i^n \sqrt{x_i} \qquad \text{(A-4)}$$

with x_i the number of mosquitoes examined for observation *i*, and *n* the total number of observations included in the analysis.

Figure A-1. Residuals of equation 3 fitted to the data plotted against mf density in the human blood (mf / 20 µL).



Results

Point-estimates of the parameters of Equation A-2/A-3 were: $a = 1.80$, $b = 0.016$ and $c = 1.14$. The fitted curve is shown elsewhere (Section 10.1.3, Figure 10-1). The plot of residuals indicates a reasonably good fit, although there are some outliers (Figure A-1).

References

Boakye DA, Wilson MD, Appawu MA and Gyapong J (2004). Vector competence, for *Wuchereria bancrofti*, of the *Anopheles* populations in the Bongo district of Ghana. *Ann Trop Med Parasitol* **98**: 501-508.

Bryan JH and Southgate BA (1988a). Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 1. Uptake of microfilariae. *Trans R Soc Trop Med Hyg* **82**: 128-137.

Bryan JH and Southgate BA (1988b). Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 2. Damage to ingested microfilariae by mosquito foregut armatures and development of filarial larvae in mosquitoes. *Trans R Soc Trop Med Hyg* **82**: 138-145.

Duerr HP, Dietz K and Eichner M (2005). Determinants of the eradicability of filarial infections: a conceptual approach. *Trends Parasitol* **21**: 88-96.

Snow LC and Michael E (2002). Transmission dynamics of lymphatic filariasis: density-dependence in the uptake of *Wuchereria bancrofti* microfilariae by vector mosquitoes. *Med Vet Entomol* **16**: 409-423.

Southgate BA and Bryan JH (1992). Factors affecting transmission of *Wuchereria bancrofti* by anopheline mosquitoes. 4. Facilitation, limitation, proportionality and their epidemiological significance. *Trans R Soc Trop Med Hyg* **86**: 523-530.

Appendix B. Quantification of LYMFASIM for *Anopheles*-transmitted infection in Africa

Model design and quantification of fixed parameters

We set out to develop a model for *Anopheles*-transmitted infection in Africa. Following the conclusions of chapter 6 of this thesis, we aimed at development of a model without acquired immunity, because this would be the most parsimonious model for the observed age-patterns in the region. As far as possible, the model parameters were quantified based on literature, expert opinion, and analysis of local data (Tables B-1 and B-2). The value of only two parameters was not fixed, namely α_E (i.e. the not age-related variation in exposure to mosquitoes) and the success ratio *sr* (i.e. the proportion of inoculated larvae that develops successfully into male or female adult worms in the human body). The value of these 'free' parameters (Table B-3) was estimated by fitting the model to the reference data as described below.

Reference data

Reference data were taken from our earlier literature review of age-prevalence patterns in India and Africa (chapter 6). We updated this database with recently published studies (Njenga *et al.* 2000; Boakye *et al.* 2004; Meyrowitsch *et al.* 2004). Since estimates of the variability of mf counts are already available for 20 µl blood smears (Subramanian *et al.* 2004), but not for other mf diagnostic tests, we considered only the 9 African studies that used this test to estimate mf prevalence or intensity in model fitting[1]. Age-specific data on mf prevalence were available for 29 locations, but age-specific data on mf intensity only for 9. These data are shown in section 10.2.1, Figure 10-2 (A-E).

To get an indication of the relationship between mf prevalence and biting rate and of the variability in biting rates, we also searched the literature for paired data on mosquito biting rates and mf prevalence levels in Africa. For 12 locations, mosquito biting rate data were based on repeated all-night man landing catches[2]; in 10 other locations, other methods were used or it was not clear how biting rates were determined[3]. The studies differed with respect to the diagnostic test that was used to measure mf prevalences, which blurs the relationship between biting rate and mf prevalence. The data are shown in section 10.2.1, Figure 10-2-F.

---

1 (McGregor *et al.* 1952; McFadzean 1954; Brengues *et al.* 1969; Brengues 1975; Brunhes 1975; Ripert *et al.* 1982; Akogun 1991; Gyapong *et al.* 1998; Boakye *et al.* 2004)

2 (Maasch 1973; Wijers 1977; Wijers & Kiilu 1977; Wijers & Kinyanjui 1977; Kuhlow & Zielke 1978; McMahon *et al.* 1981; Southgate 1992)

3 (Maasch 1973; Udonsi 1988ab; Appawu *et al.* 2001; Onapa *et al.* 2001; Pedersen & Mukoko 2002; Mukoko *et al.* 2004; Rwegoshora *et al.* 2005)

Parameter estimation

Fitting was done by manually adapting the free model parameters and comparison of simulation results with observations using a grid search. Each simulation started with a "warming-up" period, during which the population grows from 300 to 7000-8000 persons and a stable endemic situation develops. Simulations were always done for three different values of the monthly biting rate (corresponding to the median, the 16.7th and 83.3th percentile of the observed frequency distribution of biting rates). Our aim was to identify a model that, with these different values of monthly biting rate, was able to describe the range of observations from Africa. The goodness of fit was assessed by visual inspection of plots of observations and predictions.

Table B-1. List of fixed biological parameters of the LYMFASIM model (symbols as chapter 1 of this thesis).

Parameter and description		Value	Source
a	Parameter of the uptake curve	1.80	This thesis, App. A
b	,,	0.016	,,
c	,,	1.14	,,
v	Fraction of the L3 larvae, resulting from a single blood meal, that is released by a mosquito	0.1	Fixed[a]
Tl	Average lifespan of adult parasites (years)	10	(Subramanian *et al.* 2004)[b]
α_{Tl}	Shape parameter of the Weibull distribution that describes the variation in the worm lifespan	2.0	Expert opinion
Ti	Duration of the immature stage of the parasite in the human host (months)	8	(World Health Organization 1992)
s	Proportion of mf surviving per month	0.9	(Plaisier *et al.* 1999)
r_0	Number of mf produced per female parasite (per month per 20 µl of peripheral blood) in the presence of at least one worm, in the absence of acquired immunity and treatment	0.58	(Subramanian *et al.* 2004)[a, b]
E_0	Relative exposure at birth as fraction of exposure in adults	0	Fixed
a_{max}	Age at which exposure to mosquitoes reaches its maximum level	20	(Subramanian *et al.* 2004)[b]
k_m	Overdispersion parameter of the Negative Binomial distribution describing the variation in mf counts in 20 µl blood smears for an individual	0.33	,,

[a] Parameter v, r_0, and the 'success ratio (sr)' (see Table B-2) are linear multiplication factors in the same sequence of calculations in this model without immunity. We have no sound knowledge on either of these parameters. We therefore fixed v and r_0 at the given values, and only estimate the success ratio.

