Brij Kishore Tyagi *Editor*

Lymphatic Filariasis

Epidemiology, Treatment and Prevention - The Indian Perspective

Springer

Lymphatic Filariasis

Brij Kishore Tyagi
Editor

Lymphatic Filariasis

Epidemiology, Treatment and Prevention – The Indian Perspective

Springer

Editor
Brij Kishore Tyagi
Advisor, SpoRIC
VIT University
Vellore, Tamil Nadu, India

ISBN 978-981-13-1390-5 ISBN 978-981-13-1391-2 (eBook)
https://doi.org/10.1007/978-981-13-1391-2

Library of Congress Control Number: 2018954897

© Springer Nature Singapore Pte Ltd. 2018
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Dedicated to
my colleagues
and
India's two unparalleled filariasis experts
Dr. V. Kumaraswamy and Dr. R. Rajendran

Foreword

This timely book conveys the scale and scope of contemporary expertise on human lymphatic filariasis (LF) in India. Professor Tyagi brings together 24 diverse chapters by 54 authors whose knowledge and research span much wider range of interests than are being applied in the Global Programme to Eliminate Lymphatic Filariasis (GPELF) launched in year 2000, technically led by the World Health Organization (WHO) following World Health Assembly Resolution 50.29 "Elimination of lymphatic filariasis as a public health problem" supported by the Global Alliance to Eliminate Lymphatic Filariasis (GAELF).

Among more than 80 countries where LF was endemic until the end of the twentieth century, India had the most human cases and continues with the greatest national LF burden, while LF has already been eliminated as a public health problem in at least 20 countries, including neighbouring China and Sri Lanka. The GPELF strategy relies upon complete coverage of the human population with annual free mass drug administration (MDA) for at least 5 years, by giving oral diethylcarbamazine citrate (DEC 6 mg/kg) together with ivermectin (200 mcg/kg) or albendazole (400 mg) yielding general anthelmintic benefits. Also therapeutic treatment (morbidity management) is provided for patients with symptomatic elephantiasis, sometimes involving surgical alleviation. Indian ayurvedic traditions bring additional benefits to LF sufferers but may reduce compliance with effective MDA by the community, perhaps accounting for disappointing coverage rates in many districts. Even so, by year 2016, successful coverage was withdrawn from 100/130 implementation units nation-wide, harbouring 272/360 million Indian population where LF prevalence had been suppressed below the target 1% threshold.

Hence, Indian progress of the current PELF greatly exceeds the gains from the original National Filariasis Control Programme (NFCP, launched 1955) based on DEC plus vector control against *Culex quinquefasciatus* transmitted *Wuchereria bancrofti* (Bancroftian filariasis) and *Mansonia* mosquito vectors of *Brugia malayi* (Brugian filariasis). Since 2003, NFCP was incorporated into the National Vector-Borne Diseases Control Programme (NVBDCP) with Integrated Vector Management (IVM) having multi-disease impact. Chapters in this book augment the efforts and accomplishments of dedicated Filariasis Research Cells in two centres of the Indian Council of Medical Research (ICMR), namely the Centre for Research in Medical Entomology (CRME at Madurai, established 1985) and the Vector Control Research Centre (VCRC at Puducherry, established 1975).

Bringing innovative scientific technologies and state-of-the-art sociological solutions to the fore, the contents of this book are intended to illuminate some neglected aspects of the LF situation in India, including its unusual epidemiology in Andaman and Nicobar archipelagos; to describe some success stories where interventions have made exceptional gains; and thus to encourage and expedite more efficient implementation of the NPELF to the level where LF cases will die out from all Indian foci, transmission will be suppressed below the threshold for new cases to occur and LF will be eliminated as a public health problem in all states of India.

Our focus on filariasis remains vital during this decade while neglected tropical diseases are being bundled into multi-disease control programmes that should enhance operational efficiency, but risk losing specific expertise for dealing with such an intractable infection and its epidemiology.

Graham B. White
Emeritus Professor, Entomology & Nematology
University of Florida IFAS,
Gainesville, FL, USA
22nd May, 2018

Preface

Lymphatic filariasis (LF) is a group of human and animal infectious diseases caused by nematode parasites of the order Filariidae, commonly called filariae. This disease is one of the oldest and the most debilitating neglected tropical diseases (NTDs) transmitted from man to man by the bites of mosquitoes, particularly the brown-black one called *Culex quinquefasciatus*. LF is a major public health problem in many parts of the tropics, especially India which contributes as much as 40% of the total global disease burden. As a leading cause of permanent and long-term disability worldwide, the parasite infection imposes a severe physical and socioeconomic burden. In terms of economics, the burden lymphatic filariasis exacts worldwide is painfully astronomical. The World Health Organization (WHO) estimates that nearly 1.4 billion people live in high-risk areas, 120 million of which already infected with LF, and 76 million people suffering from damaged lymphatic and renal systems. Painfully, about 22 million children below 15 years of age are infected with the disease.

Southeast Asia region harbouring India as a major endemic nation is estimated with approximately 700 million people living in endemic areas constituting about 64% of the global burden with about 60 million persons (50% of the global burden) either harbouring microfilaria or suffering from clinical manifestation. All the three LF parasites, viz. *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori* are prevalent in the region. *Bancroftian filariasis* transmitted by the ubiquitous principal vector, *Culex quinquefasciatus*, is the most predominant infection in the continental Asia, while Malayan infections caused by *Brugia malayi* and transmitted by *Mansonia* and *Anopheles* species are largely endemic currently in the Kerala State, peninsular India. *Brugia timori*-related filariasis does not occur in India and is predominantly present in the Indonesian Archipelago region.

Following the epoch-making advances in the field of diagnosis and treatment of the disease, the World Health Organization classified in 1997 lymphatic filariasis, along with five other infectious diseases, as eradicable or potentially eradicable. In the same year, the World Health Assembly adopted Resolution WHA 50.29, which called on Member States to initiate steps to eliminate lymphatic filariasis as a public health problem. In response to this call, the WHO launched in 2000 the Global Programme to Eliminate Lymphatic Filariasis (GPELF), of which India was a signatory, to eliminate LF as public health problem by 2020 with the following twin strategy:

1. Interrupting transmission through annual large-scale treatment programme, known as mass drug administration, implemented to cover the entire at risk population
2. Alleviating the suffering caused by LF through morbidity management and disability prevention

Being a major endemic country, the Government of India launched mass drug administration (MDA), with diethylcarbamazine citrate (DEC), foremost as a pilot project in 13 districts of 7 states in 1996. The Government of India's National Vector Borne Disease Control Programme (NVBDCP), which monitored the pilot study, upscaled the MDA to cover a population of 77 million in 2002 from 41 million during 1996–1997. Morbidity management and disability prevention are of vital public health importance and largely focus on training healthcare workers and community to dispense proper care and treatment. Only nocturnally periodic *W. bancrofti* in most endemic urban areas is being transmitted through *Cx. quinquefasciatus* bites, and it was estimated that about 2,765 infective bites would be the average exposure leading to infection in humans.

Challenges that beset India were manifold: (1) It pronounced to eliminate disease under the National Programme to Eliminate Lymphatic Filariasis (NPELF) 5 years in advance to the global scheme of elimination, i.e. by 2015 (which, in spite of achieving phenomenal success in most parts of the country, could not be eventually achieved as envisaged originally, and the next most practical date was fixed as 2018). (2) The MDA did not progress to show higher than 50% drug intake albeit appreciably high rate of drug distribution and community coverage. (3) All States/UTs did not follow uniform, regular MDAs, consequently leaving big and frequent gaps of non-distribution of the drug. (4) Populations that were endemic but left out or under-drugged for inexplainable reasons resultantly formed the 'hot spots' that manifested far higher microfilaria (*mf*) rate than the national cut-off level (1%). India demonstrated enormous progress in course of its endeavour to eliminate disease before 2015, but, sadly enough, fell far short of expectation. Close to 100 districts with huge populations live in districts highly endemic for lymphatic filariasis of 'hot spots' which proved the biggest handicap in the country's efforts to declare it free from the disease under the Global Programme to Eliminate Lymphatic Filariasis (GPELF)! To overcome this constraint, India had launched Technical Assessment Survey (TAS) to enumerate high *mf*-endemic areas in the country and implemented yet another MDA on 10 August 2016. Would this be the starting point of Ariadne's thread that would eventually lead to the exit from the labyrinth of human filaria and lay the foundations for scientifically based disease elimination, likely with the support of vector control, in the country?

Although continuously affected adversely, nevertheless, India has made great strides on many grounds in order to exercise elimination of the disease. These advancements are of global significance be it a mechanism to early diagnose a case detection, vaccine product development, and detection of parasites in the vector to study trend of the parasite evolution in nature as a warning signal. All these failures and successes need to be recounted so as to take stern measures in the future to not

repeat the disaster once again and see a smooth transition of India's endemicity to the elimination of lymphatic filariasis.

This book, which brings in here highly authentic contributions on various different subjects of lymphatic filariasis, will be an attempt to update all our knowledge on the diverse scientific inputs that have helped the nation to achieve this milestone which will be a guiding force to many other countries constantly struggling against this highly debilitating and incapacitating disease. Lymphatic filariasis, one of the most incapacitating and disfiguring vector-borne infections to the human being, will thus be the first mosquito-borne disease which will soon be declared eliminated from India and the rest of the world.

Vellore, Tamil Nadu, India
31 August 2016

Brij Kishore Tyagi

Acknowledgement

Foremost, I immensely thank the various contributors of different articles for this book, for without their timely and strong sense of commitment, it would not have been possible to give the book its present shape – highly interesting both in its content and the novelty!

In completing the rare task of editing this book, *Lymphatic Filariasis: Epidemiology, Treatment and Prevention – The Indian Perspective*, I have received generous help from many scientists and academicians from all over the world, and I feel extremely grateful to all of them.

As always in the past, I have received enormous moral support from my wife, Ajita, who has been by my side in all the difficult times that such a rare task is naturally bound to encounter!

Last but not the least, I am highly thankful to my publisher, Springer, who has put in inexorable sense of trust in my potential to complete the book in time and for bringing out the book sooner.

Contents

About the Editor

Professor Dr. B. K. Tyagi (b. 23rd July, 1951) completed M.Sc. (Zoology) from Meerut University in 1974 and Ph.D. (Entomology/Zoology) from Garhwal University in 1978. He emeritated from ICMR in 2013, after more three decades' highly distinguished career, as Scientist 'G' (Director) & Director in-Charge at the Centre for Research in Medical Entomology, Madurai. Post-emeritation he has served as a Visiting Fellow & Professor at Bharathidasan University, Tiruchirapalli, TN, and Punjabi University, Patiala (Punjab). *Inter alia* he has been also serving as a Core Faculty of the DST-SERB School of Insect Biology at Hyderabad University, Hyderabad, and a Guest Faculty, MLS University, Udaipur. Currently, Dr. Tyagi is serving as the Advisor, SpoRIC, VIT University, Vellore, TN.

Dr. Tyagi has many achievements to his credit. With over 605 scientific titles, plus 33 books, including a WHO's Training Manual, *"Biosafety for human health and the environment in the context of the potential use of genetically modified mosquitoes (GMMs): A tool for biosafety training based on courses in Africa, Asia and Latin America, 2008–2011"*, he also holds a patent for the invention of a 'mechanical mosquito sampler' (Indian Patent # 191635). He has won the WHO-TDR First Prize in a worldwide competition in 1995 and the ICMR's prestigious Dr. MOT Iyengar Memorial Award for 2008. He has also served as the First National Representative in-Charge, National Office in India in 1981 onwards for the International Odonatological Society and as a Member in-Charge of the Odonata Specialist Group, Species Survival Commission (IUCN) in 1985 onwards. He is an editor or member of Editorial Board for >3 dozen international/national journals. He has researched extensively on all important vector-borne diseases prevalent in India and was rated amongst the top 15 most productive authors on dengue for the period 2004–2012. He is an expert member on various research committees of ICMR, DRDO, DST, DBT-RCGM etc. He was an Expert Member of the Performance Evaluation Team for the NVBDCP (GOI) in 2008 and 2013. Currently, he is President of (i) Society of Medical Arthropodology, (ii) Jodhpur Natural History Society, and (iii) Indian Dragonfly Society. He is credited to originate serial annual Conferences of Medical Arthropodology, commencing 2007, for the first time in the world. He represented India in preparing the OECD's Consensus Document on the Dengue/Yellow Fever vector mosquito, *Aedes aegypti* Linn.

Epidemiology of Lymphatic Filariasis

1

P. L. Joshi

Abstract

Human lymphatic filariasis (LF), commonly known as elephantiasis due to appearance of elephantoid lower limbs, is caused by nematode parasites (roundworms) of the order Filariidae comprising thread-like structure. Lymphatic filariasis is transmitted from man to man by the bites of mosquitoes, particularly the brown-black common house mosquito, *Culex quinquefasciatus* Say, 1823. Categorized as an NTD (neglected tropical disease), it nevertheless is a major public health problem in many parts of the tropics where it imposes a severe physical, psychological and socioeconomic burden in approximately 1.4 billion people living in endemic regions. About 120 million are currently infected with LF, while nearly 76 million people suffer from damaged lymphatic and renal systems, besides 44 million people suffering symptomatically with lymphoedema, hydrocele and elephantiasis. More than 50 countries are running programmes with the objective to reduce parasite transmission and decrease the risk of infection for people living in or visiting these communities in endemic countries. India is a signatory to the World Health Assembly resolution to achieve the elimination of lymphatic filariasis by 2020 through a nationwide mass drug administration (MDA) commencing in 250 endemic districts, covering 600 million people and deploying initially diethylcarbamazine citrate (DEC) alone in 2004 and subsequently co-administering DEC with albendazole (Alb) in 2007. So far ten rounds of MDA have been implemented in the endemic states, though not without many pitfalls. The success achieved thus far is outstanding and has kindled a hope to eliminate the disease in the next few years.

P. L. Joshi (✉)
National Vector Borne Diseases Control Programme, Ministry of Health and Family Welfare, Government of India, Delhi, India

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_1

1.1 Introduction

Mosquito-borne diseases such as malaria, filariasis, dengue, Zika virus, chikungunya, yellow fever and encephalitides of various kinds are responsible for death of over one million people every year, while hundreds of millions are left to experience pain and suffering from these illnesses that not only emaciate health of people and impoverish economy of nations but also sap off the intelligentsia from their youth (Tyagi 1994). These diseases affect poor the most for various reasons. Among all the known mosquito-borne diseases, lymphatic filariasis (LF), a neglected tropical disease, is one of the most debilitating and economically draining infections. Although the disease rarely kills, it is nevertheless a single major cause of acute and chronic morbidity and disability. It is thus a major impediment worldwide in the socioeconomic development in as many as 73 countries in Africa, Southeast Asia, the Americas and the Pacific region. As of 2016, preventive chemotherapy (PC) to eliminate the transmission of LF infection was considered required in 53 out of 72 endemic countries. Due to the tremendous efforts of national programmes, more than 6.7 billion treatments have been delivered worldwide in 64 countries since the Global Programme to Eliminate Lymphatic Filariasis (GPELF) was launched in 2000 (WHO 2013). Twenty countries have reduced infection prevalence to levels at which transmission is assumed not to be sustainable. Nine of these countries have now been acknowledged as achieving the elimination of LF as a public health problem. As far as India is concerned, despite a huge progress made in the reduction of infection (microfilariae) prevalence, the disease is still rampant and considered highly important.

Lymphatic filariasis infection occurs through prolonged exposure to mosquito bites from several species belonging to genera, such as *Culex*, *Mansonia*, *Aedes* and even *Anopheles*. Among all known vectors, *Culex quinquefasciatus* is the most important being genetically highly susceptible to the parasites of lymphatic filariasis, ubiquitous in distribution in tropics and subtropics, and its adaptation to breed in polluted waters resultant mainly of unplanned urbanization, growing population pressure and lack of sanitation. Three parasites cause human lymphatic filariasis, viz. *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*, but it is particularly *W. bancrofti* which is responsible for over 90% of infections globally, while *B. malayi* is mostly involved in transmission of the remainder. The third parasite, *B. timori*, is but restricted to a few countries in Southeast Asia. Table 1.1 shows prevalence of the infection in the WHO's endemic regions or countries.

Table 1.1 Prevalence of infection in the WHO endemic regions or countries

S. No.	WHO's LF endemic regions/countries	Prevalence (%)
1.	WHO Southeast Asia region	66
2.	African region	33
3.	Four south American endemic countries, viz. Haiti, the Dominican Republic, Guyana and Brazil	1

About 66% of those at risk of infection live in the WHO Southeast Asia region and 33% in the African region. Four South American endemic countries, viz. Haiti, the Dominican Republic, Guyana and Brazil, together contribute about 1%.

The filarial parasite is transmitted from person to person through mosquito bites, particularly those of *Cx. quinquefasciatus* Say, 1823. Lymphatic vessels harbour the adult worms who live for an average of 5–7 years and ultimately disrupt the normal function of the lymphatic system. Opportunistic mating of these worms may produce during their lifetime millions of microfilariae (immature larvae) that circulate in the blood. During the night-time, *W. bancrofti* microfilariae are evenly distributed throughout the blood, and they are thus available for ingestion and transmission by mosquitoes. The 24 h cycle of the microfilariae is orientated to the 24 h cycle of the host; and some rhythmic change in the host acts as a cue to the microfilariae. Each microfilaria has a weak endogenous circadian rhythm of its own, but the rhythms of the individual microfilariae are dominated by that of the host, so that all the different individuals do approximately the same thing at the same time, and they do it at the right time (i.e. conducive for transmission). *Wuchereria bancrofti* microfilariae respond appropriately to the stimuli, and they depend on different arrangements for the maintenance of their rhythms. *Wuchereria bancrofti* and *B. malayi* microfilariae depend upon the absolute size of the venous-arterial (VA) difference in oxygen tension ('oxygen barrier') which is lower by night (e.g. 40 mmHg) than it is by day (e.g. 55 mmHg), and so the microfilariae pass through the lungs by night but accumulate there by day. If at night the patient is caused to breathe oxygen, the arterial oxygen rises; or if he is caused to take vigorous muscular exercise, the venous oxygen tension falls; in both cases the VA difference becomes greater, and the microfilariae accumulate in the lungs. The behaviour of microfilariae in blood is adapted to promote transmission by arranging the maximum number of microfilariae in the peripheral blood at times when the arthropod vector, precisely the mosquito *Cx. quinquefasciatus*, is likely to bite. The most sophisticated arrangement to achieve this is by a 24-h rhythm – the classical 'periodicity'. These microfilariae circulate for many weeks in the infected person's blood and infect the mosquito when she bites this infected person. In the mosquito microfilaria further complete their growth and development as L_1, L_2, and L_3 in a time period little over a week, without undergoing any multiplication process. The L_3 stage of the parasite is the infective stage resting in wait in the buccal cavity before transfer to a human host. When the mosquito bites another person, the larval worm, L_3, traversing the integument, finally reach the lymph vessels where they develop into adult worms through approximately 6 months or more. Microfilarimic humans can serve as a source of infection to others through bites of vector mosquito, *Cx. quinquefasciatus*.

1.2 Filariasis Control Globally and in India

The Global Alliance to Eliminate Lymphatic Filariasis (GAELF), established in 2000, was formally raised with the sole purpose of providing fundraising, advocacy, communications and technical assistance to the World Health Organization-based

Global Programme to Eliminate Lymphatic Filariasis (GPELF), with two goals: (1) the elimination of LF as a public health problem by 2020 and (2) the alleviation of physical, social and economic hardship in individuals who have LF-induced disability. The GPELF has proposed two comprehensive strategies in the control of LF, namely, as follows:

1. *Mass drug administration* (MDA): A combination of two medicines was delivered to entire populations at risk and morbidity management and disability prevention. This involves four steps: (1) mapping, (2) mass drug administration (MDA), (3) post-MDA surveillance, and (4) verification of elimination.

   The main intervention strategy comprised mass drug administration involving combination of diethylcarbamazine (DEC) (or ivermectin in countries where onchocerciasis is endemic) with the anthelminthic albendazole (Alb) to entire communities in districts where the microfilaria (*mf*) prevalence is equal or more than 1% and morbidity management to alleviate suffering and prevent disability of those affected by the disease. During 2000–2011, a cumulative targeted population of 952 million people, including 22 million children below 15 years of age who are also infected with the disease was administered more than 3.9 billion doses of medicine. In many countries, particularly those in Africa, the MDA was supplemented by vector control with the aim to reduce exposure to mosquitoes and prevent any possible reversal in areas where the disease was eliminated.
2. *Vector control*: Despite significant success in reducing vector population, on one hand, and depleting microfilaraemia, on the other, as maximally exemplified by long-term investigations in India (Sunish et al. 2006) and many of endemic African nations, the national programmes, particularly so in India, nevertheless lack uniform guidance on the use of vector control as an supplemental strategy along with the MDA.

In 1955 India launched its National Filaria Control Programme (NFCP) with the objective of delimiting the problem, to undertake control measures in endemic areas. The NFCP was assessed through Assessment Committee at different points of time, and the revised programme was launched in 1996–1997 in 13 districts in 7 endemic states, viz. Andhra Pradesh, Bihar, Kerala, Orissa, Uttar Pradesh, Tamil Nadu and West Bengal. The case of Tamil Nadu (where the NFCP started in 1957) draws special attention due to its spectacular achievements in eliminating the disease much earlier than many of other equally endemic states.

During the last decade, India has made a big leap forwards in developing some more effective means and tools for diagnosis and treatment of filariasis. Consequently, the current strategy for filariasis elimination aims at (1) infection transmission control through MDA and (2) individual patient management for disease control. It has been estimated that annual single-dose co-administration of two drugs (diethylcarbamazine + albendazole) reduces blood microfilariae by 99% for a full year. In this manner nearly 500 million people at risk of LF are intended to be made free from the disease by the end of 2018.

Table 1.2 MDA coverage of population endemic to LF

Sl. No.	Coverage of endemic countries (total 53/72)	MDA rounds completed	Status
1	37	5 or more	Good
2	12	5 or more	Moved to post-MDA surveillance phase
3	Several countries (like Japan, China, South Korea, the Solomon Islands, Egypt and Togo)	5 or more	Brought down the microfilaraemia lower than 1%

At international scene this initiative of India, advertised as 'Hathipaon Mukt Bharat' (Filaria Free India), is considered the largest ever wherein 400 million people will be provided with the prophylactic drugs under the supervision of the Ministry of Health and Family Welfare in partnership with the Global Network for Neglected Tropical Diseases (an initiative of the Sabin Vaccine Institute, Washington, USA). The online version of this campaign including a film entitled 'Giant footprints!' was launched in January 2015. Now, this online-based campaign is being disseminated across India through regional media channels and print-based advertisements. The achievement of drug administration programme against LF depends on the upgrading of coverage and compliance of the drugs which revealed the necessity of awareness campaign that can disseminate the message in the society. This campaign will support India to successfully implement MDA initiatives by delivering preventive medication to high-risk population in 17 states. The 'clean India' movement, emphasizing the importance of cleanliness and sanitary conditions in India, also supports the control of diseases including LF associated with poor sanitations.

More than 50 countries across the world are carrying on programmes to eliminate lymphatic filariasis and reduce the transmission of the filarial parasites as well as decrease the risk of infection. However, only China and South Korea have been verified by WHO for achieving elimination of LF (WHO 2008), besides Maldives and Sri Lanka within the WHOSEA Region who have also recently eliminated lymphatic filariasis. Several rounds of mass drug administration has been ascertained to decrease parasite infection in human, with optimum human population coverage under the MDA, and mosquito population (Chu et al. 2013). India, having brought down microfilaraemia below 1% in many states, is currently very much there on its way to achieve the goal of elimination in a couple of years (Table 1.2).

1.3 Geographical Distribution

More than 90% of 120 million people in 73 countries in sub-Saharan Africa, Southeast Asia, the Indian subcontinent, Pacific islands and focal areas of Latin America and the Caribbean (including Haiti) are infected with *W. bancrofti*, whereas *Brugia malayi* occurs mainly in China, India, Malaysia, the Philippines, Indonesia and various Pacific islands. The third parasite *B. timori*, the least common of all,

Table 1.3 Top five endemic states and union territories in India

Sl. no.	State/UT	% share of cases
1	Bihar	>17
2	Kerala	15.7
3	Uttar Pradesh	14.6
4	Andhra Pradesh and Tamil Nadu	10
5	Assam	5

occurs outside India on the Timor island of Indonesia. More than 40 million infected individuals are debilitated by this disease (GPELF 2011), including approximately 25 million (60%) suffering with genital diseases, such as hydrocele, and remaining 15 million (40%), mostly women, have lymphoedema or elephantiasis of the leg. Disease burden due to lymphatic burden has been estimated on the global level, but fewer attempts have been made with reference to India which accounts for nearly 40% of the global prevalence of infection and over US$ 840 million (INR 58,000 mill.) invested annually on treatment and caring working days which could be as high as 29 days per year (Ramaiah et al. 2000; Babu et al. 2006).

Cases of filariasis have been recorded from Andhra Pradesh, Assam, Bihar, Chhattisgarh, Goa, Jharkhand, Karnataka, Gujarat, Kerala, Madhya Pradesh, Maharashtra, Orissa, Tamil Nadu, Telangana, Uttar Pradesh, West Bengal, Pondicherry, Andaman and Nicobar Islands, Daman and Diu, Dadra and Nagar Haveli and Lakshadweep. The North-Western States/UTs, namely, Jammu and Kashmir, Himachal Pradesh, Punjab, Haryana, Chandigarh, Rajasthan, Delhi and Uttaranchal and North-Eastern States, viz. Sikkim, Arunachal Pradesh, Nagaland, Meghalaya, Mizoram, Manipur and Tripura, are known to be free from indigenously acquired filarial infection. About 553 million people (146 million urban and 407 million rural) in 257 districts (62% of the surveyed) in 17 states and 6 union territories were found endemic. Parasitologically about 31 million people carried *mf*, and over 23 million suffered from some kind of filarial disease (WHO 2005). In India, 99.4% of the cases are caused *Wuchereria bancrofti*, whereas *Brugia malayi* is responsible for 0.6% of the infection. *Brugia malayi* is mostly prevalent in Kerala Trichur (Thrissur), Ernakulum, Alleppey (Alappuzha), Quilon and Trivandrum (Thiruvananthapuram), besides Tamil Nadu, Andhra Pradesh, Orissa, Madhya Pradesh, Assam and West Bengal. Table 1.3 depicts some of the endemic districts with high proportion of *mf* cases.

The *W. bancrofti* driven endemicity is low in Goa (<1%), followed by Lakshadweep (1.8%) and Madhya Pradesh (above 3%).

India is signatory to the World Health Assembly resolution under which it has resolved to achieve the national goal of LF elimination by 2015 (now 2018). Therefore, it launched a nationwide MDA programme in 2004 covering all the 250 endemic districts. The target population was about 600 million. Annual MDA was started with diethylcarbamazine citrate (DEC) alone and subsequently co-administration of DEC with albendazole was followed since 2007. Baseline (2004) microfilariae (mf) prevalence assessed in the sentinel and spot-checks sites showed that it ranged from 0.06 to 4.2% in different districts selected for MDA, the average being 1.3%. There were 161 (68%) districts with >1% *mf* prevalence (district

average), and the remaining recorded <1%. In 2013, the overall microfilaria rate was reduced to 0.29%. However, 51 districts are still recording >1% *mf* prevalence. The extrapolation of *mf* prevalence to the total population at risk in different states showed that there were 10.37 million *mf* carriers in 2004, and in 2013 it was only 4.23 million, with a reduction of 59.2%. The reduction in parasite load in the community which is the primary objective of MDA in interrupting transmission can be considered as the impact of the programme. Twelve districts completed more than five rounds of MDA with an effective coverage of >65% and *mf* prevalence <1% in the entire sentinel and spot-check sites which were subjected to transmission assessment survey (TAS) whereby all of them qualified for stopping MDA (Sabesan et al. 2000; VCRC 2014).

1.4 Transmission Cycle

Worldwide over two dozen mosquito species belonging to a variety of genera are vectors of *W. bancrofti* filariasis depending on geographical distribution, namely, *Culex* (*C. annulirostris*, *C. bitaeniorhynchus*, *C. quinquefasciatus* and *C. pipiens*), *Anopheles* (*An. arabiensis*, *An. bancroftii*, *An. farauti*, *An. funestus*, *An. gambiae*, *An. koliensis*, *An. melas*, *An. merus*, *An. punctulatus* and *An. wellcomei)*, *Aedes* (*Ae. aegypti*, *Ae. aquasalis*, *Ae. bellator*, *Ae. cooki*, *Ae. darlingi*, *Ae. kochi*, *Ae. polynesiensis*, *Ae. pseudoscutellaris*, *Ae. rotumae*, *Ae. scapularis* and *Ae. vigilax*), *Mansonia* (*Ma. pseudotitillans*, *Ma. uniformis*) and *Coquillettidia* (*Co. juxtamansonia*). While mosquito is an intermediate host of bancroftian and Malayan types of filariasis, man is the definite host in whom pathology exhibits.