[b] Chapter 2 of this thesis.

Table B-2. Life table and fertility rates for the African region that were used as input specifications for LYMFASIM.

Age group	Life table (probability to survive until the upper limit of the age-range) [a]	Fertility [b] (birthrate per female per year)
0-5	0.804	0.000
5-15	0.780	0.000
15-20	0.755	0.116
20-25	0.730	0.230
25-30	0.707	0.245
30-35	0.654	0.207
35-40	0.605	0.147
40-45	0.560	0.077
45-50	0.506	0.031
50-60	0.407	0.000
60-70	0.255	0.000
70-80	0.051	0.000
80-99	0.000	0.000
Total fertility rate		5.3

[a] The lifetable gives the probability to survive, assuming that age-specific mortality risks observed in the year 2002 apply during the entire life time of a hypothetical cohort of people. Source of mortality risks: Global Burden of Disease study, 2002 for the WHO-AFRO region; available from the WHO website (www.who.int)

[b] Source: age-specific fertility rates for Sub-Sahara Africa, available on the internet from the US Census Bureau (U.S. Census Bureau 2004). Total fertility rate: number of children per women, who survives throughout the fertile period.

Table B-3. Estimated value of the 'free' model parameter (symbols as in chapter 2 of this thesis).

Parameter	Description	Value
sr	Success ratio: fraction of inoculated L3 larvae developing into an adult male or female worm (in the absence of immune regulation)	0.10
α_E	Shape parameter of the gamma-distribution describing individual variation in exposure to mosquitoes (mean=1)	0.3

Results

Table B-3 gives the estimates of the free model parameters. The goodness-of-fit is shown in Figure 10-2. Results are discussed in section 10.2.1.

References

Akogun OB (1991). Filariasis in Gongola State Nigeria. I: Clinical and parasitological studies in Mutum-Biyu district. *J Hyg Epidemiol Microbiol Immunol* **35**: 383-393.

Appawu MA, Dadzie SK, Baffoe-Wilmot A and Wilson MD (2001). Lymphatic filariasis in Ghana: entomological investigation of transmission dynamics and intensity in communities served by irrigation systems in the Upper East Region of Ghana. *Trop Med Int Health* **6**: 511-516.

Boakye DA, Wilson MD, Appawu MA and Gyapong J (2004). Vector competence, for *Wuchereria bancrofti*, of the *Anopheles* populations in the Bongo district of Ghana. *Ann Trop Med Parasitol* **98**: 501-508.

Brengues J, Subra R and Bouchite B (1969). Etude parasitologique, clinique et entomologique sur la filariose de Bancroft dans le sud du Dahomey et du Togo. *Cah ORSTOM sér Ent méd et Parasitol* **7**: 279-305.

Brengues J (1975). La filariose de bancroft en Afrique de l'Ouest. Office de la Recherche Scientifique et Technique Outre-Mer (ORSTOM). **79:** 299pp.

Brunhes J (1975). La filariose de bancroft dans la sous-région Malgache (Comores - Madagascar - Reunion). Office de la Recherche Scientifique et Technique d'Outre-mer (ORSTOM). **81:** 212pp.

Gyapong JO, Omane-Badu K and Webber RH (1998). Evaluation of the filter paper blood collection method for detecting Og4C3 circulating antigen in bancroftian filariasis. *Trans R Soc Trop Med Hyg* **92**: 407-410.

Kuhlow F and Zielke E (1978). Dynamics and intensity of *Wuchereria bancrofti* transmission in the savannah and forest regions of Liberia. *Tropenmed Parasitol* **29**: 371-381.

Maasch HJ (1973). Quantitative Untersuchungen zur Ubertragung von *Wuchereria bancrofti* in der Kustenregion Liberias. *Z Tropenmed Parasitol* **24**: 419-434.

McFadzean JA (1954). Filariasis in Gambia and Casamance, West Africa. *Trans R Soc Trop Med Hyg* **48**: 267-273.

McGregor IA, Hawking F and Smith DA (1952). The control of filariasis with hetrazan. A field trial in a rural village (Keneba) in the Gambia. *Br Med J* **ii**: 908-.

McMahon JE, Magayauka SA, Kolstrup N, Mosha FW, Bushrod FM, Abaru DE and Bryan JH (1981). Studies on the transmission and prevalence of Bancroftian filariasis in four coastal villages of Tanzania. *Ann Trop Med Parasitol* **75**: 415-431.

Meyrowitsch DW, Simonsen PE and Magesa SM (2004). A 26-year follow-up of bancroftian filariasis in two communities in north-eastern Tanzania. *Ann Trop Med Parasitol* **98**: 155-169.

Mukoko DA, Pedersen EM, Masese NN, Estambale BB and Ouma JH (2004). Bancroftian filariasis in 12 villages in Kwale district, Coast province, Kenya - variation in clinical and parasitological patterns. *Ann Trop Med Parasitol* **98**: 801-815.

Njenga SM, Muita M, Kirigi G, Mbugua J, Mitsui Y, Fujimaki Y and Aoki Y (2000). Bancroftian filariasis in Kwale district, Kenya. *East Afr Med J* **77**: 245-249.

Onapa AW, Simonsen PE, Pedersen EM and Okello DO (2001). Lymphatic filariasis in Uganda: baseline investigations in Lira, Soroti and Katakwi districts. *Trans R Soc Trop Med Hyg* **95**: 161-167.

Pedersen EM and Mukoko DA (2002). Impact of insecticide-treated materials on filaria transmission by the various species of vector mosquito in Africa. *Ann Trop Med Parasitol* **96 Suppl 2**: S91-95.

Plaisier AP, Cao WC, van Oortmarssen GJ and Habbema JD (1999). Efficacy of ivermectin in the treatment of *Wuchereria bancrofti* infection: a model-based analysis of trial results. *Parasitology* **119**: 385-394.

Ripert C, Eono P, Eono D, Tribouley J, Appriou M and Issoufa H (1982). [Epidemiological study of bancroftian filariasis in the Logone Valley (North Cameroon) (author's transl)] Etude epidemiologique de la bancroftose dans la Vallee du Logone (Nord Cameroun). *Med Trop (Mars)* **42**: 59-66.

Rwegoshora RT, Pedersen EM, Mukoko DA, Meyrowitsch DW, Masese N, Malecela-Lazaro MN, Ouma JH, Michael E and Simonsen PE (2005). Bancroftian filariasis: patterns of vector abundance and transmission in two East African communities with different levels of endemicity. *Ann Trop Med Parasitol* **99**: 253-265.

Southgate BA (1992). Intensity and efficiency of transmission and the development of microfilaraemia and disease: their relationship in lymphatic filariasis. *J Trop Med Hyg* **95**: 1-12.

Subramanian S, Stolk WA, Ramaiah KD, Plaisier AP, Krishnamoorthy K, Van Oortmarssen GJ, Dominic Amalraj D, Habbema JD and Das PK (2004). The dynamics of *Wuchereria bancrofti* infection: a model-based analysis of longitudinal data from Pondicherry, India. *Parasitology* **128**: 467-482.

Udonsi JK (1988a). Bancroftian filariasis in the Igwun Basin, Nigeria. An epidemiological, parasitological, and clinical study in relation to the transmission dynamics. *Acta Trop* **45**: 171-179.

Udonsi JK (1988b). Bancroftian filariasis in the Igwun basin, Nigeria: an epidemiological, parasitological, and clinical study in relation to the transmission dynamics. *Folia Parasitol* **35**: 147-155.

Wijers DJ (1977). Bancroftian filariasis in Kenya. IV. Disease distribution and transmission dynamics. *Ann Trop Med Parasitol* **71**: 452-463.

Wijers DJ and Kiilu G (1977). Bancroftian filariasis in Kenya III. Entomological investigations in Mambrui, a small coastal town, and Jaribuni, a rural area more inland (Coast Province). *Ann Trop Med Parasitol* **71**: 347-359.

Wijers DJ and Kinyanjui H (1977). Bancroftian filariasis in Kenya II. Clinical and parasitological investigations in Mambrui, a small coastal town, and Jaribuni, a rural area more inland (Coast Province). *Ann Trop Med Parasitol* **71**: 333-345.

World Health Organization (1992). Lymphatic filariasis: the disease and its control. Fifth report of the WHO Expert Committee on Filariasis. *World Health Organ Tech Rep Ser* **821**: 1-71.

Summary

Lymphatic filariasis: infection, disease and elimination

Lymphatic filariasis is a disfiguring and debilitating parasitic disease that is endemic in many tropical and subtropical countries. This mosquito-borne disease is caused by different species of thread-like, filarial worms, of which *Wuchereria bancrofti* is most widespread. The worms live in the human lymphatic system and have a lifespan of 5 to 10 years. During their lifespan, female worms can bring millions of immature microfilariae (mf) into the blood. These mf can stay alive for about one year, but cannot develop into adult worms, unless they are engorged by mosquitoes taking a blood meal. Inside a mosquito, mf develop via several stages into L3 larvae. These can be transmitted to humans when the mosquito bites, where they can develop further into adult worms. Many different mosquito species can transmit the infection.

Many people may be infected without even knowing it. However, the infection causes damage to the lymphatic system, impairing the lymph drainage in the body. This can eventually lead to gross swelling of extremities and external genitalia (lymphoedema or, in the end stage, elephantiasis) or, in males, to enlargement of the scrotum due to serous fluid accumulation (hydrocele). A hydrocele can be removed surgically, but advanced lymphoedema and elephantiasis cannot be treated. These chronic manifestations are an important cause of disability and reduced quality of life. Approximately 120 million people are affected worldwide, with more than 40 million people suffering from the chronic manifestations.

Public health interventions aim at prevention of the chronic manifestations by reducing the infection load in the population. Commonly used indicators for the infection load are the proportion of people with mf in the blood (mf prevalence) and the mean concentration of mf in the blood (mf intensity). Mf prevalence and intensity can be brought down by reducing infection transmission through mosquito control measures. However, nowadays the preferred strategy is regularly repeated treatment of all individuals in the population with antifilarial drugs. For this purpose, diethylcarbamazine (DEC) or ivermectin can be used, given alone or in combination with albendazole. A single dose of these drugs leads to a sustained reduction of mf intensity in the blood. These drugs are safe, so that is is possible to treat all individuals in an area at the same time, without determining who is infected and who is not ('mass treatment'). This is easier, cheaper and more effective than screening followed by selective treatment of infected individuals. Yearly repeated mass treatment causes such a strong decline in mf prevalence and intensity, and thereby also in transmission, that it is thought possible to eliminate the infection completely. Recognizing this, the World Health Assembly called in 1997 for the 'elimination of lymphatic filariasis as a public health problem' and mass treatment

programmes are being initiated worldwide under the umbrella of the Global Programme to Eliminate Lymphatic Filariasis.

This thesis

There is much optimism about the possibility of eliminating lymphatic filariasis. However, it is uncertain how long mass treatment would have to be continued to achieve this goal and how the required duration depends on the proportion of the population that receives treatment (coverage), the treatment regimen, the pretreatment mf prevalence, and the local vector species. These questions are addressed in this thesis. Much of the reported work was done in close collaboration with the Vector Control Research Centre in Pondicherry (Indian Council of Medical Research), India.

Elimination prospects

In the first part of this thesis, we used a mathematical simulation model for transmission and control of lymphatic filariasis, to predict the long-term effects of mass treatment and to estimate the duration of mass treatment required for elimination. The employed model, which is called LYMFASIM, was previously developed at our department in collaboration with researchers of the Vector Control Research Centre in Pondicherry, India, and the Centro de Pesquisas Aggeu Magalhães in Recife, Brazil.

We quantified the LYMFASIM model so that it reflects the situation in Pondicherry, India, where lymphatic filariasis is transmitted by mosquitoes of the species *Culex quinquefasciatus* (**chapter 2**). For this purpose, we used the wealth of data that were collected for evaluation of a 5-year 'integrated vector management' programme, which ran in Pondicherry from 1981 to 1986 and aimed to reduce the transmission by lowering the number of mosquitoes. We could use individual level data on mf intensity before and after the control programme. In addition, data were available on the mean number of mosquito bites per person per month for the whole programme period. From these data, we estimated that adult parasites live for about 10 years. We further deduced that individuals after prolonged exposure acquire some kind of immunity that protects against new infections or reduces the mf production. Predictions of the resulting matched well with the observations from Pondicherry.