The process of LF transmission begins with the blood meal of an infected mosquito on human being. An infected mosquito injects third-stage larvae onto the skin of the human host, where they penetrate through bite wound to the circulatory system. Larvae from mosquito develop into adults: females measuring 80–100 mm in length and 0.24–0.30 mm in diameter, while the males being about 40 mm long and by 0.1 mm wide. The adult worms of both *W. bancrofti* and *B. malayi* live in the lymph canals and lymph nodes of man from 5 to 18 years or even 40 years (Carme and Laigret 1979; Vanamail et al. 1989, 1996). Adult female produce ova from which emerge sheathed microfilariae (*mf*), measuring 244–296 μm long 7.5–10 μm wide, in the peripheral blood 6 months to 1 year after infection (Mak 1987). Females are viviparous and give birth to as many as 50,000 *mf* per day which finds their way to blood circulation. The *mf* can live for probably 1 year or more. The *mf* remains in the arterioles of the lungs during the day and come out at night (nocturnally periodic). At this time if a mosquito feeds on an infected person, then it also becomes infected. The larvae work their way through the wall of the proventriculus and cardiac portion of the mosquito's midgut and reach the thoracic muscles. There the microfilariae develop into first-stage larvae and subsequently into second- and finally third-stage infective larvae which is also the infective stage which migrate through the hemocoel to the mosquito's proboscis ready to infect another human when the mosquito takes a blood meal.

Table 1.4 Duration of development of *mf* to infective stage varies between species

Parasite	Duration of development of *mf* (in days)
W. bancrofti	10–14
B. Malayi	7–0

The following stages of development take place in the vector:

(a) *Exsheathing*: After reaching midgut the process of exsheathing comes about whereupon larva comes out of the sheath in which it is enclosed within 1 to 2 h of ingestion. This is known as exsheathing which takes place in the stomach of the mosquito.
(b) *First-stage larva* (L_1): The free larva, L_1, is able to penetrate the stomach wall of the mosquito where it grows and develops into a sausage-shaped (short thick) form, called L_2.
(c) *Second-stage larva* (L_2): The L_2 larva increases in length (long thick form) with the development of an alimentary canal but is relatively inactive, the L_3.
(d) *Third-stage larva* (L_3): This is the final stage of infective larva (long, thin form) which may be found in any part of the insect. It is highly active or motile. When it migrates to the proboscis of the mosquito it is ready to be transmitted in a new host, and the mosquito is said to be infective. The duration of development of *mf* to infective stage varies between species, as shown in the Table 1.4.

The shortest prepatent period is estimated at about 9 months for *W. bancrofti*, while 3 months for *B. malayi*. The appearance of *mf* depends upon the mating probability of adult worms, prepatent period and the intensity of transmission.

1.5 Reservoir of Infection

The reservoir or source of infection is a person with circulating *mf* in peripheral blood. The microfilarial carriers are usually without any recognizable symptoms of illness. As a rule, while *Cx. quinquefasciatus* transmitted microfilariae of *W. bancrofti* appear nocturnally in the peripheral blood (e.g. mainland India), those which are transmitted by *Ochlerotatus* (*Aedes*) *niveus* orient in the peripheral blood only diurnally (e.g. Andaman and Nicobar Islands). Those with advanced disease (occult cases) often turn out to be negative for *mf*.

1.6 The Vectors

Depending on the geographic area, an array of mosquitoes are able to transmit the parasite, e.g. in Africa, the most common vector is an *Anopheles* species and in the Americas and Asia, it is *Cx. quinquefasciatus*, whereas in Pacific as well as Asia, many species of the genera *Aedes* and *Mansonia* can transmit infection. In India it is *Cx. quinquefaciatus* which is the main vector of bancroftian filariasis. Although

this mosquito species breeds profusely in dirty and polluted waters, it can also breed in clear water when denied polluted water. *Mansonia* mosquitoes, such as *Ma. annulifera* and *Ma. uniformis* are the chief vectors of Malayan filariasis. The breeding of these mosquitoes is associated with certain aquatic plants such as *Eichhornia crassipes* and *Pistia stratiotes*, since in the absence of such aquatic plants these mosquitoes cannot survive.

Unlike malaria and many viral infections where even single bite is having huge probability to infect a human being, in case of lymphatic filariasis a large quantum of bites over several months are needed to get the infection of lymphatic filariasis.

1.7 Host Factors

Man is the natural host, with a range of factors:

1. *Age*: Lymphatic filariasis (LF), recognized as a widespread, seriously debilitating disease of adults, was generally thought to occur only sporadically in children. All ages are however susceptible to infection in endemic areas; filarial infection has been found even in the infants aged 6 months, but the infection rates have been found to rise with age of 20–40 years and not consistently thereafter. This is attributed partly to the fact that some of the persons developing disease become negative for *mf*. Filarial disease appears only in small percentage of infected individuals. New, highly sensitive diagnostic tests now reveal, however, that lymphatic filariasis is first acquired in childhood. It has been suggested that as many as one-third of children are asymptomatically infected before age five (Witt and Ottesen 2001). The risk of infection in childhood may be related to the maternal immune response during pregnancy. In Kenya, among 159 pregnant women with active *W. bancrofti* infection, neonates lacking filarial-specific T cell responses in cord blood at birth had a 13-fold increased risk of developing childhood infection as compared with uninfected controls (Malhotra et al. 2006).

   Most people who get infection do not exhibit any symptom and may never develop clinical symptoms. Yet, many a people develop symptoms many years later after the parasite will have damaged the lymph system triggering stress on the lymphatic vessels by their accumulation in bulk and solidification. These people do not know if they had infection and need be tested to be informed. After initial subclinical symptomatology which might run into some non-specific presentations of adenitis/adenopathy, the infected person, especially after puberty, begins to show up characteristic clinical features disease syndromes like lymphoedema and hydrocele. The disease mostly affects the lower limbs (elephantiasis) but may involve other parts of the body such as arms, breasts, and genitalia (e.g. hydrocele in men). Filarial infection can also cause tropical (mostly in Asians) pulmonary eosinophilia syndrome, characterized often with high levels of IgE (Immunoglobulin E) and antifilarial antibodies, and develop symptoms of cough, shortness of breath and wheezing.

Generally children get the first infection, as many as one-third of them being asymptomatic before age of 5 years. This information carries high epidemiological significance since the risk of infection in childhood may be related to the maternal immune response during pregnancy. Thus, having become the target population in order for the programme to achieve its twin goals of (i) interrupting transmission and (ii) preventing disease in the community, they become the principal beneficiary of LF elimination as well.

2. *Sex*: The sex does not apparently associate with the infection rate.
3. *Density of infection*: The minimum level of *mf* infection in infected individual which will permit infection of mosquitoes is not known. It was reported that an individual with one *mf* per mm^3 of blood was infective to 2.6% of the mosquitoes fed on him (Hawking 1962). On the other hand, when mosquitoes were fed on carriers having as many as 80 or more mf per 20 mm^3 of blood, the heavily infected mosquitoes did not survive when a number of mf begin to reach maturity (Omri 1962).
4. *Migration of people*: The movement of people from one place to another has led to extension to filariasis into areas previously nonendemic.
5. *Immunity*: Epidemiological studies indicate that individual may develop resistance to infection and superinfection. The immunological basis of this resistance is not known.
6. *Social factors*: Urbanization, industrialization, migration of people and sleeping habits are some of the social factors associated with filariasis. The lymphoedema of the legs and other parts of the body causes suffering and reduction in the working capacity of the patients. The disease is also associated with social stigma.

1.8 Environmental Factors

Climate is an important factor in determining the endemicity of filariasis. It influences the breeding of mosquitoes, their longevity and development of the parasite in the insect vector. The maximum prevalence of *Cx. quinquefaciatus* was observed where the temperature was between 22 and 30 °C and optimum longevity when the relative humidity was 70 per cent. Breeding of the vector is associated with the nature of drainage system in the area. The vector breeds profusely in polluted water. Inadequate sewage disposal and lack of town planning have aggravated the problem of filariasis in most of the developing countries like India by increasing the facilities for the breeding of vector mosquito such as cesspools, soakage pits, ill-maintained drains, septic tanks, open ditches, burrow pits, etc.

1.9 Pathogenesis

Why is lymphatic filariasis referred to as a neglected tropical disease (NTD)? The category, NTD, itself clearly conveys the message that though LF is highly debilitating and restricting vital activities a great deal, it does not generally kill and, at the

same time in view of its severity, is therefore also given importance next to a disease like malaria that kills. The infection of lymphatic filariasis, which could exhibit asymptomatic (most common; showing no external signs of infection), acute and chronic conditions, is painful and carries significant socio-psychological impact on both the individual and the community. Ironically asymptomatic infections may still harm the lymphatic system and the kidneys and also alter the body's immune system especially the physiological function of lymphatic vessels wherein lymphatics' tortuosity or 'incompetent lymph valves' condition is brought about by the infiltration of plasma cells, eosinophils and macrophages around the infected vessels, along with endothelial and connective tissue proliferation. Additionally, following repeated episodes of inflammation (principally by adult worms causing lymphatic dilatation and thickening of vessel walls) and lymphoedema, the damage can lead to lymphatic damage, chronic swelling and elephantiasis of the legs, arms, scrotum, vulva and breasts.

The most pathetic and ignominious impact of the infection by lymphatic filariasis is the onset of lymphoedema, elephantiasis and scrotal swelling – infirmities which dawn on an infected person much later in life and can lead to permanent disability. Repeated episodes are debilitating, may last for weeks and may prevent earn wages to sustain livelihood. Uncared the infection of lymphatic filariasis may develop chronically into a condition called lymphoedema (tissue swelling) or elephantiasis (skin/tissue thickening of limbs) and hydrocele (scrotal swelling). Breasts and genital organs may also be involved. Such body deformities reflect much on the sociological and psychological burden the infected persons, particularly females, have to live with; young men are not enrolled for any respectful employment or even wages, and the girls in the age of being married are avoided to be knotted with. Those with chronic lymphoedema or ADL attract secondary infection with bacteria, and in the process, a repulsive look on the grotesqueness of the limb could be boycotted socially and pushed to live in isolation. The consequences of disease and impoverishment are immense. Major signs and symptoms include fever, inguinal or axillary lymphadenopathy, testicular and/or inguinal pain, skin exfoliation and limb or genital swelling.

1.10 Clinical Features

Lymphatic filariasis presents a wide range of clinical manifestations, and visibly most common of which is elephantiasis. These symptoms are dependent on species, such as *W. bancrofti* and *B. malayi*, and body type and can be acute or chronic in nature. Asymptomatic carriers can constitute up to 70% of infected individuals. Even though infection may be a childhood phenomenon these usually do not manifest until adolescence or adulthood, when worm burden is generally the highest. Asymptomatically (or subclinically) LF exhibits following microfilaremic presentations:

(1) Hydrocele.
(2) Acute adenolymphangitis (ADL), characterized by high fever, lymphatic inflammation and transient local oedema.
(3) Chronic lymphatic disease.

The lymphangitis, i.e. inflammation of the walls of the lymphatic vessels, begins to flow backwards indurated and inflamed vessels cascading the area where the adult parasites are lodged. Inflamed lymphatic nodes and lymphatic tract may concomitantly develop local thrombophlebitis resulting subsequently into rupture to the surface. Swelling of both extremities of the limb involving lymphadenitis and lymphangitis is common with both bancroftian and brugian filariasis, but *W. bancrofti* infection is nearly always the only cause for genital lymphatics which can be manifested by funiculitis, epididymitis and scrotal pain and tenderness.

Dermatolymphangioadenitis (DLA), characterized by oedematous inflammatory integumentary plaques, is a syndrome that includes high fever, chills, myalgias and headache and is another type of acute disease in endemic areas manifested by vesicles, ulcers and hyperpigmentation. Filarial fever is characterized by fever without associated adenitis, whereas the DLA is often diagnosed as cellulitis.

1.11 Infection/Disease Surveillance

Disease surveillance is the backbone in designing control programmes. This is done by detecting microfilaraemia in the circulating peripheral blood or by detecting antigen in the human by rapid immunodiagnostic tests (ICT filariasis test) or the ICT kits. Xenomonitoring, i.e. using DNA probe, of infective/infected vector population is another fast emerging methodology to be profitably employed during post-elimination monitoring. Such novel and innovative intervention measures have still not been put into use in routine surveillance owing to logistic reasons and cost constraints pertaining to such modern tools and techniques, e.g., concentration techniques or the nucleopore membrane technique and parasite DNA, using polymerase chain reaction (PCR) and circulating filarial antigens (immunochromatographic test and ELISA Og4C3) kits. The classical and most conventional method followed under national programme in selected endemic foci for specific purposes, of course, is microscopic detection of *mf* in carriers through night blood sample examination and by line listing of carriers (http://nvbdcp.gov.in/filariasis-new.html; cited on 13th January 2010). Unfortunately, this kind of regular/active surveillance is wanting in the programme and new tools, for reasons mentioned above are not yet fully commissioned in a long-term activity.

1.12 Current Control Strategy of Lymphatic Filariasis

Worldwide more than 50 countries are engaged in executing a national programme to eliminate lymphatic filariasis by focusing on (1) reducing transmission of the filarial parasites and (2) decreasing the risk of infection for those people living in or visiting endemic countries.

In India, National Vector Borne Disease Control Programme (NVBDCP) – the premier body programmed to control or eliminate a vector-borne disease, i.e. LF since 2003, under the National Health Policy 2002 – has set a target for LF

elimination by 2018 through an annual MDA with single dose of DEC (6 mg/kg body wt.) for at least 5 years to the entire population of an endemic district (excluding children under 2 years, pregnant women and severely ill patients) and home-based management of lymphoedema cases and hydrocelectomy operations in identified Community Health Centres (CHCs) and hospitals. To achieve success nationwide, a pilot project was launched in 1996 deploying mass drug administration (MDA) with DEC in 13 districts of 7 states (http://www.namp.gov.in/filariasis.html; cited on 12th January 2010). Subsequently, in 2002, the NVBDCP scaled up the MDA to cover a population of 77 million; approximately 53% increase when compared to the population in 1996–1997 (41 million). It further upgraded its coverage with the MDA in 2004 covering about 468 million population from 202 districts, now combined with albendazole (Alb), albeit conflicting opinion on the use of the latter in the MDA (Pani et al. 2002; Sabesan 2006). In order to resolve the issue, a large-scale trial with the support of Indian Council of Medical Research (ICMR) Task Force was carried out on the feasibility and impact of co-administration of DEC and Alb in selected districts in the country in 2000–2005, and it was recommended that DEC 6 mg/kg/ body wt. and Alb 400 mg should be co-administered in all endemic districts. Thus, for the first time, by 2007, the entire population in all the known endemic districts was brought under MDA administration.

1.13 Conclusion

India was once contributing the maximum filariasis cases in the southeastern Asian region. The scenario is fast changing since the country has achieved threshold level of reduction of microfilaraemia in most endemic zones, while antegenaemia too is steadily decreasing in most intervened areas. Already MDA (DEC + Alb) has been delivered for more than 5–8 times in most endemic areas, with both delivery and community acceptability appreciably rising. Following TAS inputs, the country will be looking forwards to make a conclusive impact on the disease prevalence in the country so as to be able to declare elimination of the disease within a couple of years from now!

References

Babu BV, Swain BK, Rath K (2006) Impact of chronic lymphatic filariasis on quantity and quality of productive work among weavers in an endemic village from India. Tropical Med Int Health 11:712

Carme B, Laigret J (1979) Longevity of *Wuchereria bancrofti* var. *pacifica* and mosquito infection acquired from a patient with low level parasitemia. Am J Trop Med Hyg 28:53–55

Chu BK, Deming M, Biritwum NK, Bougma WR, Dorkenoo AM, El-Setouhy M et al (2013) Transmission assessment surveys (TAS) to define endpoints for lymphatic filariasis mass drug administration: a multicenter evaluation. PLOS Negl Trop Dis 7(12):e2584. https://doi.org/10.1371/journal.pntd.0002584

Global Programme to Eliminate Lymphatic Filariasis: progress report on mass drug administration, 2010 (2011) WklyEpidemiol. Rec No 35(86):377–388

Govt. of India, Ministry of Health and Family Welfare: National vector borne diseases control programme; Problems and elimination of lymphatic filariasis in India (2005). Available from: http://www.namp.gov.in/filariasis.html. Cited 12 Jan 2010

Hawking F (1962) A review of progress in the chemotherapy and control of filariasis since 1955. Bull World Health Org 27(4–5):555–568

Mak JW (1987) Epidemiology of lymphatic filariasis. Ciba Found Symp 127:5–14

Malhotra I, Mungai PL, Wamachi AN, Tisch D, Kioko JM et al (2006) Prenatal T cell immunity to *Wuchereria bancrofti* and its effect on filarial immunity and infection susceptibility during childhood. J Infect Dis 193:1005–1013

Omori N (1962) A review of the role of mosquitos in the transmission of Malayan and Bancroftian filariasis in Japan. Bull World Health Org 27:585–594

Pani SP, Subramanyam Reddy G, Das LK, Vanamail P, Hoti SL, Ramesh J, Das PK (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 1:1–11

Ramaiah KD, Das PK, Michael E, Guyatt H (2000) The economic burden of lymphatic filariasis in India. Parasitol Today 16(6):251–253

Sabesan S (2006) Albendazole for mass drug administration to eliminate lymphatic filariasis. Lancet Infect Dis 6:684–685

Sabesan S, Palaniyandi M, Das PK, Michael E (2000) Mapping of lymphatic filariasis in India. Ann Trop Med Parasitol 94:591–606

Sunish IP, Rajendran R, Mani TR, Munirathinam A, Dash AP, Tyagi BK (2006) Vector control complements mass drug administration against bancroftian filariasis in Tirukoilur, India. Bull World Health Organ 85(2):138–145

Tyagi BK (1994) Distribution of arthropod vector-borne communicable diseases and control of their vectors in India. Indian Rev Life Sci 14:223–243

Vanamail P, Subramanian S, Das PK, Pani SP, Rajagopalan PK, Bundy DA et al (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, 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 (2014) Report on independent appraisal of elimination of lymphatic FilariasisProgramme in India. Vector Control Research Centre, Indian Council of Medical Research, Puducherry

WHO (2005) Sixth meeting of the technical advisory group on the global elimination of lymphatic Filariasis, Geneva, Switzerland. Wkly Epidemiol Rec 80:401–408

WHO (2008) Global programme to eliminate lymphatic filariasis. Wkly Epidemiol 83(37):333–341

WHO (2013) Global programme to eliminate lymphatic filariasis. Progress report for 2012. Wkly Epidemiol Rec 88(37):389–399

Witt C, Ottesen EA (2001) Lymphatic filariasis: an infection of childhood. Tropical Med Int Health 6(8):582–606

Lymphatic Filariasis Elimination: Update for Mission Possible

2

P. K. Srivastava and A. C. Dhariwal

Abstract

In India, lymphatic filariasis is reported to be endemic in 250 districts (presently 256) in 16 states and 5 UTs. This disease is targeted for elimination, and to achieve this goal, the Government of India, in year 2004, launched the strategy of annual mass drug administration (MDA) with a single dose of diethylcarbamazine citrate (DEC) to the population living at risk of filariasis except for children below 2 years, pregnant women and seriously ill persons. The co-administration of DEC and albendazole was introduced for MDA in the country since 2007. The current strategy of the annual mass drug administration (MDA) is to reduce the microfilaria rate to below 1% so that the transmission is interrupted and the new generation will not get the infection. The affected people with this disease mainly known as elephantiasis will be provided home-based lymphoedema management procedure by simple washing and drying. The people affected with hydrocele will be motivated for surgical intervention. The programme has already listed more than 800,000 lymphoedema and 400,000 hydrocele cases. About 130,000 hydrocele cases have been operated.

The programme scaled up to reach about 600 million population achieving 100% geographical coverage in all 256 endemic districts. Coverage rates also improved from 73% in 2004 to 88% in 2015. The efforts to scale up MDA to reduce mf rate have shown promising results as 222 out of 256 endemic districts have reported microfilaria prevalence below 1% bringing them for validation through transmission assessment survey (TAS). Seventy-two districts have already cleared the first round of TAS.

It is estimated that the first round of TAS will be taken up in all endemic districts by the end of 2017. However, as per WHO guidelines, three rounds of TAS at intervals of 2–3 years have to be conducted to ensure elimination. Therefore, in spite of the achievement of microfilaria rate below 1% in all the

P. K. Srivastava (✉) · A. C. Dhariwal
National Vector Borne Disease Control Programme (NVBDCP), New Delhi, India

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_2

255 districts, the elimination process will start after 3–4 years. However, all efforts are to be made to achieve the target of reducing microfilaria rate to below 1% by the end of 2017.

The programme has also been evaluated by the Joint Monitoring Mission with national and international experts in few districts. The independent appraisal has also been done separately by ICMR which indicated in its report about strong commitment and leadership at national level for LF elimination. Adequate funds have been ensured so as to facilitate continuation of annual cycles of MDA implementation in all the endemic districts. Guidelines have been made available to the programme managers. The Directorate of NVBDCP receives adequate and timely scientific support from research organizations. A good rapport is maintained with the WHO Regional Office, and regular supply of albendazole and diagnostics is received from the donor. Technical support and additional financial requirements for capacity building and diagnostics are received from the WHO.

There are last mile challenges like survey of nonendemic districts for current status of LF endemicity, liquidating foci in nonendemic districts with treatment and vector control under integrated vector management as the whole district may not require MDA, sustaining the achievements gained so far and preventing failure in TAS, etc.

2.1 Introduction

Lymphatic filariasis (LF), despite being nonfatal, is considered one of the most serious vector-borne infections, next only to malaria in its severity measured as disability-adjusted life years (DALYs). The infected person remains apparently healthy during the early phase of infection but serves as a source of infection for transmission. This stage may continue for 5–7 years and can be treated with microfilaricidal drug, diethylcarbamazine citrate (DEC), when detected. Subsequently, manifestations like swellings of limbs and genitals develop which increase slowly, and the person becomes incapacitated and also suffers from social stigma. The affected persons also suffer from frequent attacks of lymphangitis, high fever, swelling and pain. There is no cure at this stage, and they are forced to live with huge swellings exposed to secondary infections. Control of lymphatic filariasis is, therefore, immensely important due to personal trauma and associated social stigma among the affected persons, which are seen as reason of boycott from the community in many parts of the world.

2.2 National Filaria Control Programme

The Government of India in 1955 launched the National Filaria Control Programme (NFCP) for the control of Bancroftian filariasis with three objectives, viz. (i) delimitation surveys in known endemic areas, (ii) large-scale control operations in selected

areas and (iii) training of personnel required for programme management. From 1955 to 1960, the three conventional methods of control, namely, mass therapy with diethylcarbamazine (DEC), anti-adult mosquito measures with residual insecticides in rural areas and recurrent anti-larval measures at weekly intervals in urban areas, were adopted.

Four independent assessments of the programme were carried out by the expert committees of the Indian Council of Medical Research (ICMR), Ministry of Health and Family Welfare, Government of India. Some of the salient recommendations of these assessments are given hereunder:

1. *First Assessment* (1960): This observed stark failure of MDA due to side effects and ineffectiveness of indoor residual spray with insecticide owing to the development of high resistance among vector. Based on these, certain recommendations were made, viz. (i) to reorganize all the control units on the basis of population, (ii) to continue anti-larval measure in urban areas, (iii) to withdraw mass drug administration of DEC and indoor residual insecticidal spray and (iv) to establish new control units (ICMR 1961).
2. *Second Assessment* (1970): This recommended (i) therapy of identified microfilarae *(*mf) carrier as a compliment to anti-larval measure, (ii) delimitation survey to know the problem in unsurveyed districts and (iii) reorganization of control measures in contiguous areas (ICMR 1971).
3. *Third Assessment* (1984): Recommendations were (i) the NFCP to be made 100% centrally sponsored scheme, (ii) the Village Health Guide (VHG) and Multipurpose Worker (MPW) may distribute DEC for the treatment of filariasis, (iii) establishment of a filaria unit in a town with a minimum of 20,000 population and 4% *mf* rate and (iv) the survey unit should be engaged in resurveying each of old-surveyed districts after completion of routine survey (ICMR 1984).
4. *Fourth Assessment* (1995): Guided by a committee headed by Dr. R.S. Pandey, Ex-Additional Director, Uttar Pradesh, and the Director, NMEP as Member Secretary, this assessment was executed with the assistance of the World Health Organization (WHO). It recommended (i) launching a project in 1996 on eradication of *Brugia malayi* infection, (ii) 100% central assistance for all vector-borne diseases, (iii) integrated vector control for all vector-borne diseases and (iv) model by-law adoption for effective control of vectors in domestic situation. It was further recommended that (a) antigen- and DNA-based detection of microfilaria be adopted, (b) fresh delimitation surveys in rural areas be initiated and (c) medicated salt introduction in a phased manner and pyrethrum extract (2%) be supplied to filaria control towns where high malaria cases are recorded to tackle malaria transmission (ICMR 1995).

2.3 Organization Set-Up Recommended for NFCP

The organizational structure at central and state level was created for programme implementation, as indicated below (Sharma et al. 1995).

(i) *Central*: The Directorate NVBDCP is responsible for planning, issuing guidelines, coordinating and evaluating activities under the programme with the active assistance of regional offices of health and family welfare organizations. The research and training of filariasis for NFCP staff has been with the NICD, Delhi, and its three branches located at Calicut (Kerala), Rajahmundry (A.P.) and Varanasi (U.P.).

(ii) *State level*: At state level, the programme is looked after by a deputy director/ assistant director, under a state programme officer for vector-borne diseases.

(iii) *Unit level*: The filaria control units are manned by the following staff for the protection of a specific quantum of population by each unit:
- One biologist/medical officer for every 5 lakh population
- One field worker for every 5000 population
- One superior field worker for every 25,000 population
- One inspector for every 50,000 population
- One technician for every 1.50 lakh population
- One insect collector for every 50,000 population

In addition to this are provided one peon for every gazetted officer, one clerk for every 1.50 lakh population, one store keeper for every 75,000 population and one tinsmith for every 1 lakh population. One chowkidar per unit is also allowed. The staff strength allowed for each filaria clinic and the survey unit is as follows:

For Filaria Clinic
- Filaria inspector – 1
- Laboratory technician – 1
- Field worker – 1
- Driver – 1

For Survey Unit
- Biologist/filaria officer – 1
- Filaria inspector – 2
- Technician −2
- Inspect collector −2
- Field worker −3
- Clerk/typist – 1
- Driver −1
- Chowkidar – 1

Most of the units and clinics are however short of staff, and in the 11th Plan as well as the 12th Plan, states have been advised to integrate NFCP, malaria and other vector-borne diseases staff at state and district levels for optimum utilization of human resource.

2.4 Disease Endemicity

In mainland of India, the most predominant causative organism for filaria infection is *Wuchereria bancrofti* which is transmitted by the vector *Culex quinquefasciatus*. Bancroftian infection contributes to 99.4% of the LF problem in the country which is endemic in 21 states and union territories. The Bancroftian infection is prevalent in both urban and rural areas. *Brugia malayi* infection, earlier mainly restricted to rural areas of six states, is now reported only in one district of Kerala and recently one district of Odisha. *Mansonia annulifera* is the principal vector, while *Ma. uniformis* and *Ma. indiana are* the secondary vectors for the transmission of *B. malayi* infection. The nocturnal periodicity of microfilaraemia by both *W. bancrofti* and *B. malayi* infections are known in mainland of India (Sasa 1976).

The diurnal subperiodic *W. bancrofti* infection was discovered in 1974–1975, among aborigines inhabiting Nicobar Group of Andaman and Nicobar Islands. *Ochlerotatus* (*Aedes*) *niveus* group of mosquitoes were incriminated as the vectors for the infection (Kalra 1974).

Indigenous lymphatic filaria cases as per NFCP were reported from 18 states/union territories (UTs), namely, Andhra Pradesh, Assam, Bihar, Goa, Gujarat, Karnataka, Kerala, Karnataka, Madhya Pradesh, Maharashtra, Odisha, Tamil Nadu, West Bengal, Puducherry, Andaman and Nicobar islands, Daman and Diu, Dadra and Nagar Haveli and Lakshadweep. Due to the division of Andhra Pradesh, Madhya Pradesh and Bihar, the newly created state entities of Telangana, Chhattisgarh and Jharkhand have also been included in endemic areas making the total of 21 endemic states/UTs (Fig. 2.1a, b).

The remaining states of India, viz. Arunachal Pradesh, Nagaland, Meghalaya, Mizoram, Manipur, Sikkim and Tripura in the north-east and Jammu and Kashmir, Himachal Pradesh, Punjab, Haryana, Rajasthan, Uttarakhand and UTs like Delhi and Chandigarh in the north-west, are known to be free from indigenously acquired filarial infection.

2.5 Control Measures

Under the National Filaria Control Programme, the control measures are mainly based on:

(i) Recurrent anti-larval measures at weekly intervals with approved larvicides.
(ii) Antiparasitic measures by detection and treatment of filaria cases and microfilaria carriers with diethylcarbamazine (DEC).