The model was subsequently used to predict the long-term effects of mass treatment (**chapters 3** and **4**). We found that the duration of mass treatment required for elimination depends strongly on the proportion of the population treated per round, the efficacy of the applied treatment regimen, and the pretreatment endemicity level. In the Pondicherry-situation, with a pretreatment mf prevalence of about 8.5%, four yearly rounds of mass treatment with the recommended drug-combination DEC plus albendazole are sufficient to achieve elimination if 80% of the population is treated per round. Such high coverage levels are not always easy to achieve, though. If a more

realistic 65% of the population is treated per round, mass treatment has to be continued for 6 years. We assumed in these simulations that the DEC-albendazole combination kills on average 65% of worms and 70% of mf per individual treatment. Mass treatment needs to be continued for a longer period, if the treatment regimen is less effective or if pretreatment mf prevalence is higher. Although a 1-year interval between treatments may be practical, more frequent treatment could reduce the total duration of the programme.

We compared LYMFASIM's predictions with those from another model for lymphatic filariasis, EPIFIL, which was developed by Michael c.s. from the United Kingdom. The two models make similar predictions of trends in mf prevalence and intensity during mass treatment. However, LYMFASIM simulates the transmission of infection in more detail and is better adapted for assessing the risk of recurrent infection after stopping mass treatment (**chapter 5**).

Overall, prospects for elimination of lymphatic filariasis by mass treatment in Pondicherry seem good, provided that the level of population coverage is sufficiently high. Qualitative conclusions on the impact of coverage, treatment efficacy, and pretreatment mf prevalence on the elimination prospects can be generalized to other areas. Quantitative estimates of the required duration should however be interpreted with some care. Our predictions may be optimistic, because we did not take account of variability in treatment effects between individuals, reintroduction of infection by infected immigrants, or the possible development of parasite resistance against treatment. Our estimates are further influenced by uncertainty about treatment effects and about the processes that play a role in parasite transmission. Results cannot simply be generalized to areas with other vector species: differences between species in transmission efficiency may be important determinants of the elimination prospects.

Transmission dynamics

For more accurate prediction of the long-term impact of mass treatment, better understanding of the processes involved in parasite transmission is required. We studied two of these processes in more detail.

First, we investigated the role of acquired immunity (**chapter 6**). In Pondicherry, we observed that mean mf density and mf prevalence in the blood declined in older age groups. We explained this by assuming that individuals acquire some kind of immunity against infection after prolonged exposure, which protects against new infections or otherwise reduces the mf density in the blood. Evidence for the operation of such immunity has come from animal studies. However, when we reviewed age-patterns of lymphatic filariasis infection in other areas, we found that such patterns with a decline in older age groups are not common at all. Usually, the mf prevalence increases with age until a stable level is reached at about 20 years of age in India and 10 to 15 years later in Africa. In fact, the pattern in Pondicherry was rather exceptional. This raises doubts about the assumed role of acquired immunity in lymphatic filariasis.

Second, we examined how the mean number of infectious L3 larvae developing in mosquitoes depends on the mean mf density in human blood. This relationship differs between mosquito species and is an important determinant of elimination prospects. We compared the uptake and development of mf in *Culex quinquefasciatus*, the main vector of lymphatic filariasis in India, and *Aedes polynesiensis*, the main vector in French Polynesia. We found for both species that transmission is most efficient at the lowest mf densities: a large proportion of mf can develop into L3 larvae inside the mosquito. However, the number of L3 larvae in mosquitoes does not increase linearly with mf density in the blood, but approximates a maximum at higher mf densities ('limitation'). In other words, the proportion of mf that develops into L3 declines. The maximum number of L3 larvae developing in the mosquito was higher for *Aedes* (~23) than for *Culex* (~4). A different type of relationship has been hypothesized for the African *Anopheles* vector: the probability that mf develop into L3 larvae is lowest at low mf densities and increases with mf density ('facilitation'). Only at the highest mf densities, limiting mechanisms would get the upper hand. We did an explorative analysis, combining data from the few available studies, to quantify the uptake curve for *Anopheles* (**chapter 10.2**).

The differences between mosquito species are important when it comes to elimination. In the case of 'facilitation', mass treatment will have relatively strong impact on transmission intensity, because worm burdens and mean mf load are reduced and, in addition, a lower proportion of mf will develop successfully into adult worms. This helps elimination. The opposite is true in case of 'limitation', because transmission becomes more efficient at the lower levels.

Treatment efficacy

For realistic prediction of the long-term effects of mass treatment, accurate estimates of treatment effects on mf and adult worms are required. It is particularly relevant to know how the adult worms are affected, because any effect on transmission will be only temporary if adult worms survive and continue to produce mf. With currently available diagnostic methods, we cannot directly investigate the treatment effects on adult worms. We estimated these effects indirectly, by analyzing trends in mf density after treatment. We first investigated the efficacy of DEC and ivermectin (**chapter 8**). From the immediate reduction in mf intensity after treatment, we estimated that a single dose of DEC or ivermectin on average kills, respectively, 57% or 96% of mf. From the slow mf recurrence in the blood in the post-treatment period, we further concluded that these drugs reduce mf production by 67% and 82% on average, probably due to killing (DEC) or sterilization (ivermectin) of part of the adult worms. Especially the effects of DEC varied strongly between individuals, with some people responding poorly. Similar estimates for the currently used combinations of these drugs with albendazole were higher (**chapter 9**). The estimated reduction in mf production may be somewhat higher than the proportion of worms affected, because the mating probability of male and

female worms declines along with the number of worms. Our estimates are influenced by uncertainty about the mf life span and about the rate at which mf would recur in the blood if adult worms were not affected.

Conclusion and recommendations

We found that the prospects of lymphatic filariasis elimination by mass treatment are good for Pondicherry, India, especially if the very effective combination of DEC and albendazole is used. The for elimination required duration of mass treatment, however, depends strongly on the population coverage: 4 yearly rounds would be sufficient for elimination if 80% of the population is treated per round, but 2 more rounds would be required if only 65% can be treated. The accuracy of our predictions is influenced by uncertainty about the role of immunity and the efficacy of the treatment regimen. The efficiency of transmission differs markedly between mosquito species, which may have important implications for elimination. The results for Pondicherry should therefore not be generalized to other areas.