Ironically, due to limited resources, the activities are at present confined to 227 towns spread over 13 states and 4 union territories (Table 2.1). These units are in the urban areas for control and other larvicides. Although 27 filaria survey units (FSU) were established in order to delimit the problem of filariasis in the unsurveyed districts in known endemic states, however, 22 are now functioning. Besides, 224

filaria clinics (detection-cum-treatment teams) mainly attached to the control units were established in order to reduce the reservoir of infection. About 52 million people were protected in urban areas under 227 control units through anti-larval and antiparasitic measures throughout the country. The microfilaria rates and disease rates have also come down considerably in areas covered by most of the units. The parasitological information is recorded regularly; however, due to lack of entomological staff, the entomological surveillance is weak and needs to be strengthened.

Based on the screening of limited population in urban areas carried out by filaria control units, the microfilaria rate has reduced from 0.7 in 2000 to 0.2 in 2014. However, the data of NFCP reflects surveys in urban areas and does not reflect magnitude of the problem in districts or states. The year-wise microfilaria rate and disease rate reported by NFCP units are given in Table 2.2. The endemicity map of the

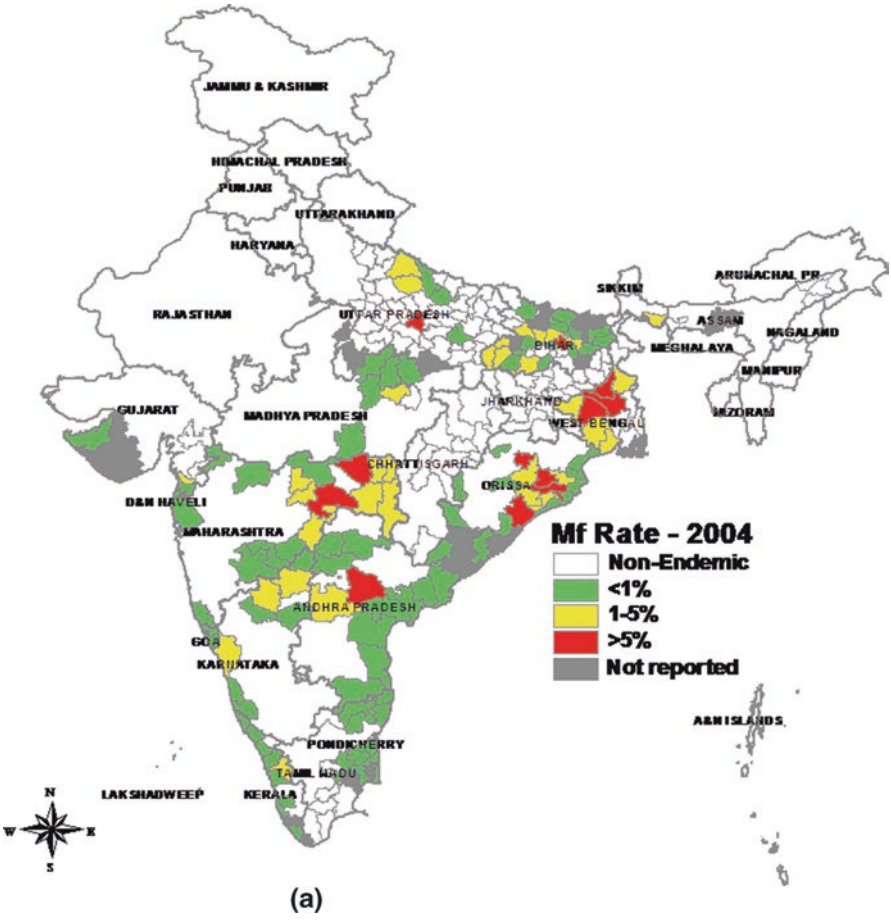


Fig. 2.1 Maps of different endemicity scenarios at different years, (**a**) 2004 and (**b**) 2010, in India. (Source: P.K. Srivastava/NVBDCP; personal communication)

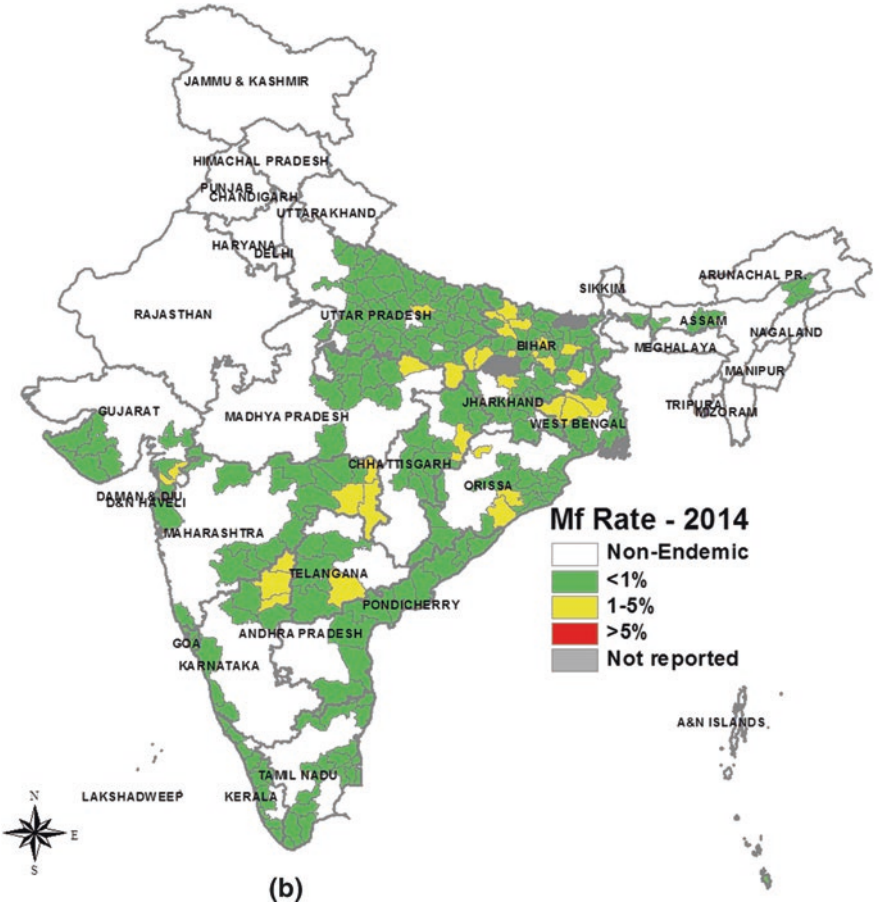


Fig. 2.1 (continued)

country for 1958, 1969 and 1995 are presented in Fig. 2.1. These historical data and surveys by other research institutions became the basis of classifying the country in endemic and nonendemic districts for implementation of revised strategy towards elimination of lymphatic filariasis. Figure 2.1 shows endemic and nonendemic districts requiring preventive chemotherapy (PCT) during MDA.

2.6 Genesis of Elimination of Lymphatic Filariasis (ELF)

The International Task Force for Disease Eradication identified lymphatic filariasis as one of the six infectious diseases to be "eradicable" or "potentially eradicable" (WHO 1995). The World Health Assembly (WHA) in 1997 adopted resolution WHA 50.29 for the elimination of lymphatic filariasis (ELF) as a global public health problem by 2020. India is a signatory to this resolution. Revised strategy for

Table 2.1 Statewise breakup of NFCP infrastructure

Sl. no.	State/UT	Population protected (in million)	Filaria control units	Survey units	Filaria clinics
1	Andhra Pradesh	5.90	29	2	4
2	Assam	0.30	1	1	0
3	Bihar	6.00	23	2	28
4	Jharkhand	2.22	7	0	7
5	Goa	0.35	4	0	6
6	Gujarat	3.82	8	0	9
7	Karnataka	0.69	8	1	25
8	Kerala	4.35	16	2	9
9	Madhya Pradesh	0.70	9	2	2
10	Maharashtra	6.39	16	6	34
11	Orissa	2.47	15	0	15
12	Tamil Nadu	9.24	47	1	43
13	Uttar Pradesh	7.17	29	0	34
14	West Bengal	1.48	9	4	3
15	Puducherry	0.53	2	0	2
16	Daman and Diu	0.20	2	0	2
17	Lakshadweep	0.01	1	0	0
18	A and N Islands	0.05	1	1	1
	Total	**51.87**	**227**	**22**	**224**

Table 2.2 Year-wise parasitological indices based on NFCP units/clinics

Year	No. of persons examined	Mf rate %	Disease rate %
2000	2,937,625	0.71	1.12
2001	2,970,195	0.55	0.93
2002	4,023,562	0.47	1.01
2003	3,293,877	0.41	1.18
2004	3,179,797	0.49	0.99
2005	2,802,968	0.41	0.97
2006	1,747,521	0.35	1.06
2007	2,095,856	0.28	1.05
2008	2,308,942	0.22	0.8
2009	2,319,888	0.30	1.15
2010	2,789,812	0.17	0.47
2011	2,466,824	0.12	0.25
2012	1,777,025	0.15	0.96
2013	1,927,509	0.17	0.94
2014	2,279,461	0.24	0.43

the control of lymphatic filariasis in India was deliberated in a workshop held at the National Institute of Communicable Diseases (now known as National Centre for Disease Control) and National Malaria Eradication Programme (now known as National Vector Borne Diseases Control Programme) (Biswas 1996).

Following the recommendations of the workshop, a pilot project was initiated in 1996–1997 with annual mass drug administration (MDA) of a single dose of DEC in 13 identified districts of 7 states, namely, East Godavari and Srikakulam (Andhra Pradesh), Darbhanga and Siwan (Bihar), Kozhikode and Alappuzha (Kerala), Puri and Khurda (Odisha), North Arcot (Vellore and Thiruvannamalai) and South Arcot (Cuddalore and Villupuram) (Tamil Nadu), Gorakhpur and Varanasi (Uttar Pradesh) and Purulia (West Bengal), covering about 41 million population. The MDA was extended to 31 districts during 2002 including 11 districts with co-administration of DEC + albendazole (4 in Odisha, 1 in Kerala and 6 in Tamil Nadu).

2.7 National Goal and Strategy for ELF

Elimination of lymphatic filariasis by 2020 has been set as a global goal. The strategy to achieve the goal of elimination as per WHO recommendation has two components (Ottesen et al. 1997):

(i) Annual mass drug administration (MDA) of a single dose of DEC (diethylcarbamazine citrate) and albendazole for 5 years or more to population at risk to interrupt transmission of the disease. The MDA will exclude pregnant women, children below 2 years of age and seriously ill persons.
(ii) Home-based management of lymphoedema cases and upscaling of surgical intervention for hydrocele cases in identified CHCs/district hospitals/medical colleges.

2.8 Mass Drug Administration

Based on various consultative meetings and reviews and considering the national goal of elimination set in NHP-2002, it was decided to start a nationwide programme to eliminate LF in 2004 (Anon. 2003; NVBDCP 2004, 2005, 2009). The districts were selected as implementation unit on the basis of historical evidence of filarial endemicity, presence of lymphoedema and hydrocele cases and/or presence of microfilaria carriers. The details of each round of MDA are detailed below:

(i) *MDA 2004 (first round)*: The MDA campaign was launched by the Union Minister for Health and Family Welfare at Thane District, Maharashtra, and by the Union Minister of State for Health and Family Welfare at Rai Bareilly, Uttar Pradesh, on 5th June 2004. The MDA was undertaken in 202 districts of 20 endemic states/UTs of the country. Home-based morbidity management

and scaling up of hydrocelectomy were initiated. A population of 276 million was covered against the target of 382 million giving a coverage rate of about 73%. The overall microfilaria rate in LF endemic states/UTs reported during the year was 1.24%.

(ii) *MDA 2005 (second round)*: Based on the experience of MDA in June 2004 (when the temperature essentially soars high), the date of the National Filaria Day was changed to 11th November in consultation with the states. The MDA campaign was launched on 11th November in the Jubilee Hall at Hyderabad by the Union and State Ministers for Health and Family Welfare along with the Director of NVBDCP and the State Programme Officers. During the year 2005, a total of 243 filaria endemic districts with a population of over 564 million were targeted, but MDA could not be implemented in 14 IUs (about 30 million population) of Tamil Nadu due to unprecedented rains and flood. The total units covered under MDA were 229 with coverage of 76%. The microfilaria rate reported during the year was 1.02%.

(iii) *MDA 2006 (third round)*: This was targeted in 243 districts. Based on experiences of MDA in 2004 and 2005, supply of DEC tablets was made in strip packing. Further, during this year, based on the 5-year pilot project of ICMR, the National Task Force (NTF) recommended global strategy of co-administration of DEC and albendazole in all the LF endemic states for annual MDA. The World Health Organization was requested to enhance the supply of albendazole, and the states were requested to plan for co-administration during MDA 2007. The supply of DEC tablets in strip packing was staggered leading to unsteady dates for MDA observance. More so, MDA could not be observed in 5 states, namely, Chhattisgarh (16 million population and 9 IUs), Tamil Nadu (30 million population and 14 IUs), Kerala (31 million population and 11 IUs), Puducherry (1 million population and 1 IU) and West Bengal (62 million population and 12 IUs). The population of 302 million in 196 districts was covered against eligible population of 369 million (82%) in 15 states/UTs. The microfilaria rate reported during the year was 0.98%.

(iv) *MDA 2007 (fourth round)*: During 2007, 7 new districts (6 of Tamil Nadu and 1 of Gujarat) were brought under MDA making a total of 250 districts under MDA. The MDA started on 15th November 2007 due to the festival of Deepavali falling on 10th November. During the year, the MDA campaign was launched on 15th November 2007 by the Union Minister for Health and Family Welfare at Nirman Bhawan, New Delhi, himself setting an example by consuming DEC tablets. All the 250 filaria endemic districts with population of 574 million at risk were brought under MDA. During this round of MDA, 421 million population was covered against the eligible population of 509 million (83%). During this round, the global strategy of co-administration of DEC with albendazole during MDA approved by the National Task Force on elimination of lymphatic filariasis was expanded from two pilot districts each in Tamil Nadu and Kerala to all the LF endemic districts of these states. The overall microfilaria rate in the country reported during the year was 0.64%.

(v) *MDA 2008 (fifth round)*: During 2008, all the 250 districts were targeted, but Bihar could not observe MDA in 38 districts with a population of 99 million at risk initially due to general election and thereafter due to excessive rains and floods. Therefore, a population of 374 million was covered against the eligible population of 433 million indicating a coverage of 86% in 19 states/UTs. The total units covered were 212. The supply of albendazole was increased to 130 million tablets by the WHO and during this round, therefore, the co-administration was expanded to 20 districts of Tamil Nadu, 11 districts of Kerala, 8 districts of Karnataka and 16 districts of Andhra Pradesh. At this stage the Government of India took a decision to procure balance albendazole from domestic sources and also requested the WHO to enhance quantity of albendazole. The microfilaria rate reported during the year was 0.53%.

(vi) *MDA 2009 (sixth round)*: All the endemic districts were brought under strategy of co-administration of DEC with albendazole during MDA. Mass drug administration could not be observed in 2 states, namely, Uttar Pradesh (138 million population in 50 IUs) and Assam (11 million population and 7 IUs). Thus, only 337 million population was covered against eligible population of 388 million, indicating the coverage of 87%. The total units covered were 193, and the microfilaria rate reported during the year was 0.65%.

(vii) *MDA 2010 (seventh round)*: A national review was done on 15th October 2010 at the Directorate, NVBDCP. The challenges in achieving coverage of >85%, delay in submission of SOE hampering financial support and delayed reporting by states were discussed. States were requested to improve MDA coverage and intensify morbidity management. During 2010, all 250 districts were targeted for MDA. But Tamil Nadu (31 million population in 20 IUs) and West Bengal (69 million population in 12 IUs) could not conduct the same. Therefore, a population of 380 million against the eligible population of 451 million was covered indicating the coverage of 84% in 18 states. The total units covered were 218. The microfilaria rate reported during the year was 0.41%.

(viii) *MDA 2011 (eighth round)*: During 2011, all the 250 districts were targeted, but Bihar (104 million population in 38 IUs) and Uttar Pradesh (100 million population in 36 IUs) could not observe MDA. Therefore, the population of 313 million against the eligible population of 356 million was covered (coverage of 88%). The total units covered were 176. The microfilaria rate reported during the year was 0.37%.

(ix) *MDA 2012 (ninth round)*: The progress on ELF was reviewed on 5th November 2012 under the chairmanship of the Special Director General of Health Services at the Directorate, National Vector Borne Diseases Control Programme (NVBDCP). Experts from Indian Council of Medical Research (ICMR); National Centre for Disease Control (NCDC); World Health Organization (WHO), Geneva, and its Southeast Asia Regional Office (SEARO), New Delhi; and the non-government organization Church's Auxiliary for Social Action (CASA) also participated. The Vector Control Research Centre (VCRC) of ICMR was given the task for detailed assessment

of the programme. The NCDC also intensified training as per the programme. States were requested to improve compliance so as to achieve target of elimination by 2015 (the proposed date of pronouncing elimination which has now been shifted to 2018). Odisha (29 million population in 20 IUs), Chhattisgarh (18 million population in 9 IUs) and Lakshadweep (1 million population in 1 IU) had missed the MDA 2012 round. Goa (1.5 million population in 2 IUs – TAS), Puducherry (1 million population in 1 IU), Tamil Nadu (25 million population in 16 IUs) and Assam (5 million population in 2 IUs – TAS) showed remarkable success leading to start of validation through transmission assessment surveys (TAS). A population of 375 million against the eligible population of 437 million was covered indicating the coverage of 85%. The total units covered were 200. The microfilaria rate reported during the year was 0.45%.

(x) *MDA 2013 (tenth round)*: The MDA 2013 round was initiated since November 2013. Lakshadweep, Dadra and Nagar Haveli, Odisha, Andaman and Nicobar islands, Assam and 15 districts of UP completed the round during November. Further MDA was staggered, and the rest of the states completed MDA in December 2013 to March 2014. The population under MDA started reducing due to successful TAS and stoppage of MDA in such districts. The total coverage was 82% with microfilaria rate of 0.4%.

(xi) *MDA 2014 (eleventh round)*: The MDA 2014 round was continued in districts reporting microfilaria above 1% in any of the sites surveyed. During this round, MDA was observed from 14th December onwards in consultation with health secretaries of the respective states. This year was emphasized as priority year to states for MDA, and period of MDA was extended for 1 week from 14th December to 20th December. The phasing out was already started, and only 184 districts observed MDA with 86% coverage and *mf* rate of 0.4%. Remaining districts were preparing for TAS. The endemicity map of India as based on microfilaria prevalence since 2004 till 2014 is shown in Fig. 2.1.

2.9 Drug Compliance Assessment

Assessment of MDA was initially done to assess the actual compliance of drug which revealed large gap between actual drug compliance during MDA. This activity was made as inbuilt component of the programme by involving medical colleges and research institutes to give insight of the programme which also indicated the gap (Regu 2006; Showkath Ali et al. 2007, 2008; Kumar et al. 2008, 2009; Lahariya and Mishra 2008; Mukhopadhyay 2010, Mukhopadhyay and Patnaik 2007, Mukhopadhyay et al. 2008a, b; Aswathy et al. 2009; Vaishnav and Patel 2006; Gunawardena 2007; Ramaiah et al. 2007; Dixit et al. 2009; Cantey 2008; de Rochars et al. 2005; Amarillo et al. 2008; Weerasooriya et al. 2007; Talbot et al. 2008; Boyd et al. 2010; Vanamail et al. 2005; Sharma et al. 1999; Srivastava and Dhillon 2008; Srivastava et al. 2013, 2014a, b, c; Vaishnav et al. 2012; Dhariwal et al. 2015), and accordingly the programme improved the compliance in subsequent years.

2.10 Transmission Assessment Survey (TAS)

It has been observed that all the districts have completed more than five rounds of MDA till 2014, and therefore these were required to be assessed for stoppage or continuance of MDA in these districts. As per WHO guidelines, the districts having observed minimum five rounds of MDA with more than 65% coverage against total population at risk in implementation unit (population of district covered under MDA) need to be subjected for transmission assessment survey (TAS) using immuno-chromatographic test (ICT). These tests are done among the children born after initiation of MDA for presence of circulating antigenaemia to know the current infection. As per WHO guidelines (2011), MDA may be stopped, if the number of positive children is below the critical cut-off during TAS. This is the first step of elimination. For the assessment, the area is considered as evaluation unit with maximum population of 2 million. The WHO conducted a regional workshop on capacity building on TAS at Puducherry (India) for all member countries of Southeast Asia Region which was followed by various national workshops (Srivastava et al. 2014a, b, c). Since 2013, a total of 9 batches of training have been conducted on TAS, and about 290 officials were trained. Seventy-two districts have successfully cleared TAS till March 2016, but many other districts are under evaluation.

2.11 Capacity Building

The different categories of state officials were trained on various aspects of strategies for elimination of lymphatic filariasis every year (Fig. 2.2). In addition, the senior faculties from various 79 medical colleges (544 faculty members belonging to medicine, community medicine, pharmacology, microbiology and paediatrics) were trained.

Fig. 2.2 Capacity building is essential for the programme implementation. (Source: P.K. Srivastava/NVBDCP)

Training was imparted in states/UTs for health personnel including medical officers, paramedicals, drug distributors, laboratory technicians, etc. This is an ongoing activity.

2.12 Social Mobilization

States/UTs have involved political/opinion leaders, local leaders and community for awareness (Fig. 2.5). Besides advocacy at political level, the decision makers were involved in review and social mobilization (Figs. 2.3 and 2.4).

2.13 Morbidity Management

Towards disability alleviation, the programme has envisaged home-based care for lymphoedema through simple hygiene and surgical intervention for hydrocele cases. The advisories on scaling up for home-based lymphoedema management practices and surgical interventions for hydrocele have been circulated to states/UTs. The process included:

1. Complete line-listing of lymphoedema and hydrocele cases in each district
2. Training on simple washing and drying for foot hygiene to affected persons for doing the same practice at home
3. Promoting surgical intervention for hydrocele cases

Fig. 2.3 Social mobilization involving enaction of mass drug administration. (Source: P.K. Srivastava/NVBDCP)

Fig. 2.4 Social mobilization involving enaction of mass drug administration. (Source: P.K. Srivastava/NVBDCP)

Fig. 2.5 Management of lymphoedema cases. (Source: P.K. Srivastava/NVBDCP)

States and UTs have been able to line-list about 8 lakh lymphoedema cases (swelling of limbs) and 4 lakh hydrocele cases (Srivastava and Dhariwal 2010). Out of 4 lakh hydrocele cases, about 1.3 lakh patients have been operated for hydrocele. The training on foot hygiene method to patients by simple washing and drying is also given for management of lymphoedema cases (Fig. 2.5).

2.14 Future Plan

The programme has fixed the priority to validate all such districts which are qualified for TAS in 2016–2017. Additional round of MDA will be continued in hardcore districts along with few other districts with microfilaria prevalence above 1%. Nonendemic districts will be resurveyed, and all measures will be taken to liquidate any LF foci reported during resurvey. Post-MDA surveillance as per WHO guidelines will be continued for 5–6 years in the districts after clearing TAS. During this period, these districts will undergo two more TAS after every 2 years to ensure that there is no recrudescence. The dossier submission for certification of elimination will start on completion of successful three transmission assessment surveys.

References

Amarillo MLE, Belizario VY Jr, Sadiang-abay JT, Sison SAM, Dayag AMS (2008) Factors associated with the acceptance of mass drug administration for the elimination of lymphatic filariasis in Agusan del Sur, Philippines. Parasit Vectors 1:14

Aswathy S, Beteena K, Leelamoni K (2009) Mass drug administration against filariasis in India: perceptions and practices in a rural community in Kerala. Ann Trop Med Parasitol 103:617–624

Biswas G (1996) Revised strategy for the control of lymphatic filariasis in India, New Delhi. National Institute of Communicable Diseases and National Malaria Eradication Program, Delhi

Boyd A, Won KY, McClintock SK, Donovan CV, Laney SJ, Williams SA, Pilotte N, Streit TG, Beau de Rochars MVE, Lammie PJ (2010) A community-based study of factors associated with continuing transmission of lymphatic filariasis in Leogane, Haiti. PLoS Negl Trop Dis 4:e640

Cantey PT (2008) Predictors of compliance with a mass drug administration programme for lymphatic filariasis in Orissa State, India 2008. Trop Med Int Health 15:224–231

de Rochars MB, Kanjilal S, Direny AN, Radday J, Lafontant JG, Mathieu E, Rheingans RD, Haddix AC, Streit TG, Beach MJ, Addiss DG, Lammie PJ (2005) The Leogane, Haiti demonstration project: decreased microfilaremia and program costs after three years of mass drug administration. Am J Trop Med Hyg 73:888–894

Dhariwal AC, Srivastava PK, Bhattacharjee J (2015) Elimination of Lymphatic Filariasis in India: an update. J Indian Med Assoc 113(12):189–190

Dixit V, Gupta AK, Prasad GB (2009) Interruption of annual single dose DEC regimen administration: impact on *Wuchereria bancrofti* microfilaraemia, vector infection and infectivity rates. J Commun Dis 4:25–31

Gunawardena S (2007) Factors influencing drug compliance in the mass drug administration programme against filariasis in the Western province of Sri Lanka. Trans R Soc Trop Med Hyg 10:445–453

I.C.M.R (1961) Report of the Assessment Committee on the National Filaria Control Programme, India. Reprinted 1967. Indian Council of Medical Research, New Delhi

I.C.M.R (1971) Assessment of the National Filaria Control Programme (India) (1961–1970). Indian Council of Medical Research, New Delhi

I.C.M.R (1984) Third Assessment of the National Filaria Control Programme (1971–1980). Indian Council of Medical Research

I.C.M.R (1995) Fourth Assessment of the National Filaria Control Programme (1981–1990). Indian Council of Medical Research

Kalra NL (1974) Filariasis among the aborigines of Andaman and Nicobar Islands. J Commun Dis 6:40–56

Kumar P, Prajapati PB, Saxena D, Kavishwar AB, Kurian G (2008) An evaluation of coverage and compliance of mass drug administration 2006 for elimination of lymphatic filariasis in endemic areas of Gujarat. Indian J Commun Med 33:38–42

Kumar A, Kumar P, Nagaraj K, Nayak D, Ashok L, Ashok K (2009) A study on coverage and compliance of mass drug administration programme for elimination of filariasis in Udupi district, Karnataka, India. J Vector Borne Dis 46:237–240

Lahariya C, Mishra A (2008) Strengthening of mass drug administration implementation is required to eliminate lymphatic filariasis from India: an evaluation study. J Vector Borne Dis 45:313–320

Mukhopadhyay AK (2010) Lymphatic filariasis in Andhra Pradesh Paper Mill Colony, Rajahmundry, India after nine rounds of MDA programme. J Vector Borne Dis 47:55–57

Mukhopadhyay AK, Patnaik SK (2007) Effect of mass drug administration programme on microfilaria carriers in East Godavari district of Andhra Pradesh. J Vector Borne Dis 44:277–280

Mukhopadhyay AK, Patnaik SK, Satya Babu P, Rao KNMB (2008a) Knowledge on lymphatic filariasis and mass drug administration (MDA) programme in filaria endemic districts of Andhra Pradesh. J Vector Borne Dis 45:73–75

Mukhopadhyay AK, Patnaik SK, Satya Babu P, Rao KNMB (2008b) Knowledge on lymphatic filariasis and mass drug administration programme in filarial endemic district of Andhra Pradesh, India. J Vector Borne Dis 45:73–75

National Health Policy (2002) New Delhi: Ministry of Health and Family Welfare, Government of India, pp 1–39

National Vector Borne Disease Control Programme (2004) Strategic plan of elimination of Lymphatic Filariasis, India. Communication to all endemic states

National Vector Borne Disease Control Programme (2005) Operational guidelines on elimination of lymphatic filariasis, India. Directorate of National Vector Borne Disease Control

National Vector Borne Disease Control Programme (2009) Guidelines on elimination of lymphatic filariasis, India

NFCP (1955) National Filaria Control Programme, Government of India

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

Ramaiah KD, Vanamail P, Das PK (2007) Changes in *Wuchereria bancrofti* infection in a highly endemic community following 10 rounds of mass administration of diethylcarbamazine. Trans R Soc Trop Med Hyg 101:250–255

Regu K (2006) Mass drug administration against lymphatic filariasis: experiences from Kozhikode district of Kerala State. J Commun Dis 38:333–338

Sasa M (1976) Human filariasis: a global survey of epidemiology and control. University of Tokyo, Tokyo, p 819

Sharma RS et al. (1995) National Filaria Control Programme, Operational manual. National Malaria Eradication Programme, Delhi

Sharma SN, Srivastava PK, Kaul SM, Lal S (1999) Filariasis-epidemiology and control. Family Med India (Special Issue) 3:59–62

Showkath Ali MK, Rajendran R, Regu K, Mohanan MK, Dhariwal AC, Lal S (2007) Study on the factors affecting the MDA programme in Kerala state. J Commun Dis 39:51–56

Showkath Ali MK, Regu K, Rajendran R, Mohanan MK, Ganesh B (2008) Awareness of health personnel about lymphatic filariasis and mass drug administration in Kerala state. J Commun Dis 40:37–40

Srivastava PK, Dhariwal AC (2010) Progress towards morbidity management under elimination of Lymphatic Filariasis Programme in India. J Indian Med Assoc 108(12):854–862

Srivastava PK, Dhillon GP (2008) Elimination of lymphatic filariasis in India – a successful endeavour. J Indian Med Assoc 106:673–674

Srivastava PK, Dhariwal AC, Bhattacharjee J (2013) Status of Lymphatic Filariasis in India. Health action, p 19

Srivastava PK, Bhattacharjee J, Dhariwal AC, Krishnamoorthy K, Dash AP (2014a) Elimination of lymphatic filariasis – current status and way ahead. J Commun Dis 46:85–94

Srivastava PK, Sharma SN, Bhattacharjee J, Dhariwal AC, Krishnamoorthy K (2014b) A tool for monitoring epidemiological impact of Mass Drug Administration (MDA) in the elimination of lymphatic filariasis- an Indian experience. J Commun Dis 46(2):1–6

Srivastava PK, Krishnamoorthy K, Govenkar S, Perni S, Sanjeev D, Subramanain S, Dhariwal AC, Bhattacharjee J, Dash AP (2014c) Elimination of Lymphatic Filariasis in Goa: first successful transmission assessment survey in India. J Commun Dis 46(2):7–16

Talbot JT, Viall A, Direny A, de Rochars MB, Addiss D, Streit T, Mathieu E, Lammie PJ (2008) Predictors of compliance in mass drug administration for the treatment and prevention of lymphatic filariasis in Leogane, Haiti. Am J Trop Med Hyg 78:283–288

Vaishnav KG, Patel IC (2006) Independent assessment of Mass Drug Administration in filariasis affected Surat city. J Commun Dis 38:149–154

Vaishnav KG, Desai HS, Srivastava PK, Joshi PT, Kurian G, Thakor HG, Dhariwal AC (2012) Impact of mass drug administration on elimination of lymphatic filariasis in Surat city, India. J Commun Dis 44(4):251–259

Vanamail P, Ramaiah KD, Subramanian S, Pani SP, Yuvaraj J, 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

Weerasooriya MV, Yahathugoda CT, Wickramasinghe D, Gunawardena KN, Dharmadasa RA, Vidanapathirana K, Weerasekara SH, Samarawickrema WA (2007) Social mobilisation, drug coverage and compliance and adverse reactions in a Mass Drug Administration (MDA) Programme for the elimination of Lymphatic Filariasis in Sri Lanka. Filaria J 6:11

World Health Organization (1995) Bridging the gap. The World Health Report 1995. WHO, Geneva

World Health Organization (2011) Monitoring and epidemiological assessment of Mass Drug Administration. A manual for National elimination programmes WHO/HIM/NTD/PCT/2011.4

3 Next Step Lymphatic Filariasis Eradication: Current Status in the Development of a Vaccine Against Lymphatic Filariasis

Ramaswamy Kalyanasundaram

Abstract

Lymphatic filariasis (LF) is a mosquito-transmitted neglected tropical parasitic infection that causes most gruesome clinical outcome in the patients resulting in severe physical disability. Mass drug administration (MDA), spearheaded by the World Health Organization (WHO), is one of the most successful elimination strategies for any infection worldwide. India started implementing MDA since 2004 and has successfully reduced the incidence of the infection to a level that can be now easily managed. Few reports of non-compliance to MDA still remain. This has resulted in the presence of residual infections in some areas designated as "hotspots." Transmission assessment surveys need to identify these "hotspots." Before the momentum of MDA wanes, it is critically important to capitalize the success gained by MDA by planning toward an eradication strategy for LF in India. Eradication will not be possible with MDA alone. There is a need to develop a more sustainable strategy such as vaccine and vector control along with MDA. This paper reviews the current status of vaccine development against lymphatic filariasis and future prospects.

3.1 Introduction

Infections with lymphatic filariasis (LF) lead to a spectrum of disease conditions that primarily affects the lymphatic system of patients. Patients with chronic LF show severe disability (WHO fact sheet 2012). According to the World Health Organization (WHO), LF is the second leading cause of physical disability in the world (WHO fact

R. Kalyanasundaram (✉)
Department of Biomedical Sciences, College of Medicine, University of Illinois, Rockford, IL, USA
e-mail: ramswamy@uic.edu

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_3

sheet 2012; Zeldenryk et al. 2011). Reversal of chronic cases is often difficult and even with treatment the patient may not reach to complete normalcy. Therefore, it is important to diagnose the infection early on, so appropriate treatment can be instituted. Acute phase of the infection is complicated by the fact that majority of the patients do not show any symptoms (Kurniawan et al. 1993; Sabesan 2007). These asymptomatic carriers are thus a major source of disease transmission in a community. Attempts to accurately diagnose asymptomatic carriers in an endemic population have been challenging and continue to be a daunting task.

Mass administration of the drug diethylcarbamazine (DEC) to the entire population in Cherthala, Kerala State, and in Puducherry in India during 1990–1992 was successful in interrupting the transmission of LF in these regions (Raju et al. 2010; Kimura 2011). Based on the success achieved at Cherthala and Puducherry, the 50th World Health Assembly passed a resolution in 1997 to eliminate LF by 2020. One of the strategies approved was annual mass drug administration (MDA) of the antifilarial drugs, diethylcarbamazine, albendazole, and ivermectin, to approximately 3.5 billion people living in the endemic areas. Antifilarial drugs needed for MDA are supplied free of cost by GlaxoSmithKline, Merck & Co. Inc., and Eisai Inc. India implemented the MDA strategy in 2004 (WHO fact sheet 2012). After 10 years of MDA coverage, the incidence of LF infection has drastically reduced in India and reached to a level where the chances of broader transmission become negligible (Hussain et al. 2014). This is a great achievement for India. Certain focal areas of transmission – "hotspots" – continue to remain due to non-compliance of MDA (Sinha et al. 2012; Hussain et al. 2014; Gosh et al. 2013), which can be easily controlled. Before the momentum gained by MDA campaign is reduced, it is important to capitalize the progress achieved so far in India by planning toward eradication strategies. MDA alone will not be effective as an eradication strategy against LF because antifilarial drugs are only effective as a treatment for existing infection. For eradication of the disease, there is a need to build herd immunity in the endemic areas, so re-emergence of any residual infection can be prevented (Babayan et al. 2012; Mariapillai et al. 2015). This can only be achieved by vaccinating the endemic population against LF.

Significant progress has been made in the last two decades toward developing an effective vaccine for LF (Anand et al. 2007; Arumugam et al. 2014; Chenthamarakshan et al. 1995; Dakshinamoorthy et al. 2014; Dakshinamoorthy and Kalayanasundaram 2013; Kushwaha et al. 2012; Madhumathi et al. 2010b; Samykutty et al. 2010). This article will review the current status of vaccine development against LF and detail some of the major achievements in this area.

3.2 Development of Immunity Against LF

3.2.1 Concept of "Endemic Normal" and Natural Immunity to LF

Certain subjects living in the endemic regions develop natural immunity to LF (Ahmed 1967; Denham et al. 1983). These individuals are called "endemic

normals" or "EN" (Day 1991). EN individuals in general have high titer of antibodies against LF in their peripheral circulation (Gnanasekar et al. 2004). This is believed to be due to repeated exposure to low-level infection resulting in the development of immunity against the infection. The circulating antibodies in the periphery of EN subjects were shown to be protective in that under in vitro conditions these antibodies can kill infective larvae of LF through an antibody-dependent cellular cytotoxicity (ADCC) mechanism (Gnanasekar et al. 2004; Freedman et al. 1989). These findings clearly showed that it is possible to induce protective immune responses against LF in the human. Subsequent studies showed that the protective antibodies in the human are largely of IgG1 and IgG3 isotype (Dakshinamoorthy et al. 2013a, b) and the cells that kill the larvae through ADCC mechanism are CD11b + macrophages that express FcγRI and produce copious amounts of myeloperoxidases and nitric oxide (Dakshinamoorthy et al. 2013a, b). Thus, we now know the potential mechanisms by which protective immunity works in the EN subjects involves IgG1 and IgG3 antibodies and FcγRI bearing macrophages.

3.2.2 The Need for Developing a Vaccine Against LF in the Human

India has achieved over 90% of MDA coverage, and this has substantially reduced the incidence of the infection (Raju et al. 2010; Nujum 2014; Anil 2012; Lahariya and Mishra 2008). However, there are few reports of non-compliance to MDA in some areas. The next phase of transmission assessment survey can potentially identify these "hotspots" where the infection still persists and implement targeted control approaches. Unfortunately the acutely infected individuals do not show any clinical symptoms (Sabesan 2007). These individuals will have adult LF parasites in their lymphatic system and produce microfilaria that appears in the peripheral blood for transmission to mosquitoes. If these individuals fail to take the anti-LF medication administered through MDA, they can be a nidus for transmission in their locality leading to formation of "hotspots" (Mariapillai et al. 2015). A recent survey to determine the presence of LF infection in mosquitoes trapped from 11 districts in the state of Maharashtra in India showed that nearly all of the villages surveyed had infected mosquitoes, especially in and around the locality where infected subjects were present (Ramaswamy 2016). This clearly suggests that transmission of infection can potentially occur in areas where there is non-compliance to MDA. Therefore it is very critical that all individuals receive routine MDA treatment in a locality where LF infected patients live.

A second major fact to consider is that the drugs used in MDA will only treat an infection; the drugs will not prevent future infections. Therefore, repeated MDA treatment is essential to ensure that no reinfection occurs (Hussain et al. 2014). In addition, since the disease is transmitted by mosquitoes, vector control should be a priority especially in communities where clinical cases of LF are diagnosed (Sabesan et al. 2010; Mariapillai et al. 2015; Sunish et al. 2007, 2016). Despite

stringent MDA coverage and effective vector control measures, it is often difficult to totally eliminate a chronic infection such as LF from a community (Mariapillai et al. 2015). There is a need for a more stringent approach such as an effective vaccine against LF that when combined with targeted treatment of infected patients and an effective mosquito vector control can achieve total elimination.

3.3 Developing a Vaccine Against LF

3.3.1 Early Attempts to Develop a Vaccine Against LF

Initial attempts to develop a vaccine against LF used live attenuated infective larvae of *B. malayi* that were attenuated using 20kR X-ray (Wong et al. 1969; Yates and Higashi 1985; Denham 1980). When seven rhesus macaques were immunized with the radiation-attenuated larvae and challenged, five macaques (71%) failed to show any signs of infection for up to 6 months after challenge (Wong et al. 1969). Similarly, Yates and Higashi (1985) showed that infective *B. malayi* L_3 attenuated using 15 kR irradiation with cobalt-60 gave as high as 91% protection in jirds against challenge infection (Yates and Higashi 1985; Denham 1980). In another study, Oothuman et al. (1979) showed that 10 kR gamma-irradiated *B. pahangi* L3 conferred significant protection in cats. Subsequently, several attempts were made using dead larvae (Abraham et al. 1989; Carlow and Philipp 1987; Hayashi et al. 1984, 1989). These studies strongly suggested the possibility of developing an effective vaccine against LF for human. However, live attenuated or dead parasites are not suitable for human vaccination. Although these studies did not result in a vaccine, these earlier studies provided important evidence that significant protective immunity can be induced against LF. These reports thus served as a strong platform for subsequent studies aimed at developing a vaccine against LF and search for suitable subunit antigens as vaccine candidates against human LF infections.

3.3.2 Strategies for Selecting the Vaccine Antigens Against LF

Immunization with attenuated parasites and dead parasites conferred significant protection. This suggested that antigens expressed on the surface of the parasite may be potential targets for protective immune responses. This led to the screening of the genome and proteome of the parasite for identifying potential soluble and surface-expressed antigens of the parasite (Bennuru et al. 2009, 2011; Luck et al. 2015; Bal and Das 1999; Armstrong et al. 2014; Dash et al. 2011). When soluble antigens of microfilaria (Hammerberg et al. 1989; Pearlman et al. 1993) and whole worm extracts of adult worms (Hayashi et al. 1989) were used as vaccine antigens, varying degree of protection was observed against challenge infections in mice. The results were not encouraging. Completion of the genome of *B. malayi* opened up new opportunities for screening the genome for potential vaccine candidates.

3.3.3 LF Parasites Modulate Host Immune Responses

It is still a mystery how the parasite goes through the circulatory system of the host and resides inside the lymphatic vessels where they are bathed by millions of immune cells daily for over 30 years yet trigger little or no protective immune responses (Semnani et al. 2004; Lawrence and Devaney 2001). Several studies demonstrated that the parasite may be producing host immunomodulatory molecules to escape host immune responses (Rajamanickam and Babu 2013; Babu and Nutman 2014; Hoerauf et al. 2005). Diethylcarbamazine (DEC) treatment partially reversed the host immunosuppression induced by lymphatic filarial parasite (Piessens et al. 1981). Thus, these molecules were targeted for developing as vaccine candidates by several groups (Kwa and Mak 1980; Nutman et al. 1987; Piessens et al. 1980a, b, 1982; Portaro et al. 1976, 1977; Weller 1978; King et al. 1992; Sharma et al. 2012; Lal et al. 1990; Sharmila et al. 2011; Dakshinamoorthy et al. 2012; Madhumathi et al. 2010a; Veerapathran et al. 2009). Extensive analyses of the host immunomodulatory molecules resulted in the identification of several promising vaccine candidates. Despite identifying these promising vaccine candidates, a major bottleneck to developing a vaccine against LF is the lack of a good animal model.

3.3.4 Animal Models and Limitations to Developing a Vaccine Against LF

Experimental rodents (mouse, jird, and mastomys) were extensively used to characterize potential vaccine candidates and develop a vaccine against LF (WHO 1981; Carlow and Philipp 1987; Cross and Hsu 1987; Ewert and Folse 1984). Unfortunately, experimental rodents are refractive to *W. bancrofti* infections (Vincent et al. 1982). Therefore, *B. malayi* and *B. pahangi* are routinely used as experimental models in rodents to define immunity to LF. Mouse is nonpermissive to *Brugia* infections, meaning the parasites do not develop into mature adult parasites in immunocompetent mice (Ewert and Folse 1984). Although several attempts were made to develop the parasite in nude mouse models, they have not been explored as a model for vaccine development (Vickery and Nayar 1987). Jird and mastomys are permissive hosts, meaning the *Brugia* parasites can develop into adult parasites in these animals and produce microfilariae that appear in the peripheral circulation similar to that in the human. Therefore, these are excellent models to study challenge parasite establishment following vaccination. A major advantage of using mastomys and jird models is that the mouse immunological reagents (antibodies to immunoglobulins and cytokines) can be used to define immune responses in these animals. However, cross-reactivity of mouse reagents with jirds' immune system is not fully established although several publications have used these (Anand et al. 2011; Thirugnanam et al. 2007; Kalyanasundaram and Balumuri 2011; Joseph and Ramaswamy 2013; Gnanasekar et al. 2004). Despite the fact that jirds and mastomys are permissive hosts, a major drawback to using these models is that these animals do not develop

the typical pathology that is seen in the human. Infections with both *W. bancrofti* and *B. malayi* in the nonhuman primates resemble human infections, and they can develop the typical pathology. Therefore, nonhuman primates are ideal models to study host-parasite relationship and immunity to LF (Wong et al. 1969; Dakshinamoorthy et al. 2014). Major drawbacks to using nonhuman primates are the cost and problems with handling. Unfortunately, in some countries, use of nonhuman primates in medical research is banned. Recent studies have attempted to understand the host-parasite relationship in nonhuman primates and develop a vaccine against LF in nonhuman primates (Dakshinamoorthy et al. 2014; Wong et al. 1969).

3.3.5 Screening Filariasis Genome to Identify Vaccine Candidates

EN subjects have circulating IgG1 and IgG3 antibodies against parasite antigens that are protective and can participate in the killing of *B. malayi* infective larvae in an ADCC reaction (Dakshinamoorthy et al. 2013a; Hitch et al. 1991; Chandrashekar et al. 1985). Thus, antigens or peptides that elicited these protective antibodies in the EN subjects will be an attractive target for prophylactic vaccine development (Gnanasekar et al. 2004; Dakshinamoorthy et al. 2012, 2013b). One of the earlier studies exploited this approach by screening a phage displayed expression library of the infective stages of LF with sera from EN subjects who had high titer of protective antibodies (Gnanasekar et al. 2004). This approach resulted in the identification of several potential vaccine antigens including some of the antigens that were identified earlier as promising vaccine antigens such as abundant larval transcript 2 (ALT2) (Gregory et al. 2000), thioredoxin peroxide (TPX2) (Gnanasekar et al. 2004), collagen 4 (Col4) (Gnanasekar et al. 2004), heat shock protein (HSP) (Dakshinamoorthy et al. 2012), and glutathione S-transferases (GST) (Veerapathran et al. 2009). Using a bioinformatics approach, those antigens that are expressed on the surface of the parasite and have minimum or no homology to human proteins were selected for further development as vaccine candidates.

3.3.6 Improving the Vaccine Efficacy

Nearly all attempts to develop a vaccine against LF with a single antigen (monovalent) gave unsatisfactory results (Vanam et al. 2009; Vedi et al. 2008; Singh et al. 2014; Kazura et al. 1990; Gnanasekar et al. 2005; Anand et al. 2007; Dixit et al. 2006; Kushwaha et al. 2013; Li et al. 1993). LF is a multicellular organism that uses multiple approaches to evade host immune responses and survive in the host. Therefore, targeting a single critical antigen will not have the desired effect as the LF parasite is notorious for using redundant mechanisms to escape host insults. Subsequent approaches to combine potential vaccine antigens as cocktail vaccines (Anand et al. 2008), chimeric

antigen (Anugraha et al. 2013), multisubunit vaccines (Shrivastava et al. 2013), or multivalent vaccines (Samykutty et al. 2010; Kalyanasundaram and Balumuri 2011; Dakshinamoorthy and Kalayanasundaram 2013; Chauhan et al. 2018) gave excellent results. Thus, vaccine development against LF started focusing toward developing multivalent vaccines. Various combinations of vaccine antigens were evaluated as multivalent vaccines. Among these, the combination of ALT-2, small heat shock protein (HSP) 12.6 (Dakshinamoorthy et al. 2012), and tetraspanin (TSP) large extracellular loop (Gnanasekar et al. 2008) as a recombinant multivalent fusion protein vaccine (r*Bm*HAT) gave close to 94% protection against challenge infection in rodents (Dakshinamoorthy et al. 2013b; Dakshinamoorthy and Kalayanasundaram 2013; Chauhan et al. 2018) and about 70% protection against challenge infections in rhesus macaques (Dakshinamoorthy et al. 2014; Khatri et al. 2018). To date, this is the best vaccine formulation anybody has demonstrated against LF and is currently undergoing human clinical trial. Table 3.1 summarizes some of the most successful vaccination trials in animal models.

Table 3.1 Summary of the most successful vaccination trials against *B.malayi* infection in animal models

Vaccines	Protection	Animal models	Reference
Irradiate L3	41%	Jirds	Carlow et al (1987)
Dead L3	43%	Mice	Li et al (1993)
Paramyosin	40–60%	Mice	Nanduri and Kazura (1989)
Purified fractions of extracts			
L3 (aqueous extract)	70%	Jirds	Carlow et al (1987)
BmL_3S-2	76%	Jirds	Bhandari et al (2005)
Mf			
Bm mf-7	81%	Jirds	Krithika et al (2005)
Adult worms			
BmAFII	85%	*Mastomys Coucha*	Dixit et al (2006)
F6 (54–68 kDa)	72%	*Mastomys Coucha*	Sahoo et al (2009)
Recombinant monovalent			
ALT-1	76%	Jirds	Gregory et al (2000)
ALT-2	74%	Mice	Ramachandran et al (2004)
VAH	64%	Jirds	Murray et al (2001)
TGA	30%	Jirds	Vanam et al (2009)
TPX	43%	Jirds	Vanam et al (2009)
GST	61%	Jirds	Veerapathran et al (2009)
HSP12.6	58%	Mice	Dakshinamoorthy et al (2012)
TSP	60%	Mice	Dakshinamoorthy et al (2013a, b)
CPI	48%	Jirds	Arumugam et al (2014)
TPP	71%	*Mastomys Coucha*	Kushwaha et al (2013)
DIM-1	50%	*Mastomys Coucha*	Kushwaha et al (2014)

(continued)

Table 3.1 (continued)

Vaccines	Protection	Animal models	Reference
Cocktail			
TGA+TPX	74%	Jirds	Vanam et al (2009)
VAH+ALT-2	80%	Jirds	Anand et al (2011)
TRX+TPX	71%	*Mastomys coucha*	Prince et al (2014)
Myosin+ TPP	70%	Jirds	Shrivastava et al (2013)
iPGM + TPP	70%	Jirds	Shrivastava et al (2013)
VAL-1+ALT-2	77–80%	Jirds	Kalyanasundaram and Balumuri (2011)
Bivalent			
HSP12.6+ALT-2	90%	Mice	Dakshinamoorthy et al (2012)
HSP12.6+TSP	80%	Mice	
TSP+ALT-2	82%	Mice	
Multivalent			
Multiepitope	70%	Jirds	Anugraha et al (2013, 2015)
HAT	94%	Mice	Dakshinamoorthy et al (2012)
HAT	45%	Rhesus macaque	Dakshinamoorthy et al (2014)
HAXT	88%	Mice	Chauhan et al (2018)
HAXT	70%	Rhesus macaque	Khatri et al (2018)

3.4 Future Prospects and Hurdles Before an LF Vaccine Becomes Available for the End Game to Control the Infection

Much of the current understanding on the host immune responses to LF came from excellent research by a number of groups during the last three decades. Subsequent studies that defined the various host immunomodulatory mechanisms by LF parasites and completion of the LF genome exploded the field of antigen discovery and led to the development of several vaccine antigens against LF. Preclinical vaccination trials proved that multivalent vaccine is highly effective. However, LF being a neglected tropical parasitic infection, there are several hurdles before the vaccine becomes available for individuals who are at risk in the endemic regions. Foremost among these hurdles is the lack of funding and interest among major pharmaceutical industries for manufacturing LF vaccine. Despite the roadblocks, a multivalent vaccine will potentially be available for human use soon. Combination of a prophylactic vaccine with targeted treatment of infected individuals and vector control will totally eliminate LF from potential "hotspots" in India.

3.5 Concluding Remarks

India has achieved tremendous progress in its efforts toward elimination of LF. The MDA program along with significant morbidity management and public health awareness has significantly reduced the incidence of LF in India. The public health

department and everybody involved in this effort needs to be highly commended for this outstanding achievement. With large areas cleared of infection by MDA, it becomes easy to identify the hotspots. All control efforts and resources can then be diverted into these hotspot areas to identify asymptomatic carriers. Once the presence of these individuals are identified in a community, targeted treatment, stringent mosquito control measures, and administration of prophylactic vaccination of all subjects at risk should be instituted for total elimination of LF infections from that community. This approach need to be repeated in all hotspot areas to achieve total elimination and possibly eradication of LF in India. Given the significant progress India has achieved in LF control over the past one decade, there is no doubt that LF will be totally eliminated from India.

References

Abraham D, Grieve RB, Holy JM, Christensen BM (1989) Immunity to larval *Brugia malayi* in BALB/c mice: protective immunity and inhibition of larval development. Am J Trop Med Hyg 40:598–604

Ahmed SS (1967) Studies on the laboratory transmission of sub-periodic *Brugia malayi* and *B. pahangi*. I. The resistance of Guinea-pigs, rabbits and white mice to infection. Ann Trop Med Parasitol 61:93–100

Anand SB, Gnanasekar M, Thangadurai M, Prabhu PR, Kaliraj P, Ramaswamy K (2007) Immune response studies with *Wuchereria bancrofti* vespid allergen homologue (WbVAH) in human lymphatic filariasis. Parasitol Res 101:981–988

Anand SB, Murugan V, Prabhu PR, Anandharaman V, Reddy MV, Kaliraj P (2008) Comparison of immunogenicity, protective efficacy of single and cocktail DNA vaccine of *Brugia malayi* abundant larval transcript (ALT-2) and thioredoxin peroxidase (TPX) in mice. Acta Trop 107:106–112

Anand SB, Kodumudi KN, Reddy MV, Kaliraj P (2011) A combination of two *Brugia malayi* filarial vaccine candidate antigens (BmALT-2 and BmVAH) enhances immune responses and protection in jirds. J Helminthol 85:442–452

Anil NS (2012) Assessing Coverage of Mass Drug Administration against Lymphatic Filariasis in Gulbarga District, Karnataka. Int J Med Public Health 2(3):25

Anugraha G, Jeyaprita PJ, Madhumathi J, Sheeba T, Kaliraj P (2013) Immune responses of *B. malayi* thioredoxin (TRX) and venom allergen homologue (VAH) chimeric multiple antigen for lymphatic filariasis. Acta Parasitol 58:468–477

Anugraha G, Madhumathi J, Prince PR, Prita PJ, Khatri VK, Amdare NP, Reddy MV, Kaliraj P (2015) Chimeric epitope vaccine from multistage antigens for lymphatic filariasis. Scand J Immunol 82:380–389

Armstrong SD, Babayan SA, Lhermitte-Vallarino N, Gray N, Xia D, Martin C, Makepeace BL (2014) Comparative analysis of the secretome from a model filarial nematode (*Litomosoides sigmodontis*) reveals maximal diversity in gravid female parasites. Mol Cell Proteomics 13:2527–2544

Arumugam S, Wei J, Ward D, Abraham D, Lustigman S, Zhan B, Klei TR (2014) Vaccination with a genetically modified *Brugia malayi* cysteine protease inhibitor-2 reduces adult parasite numbers and affects the fertility of female worms following a subcutaneous challenge of Mongolian gerbils (*Meriones unguiculatus*) with *B. malayi* infective larvae. Int J Parasitol 44:675–679

Babayan SA, Allen JE, Taylor DW (2012) Future prospects and challenges of vaccines against filariasis. Parasite Immunol 34:243–253

Babu S, Nutman TB (2014) Immunology of lymphatic filariasis. Parasite Immunol 36:338–346

Bal M, Das MK (1999) Antibody response to a filarial antigen fraction in individuals exposed to *Wuchereria bancrofti* infection in India. Acta Trop 72:259–274

Bennuru S, Semnani R, Meng Z, Ribeiro JMC, Veenstra TD, Nutman TB (2009) *Brugia malayi* excreted/secreted proteins at the host/parasite Interface: stage- and gender-specific proteomic profiling. PLoS Negl Trop Dis 3:e410. https://doi.org/10.1371/journal.pntd.0000410

Bennuru S, Meng Z, Ribeiro JM, Semnani RT, Ghedin E, Chan K, Lucas DA, Veensta TD, Nutman TB (2011) Stage-specific proteomic expression patterns of the human filarial parasite *Brugia malayi* and its endosymbiont *Wolbachia*. Proc Natl Acad Sci U S A 108:9649–9654

Bhandari Y, Dabir P, Krithika KN, Kannayakanahalli MD, Shouche YS, Reddy MVR (2005) Analysis of polymorphism of 18S rRNA gene in *Wuchereria bancrofti* microfilariae. Microbiol Immunol 49:909–914

Carlow CK, Philipp M (1987) Protective immunity to *Brugia malayi* larvae in BALB/c mice: potential of this model for the identification of protective antigens. Am J Trop Med Hyg 37:597–604

Chandrashekar R, Rao UR, Subrahmanyam D (1985) Serum dependent cell-mediated immune reactions to *Brugia pahangi* infective larvae. Parasite Immunol 7:633–641

Chauhan N, Khatri V, Banerjee P, Kalyanasundaram R (2018) Evaluating the Vaccine Potential of a Tetravalent Fusion Protein (rBmHAXT) Vaccine Antigen Against Lymphatic Filariasis in a Mouse Model. Front Immunol 9

Chenthamarakshan V, Reddy MV, Harinath BC (1995) Immunoprophylactic potential of a 120 kDa *Brugia malayi* adult antigen fraction, BmA-2, in lymphatic filariasis. Parasite Immunol 17:277–285

Cross JH, Hsu MY (1987) Development of *Brugia malayi* in Mongolian gerbils previously exposed to *Wuchereria bancrofti*. Southeast Asian J Trop Med Public Health 18:183–185

Dakshinamoorthy G, Kalyanasundaram R (2013) Evaluating the efficacy of rBmHATalphac as a multivalent vaccine against lymphatic filariasis in experimental animals and optimizing the adjuvant formulation. Vaccine 32:19–25

Dakshinamoorthy G, Samykutty AK, Munirathinam G, Shinde GB, Nutman T, Reddy MV, Kalyanasundaram R (2012) Biochemical characterization and evaluation of a *Brugia malayi* small heat shock protein as a vaccine against lymphatic filariasis. PLoS One 7:e34077

Dakshinamoorthy G, Munirathinam G, Stoicescu K, Reddy MV, Kalyanasundaram R (2013a) Large extracellular loop of tetraspanin as a potential vaccine candidate for filariasis. PLoS One 8:e77394

Dakshinamoorthy G, Samykutty AK, Munirathinam G, Reddy MV, Kalyanasundaram R (2013b) Multivalent fusion protein vaccine for lymphatic filariasis. Vaccine 31:1616–1622

Dakshinamoorthy G, von Gegerfelt A, Andersen H, Lewis M, Kalyanasundaram R (2014) Evaluation of a multivalent vaccine against lymphatic filariasis in rhesus macaque model. PLoS One 9:e112982

Dash Y, Ramesh M, Kalyanasundaram R, Munirathinam G, Shultz LD, Rajan TV (2011) Granuloma formation around filarial larvae triggered by host responses to an excretory/secretory antigen. Infect Immun 79:838–845