When the Global Programme to Eliminate Lymphatic Filariasis was started, it was hoped that 4-6 yearly rounds of mass treatment would be sufficient for elimination in most regions, provided that coverage is sufficiently high. However, because of differences in the mosquito species responsible for transmission and pretreatment mf prevalence levels, elimination prospects may vary widely between areas. Preliminary predictions for Africa illustrate this and give reason for concern (**chapter 10.2**). Mf prevalence in this region can be much higher (sometimes > 40%) than in India (usually < 20%). In African areas with about 10% mf prevalence, the number of treatment rounds required for elimination is similar to the Pondicherry-situation, assuming that the same drugs are used and that population coverage levels are similar. However, in areas with 30% or 40% mf prevalence, elimination prospects are not as good: even if 80% or 90% of the population is treated per round, the number of treatment rounds would be much larger than the expected 4-6. In some circumstances it may be advisable to shift focus to the more realistic goal of eliminating the disease as a public health problem, without necessarily eliminating the infection, even though this would require continuous control efforts.

Further research is required on the role of immunity, the efficacy of treatment, and the relationship between mf intensity in the human blood and the number of L3 larvae developing in different mosquito species. Future modelling-work should concentrate firstly on quantification of the model for other regions with different vectors. These models should be used to investigate issues that are of crucial importance for the ongoing Global Programme, including criteria for stopping mass treatment and cost-effective approaches to enhance programme effectiveness. Eventually, a more user-friendly version of the model should be developed and transferred to policy makers and programme managers for routine use in planning and evaluation of ongoing programmes for elimination of lymphatic filariasis.

Samenvatting

Lymfatische filariasis: infectie, ziekte en eliminatie

Lymfatische filariasis is een parasitaire infectieziekte, die veel voorkomt in tropische en subtropische gebieden en kan leiden tot ernstige misvorming en handicap. De ziekte is het gevolg van infectie met draadvormige wormpjes ('filaria'), meestal van de soort *Wuchereria bancrofti*. Deze wormen leven in het lymfestelsel van de mens en hebben een levensduur van 5 à 10 jaar. Als ze bevrucht zijn, kunnen vrouwelijke wormen gedurende hun leven miljoenen microfilariën (mf) produceren die in het bloed te vinden zijn. Deze mf kunnen ongeveer een jaar blijven leven, maar kunnen zich alleen verder ontwikkelen tot volwassen worm als ze worden opgenomen door een mug. In de mug ontwikkelen mf zich via verschillende stadia tot L3 larven. Deze L3 larven kunnen via een muggenbeet weer worden overgebracht op mensen en kunnen zich dan verder ontwikkelen tot een volwassen worm. Veel verschillende muggensoorten kunnen de infectie overbrengen.

Veel mensen zijn geïnfecteerd zonder het te weten. De infectie veroorzaakt echter schade aan het lymfesysteem waardoor de afvoer van weefselvloeistof in het lichaam verstoord wordt. Dit kan uiteindelijk leiden tot ernstige vergroting en misvorming van de ledematen of externe geslachtsorganen (lymfoedeem of, in het eindstadium, elefantiasis). Bij mannen zien we ook vaak vochtophoping in het scrotum, die daardoor ook grote afmetingen kan aannemen (hydrokèle). Een hydrokèle kan operatief verwijderd worden, maar vergevorderd lymfoedeem en elefantiasis zijn niet goed te behandelen. Deze chronische aandoeningen zijn een belangrijke oorzaak van invaliditeit en verminderde kwaliteit van leven. Naar schatting zijn wereldwijd 120 miljoen mensen geïnfecteerd met lymfatische filariasis, van wie meer dan 40 miljoen leiden aan lymfoedeem of hydrokèle.

Volksgezondheidsinterventies richten zich op het voorkomen van chronische ziekte door de infectielast in de populatie te verminderen. Daarbij kijkt men meestal naar het percentage mensen met mf in het bloed (mf prevalentie) en de gemiddelde concentratie van mf in het bloed (mf intensiteit). Mf prevalentie en intensiteit kunnen omlaag gebracht worden door de overdracht van infectie te verminderen met maatregelen tegen muggen. Tegenwoordig geeft men er echter de voorkeur aan om regelmatig alle mensen in een gebied te behandelen met medicijnen tegen de infectie. Daarbij kan men gebruik maken van diethylcarbamazine (DEC) of ivermectine, al dan niet in combinatie met albendazol. Een enkele dosis van deze medicijnen leidt al tot een langdurige reductie in de mf intensiteit in het bloed. Deze medicijnen zijn veilig, wat het mogelijk maakt om iedereen in een gebied tegelijkertijd te behandelen, zonder eerst te onderzoeken wie er geïnfecteerd zijn ('massabehandeling'). Dit is makkelijker, goedkoper en effectiever dan screening gevolgd door selectieve behandeling van geïnfecteerden. Jaarlijks herhaalde massabehandeling leidt tot zo een sterke daling in de mf prevalentie en intensiteit, dat de transmissie sterk verminderd wordt en de infectie mogelijk zelfs helemaal geëlimineerd

kan worden. Dit beseffend, nam de World Health Assembly in 1997 een resolutie aan die oproept tot de 'eliminatie van lymfatische filariasis als een volksgezondheidsprobleem' en werd een mondiaal programma voor eliminatie van lymfatische filariasis opgericht. In het kader van dit programma wordt er nu wereldwijd gestart met jaarlijkse massabehandeling.

Dit proefschrift

Men is optimistisch over de vooruitzichten op eliminatie van lymfatische filariasis. Het is echter onzeker hoe lang jaarlijkse massabehandeling door zou moeten gaan om eliminatie te bereiken en hoe de benodigde duur afhangt van het percentage daadwerkelijk behandelde mensen, de gebruikte medicijnen, de mf prevalentie voor de start van massabehandeling, en de soort mug die verantwoordelijk is voor de overdracht van infectie. Deze vragen staan centraal in dit proefschrift. Een groot deel van het werk is uitgevoerd in nauwe samenwerking met het Vector Control Research Centre (Indian Council of Medical Research) in Pondicherry, India.

Vooruitzichten op eliminatie

In het eerste deel van dit proefschrift gebruikten we een wiskundig simulatie model voor de overdracht van lymfatische filariasis om de lange termijn effecten van massabehandeling te voorspellen en te onderzoeken hoe lang massabehandeling voortgezet zou moeten worden om eliminatie te bewerkstelligen. Het gebruikte model, LYMFASIM, is eerder ontwikkeld op de afdeling Maatschappelijke Gezondheidszorg van het Erasmus MC, in samenwerking met onderzoekers van het Vector Control Research Centre in Pondicherry, India en het Centro de Pesquisas Aggeu Magalhães in Recife, Brazilië.

Ons model hebben we zo gekwantificeerd dat het de situatie in Pondicherry, India, beschrijft, waar lymfatische filariasis verspreid wordt door de muggensoort *Culex quinquefasciatus* (**hoofdstuk 2**). De modelparameters konden we kwantificeren met behulp van de vele gegevens die eerder verzameld waren voor de evaluatie van een 5 jaar durend programma voor muggenbestrijding dat in Pondicherry liep van 1981 tot 1986. Er waren individuele gegevens beschikbaar over de mf intensiteit voor en na de interventie. Daarnaast hadden we gegevens over het gemiddeld aantal muggenbeten per persoon per maand voor de hele periode van muggenbestrijding. Uit deze gegevens konden we afleiden dat volwassen wormen een gemiddelde levensduur van ongeveer 10 jaar hebben. Daarnaast vonden we dat mensen, na langdurige blootstelling aan infectie, waarschijnlijk een soort immuniteit ontwikkelen, die beschermt tegen nieuwe infecties of het gemiddeld aantal mf in het bloed sterk vermindert. Voorspellingen van het resulterende model kwamen goed overeen met waarnemingen in Pondicherry.

Het voor Pondicherry gekwantificeerde model hebben we vervolgens gebruikt om de lange termijn effecten van massabehandeling te voorspellen en om te onderzoeken hoe lang jaarlijkse massabehandeling door zou moeten gaan om eliminatie te bereiken

(**hoofdstuk 3** en **4**). We vonden dat de voor eliminatie benodigde duur sterk afhangt van het percentage mensen dat per keer behandeld wordt ('bereik'), de effectiviteit van de gebruikte medicijnen, en de mf prevalentie voor de start van de massabehandeling. In Pondicherry, waar de mf prevalentie ongeveer 8.5% was voor de start van massabehandeling, zou men 4 jaarlijkse rondes van massabehandeling met de combinatie van DEC en albendazol nodig hebben voor eliminatie als 80% van de bevolking wordt behandeld per ronde. Het bereik is echter vaak minder hoog. Bij een realistischer bereik van 65% zouden er 6 jaarlijkse rondes nodig zijn. Hierbij gaan we ervan uit dat een enkele behandeling met DEC en albendazol steeds 65% van de aanwezige volwassen wormen en 70% van de mf doodt. Massabehandeling zou langer moeten worden voortgezet als de gebruikte medicijnen minder effectief zijn of als mf prevalentie voor de eerste ronde van massabehandeling hoger is. Hoewel het misschien praktisch is om massabehandeling jaarlijks uit te voeren, zou de totale duur van een eliminatie programma sterk gereduceerd kunnen worden door het interval tussen behandelingen te verkorten.

De voorspellingen van ons LYMFASIM model hebben we vergeleken met die van een tweede model, EPIFIL, dat in Engeland is ontwikkeld door Michael c.s. De twee modellen geven vergelijkbare voorspellingen van de verandering in mf prevalentie en intensiteit tijdens een periode van massabehandeling. Het LYMFASIM model is echter meer gedetailleerd en kan daardoor meer realistische voorspellingen doen over het risico dat infectie weer terugkomt na het stoppen van massabehandeling (**hoofdstuk 5**).

Samenvattend kunnen we stellen dat de vooruitzichten op eliminatie van lymfatische filariasis door massabehandeling goed zijn voor Pondicherry, mits een voldoende groot percentage van de mensen bereikt wordt. Kwalitatieve conclusies over de invloed van het bereik van massabehandeling, behandelingseffecten, en mf prevalentie op de vooruitzichten op eliminatie zijn te generaliseren. Schattingen van het voor eliminatie benodigde aantal behandelingsrondes moeten echter voorzichtig geïnterpreteerd worden. Onze voorspellingen zijn mogelijk te optimistisch, omdat we geen rekening houden met variatie in de effectiviteit van behandeling tussen mensen, (her)introductie van infectie door geïnfecteerde immigranten, of de mogelijkheid dat de parasiet resistentie ontwikkelt tegen het geneesmiddel. Daarnaast zijn we niet zeker over de effectiviteit van de gebruikte medicijnen en over de processen en mechanismen die een rol spelen bij de transmissie van infectie. De voorspellingen kunnen niet zonder meer gegeneraliseerd worden naar gebieden waar een andere muggensoort verantwoordelijk is voor de transmissie van lymfatische filariasis, omdat verschillen in transmissie-efficiëntie tussen muggensoorten grote invloed kunnen hebben op de vooruitzichten op eliminatie.

Dynamiek van transmissie

Om de lange termijn effecten van massabehandeling nauwkeuriger te kunnen voorspellen, hebben we beter inzicht nodig in de processen die betrokken zijn bij de transmissie van infectie. Twee processen hebben we in detail onderzocht.

Ten eerste bestudeerden we de rol van immuniteit (**hoofdstuk 6**). In Pondicherry zagen we dat de gemiddelde mf intensiteit en prevalentie afnamen in oudere leeftijdsgroepen. Dit verklaarden we in ons model door aan te nemen dat oudere mensen, als gevolg van langdurige blootstelling aan infectie, een vorm van immuniteit hebben ontwikkeld die beschermt tegen nieuwe infecties of het aantal mf in het bloed vermindert. Als deze verklaring correct is, dan zouden we verwachten dat ook in andere gebieden de prevalentie en intensiteit van infectie afnemen bij oudere leeftijdsgroepen. Dit blijkt echter niet het geval te zijn. In een systematisch literatuur onderzoek vonden we dat de mf prevalentie meestal toeneemt met leeftijd totdat een min of meer stabiel niveau bereikt wordt op een leeftijd van ongeveer 20 jaar in India en 10 tot 15 jaar later in Afrika. Het leeftijdspatroon in Pondicherry was uitzonderlijk, wat tot twijfel leidt over de veronderstelde rol van immuniteit in lymfatische filariasis.