Day KP (1991) The endemic normal in lymphatic filariasis: a static concept. Parasitol Today 7:341–343

Denham DA (1980) Vaccination against filarial worms using radiation-attenuated vaccines. Int J Nucl Med Biol 7:105–111

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

Dixit S, Gaur RL, Sahoo MK, Joseph SK, Murthy PS, Murthy PK (2006) Protection against L3 induced *Brugia malayi* infection in *Mastomys coucha* pre-immunized with BmAFII fraction of the filarial adult worm. Vaccine 24:5824–5831

Ewert A, Folse D (1984) Animal model of human disease. Lymphatic filariasis. Am J Pathol 115:135–137

Freedman DO, Nutman TB, Ottesen EA (1989) Protective immunity in bancroftian filariasis. J Clin Invest 83:14–22

Gosh S, Samanta A, Kole S (2013) Mass drug administration for elimination of lymphatic filariasis: recent experiences from a district of West Bengal, India. Trop Parasitol 3:67–71

Gnanasekar M, Rao KV, He YX, Mishra PK, Nutman TB, Kaliraj P, Ramaswamy K (2004) Novel phage display-based subtractive screening to identify vaccine candidates of *Brugia malayi*. Infect Immun 72:4707–4715

Gnanasekar M, Padmavathi B, Ramaswamy K (2005) Cloning and characterization of a novel immunogenic protein 3 (NIP3) from *Brugia malayi* by immuno screening of a phage-display cDNA expression library. Parasitol Res 97:49–58

Gnanasekar M, Anand SB, Ramaswamy K (2008) Identification and cloning of a novel tetraspanin (TSP) homologue from *Brugia malayi*. DNA Seq 19:151–156

Gregory WF, Atmadja AK, Allen JE, Maizels RM (2000) The abundant larval transcript-1 and -2 genes of *Brugia malayi* encode stage-specific candidate vaccine antigens for filariasis. Infect Immun 68:4174–4179

Hammerberg B, Nogami S, Nakagaki K, Hayashi Y, Tanaka H (1989) Protective immunity against Brugia malayi infective larvae in mice. II. Induction by a T cell-dependent antigen isolated by monoclonal antibody affinity chromatography and SDS-PAGE. J Immunol 143:4201–4207

Hayashi Y, Noda K, Shirasaka A, Nogami S, Nakamura M (1984) Vaccination of BALB/c mice against *Brugia malayi* and *B. pahangi* with larvae attenuated by gamma irradiation. Jpn J Exp Med 54:177–181

Hayashi Y, Nakagaki K, Nogami S, Hammerberg B, Tanaka H (1989) Protective immunity against *Brugia malayi* infective larvae in mice. I. Parameters of active and passive immunity. Am J Trop Med Hyg 41:650–656

Hitch WL, Hightower AW, Eberhard ML, Lammie PJ (1991) Analysis of isotype-specific antifilarial antibody levels in a Haitian pediatric population. Am J Trop Med Hyg 44:161–167

Hoerauf A, Satogunia J, Saeftel M, Specht S (2005) Immunomodulation by filarial nematodes. Parasite Immunol 27:417–429

Hussain MA, Sitha AK, Swain S, Kadam S, Pati S (2014) Mass drug administration for lymphatic filariasis elimination in a coastal state of India: a study on barriers to coverage and compliance. Infect Dis Poverty 3:31

Joseph SK, Ramaswamy K (2013) Single multivalent vaccination boosted by trickle larval infection confers protection against experimental lymphatic filariasis. Vaccine 31:3320–3326

Kalyanasundaram R, Balumuri P (2011) Multivalent vaccine formulation with BmVAL-1 and BmALT-2 confer significant protection against challenge infections with *Brugia malayi* in mice and jirds. Res Rep Trop Med 2011:45–56

Kazura JW, Maroney PA, Pearlman E, Nilsen TW (1990) Protective efficacy of a cloned *Brugia malayi* antigen in a mouse model of microfilaremia. J Immunol 145:2260–2264

Khatri V, Chauhan N, Vishnoi K, von Gegerfelt A, Gittens C, Kalyanasundaram R (2018) Prospects of developing a prophylactic vaccine against human lymphatic filariasis - evaluation of protection in nonhuman primates. Int J Parasitol. https://doi.org/10.1016/j.ijpara.2018.04.002 [Epub ahead of print]

Kimura E (2011) The Global Programme to Eliminate Lymphatic Filariasis: history and achievements with special reference to annual single-dose treatment with diethylcarbamazine in Samoa and Fiji. Trop Med Health 39:17–30

King CL, Kumaraswami V, Poindexter RW, Kumari S, Jayaraman K, Alling DW, Nutman TB (1992) Immunologic tolerance in lymphatic filariasis. Diminished parasite-specific T and B lymphocyte precursor frequency in the microfilaremic state. J Clin Invest 89:1403–1410

Krithika KN, Dabir P, Kulkarni S, Anandharaman V, Reddy MVR (2005) Identification of 38kDa *Brugia malayi* microfilarial protease as a vaccine candidate for lymphatic filariasis. Indian J Exp Biol 43:759–768

Kurniawan A, Yazdanbakhsh M, van Ree R, Aalberse R, Selkirk ME, Partono F, Maizels RM (1993) Differential expression of IgE and IgG4 specific antibody responses in asymptomatic and chronic human filariasis. J Immunol 150:3941–3950

Kushwaha S, Singh PK, Gupta J, Soni VK, Misra-Bhattacharya S (2012) Recombinant trehalose-6-phosphate phosphatase of *Brugia malayi* cross-reacts with human *Wuchereria bancrofti* immune sera and engenders a robust protective outcome in mice. Microbes Infect 14:1330–1339

Kushwaha S, Singh PK, Rana AK, Misra-Bhattacharya S (2013) Immunization of Mastomys coucha with *Brugia malayi* recombinant trehalose-6-phosphate phosphatase results in significant protection against homologous challenge infection. PLoS One 8:e72585

Kushwaha V, Kumar V, Verma SK, Sharma R, Siddiqi MI, Murthy PK (2014) Disorganized muscle protein-1 (DIM-1) of filarial parasite *Brugia malayi*: cDNA cloning, expression, purification, structural modeling and its potential as vaccine candidate for human filarial infection. Vaccine 32:1693–1699

Kwa BH, Mak JW (1980) Specific depression of cell-mediated immunity in Malayan filariasis. Trans R Soc Trop Med Hyg 74:522–527

Lahariya C, Mishra A (2008) Strengthening of mass drug administration implementation is required to eliminate lymphatic filariasis from India: an evaluation study. J Vector Borne Dis 45:313–320

Lal RB, Kumaraswami V, Steel C, Nutman TB (1990) Phosphocholine-containing antigens of *Brugia malayi* nonspecifically suppress lymphocyte function. Am J Trop Med Hyg 42:56–64

Lawrence RA, Devaney E (2001) Lymphatic filariasis: parallels between the immunology of infection in humans and mice. Parasite Immunol 23:353–361

Li BW, Chandrashekar R, Weil GJ (1993) Vaccination with recombinant filarial paramyosin induces partial immunity to *Brugia malayi* infection in jirds. J Immunol 150:1881–1885

Luck AN, Anderson KG, McClung CM, VerBerkmoes NC, Foster JM, Michalski ML, Slatko BE (2015) Tissue-specific transcriptomics and proteomics of a filarial nematode and its *Wolbachia* endosymbiont. BMC Genomics 16:920–936

Madhumathi J, Pradiba D, Prince PR, Jeyaprita PJ, Rao DN, Kaliraj P (2010a) Crucial epitopes of *Wuchereria bancrofti* abundant larval transcript recognized in natural infection. Eur J Clin Microbiol Infect Dis 29:1481–1486

Madhumathi J, Prince PR, Anugraha G, Kiran P, Rao DN, Reddy MV, Kaliraj P (2010b) Identification and characterization of nematode specific protective epitopes of *Brugia malayi* TRX towards development of synthetic vaccine construct for lymphatic filariasis. Vaccine 28:5038–5048

Mariapillai K, Sunish IP, Nagaraj J, Tyagi BK (2015) Residual microfilaraemia in rural pockets of South India after five rounds of DEC plus albendazole administration as part of the LF elimination campaign. J Vector Borne Dis 52:182–184

Murray J, Gregory WF, Gomez-Escobar N, Atmadja AK, Maizels RM (2001) Expression and immune recognition of *Brugia malayi* VAL-1, a homologue of vespid venom allergens and Ancylostoma secreted proteins. Mol Biochem Parasitol 118:89–96

Nanduri J, Kazura JW (1989) Paramyosin-enhanced clearance of *Brugia malayi* microfilaremia in mice. J Immunol 143:3359–3363

Nujum ZT (2014) Coverage of, and compliance with, mass drug administration under the programme to eliminate lymphatic filariasis in India: a systematic review. Trans R Soc Trop Med Hyg 108:538–549

Nutman TB, Kumaraswami V, Ottesen EA (1987) Parasite-specific anergy in human filariasis. Insights after analysis of parasite antigen-driven lymphokine production. J Clin Invest 79:1516–1523

Oothuman P, Denham DA, McGreevy PB, Nelson GS, Rogers R (1979) Successful vaccination of cats against *Brugia pahangi* with larvae attenuated by irradiation with 10 krad cobalt 60. Parasite Immunol 1:209–216

Pearlman E, Hazlett FE Jr, Boom WH, Kazura JW (1993) Induction of murine T-helper-cell responses to the filarial nematode *Brugia malayi*. Infect Immun 61:1105–1112

Piessens WF, McGreevy PB, Piessens PW, McGreevy M, Koiman I, Saroso JS, Dennis DT (1980a) Immune responses in human infections with *Brugia malayi*: specific cellular unresponsiveness to filarial antigens. J Clin Invest 65:172–179

Piessens WF, McGreevy PB, Ratiwayanto S, McGreevy M, Piessens PW, Koiman I, Dennis DT (1980b) Immune responses in human infections with *Brugia malayi*: correlation of cellular and humoral reactions to microfilarial antigens with clinical status. Am J Trop Med Hyg 29:563–570

Piessens WF, Ratiwayanto S, Piessens PW, Tuti S, McGreevy PB, Darwis F, Dennis DT (1981) Effect of treatment with diethylcarbamazine on immune responses to filarial antigens in patients infected with *Brugia malayi*. Acta Trop 38:227–234

Piessens WF, Partono F, Hoffman SL, Ratiwayanto S, Piessens PW, Palmieri JR, Carney WP (1982) Antigen-specific suppressor T lymphocytes in human lymphatic filariasis. N Engl J Med 307:144–148

Portaro JK, Britton S, Ash LR (1976) *Brugia pahangi*: depressed mitogen reactivity in filarial infections in the jird, Meriones unguiculatus. Exp Parasitol 40:438–436

Portaro JK, Kowalski JC, Ash LR (1977) *Brugia pahangi*: effects of age and filarial infection on jird (Meriones unguiculatus) lymphocyte function. Exp Parasitol 43:122–127

Prince PR, Madhumathi J, Anugraha G, Jeyaprita PJ, Reddy MV, Kaliraj P (2014) Tandem antioxidant enzymes confer synergistic protective responses in experimental filariasis. J Helminthol 88:402–410

Rajamanickam A, Babu S (2013) Immunomodulation by filarial parasites. Int Trends Immun 1:12–20

Raju K, Jambulingam P, Sabesan S, Vanamail P (2010) Lymphatic filariasis in India: epidemiology and control measures. J Postgrad Med 56:232–238

Ramachandran S, Kumar MP, Rami RM, Chinnaiah HB, Nutman T, Kaliraj P, McCarthy J (2004) The larval specific lymphatic filarial ALT-2: induction of protection using protein or DNA vaccination. Microbiol Immunol 48:945–955

Ramaswamy K (2016) Chapter 3: Lymphatic Filariasis: current status of elimination using chemotherapy and the need for a vaccine. In: Saxena AK (ed) Tropical diseases II. Topics in medicinal chemistry. https://doi.org/10.1007/7355_2015_5002

Sabesan S (2007) Human lymphatic filariasis is not a disease, but a syndrome. Curr Sci 92:283–284

Sabesan S, Vanamail P, Raju KHK, Jambulingam P (2010) Lymphatic filariasis in India: epidemiology and control measures. J Postgrad Med 56:232–238

Sahoo MK, Sisodia BS, Dixit S, Joseph SK, Gaur RL, Verma SK, Verma AK, Shasany AK, Dowle AA, Murthy PK (2009) Immunization with inflammatory proteome of *Brugia malayi* adult worm induces a Th1/Th2-immune response and confers protection against the filarial infection. Vaccine 27:4263–4271

Samykutty A, Dakshinamoorthy G, Kalyanasundaram R (2010) Multivalent vaccine for lymphatic Filariasis. Procedia Vaccinol 3:12–18

Semnani RT, Law M, Kubofcik J, Nutman TB (2004) Filaria-induced immune evasion: suppression by the infective stage of *Brugia malayi* at the earliest host-parasite interface. J Immunol 172:6229–6238

Sharma R, Hoti SL, Meena RL, Vasuki V, Sankari T, Kaliraj P (2012) Molecular and functional characterization of macrophage migration inhibitory factor (MIF) homolog of human from lymphatic filarial parasite *Wuchereria bancrofti*. Parasitol Res 111:2035–2047

Sharmila S, Christiana I, Kiran P, Reddy MV, Kaliraj P (2011) The adjuvant-free immunoprotection of recombinant filarial protein Abundant Larval Transcript-2 (ALT-2) in *Mastomys coucha* and the immunoprophylactic importance of its putative signal sequence. Exp Parasitol 129:247–253

Shrivastava N, Singh PK, Nag JK, Kushwaha S, Misra-Bhattacharya S (2013) Immunization with a multisubunit vaccine considerably reduces establishment of infective larvae in a rodent model of *Brugia malayi*. Comp Immunol Microbiol Infect Dis 36:507–519

Singh PK, Kushwaha S, Rana AK, Misra-Bhattacharya S (2014) Cofactor independent phosphoglycerate mutase of *Brugia malayi* induces a mixed Th1/Th2 type immune response and inhibits larval development in the host. Biomed Res Int 2014:590281

Sinha N, Mallik S, Mallik S, Panja TK, Haldar A (2012) Coverage and compliance of mass drug Administration in Lymphatic Filariasis: a comparative analysis in a district of West Bengal, India. Glob J Med Pub Health 1:3–10

Sunish I, Rajendran R, Mani T, Munirathinam A, Dash A, Tyagi B (2007) Vector control complements mass drug administration against bancroftian filariasis in Tirukoilur, India. Bull World Health Organ 85:138–145

Sunish I, Kalimuthu M, Ashok Kumar V, Munirathinam A, Nagaraj J, Tyagi BK, White GB, Arunachalam N (2016) Can community-based integrated vector control hasten the process of LF elimination? Parasitol Res 115:2353–2362

Thirugnanam S, Pandiaraja P, Ramaswamy K, Murugan V, Gnanasekar M, Nandakumar K, Kaliraj P (2007) *Brugia malayi*: comparison of protective immune responses induced by Bm-alt-2 DNA, recombinant Bm-ALT-2 protein and prime-boost vaccine regimens in a jird model. Exp Parasitol 116:483–491

Vanam U, Pandey V, Prabhu PR, Dakshinamurthy G, Reddy MV, Kaliraj P (2009) Evaluation of immunoprophylactic efficacy of *Brugia malayi* transglutaminase (BmTGA) in single and multiple antigen vaccination with BmALT-2 and BmTPX for human lymphatic filariasis. Am J Trop Med Hyg 80:319–324

Vedi S, Dangi A, Hajela K, Misra-Bhattacharya S (2008) Vaccination with 73kDa recombinant heavy chain myosin generates high level of protection against *Brugia malayi* challenge in jird and mastomys models. Vaccine 26:5997–6005

Veerapathran A, Dakshinamoorthy G, Gnanasekar M, Reddy MV, Kalyanasundaram R (2009) Evaluation of *Wuchereria bancrofti* GST as a vaccine candidate for lymphatic filariasis. PLoS Negl Trop Dis 3:e457

Vickery AC, Nayar JK (1987) *Brugia pahangi* in nude mice: protective immunity to infective larvae is Thy 1.2+ cell dependent and cyclosporin A resistant. J Helminthol 61:19–27

Vincent AL, Vickery AC, Nayar JK, Sauerman DM, Yangco BG (1982) Nondevelopment of *Wuchereria bancrofti* in nude (congenitally athymic) mice. Am J Trop Med Hyg 31:1062–1064

Weller PF (1978) Cell-mediated immunity in experimental filariasis: lymphocyte reactivity to filarial stage-specific antigens and to B- and T-cell mitogens during acute and chronic infection. Cell Immunol 37:369–382

WHO Fact Sheet (2012) Lymphatic filariasis. 102

WHO Scientific Working Group on Filariasis (1981) The immunology of filariasis. Bull World Health Organ 59:1–8

Wong MM, Fredericks HJ, Ramachandran CP (1969) Studies on immunization against *Brugia malayi* infection in the rhesus monkey. Bull World Health Organ 40:493–501

Yates JA, Higashi GI (1985) Brugia malayi: vaccination of jirds with 60cobalt-attenuated infective stage larvae protects against homologous challenge. Am J Trop Med Hyg 1985 Nov 34(6):1132–1137

Zeldenryk LM, Gray M, Speare R, Gordon S, Melrose W (2011) The emerging story of disability associated with lymphatic filariasis: a critical review. PLoS Negl Trop Dis 5:e1366

Progress in the Treatment and Control of Lymphatic Filariasis

4

Shailja Misra-Bhattacharya and Mohd. Shahab

Abstract

Lymphatic filariasis caused by tissue nematodes is a nonfatal public health disease which causes huge deformities in several tropical and subtropical countries including India leading to severe man-hour loss. Mass drug administration using single-dose treatment with diethylcarbamazine or ivermectin in combination with albendazole has brought down filarial prevalence in several geographical regions; however, reports on development of resistance to mainstay drugs especially in veterinary settings are alarming. Discovery of *Wolbachia* endosymbionts in filarial parasites and dependence of filariids on these microorganisms for their fertility, growth and survival suggest these bacteria to be an attractive drug target. Knowledge on *Brugia malayi* genome has given huge impetus on the search for new drug molecules and novel drug targets. Modern bioinformatics approaches such as in silico drug designing and screening of compounds/target enzyme inhibitors or compound libraries as well as new assay development would facilitate the new drug discovery and development programme.

4.1 Introduction

Lymphatic filariasis (LF) is a mosquito-borne parasitic infection, caused by the filarial nematodes, *Wuchereria bancrofti*, *Brugia malayi* or *Brugia timori*. It remains a major public health problem causing significant disability as well as loss of productivity in developing nations (Ottesen 1992). Currently the disease affects ~130 million people globally, appending risk to other ~1.3 billion ones (WHO 2011a, b).

S. Misra-Bhattacharya (✉) · M. Shahab
Division of Parasitology, CSIR-Central Drug Research Institute,
Lucknow, Uttar Pradesh, India
e-mail: shailjacdri@yahoo.com

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_4

India alone contributes to 40% of the global burden with 600 million people staying in filaria-endemic area in 20 endemic states (Brady 2014). The adult worms reside in nests within the dilated lymphatic vessels mostly of the extremities and male genitalia and release microfilariae which migrate to the blood stream. Severe pathology such as hydrocele affects about 21% of people, while 12% of the infected individuals suffer from lymphoedema as a result of death of adult parasites in the lymphatics (WHO 2010).

In the absence of an antifilarial vaccine, treatment and control of filariasis on such a large scale are difficult, which leaves chemotherapy as the mainstay method of control. Vector control also does not result into a sustained effect except in some small locations (Gyapong et al. 2005; McCarthy 2005). In 2000, with the generous help from international donor agencies, Global Programme to Eliminate Lymphatic Filariasis (GPELF) began its campaign. The prime motto was to interrupt transmission of infection by reducing the microfilarial load in a given population by single oral annual treatment of at-risk population with diethylcarbamazine (DEC) or ivermectin in combination with albendazole through mass drug administration (MDA) programme. In India, MDA for LF was piloted in the 1950s, with a 2002 national health policy calling for elimination by 2015 (Brady 2014), but approaches to these drug regimens do not cover fully on ground basis (Babu and Kar 2004). Combining urban and rural areas within each study, coverage ranged from 48.8% to 98.8% (NVBDCP 2014), provisional GPELF data from India in 2013 showed coverage of 71.4% of the target population (WHO 2014). These drugs are principally microfilaricidal and exhibit only limited macrofilaricidal efficacy allowing adult worms to survive in human hosts for up to decades. The adult parasites thus continue to cause pathology and further transmission when microfilarial density is rebuilt. Repeated annual treatments are therefore required with high coverage of drug administration to bring the microfilaria density to such a low level that does not permit transmission of infection (Babu and Kar 2004).

The mathematical model has assumed the reproductive life span of *W. bancrofti* to be approximately 5 years predicting the need for annual treatment for 4–6 years (Norman et al. 2000; Plaisier et al. 2000). Major constraints in MDA are adverse effects of drugs, unavailability of many of the infected individuals for 5–6 years of continuous annual treatment and nondevelopment of disease symptoms in several infected individuals throughout their life (asymptomatic carriers) with the result they consider themselves as infection-free and therefore do not accept treatment. Thus it is equally essential to change their perceptions regarding LF and its elimination. GPELF has also defined the sets of program regarding morbidity managements (WHO 2011a, b, 2013), which has provided control to some extent, but in absence of macrofilaricidal drug, the problems remain in achieving sufficient levels of control. Recently, there are the indications of development of resistance against IVM in humans infected with *Onchocerca volvulus* (Awadzi et al. 2004) which was previously confined to veterinary nematodes. Also, the presence of ALB-resistant alleles in untreated populations of *W. bancrofti* (Schwab et al. 2005) raises a great concern for ongoing and future elimination programs. Resistance against DEC has although not been reported; however studies related to DEC resistance are limited due to the poor understanding of its mechanism of action.

Vaccination might be an excellent strategy backed by the presence of individuals termed as 'endemic normals' who are resistant to infection in spite of being repeatedly exposed to the infective larvae. This approach not only appears feasible because of the presence of naturally acquired immunity in these individuals (Day 1991) but also the antigens recognized by endemic normal sera would be expected to stimulate a preferentially protective immune response in the host (Ottesen et al. 1982; Freedman et al. 1989; Dabir et al. 2006). Since LF infections are associated with strong polarization of the host immune response towards a Th2/Treg phenotype (Maizels and Yazdanbakhsh 2003), development of vaccine against these parasites therefore does not seem to be an easy task. Irradiated infective larvae derived from various species of filarial parasites offered strong protection (Kurniawan-Atmadja et al. 1998) against reinfection in rodents and non-human primate models apart from excretory-secretory (ES) antigens and mitochondria-rich (MT) fraction, and some recombinant proteins have also been reported to offer variable degree of protection (Veerapathran et al. 2009; Shahab and Misra-Bhattacharya 2012; Kushwaha et al. 2013; Singh et al. 2014).

New alternative chemotherapy with doxycycline targeting *Wolbachia* endosymbionts which are present in most human filariae gives hope to filaria elimination program. *Wolbachia* depletion is associated with reduced chronic pathology in addition to superior macrofilaricidal activity over all the standard antifilarial treatments (Taylor et al. 2013). However, in current scenario the anti-*Wolbachia* 'doxycycline' treatment requires long course of treatment which cannot be recommended on mass basis because of added contraindications.

Research is already trending for identification and validation of novel antifilarial drug or vaccine targets. New insights into the biology of nematode and host are expanding through their completed or partially annotated genome. Further, efficient molecular methods combined with in silico tools have facilitated in-depth analysis of structural and functional aspects of genes that are primarily essential for pathogen survival. In the past, the number of drugs and their targets has been reviewed extensively, based on contrasting filarial and human metabolism for novel antifilarial drug discovery (Foster et al. 2005a, b; Kumar et al. 2007; Singh et al. 2011a, b; Rana and Misra-Bhattacharya 2013). The process of validating these targets has not been up to the mark leading to drugs having poor efficacies or generating unwanted side effects. Application of RNA interference (RNAi) in filarial worms by silencing the desired gene/s has improved significantly in the last few years. In vitro and in vivo inhibitor studies against filarial or *Wolbachia* enzymes have also been employed to validate some of the filarial and *Wolbachia* drug targets recently.

4.2 *B. malayi* Targets

B. malayi serves as an excellent model for undertaking research on lymphatic filarial nematodes. The infection can be experimentally maintained in laboratory via mosquito-rodent cycle and is amenable to in vitro studies at different stages of its life cycle (Ash and Riley 1970). Comparing the genome sequences of parasitic nematodes with that of *Caenorhabditis elegans* (assisted with phenotypes resulting

from RNAi gene knockdown), potentially essential genes in *B. malayi* have been identified (Aboobaker and Blaxter 2003). Our group has evaluated few enzymes of *B. malayi* expected to be essential for life-sustaining pathways. One of them, trehalose-6-phosphate phosphatase (tpp) of trehalose biosynthesis, seems to be an ideal drug target as silencing of *tpp* gene in *B. malayi* caused the arrested growth of larvae effecting overall parasite viability (Kushwaha et al. 2012). Designing or screening (via molecular docking) of specific tpp inhibitors is thus urgently needed. Crystal structure of *B. malayi* tpp has now been recently reported (Farelli et al. 2014) which can be used for discovering anti-tpp compounds that can later be tested in in vitro and in in vivo systems. Similarly, another enzyme, RNA helicase, related to nucleic acid has also been suggested to be a novel drug target in *B. malayi* in our earlier study (Singh et al. 2011a, b).

Research is under way to screen specific inhibitors against these drug targets. Some other enzymes of interest are glutathione S-transferase (GST) (Azeez et al. 2012), heat shock protein 90 (Hsp90) (Gillan et al. 2014), topoisomerase II (Misra-Bhattacharya et al. 2004), asparagine tRNA synthetase (Yu et al. 2011), cysteine protease (Lustigman et al. 2014) and N-myristoyltransferase (NMT) (Galvin et al. 2014). All of these have been under intense investigation as specific inhibitors/drug with the hope of discovering new drug candidates. In vitro studies related to tyrosine kinase inhibitors (an FDA-approved drug) against Abl-like kinases have recently been shown to be safe and highly effective against *B. malayi* microfilariae and adults (O'Connell et al. 2015). Another drug, auranofin, was found to be effective in inhibiting *B. malayi* motility both in vitro and in vivo. Auranofin is known to inhibit enzymatic activity of thioredoxin reductase in some parasites (Bulman et al. 2015).

4.3 *Wolbachia*

Wolbachia are maternally inherited intracellular gram-negative alphaproteobacteria widely spread among arthropods and filarial nematodes exhibiting a diverse range of associations with their host. *Wolbachia* were first identified in the ovaries of *Culex* mosquitoes in 1924 (Hertig and Wolbach 1924). Strains infecting arthropods are endosymbionts or reproductive parasites where they induce a sex-ratio distortion by different mechanisms (Bandi et al. 2001). The rediscovery of bacterial sequences in filaria in the 1990s in the course of filarial genome sequencing project led to renewal of interest in filarial research. In filarial nematodes, *Wolbachia* are found in host-derived vacuoles within all life-cycle stages (Taylor et al. 2005). Female adult worms carry the highest bacterial load, concentrated mainly around the reproductive organs. The lowest density of *Wolbachia* is observed in microfilaria and mosquito-borne larval stages. Within 7 days of infection to the mammalian host, bacteria increase 600-folds (McGarry et al. 2004). They are suggested to be secreted out from the lumen of the excretory/secretory canals of worms (Slatko et al. 2014), aiding in stimulation of the immune responses, as observed in filarial pathologies (Taylor 2003). *Wolbachia* induce innate inflammatory immune

responses involving macrophages and neutrophils with a shift in host vigorous T helper type 2 (Th2)-dominated response to moderate pro-inflammatory (Th1) immune response (Babu and Nutman 2003; Shahab and Misra-Bhattacharya 2012). Alternatively, they may play an important role in protecting the nematodes against the host immune responses. The discovery of genome data of both *B. malayi* and *Wolbachia* has opened up several opportunities for exploiting the biology shared among these (Foster et al. 2005a, b; Ghedin et al. 2007).

4.4 *Wolbachia* as Antifilarial Target

The drugs currently used for the treatment of LF were first discovered in the veterinary industry (Panic et al. 2014). However, heavy reliance on anthelmintics has led to development of resistance in nematode parasites of sheep limiting their veterinary use (Coles et al. 1994; Wolstenholme et al. 2004). Resistance has assumed an alarming situation and therefore may hinder future LF elimination program. The presence of endosymbionts has opened novel ways to alternative chemotherapeutic approaches using antibiotics that may lead long-lasting reduction in pathology-inducing worm population with the loss of 90% of *W. bancrofti* adult worms (Debrah et al. 2007; Hoerauf et al. 2008; Mand et al. 2009; Turner et al. 2010). Studies have shown that depletion of *Wolbachia* in filarial parasites results into slow death of adult worms (Taylor et al. 2010) that prevents sudden release of huge amounts of *Wolbachia*, thereby decreasing the commonly encountered adverse reactions, e.g. with DEC (Supali et al. 2008). These studies in conjunction with clinical trials indicate that *Wolbachia* are indispensable for their filarial hosts and represent a promising new targeted strategy for LF control. Field trials using doxycycline have led to macrofilaricidal effect, if given for an appropriate length of time (daily, 4–6 weeks) (Johnston et al. 2014). This regime is feasible and may prove effective only in restricted populations due to contraindications in children under 8 and pregnant women. In silico virtual screening along with proteomics studies has generated eminent resources for identification of *Wolbachia* gene repertoire, essential for the survival. The process of validating them, however, is problematic as these bacteria are not amenable to genetic manipulation and also due to limitation of functional genomics technologies (RNAi for filarial *Wolbachia*).

Wolbachia bears ~1.08 Mb circular genome with extremely low number of predicted functional genes compared to all other bacteria (Foster et al. 2005a, b). Proteomic studies of the various stages of *B. malayi* identified 557 of the 805 *Wolbachia* predicted proteins. It was also analysed that out of 166 hypothetical/predicted genes, 96 were validated as producing protein during 1 or more stages of filarial development (Bennuru et al. 2011). It is believed that mutualistic *Wolbachia* provide metabolites that are essential for host reproduction, development and survival (Taylor and Hoerauf 1999). *Wolbachia* has reduced metabolic pathways, which likely makes them dependent upon their host. They are incapable of synthesizing many cofactors/vitamins and also the pathways for synthesis of Coenzyme A, NAD, lipoic acid, biotin, folate, ubiquinone and pyridoxal phosphate (Taylor et al. 2000;

Scott and Ghedin 2009; Wu et al. 2009). These bacteria have lost genes for synthesis of all amino acids except meso-diaminopimelate, a major component of peptidoglycan (Taylor et al. 2000; Scott and Ghedin 2009); however, they have retained some biosynthesis pathways for purines and pyrimidines believed to be used by their host, *B. malayi*, during high DNA requirements as *B. malayi* genome possesses incomplete pathways for these (Ghedin et al. 2007). Furthermore, *Wolbachia* encode enzymes for biosynthesis of glutathione, which might be used for reducing their or hosts' oxidative stress. A few proteins/enzymes of *Wolbachia* have been characterized which are enlisted in Table 4.1.

Pathway related to the biosynthesis of heme is of special interest encoded by *Wolbachia*. It is an essential cofactor involved in many important biological processes like oxidative phosphorylation and electron transport. *Wolbachia* encode almost all genes to synthesize this cofactor. Contrary to this, *B. malayi* lacks most of genes required to produce heme (Foster et al. 2005a, b). In vitro studies suggested that worms are apparently unable to uptake heme from their surroundings, thus depending on their endosymbiotic *Wolbachia* (Wu et al. 2009). Specific in vitro targeting wALAD of heme biosynthesis by a variety of different chemical scaffolds exhibits macrofilaricidal effects on *Wolbachia*-containing filarial nematodes. Using high-throughput cell-based screening, other albendazole-like compounds (Albendazole sulfone) were screened, and these were suggested to shorten the current duration of anti-*Wolbachia* treatments (Serbus et al. 2012). *Wolbachia* are shown to be sensitive against antibiotics of the acyldepsipeptide class which induce dysregulation of the protease ClpP that facilitates degradation of misfolded or damaged proteins (Brotz-Oesterhelt et al. 2005; Schiefer et al. 2013). In vitro, targeting of the GTPase activity of *Wolbachia* cell division protein (FtsZ) has resulted into reduced worm motility and reproduction (Li et al. 2011). Corallopyronin A (a noncompetitive inhibitor of bacterial DNA-dependent RNA polymerase activity) was

Table 4.1 Enzymes/proteins characterized in *Wolbachia*

	Wolbachia: enzymes/proteins	Type of study
1	Pyruvate phosphate dikinase (Raverdy et al. 2008)	Biochemical
2	Cofactor-independent phosphoglycerate mutase gene (iPGM) (Foster et al. 2009; Dhamodharan et al. 2012)	Biochemical
3	Filamenting temperature-sensitive (FtsZ) protein (Li et al. 2011)	Biochemical/ inhibitors
4	Transcription factors (Li and Carlow 2012)	Molecular
5	rsmD-like rRNA methyltransferase (Rana et al. 2013)	Biochemical/ inhibitors
6	Transcription elongation factor 'WolGreA' (Nag et al. 2014)	Molecular/ interactions
7	UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) (Shahab et al. 2014)	Biochemical
8	NAD^+-dependent DNA ligase (Shrivastava et al. 2015)	Biochemical/ inhibitors
9	Heat shock protein (HSP60) (Shiny et al. 2011)	Immunological
10	*Wolbachia* surface protein (Pathak et al. 2014)	Immunological

tested against *Wolbachia* in the rodent filarial nematode where it depleted *Wolbachia* in a dose-dependent manner with more ~~effective~~ affectivity than higher doses of doxycycline for a shorter duration (Schiefer et al. 2012). In cells, ribosome is one of the most common targets of antibiotics, and several mutations in the genes (23S, 16S rRNA genes, rRNA methyltransferases and other ribosomal proteins) regulating them leads to drug resistance in various bacterial strains (Weisblum 1995; Rana et al. 2013). *Wolbachia* of *B. malayi* possess five rRNA methyltransferases, of which rsmD-like methyltransferase was evaluated in vitro and in vivo for being a potent drug target in our lab recently (Rana et al. 2013). RsmD is an rRNA small-subunit methyltransferase D involved in catalysing a single target nucleotide and acts late in the assembly process and is able to modify a completely assembled 30S subunit in the process of ribosomal translation events. The specific inhibitors, which are basically heteroaryl compounds, were found to kill *B. malayi* in a few days or a week at very low concentration (0.15–3.5 μM) suggesting the enzyme to be an ideal drug target (Rana et al. 2013). Another study was also undertaken by our group using dispiro-cycloalkanone class of compounds as inhibitors to NAD^+-dependent DNA ligase of *Wolbachia* of *B. malayi* (Shrivastava et al. 2015). This enzyme is vital for prokaryotes in several fundamental processes in DNA metabolism. Prokaryotes use an NAD^+-dependant DNA ligase, whereas all human DNA ligase activities are ATP dependent providing selective enzyme targeting in *Wolbachia*. The use of these dispiro-cycloalkanones has led to female worm sterilization with significantly reduced microfilarial density in the primary screen model (*Meriones unguiculatus*). This was suggested to be due to dysfunction of DNA repair affecting transcription and subsequent protein synthesis which may result in the reduced motility, viability and survival of filarial parasite.

4.5 Moxidectin

Macrocyclic lactones such as ivermectin (IVM) and milbemycins are the fermentation products of soil-dwelling microorganisms employed to control human and animal nematode infections (Campbell et al. 1983). Moxidectin (MOX), a milbemycin, is a semisynthetic methoxime derivative of nemadectin and a product of *Streptomyces cyanogriseus* subsp. *noncyanogenus* (Shoop et al. 1995). IVM remains the sole macrocyclic lactone for treating filarioids, strongyloides and mites in humans (Omura and Crump 2004). MOX has been evaluated in several African countries against human onchocerciasis infections and has undergone Phase 1, 2 and 3 clinical trials (Prichard et al. 2012). In humans, MOX in a single dose of 3–36 mg has been shown to be safe and well tolerated (Cotreau et al. 2003), and in *B. malayi* infected animals, this drug exerted microfilaricidal activity and altered the embryo morphology. The adulticidal effects have been demonstrated to be limited to *Acanthocheilonema viteae* only. MOX shows longer persistence and higher efficacy against tissue-dwelling nematodes unlike IVM which contains more potency towards ectoparasites. Recent studies from our lab revealed noticeable antifilarial efficacy of MOX on adult *B. malayi* in rodents (Verma et al. 2014). Moreover,

in vitro, it brought about 100% reductions in adult female worm motility at 0.6 μM concentration within 7 days. A single dose of 20 mg/kg proved effective in vivo on both adult parasites and microfilariae causing death of ~49% of adult worms and ~54% sterilization of recovered live female worms warranting trial of MOX in combination with ALB to control transmission of LF with added advantage of adulticidal action (Verma et al. 2014).

4.6 Conclusion and Prospects

Our knowledge towards LF has increased tremendously over the last few years. In absence of an effective vaccine candidate, preventive chemotherapy (MDA) remains the only choice to control LF. Despite remarkable achievements of MDA and related programs, LF still remains a health problem endangering health of millions of people causing disease and economic burden. Till date, the elimination strategies for LF are backed by only three drugs (DEC, IVM and ALB), of which the latter two drugs seriously raise the concern about their future use because of emergence of drug resistance issues. Newer chemotherapeutic options are therefore urgently required to replace currently available limited tools. Conventional drug discovery methods such as in vitro and in vivo screening of compounds against whole organism with limited availability of target parasite material especially adult worms, difficult long-term in vitro maintenance and culture of filariids make it a tedious and cumbersome process. In the last decade, several advancements have been made in filarial research especially in the area of diagnosis, pathogenesis, treatment and control; however publication of *B. malayi* genome proved to be a landmark discovery that has markedly boosted up the ongoing antifilarial drug discovery and drug target research. In-depth screening via genome comparison and functional genomics has provided a list of vital targets that may be further validated. Development of an assay system is an important part of targeted drug discovery process where target-specific chemical libraries can be tested in high-/medium-throughput screens. The existing knowledge on chemistry and structure around the target may enable rapid identification of new candidate molecules. The cognition of *Wolbachia* essentiality in filarial nematodes has opened an alternative approach in drug discovery programs. A cell-based assay employing *Wolbachia*-infected insect cell line (C6/36 Wp) is already in place that may facilitate rapid and efficient high-throughput anti-wolbachial screening for pushing molecule(s) further into drug discovery pipeline (Johnston et al. 2014; Clare et al. 2015). The repurposing of the known compounds/drugs with known pharmacological/ toxicological properties would further cut the time required for trials and approvals and facilitate antifilarial drug development process.

References

Aboobaker AA, Blaxter ML (2003) Use of RNA interference to investigate gene function in the human filarial nematode parasite *Brugia malayi*. Mol Biochem Parasitol 129(1):41–51

Ash LR, Riley JM (1970) Development of subperiodic *Brugia malayi* in the jird, *Meriones unguiculatus*, with notes on infections in other rodents. J Parasitol 56(5):969–973

Awadzi K, Boakye DA et al (2004) An investigation of persistent microfilaridermias despite multiple treatments with ivermectin, in two onchocerciasis-endemic foci in Ghana. Ann Trop Med Parasitol 98(3):231–249

Azeez S, Babu RO et al (2012) Virtual screening and *in vitro* assay of potential drug like inhibitors from spices against glutathione-S-transferase of filarial nematodes. J Mol Model 18(1):151–163

Babu BV, Kar SK (2004) Coverage, compliance and some operational issues of mass drug administration during the programme to eliminate lymphatic filariasis in Orissa, India. Trop Med Int Health 9(6):702–709

Babu S, Nutman TB (2003) Proinflammatory cytokines dominate the early immune response to filarial parasites. J Immunol 171(12):6723–6732

Bandi C, Dunn AM et al (2001) Inherited microorganisms, sex-specific virulence and reproductive parasitism. Trends Parasitol 17(2):88–94

Bennuru S, Meng Z et al (2011) Stage-specific proteomic expression patterns of the human filarial parasite *Brugia malayi* and its endosymbiont *Wolbachia*. Proc Natl Acad Sci USA 108(23):9649–9654

Brady M (2014) Seventh meeting of the Global Alliance to Eliminate Lymphatic Filariasis: reaching the vision by scaling up, scaling down, and reaching out. Parasit Vectors 7:46

Brotz-Oesterhelt H, Beyer D et al (2005) Dysregulation of bacterial proteolytic machinery by a new class of antibiotics. Nat Med 11(10):1082–1087

Bulman CA, Bidlow CM et al (2015) Repurposing auranofin as a lead candidate for treatment of lymphatic filariasis and onchocerciasis. PLoS Negl Trop Dis 9(2):e0003534

Campbell WC, Fisher MH et al (1983) Ivermectin: a potent new antiparasitic agent. Science 221(4613):823–828

Clare RH, Cook DA et al (2015) Development and validation of a high-throughput anti-*Wolbachia* whole-cell screen: a route to macrofilaricidal drugs against onchocerciasis and lymphatic filariasis. J Biomol Screen 20(1):64–69

Coles GC, Giordano-Fenton DJ et al (1994) Efficacy of moxidectin against nematodes in naturally infected sheep. Vet Rec 135(2):38–39

Cotreau MM, Warren S et al (2003) The antiparasitic moxidectin: safety, tolerability, and pharmacokinetics in humans. J Clin Pharmacol 43(10):1108–1115

Dabir P, Dabir S et al (2006) Immunoprophylactic evaluation of a 37-kDa *Brugia malayi* recombinant antigen in lymphatic filariasis. Clin Microbiol Infect 12(4):361–368

Day KP (1991) The endemic normal in lymphatic filariasis: A static concept. Parasitol Today 7(12):341–343

Debrah AY, Mand S et al (2007) Macrofilaricidal effect of 4 weeks of treatment with doxycycline on *Wuchereria bancrofti*. Trop Med Int Health 12(12):1433–1441

Dhamodharan R, Hoti SL et al (2012) Characterization of cofactor-independent phosphoglycerate mutase isoform-1 (Wb-iPGM) gene: a drug and diagnostic target from human lymphatic filarial parasite, *Wuchereria bancrofti*. Infect Genet Evol 12(5):957–965

Farelli JD, Galvin BD et al (2014) Structure of the trehalose-6-phosphate phosphatase from *Brugia malayi* reveals key design principles for anthelmintic drugs. PLoS Pathog 10(7):e1004245

Foster J, Ganatra M et al (2005a) The *Wolbachia* genome of *Brugia malayi*: endosymbiont evolution within a human pathogenic nematode. PLoS Biol 3(4):e121

Foster JM, Zhang Y et al (2005b) Mining nematode genome data for novel drug targets. Trends Parasitol 21(3):101–104

Foster JM, Raverdy S et al (2009) The *Wolbachia* endosymbiont of *Brugia malayi* has an active phosphoglycerate mutase: a candidate target for anti-filarial therapies. Parasitol Res 104(5):1047–1052

Freedman DO, Nutman TB et al (1989) Protective immunity in bancroftian filariasis. Selective recognition of a 43-kD larval stage antigen by infection-free individuals in an endemic area. J Clin Invest 83(1):14–22

Galvin BD, Li Z et al (2014) A target repurposing approach identifies N-myristoyltransferase as a new candidate drug target in filarial nematodes. PLoS Negl Trop Dis 8(9):e3145

Ghedin E, Wang S et al (2007) Draft genome of the filarial nematode parasite *Brugia malayi*. Science 317(5845):1756–1760

Gillan V, O'Neill K et al (2014) A repurposing strategy for Hsp90 inhibitors demonstrates their potency against filarial nematodes. PLoS Negl Trop Dis 8(2):e2699

Gyapong JO, Kumaraswami V et al (2005) Treatment strategies underpinning the global programme to eliminate lymphatic filariasis. Expert Opin Pharmacother 6(2):179–200

Hertig M, Wolbach SB (1924) Studies on rickettsia-like micro-organisms in insects. J Med Res 44(3):329–374 7

Hoerauf A, Specht S et al (2008) *Wolbachia* endobacteria depletion by doxycycline as antifilarial therapy has macrofilaricidal activity in onchocerciasis: a randomized placebo-controlled study. Med Microbiol Immunol 197(3):295–311

Johnston KL, Ford L et al (2014) Repurposing of approved drugs from the human pharmacopoeia to target *Wolbachia* endosymbionts of onchocerciasis and lymphatic filariasis. Int J Parasitol Drugs Drug Resist 4(3):278–286

Kumar S, Chaudhary K et al (2007) Mining predicted essential genes of *Brugia malayi* for nematode drug targets. PLoS One 2(11):e1189

Kurniawan-Atmadja A, Sartono E et al (1998) Antibody responses to filarial infective larvae are not dominated by the IgG4 isotype. Parasite Immunol 20(1):9–17

Kushwaha S, Singh PK et al (2012) *In vitro* silencing of *Brugia malayi* trehalose-6-phosphate phosphatase impairs embryogenesis and *in vivo* development of infective larvae in jirds. PLoS Negl Trop Dis 6(8):e1770

Kushwaha S, Singh PK et al (2013) Immunization of *Mastomys coucha* with *Brugia malayi* recombinant trehalose-6-phosphate phosphatase results in significant protection against homologous challenge infection. PLoS One 8(8):e72585

Li Z, Carlow CK (2012) Characterization of transcription factors that regulate the type IV secretion system and riboflavin biosynthesis in *Wolbachia* of *Brugia malayi*. PLoS One 7(12):e51597

Li Z, Garner AL et al (2011) Targeting the *Wolbachia* cell division protein FtsZ as a new approach for antifilarial therapy. PLoS Negl Trop Dis 5(11):e1411

Lustigman S, Melnikow E et al (2014) Potential involvement of *Brugia malayi* cysteine proteases in the maintenance of the endosymbiotic relationship with *Wolbachia*. Int J Parasitol Drugs Drug Res 4(3):267–277

Maizels RM, Yazdanbakhsh M (2003) Immune regulation by helminth parasites: cellular and molecular mechanisms. Nat Rev Immunol 3(9):733–744

Mand S, Pfarr K et al (2009) Macrofilaricidal activity and amelioration of lymphatic pathology in bancroftian filariasis after 3 weeks of doxycycline followed by single-dose diethylcarbamazine. Am J Trop Med Hyg 81(4):702–711

McCarthy J (2005) Is anthelmintic resistance a threat to the program to eliminate lymphatic filariasis? Am J Trop Med Hyg 73(2):232–233

McGarry HF, Egerton GL et al (2004) Population dynamics of *Wolbachia* bacterial endosymbionts in *Brugia malayi*. Mol Biochem Parasitol 135(1):57–67

Misra-Bhattacharya S, Katiyar D et al (2004) 4-Methyl-7-(tetradecanoyl)-2H-1-benzopyran-2-one: a novel DNA topoisomerase II inhibitor with adulticidal and embryostatic activity against sub-periodic *Brugia malayi*. Parasitol Res 92(3):177–182

Nag JK, Shrivastava N et al (2014) *Wolbachia* transcription elongation factor "WolGreA" interacts with $\alpha 2\beta\beta'\sigma$ subunits of RNA polymerase through its dimeric C-terminal domain. PLoS Negl Trop Dis 8(6):e2930

National Vector Borne Disease Control Programme (2014) Mass drug administration. Government of India. http://nvbdcp.gov.in/mda.html

Norman RA, Chan MS et al (2000) EPIFIL: the development of an age-structured model for describing the transmission dynamics and control of lymphatic filariasis. Epidemiol Infect 124(3):529–541

O'Connell EM, Bennuru S et al (2015) Targeting filarial Abl-like kinases: orally available, Food and Drug Administration-approved tyrosine kinase inhibitors are microfilaricidal and macrofilaricidal. J Infect Dis 212(5):684–693

Omura S, Crump A (2004) The life and times of ivermectin – a success story. Nat Rev Microbiol 2(12):984–989

Ottesen EA (1992) The Wellcome Trust Lecture. Infection and disease in lymphatic filariasis: an immunological perspective. Parasitology 104(Suppl):S71–S79

Ottesen EA, Weller PF et al (1982) Endemic filariasis on a Pacific Island. II. Immunologic aspects: immunoglobulin, complement, and specific antifilarial IgG, IgM, and IgE antibodies. Am J Trop Med Hyg 31(5):953–961

Panic G, Duthaler U et al (2014) Repurposing drugs for the treatment and control of helminth infections. Int J Parasitol Drugs Drug Resist 4(3):185–200

Pathak M, Verma M et al (2014) *Wolbachia* endosymbiont of *Brugia malayi* elicits a T helper type 17-mediated pro-inflammatory immune response through *Wolbachia* surface protein. Immunology 144(2):231–244

Plaisier AP, Stolk WA et al (2000) Effectiveness of annual ivermectin treatment for *Wuchereria bancrofti* infection. Parasitol Today 16(7):298–302

Prichard R, Menez C et al (2012) Moxidectin and the avermectins: Consanguinity but not identity. Int J Parasitol Drugs Drug Resist 2:134–153

Rana AK, Misra-Bhattacharya S (2013) Current drug targets for helminthic diseases. Parasitol Res 112(5):1819–1831

Rana AK, Chandra S et al (2013) Molecular characterization of an rsmD-like rRNA methyltransferase from the *Wolbachia* endosymbiont of *Brugia malayi* and antifilarial activity of specific inhibitors of the enzyme. Antimicrob Agents Chemother 57(8):3843–3856

Raverdy S, Foster JM et al (2008) The *Wolbachia* endosymbiont of *Brugia malayi* has an active pyruvate phosphate dikinase. Mol Biochem Parasitol 160(2):163–166

Schiefer A, Schmitz A et al (2012) Corallopyronin A specifically targets and depletes essential obligate *Wolbachia* endobacteria from filarial nematodes *in vivo*. J Infect Dis 206(2):249–257

Schiefer A, Vollmer V et al (2013) The ClpP peptidase of *Wolbachia* endobacteria is a novel target for drug development against filarial infections. J Antimicrob Chemother 68(8):1790–1800

Schwab AE, Boakye DA et al (2005) Detection of benzimidazole resistance-associated mutations in the filarial nematode *Wuchereria bancrofti* and evidence for selection by albendazole and ivermectin combination treatment. Am J Trop Med Hyg 73(2):234–238

Scott AL, Ghedin E (2009) The genome of Brugia malayi - all worms are not created equal. Parasitol Int 58(1):6–11

Serbus LR, Landmann F et al (2012) A cell-based screen reveals that the albendazole metabolite, albendazole sulfone, targets *Wolbachia*. PLoS Pathog 8(9):e1002922

Shahab M, Misra-Bhattacharya S (2012) Combating mosquito-borne lymphatic filariasis with genomics technologies: enabling novel drug discovery for neglected tropical diseases. Curr Pharmacogenomics Person Med 10:148–158

Shahab M, Verma M et al (2014) Cloning, expression and characterization of UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) from *Wolbachia* endosymbiont of human lymphatic filarial parasite *Brugia malayi*. PLoS One 9(6):e99884

Shiny C, Krushna NS et al (2011) Recombinant *Wolbachia* heat shock protein 60 (HSP60) mediated immune responses in patients with lymphatic filariasis. Microbes Infect 13(14-15):1221–1231

Shoop WL, Mrozik H et al (1995) Structure and activity of avermectins and milbemycins in animal health. Vet Parasitol 59(2):139–156

Shrivastava N, Nag JK et al (2015) Homology modeling of NAD^+-dependent DNA ligase of the *Wolbachia* endosymbiont of *Brugia malayi* and its drug target potential using dispiro-Cycloalkanones. Antimicrob Agents Chemother. 59(7):3736–3747

Singh M, Singh PK et al (2011a) RNAi mediated silencing of ATPase RNA helicase gene in adult filarial parasite *Brugia malayi* impairs *in vitro* microfilaria release and adult parasite viability. J Biotechnol 157(3):351–358

Singh PK, Ajay A et al (2011b) Towards novel antifilarial drugs: challenges and recent developments. Future Med Chem 2(2):251–283
Singh PK, Kushwaha S et al (2014) Cofactor independent phosphoglycerate mutase of *Brugia malayi* induces a mixed Th1/Th2 type immune response and inhibits larval development in the host. BioMed Res Int 2014(19):590281
Slatko BE, Luck AN et al (2014) *Wolbachia* endosymbionts and human disease control. Mol Biochem Parasitol 195(2):88–95
Supali T, Djuardi Y et al (2008) Doxycycline treatment of *Brugia malayi*-infected persons reduces microfilaremia and adverse reactions after diethylcarbamazine and albendazole treatment. Clin Infect Dis 46(9):1385–1393
Taylor MJ (2003) *Wolbachia* in the inflammatory pathogenesis of human filariasis. Ann N Y Acad Sci 990:444–449
Taylor MJ, Hoerauf A (1999) *Wolbachia* bacteria of filarial nematodes. Parasitol Today 15(11):437–442
Taylor MJ, Bandi C et al (2000) *Wolbachia* bacteria of filarial nematodes: a target for control? Parasitol Today 16(5):179–180
Taylor MJ, Bandi C et al (2005) *Wolbachia* bacterial endosymbionts of filarial nematodes. Adv Parasitol 60:245–284
Taylor MJ, Hoerauf A et al (2010) Lymphatic filariasis and onchocerciasis. Lancet 376(9747):1175–1185
Taylor MJ, Hoerauf A et al (2013) Anti-*Wolbachia* drug discovery and development: safe macrofilaricides for onchocerciasis and lymphatic filariasis. Parasitology 141(1):119–127
Turner JD, Tendongfor N et al (2010) Macrofilaricidal activity after doxycycline only treatment of *Onchocerca volvulus* in an area of Loa loa co-endemicity: a randomized controlled trial. PLoS Negl Trop Dis 4(4):e660
Veerapathran A, Dakshinamoorthy G et al (2009) Evaluation of *Wuchereria bancrofti* GST as a vaccine candidate for lymphatic filariasis. PLoS Negl Trop Dis 3(6):e457
Verma M, Pathak M et al (2014) Moxidectin causes adult worm mortality of human lymphatic filarial parasite *Brugia malayi* in rodent models. Folia Parasitol 61(6):561–570
Weisblum B (1995) Erythromycin resistance by ribosome modification. Antimicrob Agents Chemother 39(3):577–585
WHO (2010) Global programme to eliminate lymphatic filariasis. Progress report 2000–2009 and strategic plan 2010–2020 of the global programme to eliminate lymphatic filariasis: halfway towards eliminating lymphatic filariasis. WHO, Geneva
WHO (2011a) Lymphatic filariasis. WHO, Geneva
WHO (2011b) Position statement on managing morbidity and preventing disability in the global programme to eliminate lymphatic filariasis. WHO, Geneva
WHO (2013) Lymphatic filariasis: managing morbidity and preventing disability: an aideme'moire for national programme managers. WHO, Geneva
WHO (2014) Global programme to eliminate lymphatic filariasis: progress report, 2013. Wkly Epidemiol Rec 89:409–418
Wolstenholme AJ, Fairweather I et al (2004) Drug resistance in veterinary helminths. Trends Parasitol 20(10):469–476
Wu B, Novelli J et al (2009) The heme biosynthetic pathway of the obligate *Wolbachia* endosymbiont of *Brugia malayi* as a potential anti-filarial drug target. PLoS Negl Trop Dis 3(7):e475
Yu Z, Vodanovic-Jankovic S et al (2011) Tirandamycins from *Streptomyces sp.* 17944 inhibiting the parasite *Brugia malayi* asparagine tRNA synthetase. Org Lett 13(8):2034–2037

5 Immunotechnological Advancements in Developing Vaccines for Lymphatic Filariasis

Prince R. Prabhu, Jayaprakasam Madhumathi, and Perumal Kaliraj

Abstract

As we head toward the elimination of LF through mass drug administration (MDA) and vector control programs, it is equally reasonable to sustain efforts on vaccine development on a long-term basis. This prophylactic approach will give a foothold for complete protection in endemic areas from future emergence of infection and will also strengthen the current MDA efforts. Currently, we do not have any vaccines to combat multicellular infectious agents leaving alone the neglected infectious diseases like filariasis. But there have been significant efforts on the part of India toward extensive basic research in the identification and preclinical animal model validation of suitable vaccine candidates. This review gives a summary of these efforts that would be pivotal for future clinical vaccine studies in lymphatic filariasis (LF).

5.1 Introduction

Lymphatic filariasis is a complex immunological disease, and hence it has been an ongoing challenge to develop vaccines against this parasitic disease. However, many vaccine candidates have been screened and tested in animal models with promising results. Here, we discuss the key issues in vaccine development for lymphatic filariasis, modes of vaccines, candidate antigens used as vaccines, and recent technologies in vaccine development.

P. R. Prabhu (✉) · J. Madhumathi · P. Kaliraj (✉)
Centre for Biotechnology, Anna University, Chennai, India

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_5

5.2 Filarial Vaccines: Possible or Impossible?

All vaccine development attempts linger on the promising observation of natural protective immunity against pathogens without which all efforts will be futile. In lymphatic filariasis (LF), there is a positive evidence of the existence of protective immune response in patients. Epidemiological studies show the existence of putative immune individuals in areas endemic for filariasis (Day et al. 1991; Steel et al. 1996). This has been attributed to resistance of infection to the incoming L3 larvae (WHO 2006). Therefore, it is not only logical to think of developing vaccines against filariasis but also feasible if approached in a strategic perspective without forgetting the fact that we target a formidable, complex parasite worm pliable for view but vicious enough to suppress human immune responses for its advantage.

5.3 Challenges

For obvious reasons of complete unavailability of a commensurate animal model, the research for vaccine development to prevent nematode parasitic infections is still in its primitive stage compared to prophylactic research seen in related parasitic and protozoan diseases like malaria and schistosomiasis. The multicellular tissue-dwelling nature of the parasite has severely hampered the progress in vaccine research for filariasis (Grieve et al. 1995). The following depict some of the major problems:

1. The prolonged/intricate life stages of parasitic worms are closely associated with complex immunological host responses.
2. The distinctive features associated with a comparatively huge number of diverse symptoms in the pathologic process.
3. The deficiency of appropriate animal model systems to study the clinical course of infections.
4. The scarcity or limitation of parasite substances to isolate antigen screening and phage library constructions.
5. The need for significant demonstration of protective immune response in host immune responses.
6. The inadequate information on the process by which protective responses are demonstrated in the host. In LF, the crucial stage for vaccine target identification happens to be the L3 and Mf stages.