Ten tweede onderzochten we hoe het gemiddeld aantal infectieuze L3 larven dat zich ontwikkelt in een mug afhangt van de mf intensiteit in het bloed. Deze relatie verschilt tussen muggensoorten en kan bepalend zijn voor de kans op eliminatie. In **hoofdstuk 7** vergeleken we *Culex quinquefasciatus*, de belangrijkste vector van lymfatische filariasis in India, en *Aedes polynesiensis*, de vector van lymfatische filariasis in Frans Polynesië. Voor beide muggensoorten vonden we dat de transmissie van infectie het meest efficiënt is bij lage mf concentraties: een groot percentage van mf ontwikkelt zich in de mug tot L3 larven. Het gemiddeld aantal L3 larven per mug neemt niet lineair toe met de concentratie van mf in het bloed, maar gaat naar een maximum ('limitatie'): het percentage van mf dat zich ontwikkelt tot L3 neemt dus af. Het maximum was aanzienlijk hoger voor *Aedes polynesiensis* (~23) dan voor *Culex quinquefasiatus* (~4). Voor *Anopheles* muggensoorten die verantwoordelijk zijn voor de verspreiding van lymfatische filariasis in Afrika is de relatie waarschijnlijk omgekeerd: de kans dat een mf zich ontwikkelt tot L3 larve is juist het laagst bij lage mf intensiteit en neemt toe bij hogere intensiteiten ('facilitatie') tot op een gegeven moment de limiterende processen ook hier de overhand krijgen. Uit de literatuur zijn te weinig data beschikbaar voor nauwkeurige kwantificatie van deze relatie voor *Anopheles*, en onze analyses op dit gebied zijn exploratief (**hoofdstuk 10.2**).

De verschillen tussen muggensoorten zijn van belang als het gaat om eliminatie. In het geval van facilitatie (*Anopheles*) zal vermindering van de mf intensiteit in het bloed een relatief groot effect hebben op transmissie, omdat de kans dat mf zich ontwikkelen tot L3 afneemt. Dit is gunstig voor eliminatie. Het omgekeerde is juist het geval bij limitatie (*Aedes*, *Culex*), omdat de transmissie juist efficiënter wordt bij lagere concentraties van mf in het bloed.

Effectiviteit van medicijnen

Om de lange termijn impact van massabehandeling goed te kunnen voorspellen, hebben we nauwkeurige schattingen nodig van de effectiviteit van de medicijnen. Vooral het

effect op volwassen wormen is van belang, omdat massabehandeling slecht een tijdelijk effect op transmissie zal hebben wanneer de volwassen wormen niet zijn aangedaan en doorgaan met het produceren van mf. Met de huidige diagnostische methoden kunnen we helaas niet direct bepalen wat er met de volwassen worm gebeurt. Daarom gebruikten we een indirecte methode om de effecten van behandeling te kwantificeren, namelijk de analyse van trends in mf intensiteit na behandeling. Eerst onderzochten we de effectiviteit van DEC en ivermectine (**hoofdstuk 8**). Uit de sterke reductie in mf intensiteit in het bloed direct na behandeling, concludeerden we dat een enkele dosis van DEC of ivermectine leidt tot de dood van, respectievelijk, 57% of 96% van de mf. Uit de langzame toename van mf intensiteit in het bloed in het eerste jaar na behandeling, leidden we verder af dat deze medicijnen de mf productie met respectievelijk 67% en 82% reduceren, wat waarschijnlijk het gevolg is van dood (DEC) of sterilisatie (ivermectine) van de volwassen wormen. Met name de effectiviteit van DEC bleek sterk te kunnen variëren tussen individuen, en bij sommige individuen lijkt het medicijn nauwelijks effect te hebben. Vergelijkbare schattingen voor de aanbevolen combinaties van DEC of ivermectine met albendazol waren hoger (**hoofdstuk 9**). Deze geschatte afname in mf productie zou wat hoger kunnen zijn dan het percentage wormen dat aangedaan is, omdat de kans op bevruchting van een vrouwelijke worm afneemt bij lagere worm aantallen. Onze schattingen worden beïnvloed door onzekerheid over de levensduur van mf en over de snelheid waarmee mf in het bloed zouden terug keren als volwassen wormen niet zouden zijn aangedaan.

Conclusies en aanbevelingen

We vonden dat de vooruitzichten op eliminatie van lymfatische filariasis door massabehandeling goed zijn voor Pondicherry, India, met name als de zeer effectieve combinatie van DEC en albendazol gebruikt wordt. De benodigde duur voor eliminatie hangt echter sterk af van het bereik: waar 4 jaarlijkse rondes voldoende zouden kunnen zijn voor eliminatie als 80% van de bevolking bereikt wordt, zouden er al 6 rondes nodig zijn als slechts 65% van de bevolking bereikt wordt per ronde. De nauwkeurigheid van deze voorspellingen wordt beïnvloed door onzekerheid over de rol van immuniteit en de effectiviteit van de gebruikte medicijnen. We vonden grote verschillen tussen muggensoorten in de efficiëntie van transmissie, wat belangrijke consequenties kan hebben voor de vooruitzichten op eliminatie. De resultaten voor Pondicherry zijn daarom niet zonder meer generaliseerbaar naar andere gebieden.

Toen het mondiale programma voor eliminatie van lymfatische filariasis van start ging, hoopte men dat 4 tot 6 jaarlijkse rondes van massabehandeling meestal genoeg zouden zijn voor eliminatie, als het populatiebereik tenminste hoog genoeg is. Het is echter duidelijk dat de vooruitzichten op eliminatie sterk kunnen variëren tussen regio's, bijvoorbeeld door verschillen in de muggensoort die verantwoordelijk is voor transmissie of de mf prevalentie voor de start van behandeling. Verkennende analyses voor Afrika

illustreren dit en zijn zorgwekkend (**hoofdstuk 10.2**). De mf prevalentie kan in deze regio veel hoger zijn (tot >40%) dan in India (meestal <20%). Bij een lage mf prevalentie van ongeveer 10% waren de vooruitzichten op eliminatie in Afrika vergelijkbaar aan die in Pondicherry, aannemend dat even effectieve medicijnen gebruikt worden en een even groot deel van de bevolking bereikt wordt. Bij hogere mf prevalenties van 30% of 40% zijn de vooruitzichten minder gunstig: zelfs wanneer 80% of 90% van de bevolking behandeld wordt per ronde, zou het aantal rondes nodig voor eliminatie veel groter zijn dan de verwachte 4 tot 6. Soms is misschien beter om te concentreren op eliminatie van de ziekte als volksgezondheidsprobleem, zonder noodzakelijkerwijs de parasiet ook te elimineren. Dit zou echter wel betekenen dat er continu maatregelen nodig blijven om te zorgen dat de infectie niet terug komt.