The implication for vaccine development has overshadowed the basic barriers to this vital research. Further, the accumulation of experimental proof from epidemiological data has positivity suggested the feasibility of filarial vaccine discovery a reality to occur in rapid pace in the recent past years. In this regard, range of attractive vaccine targets have been overexpressed in recombinant expression systems for evaluation in filarial animal models.

5.4 Animal Models for Vaccine Studies

The parasitic worms that cause LF and river blindness are shown to have stern host specificity and said to attain reproductive maturity only in primates (Maizels et al. 1988). The deficiency of convenient LF model in animal systems seems to prohibit any extensive experimental immunity studies using the human pathogens *W. bancrofti* and *O. volvulus*. Though studies with animal filarial parasites, *A. viteae*, *D. immitis*, and *S. digitata*, have been able to shed some light in the immune effector mechanisms involved, the zoophilic strain, *B. malayi*, a human parasite, will be the choice. Moreover, the stern host specificities of the human-LF nematodes do not mimic exactly all the facets of human infection in any of the existing LF animal model. An effort to gain full matured growth of *W. bancrofti* in nude mice systems (congenitally athymic mice) and immunosuppressed *Meriones* (jirds) has proved to be futile (Philipp et al. 1984). Therefore, innate mammalian hosts for nonhuman filarial parasites like *Dirofilaria immitis* of dog, *B. pahangi* of cat, or *A. viteae* of jirds are being used for vaccine studies. Further, surrogate hosts or nonpermissive hosts with differing permissiveness like BALB/c mice have been investigated to study the role of vaccine candidates as to how they exhibit protection against nematode infection.

The only reported model for *W. bancrofti* is the infection in silver leaf monkeys (Palmieri et al. 1984) and serves as a tool for immunological studies. The other lymphatic filarial parasite *B. malayi* can be sustained in small animals like rodents, and the susceptibility for the parasite infection has found to be varied in diverse laboratory hosts that range from completely susceptible jirds (gerbils) to semi-permissible mice. However, among the currently available animal models, jird or gerbil (Ash and Riley 1970) and *Mastomys* (Sanger et al. 1981; Lok and Abraham 1992) are permissive hosts for *B. malayi* and are a well-studied animal model for *B. malayi* parasite infections that completely covers the parasite life cycle from L3 stage to adult and is also widely used for studying lymphatic pathology (Klei et al. 1987).

Though male jirds are fully susceptible to infection of *B. malayi*, the sequel of pathological and immune responses is reverse of that postulated in humans. The heightened phase of parasite infection in jirds shows changes in lymphatic vessels, normally associated with the chronic state in humans exhibiting lymphadenopathy and a microfilaremia. Features observed in the interim stage of asymptomatic, microfilaremic patients characterize the chronic infection in jirds: a stable microfilaremia and antigen-specific hyporesponsiveness (Philipp et al. 1984).

Partially permissive hosts like BALB/c mice have been studied earlier to demonstrate their use as fitting experimental systems to evaluate protective immune responses against L3-infective larvae from *B. malayi* (Ash and Riley 1970). But it has to be noted that these parasites that belong to *Brugia* genus seldom reach adulthood in these mice. The relative insusceptibility to infection is hypothesized to mimic the course by which endemic normals show resistance to worm infection, mainly by antilarval immune responses. The limited development of the early larval

stages and the extended survival of *mf* in BALB/c mice have provided valuable insight into the mechanism of protective immunity as well as a model for studying the protective potential of vaccine candidates. However, remarkable outcomes have been accomplished in vaccination and in situ transfer experiments in which the recovery of viable worms on the 14th day from the mice was found to be in the range of 26% (Ash and Riley 1970).

The intrinsic problem in these animal models (semi-permissible mice models) is the gradual destruction of worms almost a hundred percent death of these implanted parasite worms that die after 30 days after infection. This makes vaccine assessment studies for nematode diseases with a limited window of evaluation and is reflected in the fact that shows significant standard deviation in nematode recoveries between individual members of the experimental groups. However, evaluation of immunological factors through splenocyte proliferation studies, immunoglobulin isotype profiling, and cytokine evaluation at transcriptional level (TR-PCR) or in the protein level (ELISA) is quite possible in mice models by the use of propriety reagents/kits that are commercially available. So a significant level of molecular basis of antigen characteristics in a natural host can be studied with immunological perspective in these models. Among filarial animal models, the one which is closest to resemble human pathology can be derived from Mongolian jirds (*Meriones unguiculatus*) infected intraperitoneally with *B. pahangi* or *B. malayi*, where macrophages present in peritoneal cavity play a significant role in clearing infection. When studied and compared during evaluation of vaccine candidates, there is significant correlation in protective immune responses between vaccinated-infected and control-infected jirds (Abraham et al. 1988; Krithika et al. 2005; Madhumathi et al. 2010).

Morphological changes and granuloma formation were also observed in the peritoneal cavities of intraperitoneally infected jirds. Thus the peritoneal cavity of jirds may serve as a model to study cell-worm interactions in filarial nematode infections. All the infected jirds showed the parasite in different stages of development (Murthy et al. 1997).

5.5 Milestones in LF Vaccination Studies

5.5.1 Parasites and Crude Antigens

Growth retardation or arrested development of the parasites in immunized host has been reported for several filarial species, including *D. viteae* (Abraham et al. 1986) and *D. immitis* (Grieve et al. 1988). The retardation of growth or development results in longer exposure of the parasite to host immune responses, leading to the clearance of the parasite. Irradiation or treatments with antiparasitic agents have been observed to retard the growth and development of the parasite, prompting the use of irradiated larvae or chemically abbreviated infections as a source of vaccination. Generally, larvae that undergo some degree of limited development and migration induce the highest levels of protective immunity. Most immunization studies are based on attenuated larvae or crude parasite antigens.

Vaccination with radiation-attenuated or chemically abbreviated larvae has been found successful in various filarial parasites such as *B. malayi* (Hayashi et al. 1984, 1989), *D. immitis* (Abraham et al. 1988), *B. pahangi* (Hayashi et al. 1984; Chusattayanond and Denham 1986), and *O. volvulus*. Live L3 larvae *D. immitis* showed partial protection (Abraham et al. 1988). *B. malayi* radiation-attenuated L3 larvae induced 69–91% protection (Yates and Higashi 1985), and irradiated L3 larvae of *O. volvulus* conferred 25% protection (Prince et al. 1992).

High levels of protective immunity in several host-parasite systems have been induced using irradiated filarial larvae, L3 of *B. malayi* in monkeys (Wong et al. 1969) and mice (Abraham et al. 1989), and using irradiated *O. volvulus* in mice (Lange et al. 1993) and ivermectin-treated *D. immitis* in dogs (Grieve et al. 1988). Irradiated L3 of *D. viteae* induced 90% protection (Lucius et al. 1986). Chemically abbreviated *D. immitis* infections conferred 63% protection (Grieve et al. 1988). Irradiation-attenuated L3 or exported larval antigens of *A. viteae* showed 60–94% protection (Lucius et al. 1991), while drug-abbreviated *B. pahangi* infection induced 19–77% protection (Horii et al. 1992). Live microfilariae of *O. lienalis* conferred 91–98% protection (Townson et al. 1985), and microfilariae of *O. volvulus* or *O. lienalis* conferred 49–96% protection (Bianco et al. 1991).

Immunization with crude antigens of microfilariae (Kazura et al. 1986; Carlow and Philipp 1987; Cheirmaraj et al. 1991), infective L3 larvae (Wenk and Mossinger 1991), or purified antigens from the different stages of parasite (Frank et al. 1996; Wang et al. 1997; Jenkins et al. 1998; Chenthamarakshan et al. 1995) has been found to provide varying levels of protection ranging from 25 to 90%.

OvB20 an antigen of *Onchocerca volvulus* preferentially recognized by sera from cattle vaccinated with irradiated infective larvae of *Onchocerca lienalis* has been observed to induce protective immunity in experimental animal models (Taylor et al. 1995). ES antigens of *Brugia pahangi* Mf induced partial protection (Rajasekariah et al. 1988). Egg antigens of various *Onchocerca* species induced 29–78% protection (Carlow and Bianco 1987). Similarly uterine contents of *O. lienalis*, *O. gutturosa*, or *O. volvulus* conferred 66–75% protection (Townson et al. 1985). 38 kDa microfilarial proteases (Bm mf S-7) could stimulate a strong protective immune response against microfilariae and infective larvae in jird model to block the transmission of filariasis (Krithika et al. 2005). A 120 kDa antigen isolated from *Brugia malayi* adult parasite has been reported to have immunoprophylactic potential, and active immunization with BmA-2 is reported to be effective against filarial infection (Chenthamarakshan et al. 1995). 175 kDa collagenase, purified from adult female *Setaria cervi*, showed 75% protection in jirds (Srivastava et al. 2004).

Immunization with one developmental stage has been shown to induce protection against the challenge of a different stage (Carlow and Phillips 1987; Hayashi et al. 1989; Kazura et al. 1986). This observation might be due to the sharing of the antigenic determinants in the different life phases of the parasite (Maizels et al. 1983). Cross-reacting surface antigens have also been observed to be shared among the same stage of several filaria species (Irvine et al. 1994; Wuhrer et al. 2000). Considerable antigenic similarity has also been demonstrated between *L. carinii*

and *W. bancrofti* (Subrahmanyam et al. 1978). Induction of protective immunity using heterologous parasites is supported by the cross-protection studies in which irradiated *B. pahangi* conferred protection against *B. malayi* (Carlow and Philipp 1987); *O. lienalis* larvae induced immunity against *O. volvulus* (Lange et al. 1993) and cross-protection between different genera (Storey and Al-Mukhtar 1982).

5.5.2 Recombinant Filarial Vaccine Candidates

Batteries of antigens from various stages of the parasite have been identified as potential vaccine candidates; the genes coding for these antigens have been cloned, expressed, and used in experimental animal models (Table 5.1). Despite the use of different expression systems, only a few recombinants induced high levels of protection against helminths. Using a phage display-based iterative screening of a *B. malayi* L3 cDNA library with immune sera, five antigens, four of which were previously reported (ALT-2, TPX-2, VAH, and COX-2) and the other one, a novel cuticular collagen (Col-4), were identified as potential vaccine candidates.

Larvae-derived recombinant paramyosin showed partial protection with 43% reduction in worm burden (Li et al. 1993, 1999), and tropomyosin showed 46% reduction in adult worm recovery (Taylor et al. 1996). Another *Brugia malayi* filarial antigen identified by screening *Brugia* L3cDNA library is designated as rBm-SL3 67–69% cytotoxicity against infective larvae in jirds (Dabir et al. 2006).

Microfilaria-specific chitinase from the brugian filarial parasite has been identified as a possible transmission-blocking vaccine candidate (Fuhrman et al. 1992). Immunization of jirds with recombinant chitinase and SXP induced partial protection against microfilaremia resulting from subsequent infection with *B. malayi*, but did not reduce adult worm burdens (Wang et al. 1997). Recombinant fructose 1,6-bisphosphate aldolase induced 50% protection (McCarthy et al. 2002), and HSP70 failed to confer protection (Peralta et al. 1999).

ALT-1 has been reported to show ~76% protection (Gregory et al. 2000). Recently *W. bancrofti* glutathione S-transferase was shown to induce 61% protection in jirds (Veerapathran et al. 2009). DNA vaccines of chitinase (Harrison et al. 1999), paramyosin (Li et al. 1999), glutathione S-transferase (Veerapathran et al. 2009; Catmull et al. 1999), tropomyosin, and OvB20 (Harrison and Bianco 2000) have been observed to induce partial protection in animal models by induction of both humoral and cellular immune response. Recombinant *B. malayi* VAL-1 has been shown to induce 64% protection (Murray et al. 2001).

Studies in our laboratory for the past three decades have identified many important recombinant proteins that showed promising results in challenge experiments. Various strategies like protein, DNA, phage-displayed antigen, prime boost, and multiple-antigen strategies have been tested in animal models. ALT-2 conferred ~64–75% protection in mice and jird models as protein, DNA, or bimodal vaccine (Ramachandran et al. 2004; Thirugnanam et al. 2007; Gnanasekar et al. 2004). BmTGA protein showed 30% protection (Vanam et al. 2009a), while the DNA vaccine showed a very poor response of 21% in jirds (Vanam et al. 2009b). Similarly

Table 5.1 List of filarial vaccine candidates and their corresponding animal validation studies

S. no	Vaccine strategy	Antigen	Species	Protection (%)	References
1.	Phage-displayed antigen	ALT-2 phage-displayed antigen	*B. malayi*	Mice – 62	Pankaj (2003)
				Jirds – 58	
2.	DNA vaccine	ALT-2	*B. malayi*	Mice – 31	Ramachandran et al. (2004)
				Jirds – 57	
				Mice – 34	Thirugnanam et al. (2007)
				Jirds – 56	
					Anand et al. (2008)
					Anand et al. (2011)
3.	DNA vaccine	Venom allergen homologue (VAH)	*B. malayi*	Jirds – 50	Anand et al. (2007, 2011)
4.	DNA vaccine	Transglutaminase (TGA)	*B. malayi*	Mice – 21	Vanam et al. (2009b)
5.	DNA vaccine	Thioredoxin peroxidase (TPX)	*B. malayi*	Mice – 37	Anand et al. (2008)
6.	Bimodal vaccine	ALT-2 DNA-protein bimodal vaccine	*B. malayi*	Jirds – 64	Thirugnanam et al. (2007)
7.	Bimodal vaccine	VAH DNA- protein bimodal vaccine	*B. malayi*	Jirds – 54	Padma (2005)
8.	Protein vaccine	ALT-2 protein	*B. malayi*	Mice – 64	Gnanasekar et al. (2004)
				Jirds – 73	
9.	Protein vaccine	ALT-2 protein	*B. malayi*	Mice – 74	Ramachandran et al. (2004)
				Jirds – 75	
				Jirds – 72	
				Jirds – 69	Thirugnanam et al. (2007)
					Anand et al. (2011)
					Vanam et al. (2009a)
10.	Protein vaccine	VAH protein	*B. malayi*	Jirds – 60	Anand et al. (2011)
11.	Protein vaccine	TGA protein	*B. malayi*	Jirds – 30	Vanam et al. (2009a)
12.	Protein vaccine	TPX protein	*B. malayi*	Jirds – 43	Vanam et al. (2009a)
13.	Protein vaccine	Thioredoxin (TRX) protein	*B. malayi*	*Mastomys* – 63	Madhumathi et al. (2010)
14.	Multiple-antigen vaccines – DNA	pVAX (ALT +LPX)	*B. malayi*	Mice – 78	Anand et al. (2008)

(continued)

Table 5.1 (continued)

S. no	Vaccine strategy	Antigen	Species	Protection (%)	References
15.	Multiple-antigen vaccines – DNA	pBUD (ALT'VAH)	*B. malayi*	Jirds – 63 and 57	Anand et al. (2011)
16.	Multiple-antigen vaccines – Protein	TGA + ALT	*B. malayi*	Jirds – 47	Vanam et al. (2009a)
17.	Multiple-antigen vaccines – Protein	TGA-TPX	*B. malayi*	Jirds – 73	Vanam et al. (2009a)
18.	Multiple-antigen vaccines – Protein	Alt+VAH	*B. malayi*	Jirds – 77 and 80	Anand et al. (2011)
19.	Peptide vaccine	TRX peptide conjugates	*B. malayi*	*Mastomys* – 71–75	Madhumathi et al. (2010)
20.	Lipid-modified antigen	Lipid-modified ALT-2	*B. malayi*	*Mastomys* – 79%	Sharmila et al. (2013)
21.	Enhanced multiple antigen	*Pichia*-expressed GP29+ VAH	*B. malayi*	*Mastomys* – 80	Prince (2011)

BmTPX protein conferred 43% protection in jirds (Vanam et al. 2009a) and mice, whereas the DNA vaccine showed 37% in mice (Anand et al. 2008). Studies of BmVAH as DNA and bimodal vaccines in this lab showed 50% and 54% protection, respectively (Padma 2005).

The combination of ALT-2 and TPX in the multiple DNA vaccine modes conferred ~78–80% protection in mice (Anand et al. 2008). Similarly, the protective efficacy of *B. malayi* transglutaminase (BmTGA) protein was enhanced significantly (74%) by immunizing the jirds in multiple-antigen mode along with BmTPX. But surprisingly, TGA showed ~47% protection when combined with ALT-2 (Vanam et al. 2009a). Madhumathi et al. (2010) have also shown protective immunity ~75% for TRX host nonhomologous peptides compared to whole recombinant TRX protein (~63%) in jird model. The combination of ALT-2 and VAH as protein and DNA vaccines conferred 78% and 68%, respectively (Anand et al. 2011).

5.6 Identification Methods for LF Vaccine Targets

Modern approaches in molecular biology and computational tools have facilitated the discovery of potential vaccine candidates against several parasitic diseases. Different high-throughput procedures have paved way for the analysis of the degree of differences in gene expression that includes relative EST (expressed sequence tag; short single-pass DNA sequences obtained from either end of cDNA clones), differential phage-displayed libraries, SAGE (serial analysis of gene expression), and microarray techniques (DNA chip technology).

ESTs offer a swift and competent means of gene detection, discovery, and categorization in silico that facilitates the labeling of eukaryotic genes for potential antigenic regions. Large-scale EST sequencing endeavors on diverse life stages of *S. mansoni* (Williams 1999), *O. volvulus* (Lizotte-Waniewski et al. 2000), and *B. malayi* (Williams 1999) and additionally a pilot EST investigation on *L. sigmodontis* (Allen et al. 2000), *Haemonchus contortus* (Hoekstra 2000), *T. canis* (Maizels 2000), and *N. americanus* (Daub 2000) have highlighted the significance of EST resources for the discovery of unique parasite vaccine candidates.

Several putative antigenic regions have been identified in the EST dataset, and some are copiously expressed (Maizels et al. 2001). The ~23 most plentiful ESTs can be assembled into five categories that includes (1) mitochondrial-based transcripts (9 groups), (2) ribosomal proteins (3 groups), (3) conserved metabolic targets and structural proteins (6 groups), (4) proteins with a potential or demonstrated role in arbitrating the host-parasite interaction (2 groups), and (5) proteins with hypothetical function at this point (3 groups). Moreover, ESTs and sequencing projects are underway for other life phases of these parasites to study the differences in the expression of gene profiles that endeavor the identification of potential stage-specific drug/vaccine candidates. The crucial functional roles of these putative protein targets are studied by comparing their sequences with earlier known functionally similar sequences and structures. Through this the relevance in their abundance among EST groups could be analyzed to ascertain their role in the survival mechanism of the parasites. Moreover, their relative variation in their abundance among various life stages may also reveal their functional role in those specific stages.

Selected ESTs known to be either a subset or a combination of the following are considered to be ideal candidate antigens: (1) major metabolic enzymes like antioxidants and those required for growth and development, (2) secreted or surface/membrane associated, (3) proteins that are expressed in to regulate specific functions pertaining to the life stage of the parasites, (4) highly expressed in infective stage, and (5) nonhost homologous novel stage-specific proteins unique for the parasite survival. Special stress has been placed on the L3-infective stages of these parasites that are essential to impart infection. Thus, they carry protective antigens and are the targets for immunoprophylaxis. Moreover, high-throughput screening (HTS) of gene expression libraries and specific protein arrays constructed from cDNA expression libraries using clinical sera is used for prompt identification of immunogenic proteins in the natural host that signify probable targets for vaccine development. Alternatively, proteomics approaches can also be utilized for the identification of putative immunoprophylactic candidates through screening of two-dimensional gels of parasite nematode extracts using endemic patient sera who are putatively immune. The screening antigens can further be characterized by mass spectra to determine their functional role by searching in the gene databases.

5.7 Different Categories of LF Vaccine Candidates

5.7.1 Host–Parasite Interface: A Niche for Vaccine Candidates

In the pursuit of parasite vaccine target identification, the natural attention would be drawn toward host-pathogen interface, because the outer boundaries of the parasites are those areas which come into direct contact with the immune system of the mammalian host and are also physiologically important as a site of nutrient acquisition (Selkirk et al. 1992). This boundary refers to the molecules that constitute both the surface of the parasite and those that are secreted by them. Investigation of the molecules from this domain will provide a wealth of information as to how these parasites skillfully defend and subvert host immune cells.

5.7.2 Metabolic Enzymes: Antioxidants

It has been proved beyond doubt that antioxidants play an important role in counteracting the immune attack from the host and protect the adult parasite at the host interface (Chiumiento and Bruschi 2009). Preliminary experiments provide in vivo evidence that antioxidant enzymes have a role in protective immunity (LoVerde 1998; LoVerde et al. 2004). Parasitic helminths, like all aerobic microbes, require antioxidant proteins to handle with reactive oxygen species (ROS) produced during cellular metabolism (Henkle-Dührsen and Kampkotter 2001). Furthermore, they have to guard themselves against ROS formed by the host. In specific, the ROS can injure diverse cellular constituents by oxidation of lipids, nucleic acids, and essential proteins. This will be pursued by the alteration of receptors, cytoskeleton components, membrane proteins, inactivation of enzymes, and ultimately a lasting damage to the genome. Interestingly, these components apart from its defensive role also engage in offensive actions by combating against the efforts of the host. Numerous antioxidant enzymes like TPX (thioredoxin peroxidase), GPX (glutathione peroxidase), and GST (glutathione *S*-transferase) have been identified from lymphatic filarial parasites.

Peroxidoxins are now being found to be distributed widely among both helminth and protozoan parasites; many have been discovered as a result of genome sequencing and expressed sequence tag (EST) projects. The group of proteins from PRX family (peroxiredoxins) takes part in an essential role in foraging the hydroperoxides that are reduced by the sulfydrilic functional groups of their PRX family. The large quantities of peroxidoxins secreted in these nematodes propose a role for these enzymes in guarding against the assault by the host in addition to an endogenous maintenance function. The peroxidoxins with 2-Cys correspond to thioredoxins (TRX) in their function of using electrons obtained from the thioredoxin system to reduce the disulfide bond derived after the oxidation (Kang et al. 1998). In a different way, compared to other antioxidant proteins, PRX lacks metals in their functional site. The foremost peroxidoxin to be reported by McGonigle (1998) was TPX (thioredoxin peroxidase) (previously named thiol-specific antioxidant (TSA)) and

was first identified in *S. cerevisiae*, where it is expressed not constitutively but in proportion in accordance with oxidative stress. The filarial thioredoxin peroxidase plays a similar role and was even found to be an effective vaccine candidate proved by challenge studies in LF animal models (Table 5.1).

5.8 Infective Stage–Specific Antigens

When the infective L3 stage of the larvae first encounters the definitive host, it exhibits a specific pattern of gene expression. Genes expressed during this stage of the parasite are considered to be potential targets for chemotherapy and immunological interventions. Further the so-called putatively immune or endemic normals (EN) have been found to recognize the antigens from larval stages of the parasite significantly better than the other patient groups, namely, the microfilaremic and chronic patients. Potentially suitable candidates for vaccines would therefore be antigens from the L3 stage that are preferentially recognized by the endemic normals. Few antigens are identified that are highly specific for L3 stage, viz., abundant larval transcript (ALT), venom allergen homologue (VAH), etc. Among this, ALT is the most abundant filarial antigen that plays a crucial role in establishing infection.

5.8.1 Abundant Larval Transcripts (ALT): A Prototype L3 Stage Candidate Antigen

In order to identify parasite gene products required for invasion and establishment in the mammalian host, highly expressed mRNAs from the infective larvae (L3 stage) were characterized, and the SL-directed technique highlighted a set of genes now designated *ALT* (abundant larval transcript), and this assignation has been supported by an analysis of filarial ESTs in which ALT-1 and ALT-2 together represent some 5% of the L3 cDNA. ALT-2 and ALT-1 represent greater than 3% and 1.5%, respectively, of all (L3) infective stage larval ESTs in the filarial genome. Similarly, Di-ALT was also reported to be seen in the ES products of L3 *D. immitis* and discharged during the molting of L3/L4. Ov-ALT (*O. volvulus*) was also found be a homologue of Bm/Wb-ALTs, and even in this case, not less than 4.6% of mRNA of *O. volvulus*L3 was also found to be homologous to alt-2. Further, Ov-ALT was found be partially protective in mouse peritoneal chamber (in situ) experiments. It has also been reported that *L. sigmodontis* and *A. viteae* (Av18, Av-ALT) ALT proteins also belong to the ALT family proteins (Allen et al. 2000). It is noted that ALT proteins are produced only in the later stages of growth in the mosquito. The biological function of ALT proteins still remains unclear. Since ALT protein expression is elevated with tight controlled expression and is also abundantly present in the ES (excreted-secreted products) of mammalian-specific nematodes, it is proposed that they may play an essential role in launching and sustaining infection in the human host (Gomez-Escobar et al. 2005). ALT is accumulated in the esophageal glands of L3-infective larvae and is secreted by them when they encounter mammalian

environment or any culture conditions resembling mammalian host. Hence, it can be noted that *alt* genes are conserved across all filarial family of nematodes that were studied till date and also show very frail similarity to a single locus in free-living nematode *C. elegans* which entails that ALT protein may play a significant role in worm physiology. It is interesting to note that ALT family of proteins shows extremely low sequence similarity to other known proteins from non-filarial organisms and currently no known homology with other mammalian proteins. ALT protein has been reported to show maximum reactivity against presumed immune sera of individuals living in endemic areas. In filarial animal model vaccination studies, recombinant ALT-1 showed ~76% protection (Gregory et al. 2000). Identification of the ALT antigen has also been connected with protective immune responses in experimental filarial animal models of *D. immitis* and *O. volvulus* infection.

ALT-2 has been extensively studied by Dr. P. Kaliraj's group in the form of recombinant protein, DNA, bimodal, phage-displayed antigen, and multiple-antigen modes. ALT-2 was the first and foremost antigen recognized by the immune sera in a classic experiment of screening vaccine antigens using novel phage-displayed library (Gregory et al. 2000; Gnanasekar et al. 2004). In the same study, it was shown that ALT-2 conferred over 73% protection against a challenge infection in the jird model and over 64% protection in the mouse model. The utility of BmALT-2 as a vaccine candidate was again tested in a murine (Ramachandran et al. 2004) and jird model (Thirugnanam et al. 2007) using either recombinant protein (74% and 75%, respectively) or a DNA vaccine construct (31% and 57%). When immunized along with VAH in jirds, it conferred 78% protection as a protein and 68% as DNA vaccine. About 78% protection was observed when immunized along with TPX as a multiple DNA vaccine (Anand et al. 2008) and 47% with TGA (Vanam et al. 2009a, b). Thus, these proteins are most strongly implicated in protective immunity.

5.8.2 Modern Approaches for LF Vaccine Development

The progress in vaccine development is closely matched with the modern-day marvels of immunological advancement and commemorates a milestone in the history of human attempts to fight diseases. Ever since the beginning of systematic smallpox prophylactic evaluation dating from 1796, there is a growing trend in the application of technology to bring drastic advancements in vaccine research to combat infectious diseases. But there are still many challenges in vaccine development, and chiefly these result from the existing methods used in vaccine production, and this includes the difficult task of in vitro culturing of pathogens such as viruses and parasites, biohazard and safety considerations, and the presence of evolving mutations in the established antigens of pathogens rendering them ineffective in their efficacy for protection. The existing vaccines mainly aim at microorganisms that show limited variability in antigen profile that is also distinctly different from the host. Hence the vaccines are able to elicit antibody-based protective response to clear infection which includes infections by polio, tetanus, etc., so the conventional methods of vaccine preparation is insufficient for complex organisms like multicellular parasite

who have a greater selective pressure when they constantly live inside the host. This makes them change their antigenic profile constantly to evade detection by the immune system of the host. Further, culturing these nematodes in vitro to study their antigenic diversity in the laboratory and, added to this, the deficiency of exact lab parasite-host model aggravates the delay in developing effective vaccines.

Novel tactics for vaccine enhancement have to focus on conquering these hurdles. Nonetheless, the advent of the genome-based revolution has drastically altered the mode of discovering vaccine targets in parasitic worm diseases. Recent progress in sequencing technical know-how along with the enhancement of tools available in bioinformatics has brought a rapid growth in exploring the use of genome data effectively for vaccine applications. Hence the application of genome data for searching candidates for vaccine study has become a specialty in itself and is often termed as "reverse vaccinology." This technique will also check the candidates screened by other methods and thus would kick off a constructive feedback mechanism for validating that target before their commencement of in vivo studies. This method also enables the study of interaction with host immune components to check for adverse/effective response. Thus reverse vaccinology when made practical can help identify the complete range of possible antigens that the parasite could express by analyzing its genome data. Thus surface proteome analysis can target on a core fraction of proteins that are expressed on its surface. Similarly, functional genomics application can shed light on the range of major proteins that play a function in survival mechanism. Advanced specialties like parasite immunomics focus on expounding host-parasite interactions and together with structural studies on vaccine targets can also help in the identification of conformation epitopes. Further, cataloguing of host-specific characteristic immune responses against presumed parasite vaccines has also enabled the use of vaccinomic approaches in advancing vaccine studies in LF (Rinaudo et al. 2009).