Er is meer onderzoek nodig naar de rol van immuniteit, de effectiviteit van behandeling, en de relatie tussen mf intensiteit in het bloed van mensen en de ontwikkeling van L3 larven voor de verschillende muggensoorten. Verder werk met het model voor lymfatische filariasis zal zich in eerste instantie moeten concentreren op de kwantificatie van het model voor regio's waar andere muggensoorten verantwoordelijk zijn voor de overdracht van infectie. Deze modellen kunnen vervolgens toegepast worden om een aantal belangrijke vragen voor de eliminatie programma's te beantwoorden. De belangrijkste vraag is wanneer massabehandeling gestopt kan worden met minimaal risico dat infectie weer terugkomt. Daarnaast is het van belang om te onderzoeken op welke manier de effectiviteit van interventie op een kosteneffectieve manier verhoogd kan worden. Uiteindelijk zou er een meer gebruiksvriendelijke versie van het model ontwikkeld moeten worden, die door beleidsmakers en programmamanagers gebruikt kan worden in de routinematige planning en evaluatie van programma's ter bestrijding van lymfatische filariasis.

Acknowledgements

The work reported in this thesis would have been impossible without the intensive collaboration with the Vector Control Research Centre (VCRC) in Pondicherry (India). I enjoyed this collaboration and my visits to Pondicherry very much. In particular, I like to thank dr. PK Das, director of VCRC and a leading force in our international collaborative project, and dr. Pani, dr. Ramaiah and dr. Subramanian. Thank you for your valuable contributions to my work and for your kind hospitality during my visits to VCRC. It was a real pleasure to work with you! I further like to thank drs. Ravi, Krishnamoorthy, Amalraj, Shanti, Vanamail, and mrs. Srividya and other staff members of VCRC for their friendly hospitality.

I further like to thank drs. Hans Remme, Gautam Biswas and Sergio Yactayo, who always supported and guided our work on modelling on lymphatic filariasis and enabled collaboration with partners from different countries. I this respect, I also like to acknowledge the contributions made by several research partners in past and present international collaborative projects, including drs. Paul Simonsen, Dan Meyrowitsch, Gerusa Dreyer, Kazuyo Ichimori, Eric Ottesen, John Gyapong, Daniel Boakye, and Gary Weil. The contact with them was always pleasant and stimulating.

The work was financially supported by a series of grants from UNDP/World Bank/WHO special Programme for Research and Training in Tropical Diseases (TDR) (ID. no's. 920743, 950247, 970817, A20652) and a technical services agreement provided by the World Health Organization (Reg file: F3/181/61, ID FIL/03/01).

Dankwoord

Alleen mijn naam prijkt trots op de kaft van dit proefschrift, maar velen hebben er aan bijgedragen. Mijn proefschrift zou niet af zijn zonder een dankwoord.

Allereerst wil ik mijn promotor, Dik Habbema, noemen. Dik, net als veel van mijn voorgangers roem ik je scherpe inzicht. Met een simpele vraag of opmerking wist je ingewikkelde problemen terug te brengen tot begrijpelijke proporties. Je liet me vrij om mijn eigen weg te zoeken in het onderzoek. Bedankt daarvoor! Minstens zo belangrijk waren Anton Plaisier, Gerrit van Oortmarssen en Sake de Vlas. De titel 'co-promotor' is uiteindelijk gegaan naar Sake, maar die eer komt hen wat mij betreft alledrie in gelijke mate toe! Anton, met je grote didactische vaardigheid heb je me ingewijd in de lymfatische filariasis en het modelleren. Gerrit, jij was de technische man achter onze modellen en methoden. Ik heb veel geleerd van jouw systematische manier van denken en probleem-oplossen. Sake, pas in de eindfase van mijn onderzoek ben ik met jou gaan samenwerken. Bedankt voor je altijd snelle reactie op mijn vragen en geschreven teksten, maar bovenal voor je betrokkenheid en grote enthousiasme!

Veel dank ben ik verschuldigd aan Caspar Looman en Gerard Borsboom, bij wie ik altijd kon aankloppen voor hulp bij de statistiek. En dankzij de goede ondersteuning van het secretariaat (in het bijzonder Mirela), de automatisering en het financieel beheer waren er nooit praktische problemen bij de uitvoering van mijn onderzoek.

Het was leuk en leerzaam om in de beginfase van mijn onderzoek samen te kunnen werken met Annemarie Terhell en Maria Yazdanbakhsh van het Leiden Universitair Medisch Centrum. Het begeleiden van studenten—Suzanne Schellekens, Marlieke de Kraker en Roya Sharafi—was minstens zo leuk en leerzaam en daarnaast bijzonder stimulerend en motiverend!

Dan zijn er mijn paranimfen, Fanny en Anita: een erebaantje voor een supercollega en -vriendin! Fanny, als oud-collega vertegenwoordig jij vandaag de fijne collega's van MGZ. Dat zijn er teveel om op te noemen, maar een aantal mensen wil ik toch in het bijzonder danken: mijn kamergenoten van de laatste jaren Sita en Esther, 'goede buur' Bram, Annelies en alle andere (oud-)collega's van de sectie infectieziekten, Elsbeth en Hein. Bij jullie kon ik altijd terecht voor goede raad of gewoon een praatje. Anita, jij vertegenwoordigt vandaag mijn vriendenclub en met jou wil ik ook Femke, Mariska, Sabine, Nadinja, Max, Jeannette, Marjon, Willem, Andries, en Mirjam bedanken. Jullie volgden mijn werk met belangstelling en hielpen me om de boel te relativeren en af en toe te vergeten. Bedankt allemaal! Er is geen paranimf nodig om de familie te vertegenwoordigen: een ereplek is al voor jullie gereserveerd! Bedankt voor jullie belangstelling en vertrouwen!

☺

Curriculum vitae

Wilma Stolk was born on January 10, 1975 in Bunnik, The Netherlands. After graduating from the Christelijk Gymnasium in Utrecht in 1993, she started the study Biomedical Sciences at the University Medical Centre Nijmegen. With a major in epidemiology, she graduated *cum laude* in 1998. In 2001, she obtained her Master of Science degree in Health Services Research at the Netherlands Institute of Health Sciences. Since 1998, Wilma has been working at the Department of Public Health of Erasmus MC in Rotterdam. She first worked on the evaluation of cervical cancer screening, before starting her project on lymphatic filariasis in 1999. Her PhD-project focused on prediction of the long-term impact of control programmes for this tropical, parasitic disease, by means of mathematical modeling. She collaborated closely with scientists at the Vector Control Research Centre in Pondicherry, India, and worked with representatives of the Global Programme to Eliminate Lymphatic Filariasis. Her present research activities at the Department of Public Health cover both lymphatic filariasis and river blindness.