The early vaccine approaches in LF animal models used whole parasites either dead or attenuated to establish protective immunity in the host. This involved the use of L3-irradiated larva as a preferred mode of attenuation in animal challenge studies (Abraham et al. 1986, 1988). The next phase is involved in the purification of L3 larval contents to generate subunit components in par with the whole parasite. At this stage, recombinant proteins from phage-displayed libraries were used. This approach was expected to reduce untoward allergic reactions with enhanced and focused immune responses in the host. Other approaches have included the use of DNA vaccination to skew immune responses for promoting cell-mediated immunity (Table 5.1). The use of multiple recombinant antigens either prepared in cocktail fashion or in the form of conjugates has also been attempted in LF animal model vaccination trials with promising results. The recent approach in LF vaccination has utilized epitope screening of already identified and characterized antigens to strengthen further the immune response by chemically stitching B- and T-cell epitopes together for making effective epitope conjugate vaccine candidates (Table 5.1). The protective immunity is expected in the host to prevent LF endeavors to focus on preventing morbidity by bringing down parasite burden to considerable level especially to a point where the host could be effectively sensitized for parasite-specific

activation of immune responses. This brings a stark contract in the type of terminal immunity that is usually expected in viral and bacterial vaccines. But in the case of nematode vaccines, a protective response that can provide anything more than 60% reduction in worm burden accompanied by symptomatic improvement in clinical pathology is considered significant.

5.9 Immunomics: Identification of Immunogenic Proteins

In every nematode parasitic infection, clearance of worms is achieved by parasite-specific protective antibodies that function by activating ADCC (antibody-dependent cellular cytotoxicity) mechanism. Hence, these protective antibodies symbolize molecular indentation of parasite antigens that provoked such protective humoral responses. Further, elucidation of these imprints involves the use of both genomic and proteomic techniques and when complimented with serological screening can result in the identification and validation of prospective vaccine candidates. That array of proteins recognized by this method constitutes the immunoproteome profile of the parasite infective arm. The development of phage-displayed libraries incorporating and representing the cDNA content of each stage of the parasite nematode will now enable the screening of the entire immunoproteome through sera obtained from the healthy individual living in endemic areas that are naturally immune to nematode infection. The identified immunogens and putative proteins can further be validated based on their in vitro levels of expression. This method of reverse vaccinology to screen antigens is more suitable for nematode pathogens with inherent limitations in their culturing and strict species specificity. The identified antigens can now be sequenced, cloned, and expressed in recombinant manner in suitable host to produce antigens for vaccine challenge studies. Alternatively, elucidation of peptide sequences can also be used in their synthesis of peptides incorporating potential epitopes for provoking protective responses in the experimental host against challenge infection. This approach ultimately tries to reconcile the way humoral wing of immunity would effectively blend with the cellular wing for coordinated responses, since the mode of epitope recognition is quite distinct in either case. In this way, we can bring in the best of epitopes under each category to provide tandem protective response. The eventual goal would be to widen the scope of antigens analyzed in each stage to provide comprehensive protection considering the multicellular nature of the infection and also have a minimalistic version of the antigen combinations without compromising protection, thereby affording specificity in response devoid of allergic or host autoimmune responses.

5.10 Peptide Vaccine Approach

The prime attempt of replacing small peptides in place of whole antigen of virus to provide T-cell-based immune response was pioneered by Townsend et al. (1986). They showed evidence of lysis among viral-infected cells by viral peptide-sensitized

Tc cells. Further report by Zinkernagel and Doherty 1979 with more details on MHC-constrained T-cell antigen detection along with the availability of major histocompatibility complex (MHC) structure by Bjorkman et al. 1987 brought forth the avenue of using peptides as an immunologically defined entity and also as an effective alternative for whole pathogens in vaccines. This trend of making use of the immuno-peptides has become an essential approach in strategically designing custom vaccines. The methodology of peptide synthesis has gradually evolved in the past decades to an extent where multiple crusts of peptides can be stitched in linear fashion in various combinations and can also be designed in multimeric patterns with branching architecture to enhance immune responses. This flexibility in peptide-synthesizing technologies down the years has also made use of the incorporation of nonprotein immunogenic conjugates to increase effectiveness in immune responses (Purcell et al. 2003).

Moreover, custom designing of antigenic peptides in rational manner results in the enhancement of the quality of these antigens in terms of its stability, scalability in large-volume production, and efficacy in immune responses. Further, there is an additional advantage of ease in storage and transport through customized manipulation of peptide combinations without compromising immune responses (Demotz et al. 2001; Ben-Yedidia and Arnon 1997).

A general survey reveals an overall demand for vaccines for multiple infectious diseases especially that involves multicellular organisms and parasites such as leishmaniasis, hookworm infections, and malaria. The current approach for some of these diseases still includes the use of an admixture of whole organisms in an attenuated or dead manner for eliciting protection. The limitation of these vaccine preparations comes with a compromise on cost-production-quality and safety issues versus long-term protection considering the industrial level production to meet the ever-rising demand. Review updates on polio vaccine production on these issues have brought grave concern among the vaccine community (Weiss et al. 2009). Further, the risk of these concerns poses a greater threat on some specific groups such as gestational women and immunosuppressed patients especially with the use of attenuated viral vaccines. Hence, a straight alternative can be sought with epitope vaccines to tackle these issues efficiently, and they provide several advantages like evasion of secondary infections, reduction of autoimmune exposure, and nil likelihood of virulence owing from decline/re-mixture linked with traditional vaccines that may use genetic combination/blending connected with DNA-based vaccines.

Moreover, peptide prophylactic agents do not need cold cargo facilities for transportation that would drastically provide economic incentives for vaccine industries and an added advantage for developing and underdeveloped countries with large populations and potential users of vaccines on a massive scale. But the dawn of gene-manipulating techniques and their application in vaccine engineering and development in general have transformed the way we make vaccines. We cannot deny the fact of saving time and efforts that were originally involved in the industrial-scale conversion of base pathogenic components available in minimal quantities to meet the huge demands of vaccine production that was squarely replaced by recombinant protein production methods for many mainline viral vaccines. Nevertheless,

recombinant technology has its limitations, like the difficulty in the expression of eukaryotic genes, solubility, and refolding and purification of proteins, especially if there is a structural modification that is required for immunogenic function that was available in native antigen. Hence, in such cases the uses of advanced peptide technology can provide the customary manufacture of polypeptides with a range capable of more than 100 residues in sufficient amounts (in mg levels). Further, secondary structural modifications through advanced disulfide bond formation can also be introduced to provide for native-fold antigen conformation. There is also provision of quality monitoring and control since the production mostly involves reactions to provide that finished product with excellent execution of GMP requirements. Moreover, there is no concern of contamination with infectious agents that might result from routine cell line infections (like VERO cell line RNA virus infections).

5.11 Multiple–Antigen Vaccine Strategy for LF

Filarial nematodes are multicellular parasites and elicit stage-specific immune responses in human host. This ability of the nematodes could have been due to the presence of effective genomic resources developed exclusively by filariae over a period of time as they struggled their way for survival in multiple hosts capturing strategies for immune evasion which can be reasoned for their long-term survival in such definitive hosts. Hence, such gene components offer a gamut of opportunities to be evolved in the molecular level by parasitic worms to trick and elude the mammalian protective immunity (Maizels et al. 1999, 2003).

Moreover, in natural course of filarial infection, there is an aspect of stage-specific antigenic distinction that confers a state of associated immunity that also possibly averts superinfection. This will be an important feature that should be considered while contemplating methods for developing effective vaccines for eradication programs. Hence, multiple-antigen vaccination will be a promising strategy for efficacious vaccine development in LF. However, extensive study should be done in terms of selecting the antigens for effective combination to warrant synergistic protective response. Although a single antigen-based vaccination could also provide protection, it may not be strong enough to cover the complex life cycle of the parasite along with its myriad of antigens. Hence, the use of multiple antigens in a cocktail mode would be necessary to achieve a high level of protection (Rainczuk et al. 2004; Mendez et al. 2005). There is consensus to this approach, which has been successfully demonstrated with hopeful results in other diseases such as leishmaniasis, hookworm infection, schistosomiasis, and malaria (Dupre et al. 1999; Coler and Reed 2005). Moreover, multivalent vaccines can also provide additional benefits of obviating the problems associated with host genetic restriction and antigenic variability. Multiple vaccination studies in LF that combine major antigens of the infective stage larvae could complement each other synergistically for inducing both humoral and cellular immune responses of the host for high efficacy. A recent study by Anand et al. (2011) showed that ALT and VAH when given as a cocktail vaccine can confer significant protection (80%). Thus the use of ALT or VAH for

multivalent combination with other L3 stage-specific antigens could be a promising choice for further vaccine studies in LF.

5.12 Conclusion

Vaccine development for multicellular parasitic nematodes is a challenging task, and so far we have come in terms of identifying and validating potential candidates through effective animal model protection studies. Among the vaccine candidate studies validated for LF, we conclude L3 stage-specific antigens such as ALT and VAH have potential to be considered for further clinical studies. Among the methods of vaccine design, development, and validation, novel techniques utilizing the advances in recombinant technology, immunomics, and informatics would be needed to develop efficacious vaccine for complex parasitic diseases like lymphatic filariasis.

References

Abraham D, Grieve RB, Holy JM, Christensen BM (1989) Immunity to larval *Brugia malayi* in BALB/c mice: protective immunity and inhibition of larval development. Am J Trop Med & Hyg 40:598–604

Abraham D, Grieve RB, Mika-Grieve M, Seibert BP (1988) Active and passive immunization of mice against larval *Dirofilaria immitis*. J Parasitol 74:275–282

Abraham D, Weiner DJ, Farrell JP (1986) Protective immune response of the jird to larval *Dipetalonema viteae*. Immunology 57:165–169

Allen JE, Daub J, Guiliano D, McDonnell A, Lizotte-Waniewski M, Taylor DW, Blaxter M (2000) Analysis of genes expressed at the infective larval stage validates utility of *Litomosoides sigmodontis* as murine model for filarial vaccine development'. Infect Immun 68:5454–5458

Anand SB, Gnanasekar M, Thangadurai M, Prabhu PR, Kaliraj P, Ramaswamy K (2007) Immune response studies with *Wuchereria bancrofti* vespid allergen homologue (WbVAH) in human lymphatic filariasis. Parasitol Res 101(4):981–988

Anand SB, Murugan V, Prabhu PR, Anandharaman V, Reddy MV, Kaliraj P (2008) Comparison of immunogenicity, protective efficacy of single and cocktail DNA vaccine of *Brugia malayi* abundant larval transcript (ALT-2) and thioredoxin peroxidase (TPX) in mice. Acta Trop 107(2):106–112

Anand SB, Kodumudi KN, Reddy MV, Kaliraj P (2011) A combination of two *Brugia malayi* filarial vaccine candidate antigens (BmALT-2 and BmVAH) enhances immune responses and protection in jirds. J Helminthol 4:1–11

Ash LR, Riley JM (1970) Development of subperiodic *Brugia malayi* in the jird, *Meriones unguiculatus*, with notes on infections in other rodents. J Parasitol 56:969–973

Ben-Yedidia T, Arnon R (1997) Design of peptide and polypeptide vaccines. Curr Opin Biotechnol 8(4):442–448

Bianco AE, Luty A, Whitworth J, Taylor D (1991) Immunity to *Onchocerca volvulus* microfilariae in mice and the induction of cross-protection with *O. lienalis*. Trop Med Parasitol 42:188–190

Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC (1987) Structure of the human class I histocompatibility antigen, HLA-A2. Nature 329(6139):506–512

Carlow CK, Bianco AE (1987) Resistance to Onchocerca lienalis microfilariae in mice conferred by egg antigens of homologous and heterologous Onchocerca species. Parasitology 94:485–496

Carlow CK, Philipp M (1987) Protective immunity to *Brugia malayi* larvae in BALB/c mice: potential of this model for the identification of protective antigens. Am J Trop Med &Hyg 37:597–604

Catmull J, Wilson ME, Kirchhoff LV, Metwali A, Donelson JE (1999) Induction of specific cell-mediated immunity in mice by oral immunization with Salmonella expressing *Onchocerca volvulus* glutathione S-transferase. Vaccine 17(1):31–39

Cheirmaraj K, Chenthamarakshan V, Reddy MVR, Harinath BC (1991) Immunoprophylaxis against filarial parasite, *Brugia malayi*: potential of excretory secretory antigens in inducing immunity. J Biosci 16:209–216

Chenthamarakshan V, Reddy MV, Harinath BC (1995) Immunoprophylactic potential of a 120 kDa*Brugia malayi* adult antigen fraction, BmA-2, in lymphatic filariasis. Parasite Immunol 17(6):277–285

Chiumiento L, Bruschi F (2009) Enzymatic antioxidant systems in helminth parasites. Parasitol Res 105(3):593–603

Chusattayanond W, Denham DA (1986) Attempted vaccination of jirds, *Meriones unguiculatus* against *Brugia pahangi* with radiation attenuated infective larvae. J Helminthol 60:149–155

Coler RN, Reed SG (2005) Second-generation vaccines against leishmaniasis. Trends Parasitol 21(5):244–249

Dabir P, Dabir S, Krithika KN, Goswami K, Reddy MV (2006) Immunoprophylactic evaluation of a 37-kDa *Brugia malayi* recombinant antigen in lymphatic filariasis. Clin Microbiol Infect 12(4):361–368

Daub J (2000) A survey of genes expressed in adults of the human hookworm, *Necator americanus*. Parasitology 120:171–184

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

Demotz S, Moulon C, Roggero MA, Fasel N, Masina S (2001) Native-like, long synthetic peptides as components of sub-unit vaccines: practical and theoretical considerations for their use in humans. Mol Immunol 38(6):415–422

DupreHerv M, Schacht AM, Capron A, Riveau G (1999) Control of *Schistosomiasis* pathology by combination of Sm28GST DNA immunization and praziquantel treatment. J Infect Dis 180(2):454–463

Frank GR, Tripp CA, Grieve RB (1996) Molecular cloning of a developmentally regulated protein isolated from excretory-secretory products of larval *Dirofilaria immitis*. Mol Biochem Parasitol 75(2):231–240

Fuhrman JA, Lane WS, Smith RF, Piessens WF, Perler FB (1992) Transmission-blocking antibodies recognize microfilarial chitinase in brugian lymphatic filariasis. Proc Natl Acad Sci U S A 89:1548–1552

Gnanasekar M, Rao KV, He YX, Mishra PK, Nutman TB, Kaliraj P et al (2004) Novel phage display-based subtractive screening to identify vaccine candidates of *Brugia malayi*. Infect Immun 72(8):4707–4715

Gomez-Escobar N, Bennett C, Prieto-Lafuente L, Aebischer T, Blackburn CC, Maizels RM (2005) Heterologous expression of the filarial nematode alt gene products reveals their potential to inhibit immune function. BMC Biol 3:8

Gregory WF, Atmadja AK, Allen JE, Maizels RM (2000) The abundant larval transcript −1 and −2 genes of *Brugia malayi* encode stage-specific candidate vaccine antigens for filariasis. Infect Immun 68:4174–4179

Grieve RB, Abraham D, Mika-Grieve M, Seibert BP (1988) Induction of protective immunity in dogs to infection with *Dirofilaria immitis* using chemically-abbreviated infections. Am J Trop Med & Hyg 39:373–379

Grieve RB, Wisnewski N, Frank GR, Tripp CA (1995) Vaccine research and development for the prevention of filarial nematode infections. Pharm Biotechnol 6:737–768

Harrison RA, Bianco AE (2000) DNA immunization with *Onchocerca volvulus* genes, Ov-tmy-1 and OvB20: serological and parasitological outcomes following intramuscular or GeneGun delivery in a mouse model of Onchocerciasis. Parasite Immunol 22:249–257

Harrison RA, Wu Y, Egerton G, Bianco AE (1999) DNA immunisation with *Onchocercavolvulus*chitinase induces partial protection against challenge infection with L3 larvae in mice. Vaccine 18:647–655

Hayashi Y, Noda K, Shirasaka A, Nogami S, Nakamura M (1984) Vaccination of BALB/c mice against *Brugia malayi* and *B. pahangi* with larvae attenuated by gamma irradiation. Jpn J Exp Med 54(4):177–181

Hayashi Y, Nakagaki K, Nogami S, Hammerberg B, Tanaka H (1989) Protective immunity against *Brugia malayi* infective larvae in mice. I parameters of active and passive immunity. Am J Trop Med & Hyg 41:650–656

Henkle-Dührsen K, Kampkotter A (2001) Antioxidant enzyme families in parasitic nematodes. Mol Biochem Parasitol 114:129–142

Hoekstra R (2000) EST sequencing of the parasitic nematode *Haemonchus contortus* suggests a shift in gene expression during transition to the parasitic stages. Mol & Biochem Parasitol 110:53–68

Horii Y, Nakanishi H, Mori A, Ueda M, Kurokawa K, Zaitsu M, Oda T, Fujita K (1992) Induction of protective immunity to *Brugia pahangi* in jirds by drug-abbreviated infection. J Helminthol 66:147–154

Irvine M, Huima T, Prince AM, Lustigman S (1994) Identification and characterization of an *Onchocerca volvulus* cDNA clone encoding a highly immunogenic calponin-like protein. Mol Biochem Parasitol 65:135–146

Jenkins RE, Taylor MJ, Gilvary NJ, Bianco AE (1998) Tropomyosin implicated in host protective responses to microfilariae in onchocerciasis. Proc Natl Acad Sci U S A 95:7550–7555

Kang Y, Calvo PA, Daly TM, Long CA (1998) Comparison of humoral immune responses elicited by DNA and protein vaccines based on merozoite surface protein-1 from *Plasmodium yoelii*, a rodent malaria parasite. J Immunol 161:4211–4219

Kazura JW, Cicirello H, McCall JW (1986) Induction of protection against *Brugia malayi* infection in jirds by microfilarial antigens. J Immunol 136:1422–1426

Klei TR, McDonough KC, Coleman SU, Enright FM (1987) Induction of lymphatic lesions by *Brugia pahangi* in jirds with large and small pre-existing homologous intraperitoneal infections. J Parasitol 73:290–294

Krithika KN, Dabir P, Kulkarni S, Anandharaman V, Reddy MV (2005) Identification of 38kDa *Brugia malayi* microfilarial protease as a vaccine candidate for lymphatic filariasis. Indian J Exp Biol 43(9):759–768

Lange AM, Yutanawiboonchai W, Lok JB, Trpis M, Abraham D (1993) Induction of protective immunity against larval *Onchocerca volvulus* in a mouse model. Am J Trop Med & Hyg 49:783–788

Li BW, Chandrashekar R, Weil GJ (1993) Vaccination with recombinant filarial paramyosin induces partial immunity to *Brugia malayi* infection in jirds. J Immunol 15:1881–1885

Li BW, Zhang S, Curtis KC, Weil GJ (1999) Immune responses to *Brugia malayi* paramyosin in rodents after DNA vaccination. Vaccine 18:76–81

Lizotte-Waniewski M, Tawe W, Guiliano DB, Lu W, Liu J, Williams SA, Lustigman S (2000) Identification of potential vaccine and drug target candidates by expressed sequence tag analysis and immunoscreening of *Onchocerca volvulus* larval cDNA libraries. Infect Immun 68:3491–3501

Lok JB, Abraham D (1992) Animal models for the study of immunity in human filariasis. Parasitol Today 8(5):168–171

LoVerde PT, Carvalho-Queiroz C, Cook R (2004) Vaccination with antioxidant enzymes confers protective immunity against challenge infection with *Schistosoma mansoni*. Mem Inst Oswaldo Cruz 99(Suppl. I):37–43

LoVerde PT (1998) Do antioxidants play a role in Schistosome host–parasite interactions? Parasitol Today 14(7):284–289

Lucius R, Ruppel A, Diesfeld HJ (1986) *Dipetalonema viteae*: resistance in *Meriones unguiculatus* with multiple infections of stage-3 larvae. Exp Parasitol 62:237–246

Lucius R, Textor G, Kern A, Kirsten C (1991) *Acanthocheilonema viteae*: vaccination of jirds with irradiation-attenuated stage-3 larvae and with exported larval antigens. Exp Parasitol 73:184–196

Madhumathi J, Prince PR, Anugraha G, Kiran P, Rao DN, Reddy MVR, Kaliraj P (2010) Identification and characterization of nematode specific protective epitopes of *Brugia malayi* TRX towards development of synthetic vaccine construct for lymphatic Filariasis. Vaccine 28(31):5038–5048

Maizels RM (2000) *Toxocara canis* genes expressed by the arrested infective larval stage of a parasitic nematode. Int J Parasitol 30:495–508

Maizels RM, Balxter ML, Scott AL (2001) Immunological genomics of *Brugia malayi*: filarial genes implicated in immune evasion and protective immunity. Parasite Immunol 23:327–344

Maizels RM, Morgan TM, Gregory WF, Selkirk ME, Purnomo S, Partono F (1988) Circulating antibodies and antigens in Presbytis monkeys infected with the filarial parasite *Wuchereria bancrofti*. Trop Med Parasitol 39:214–220

Maizels RM, Partono F, Oemijati S, Denham DA, Ogilvie BM (1983) Cross reactive surface antigens of three stages of *Brugia malayi, B Pahangi* and *B timori*. Parasitology 87:249–263

Maizels RM, Holland MJ, Falcone FH, Zang XX, Yazdanbakhsh M (1999) Vaccination against helminth parasites--the ultimate challenge for vaccinologists? Immunol Rev 171:125–147

Maizels RM, Yazdanbakhsh M (2003) Immune regulation by helminth parasites: cellular and molecular mechanisms. Nat Rev Immunol 3(9):733–744

McCarthy JS, Wieseman M, Tropea J, Kaslow D, Abraham D, Lustigman S, Tuan R, Guderian RH, Nutman TB (2002) *Onchocerca volvulus* glycolytic enzyme fructose-1,6-bisphosphate aldolase as a target for a protective immune response in humans. Infect Immun 70(2):851–858

Mcgonigle S, Dalton JP, James ER (1998) Peroxidoxins: a new antioxidant family. Parasitol Today 14:139–145

Mendez S, Zhan B, Goud G, Ghosh K, Dobardzic A, Wu W, Liu S, Deumic V, Dobardzic R, Liu Y, Bethony J, Hotez PJ (2005) Effect of combining the larval antigens Ancylostoma secreted protein 2 (ASP-2) and metalloprotease 1 (MTP-1) in protecting hamsters against hookworm infection and disease caused by *Ancylostoma ceylanicum*. Vaccine Vol. 23(24):3123–3130

Murray J, Gregory WF, Gomez-Escobar N, Atmadja AK, Maizels RM (2001) Expression and immune recognition of *Brugia malayi*VAL-1, a homologue of vespid venom allergens and Ancylostoma secreted proteins. Mol Biochem Parasitol 118(1):89–96

Murthy PK, Murthy PS, Tyagi K, Chatterjee RK (1997) Fate of infective larvae of *Brugia malayi* in the peritoneal cavity of *Mastomys natalensis* and *Meriones unguiculatus*. Folia Parasitol (Praha) 44(4):302–304

Padma B (2005) Protective efficacy of Brugia malayi Venom Allergen Homologue (VAH) AND Wuchereria bancrofti Thioredoxin (TRX) homologue as a DNA and bimodal vaccine in animal models. Ph.D Dissertation, Anna University

Palmieri JR, Van Dellen AF, Tirtokusumo S, Masbar S, Rusch J, Connor DH (1984) Trapping, care, and laboratory management of the silvered leaf monkey (*Presbytis cristatus*). Lab Anim Sci 34:194–197

Pankaj KM (2003) Cellular immune responses and the protective efficacy after immunization with Brugia Malayi abundant Larval transcritpion - 2 [ALT - 2] recombinant protein phage displayed Antigen or As a DNA vaccine in mice and gerbils models. Ph.D Dissertation, Anna University

Peralta ME, Schmitz KA, Rajan TV (1999) Failure of highly immunogenic filarial proteins to provide host-protective immunity. Exp Parasitol 91(4):334–340

Philipp M, Worms MJ, Maizels RM, Ogilvie BM (1984) Rodent models of filariasis. Contemp Top Immunobiol 12:275–321

Prince RP (2011) Structural elucidation and immunoprophylactic studies of recombinant parasitic proteins for therapeutic intervention in human lymphatic filariasis. Ph.D Dissertation, Anna University

Prince AM, Brotman B, Johnson EH, Smith A, Pascual D, Lustigman S (1992) *Onchocerca volvulus*: immunization of chimpanzees with X-irradiated third-stage (L3) larvae. Exp Parasitol 74:239–250

Purcell AW, Zeng W, Mifsud NA, Ely LK, Macdonald WA, Jackson DC (2003) Dissecting the role of peptides in the immune response: theory, practice and the application to vaccine design. J Pept Sci 9(5):255–281

Rainczuk A, Scorza T, Spithill TW, Smooker PM (2004) A bicistronic DNA vaccine containing apical membrane antigen 1 and merozoite surface protein 4/5 can prime humoral and cellular immune responses and partially protect mice against virulent *Plasmodium chabaudi adami* DS malaria. Infect Immun 72(10):5565–5573

Rajasekariah GR, Puri PM, Chandrashekar R, Subrahmanyam D (1988) Clearance of *Brugia pahangi* microfilariae in immunized mice. Immunol Cell Biol 66:331–336

Ramachandran S, Kumar MP, Rami RM, Chinnaiah HB, Nutman T, Kaliraj P et al (2004) The larval specific lymphatic filarial ALT-2: induction of protection using protein or DNA vaccination. Microbiol Immunol 48:945–955

Rinaudo CD, Telford JL, Rappuoli R, Seib KL (2009) Vaccinology in the genome era. J Clin Invest 119(9):2515–2525. https://doi.org/10.1172/JCI38330

Sänger I, Lämmler G, Kimmig P (1981) Filarial infections of *Mastomys natalensis* and their relevance for experimental chemotherapy. Acta Trop 38(3):277–288

Selkirk ME, Maizels RM, Yazdanbakhsh M (1992) Immunity and the prospects for vaccination against filariasis. Immunobiology 184(2–3):263–281

Sharmila S, Christiana I, Kiran P, Reddy MVR, Sankaran K, Kaliraj P (2013a) Bacterial lipid modification enhances immunoprophylaxis of filarial abundant larval transcript-2 protein in Mastomys model. Parasite Immunol 35(7):201–213. https://doi.org/10.1111/pim.12034

Srivastava Y, Rathaur S, Bhandari YP, Reddy MV, Harinath BC (2004) Adult 175 kDa collagenase antigen of *Setaria cervi* in immunoprophylaxis against *Brugia malayi* in jirds. J Helminthol 78(4):347–352

Steel C, Guinea A, Ottesen EA (1996) Evidence for protective immunity to Bancroftian Filariasis in the Cook Islands. J Infect Dis 174:598–605

Storey DM, Al-Mukhtar AS (1982) Vaccination of jirds, *Meriones unguiculatus*, against *Litomosoides carinii* and *Brugia pahangi* using irradiated larvae of *L carinii*. Trop Med Parasitol 33:23–24

Subrahmanyam D, Mehta K, Nelson DS, Rao YV, Rao CK (1978) Immune reactions in human filariasis. J Clin Microbiol 8:228–232

Taylor MJ, Abdel WN, Wu Y, Jenkins RE, Bianco AE (1995) *Onchocerca volvulus* larval antigen, OvB20, induces partial protection in a rodent model of onchocerciasis. Infect Immun 63:4417–4422

Taylor MJ, Jenkins RE, Bianco AE (1996) Protective immunity induced by vaccination with *Onchocerca volvulus* tropomyosin in rodents. Parasite Immunol 18:219–225

Thirugnanam S, Pandiaraja P, Ramaswamy K, Murugan V, Gnanasekar M, Nandakumar K, Reddy MV, Kaliraj P (2007) *Brugia malayi*: comparison of protective immune responses induced by Bm-alt-2 DNA, recombinant Bm-ALT-2 protein and prime-boost vaccine regimens in a jird model. Exp Parasitology 116(4):483–491

Townsend AR, Rothbard J, Gotch FM, Bahadur G, Wraith D, McMichael AJ (1986) The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. Cell 44(6):959–968

Townson S, Nelson GS, Bianco AE (1985) Immunity to *Onchocerca lienalis* microfilariae in mice. II. Effects of sensitization with a range of heterologous species. J Helminthol 59:337–346

Vanam U, Pandey V, Prabhu PR, Dakshinamurthy G, Reddy MV, Kaliraj P (2009a) Evaluation of immunoprophylactic efficacy of *Brugia malayi* transglutaminase (BmTGA) in single and multiple antigen vaccination with BmALT-2 and BmTPX for human lymphatic filariasis. Am J Trop Med Hyg 80(2):319–324

Vanam U, Prabhu PR, Pandey V, Dakshinamurthy G, Reddy MV, Perumal K (2009b) Immune responses generated by intramuscular DNA immunization of Brugia malayi transglutaminase (BmTGA) in mice. Parasitology 136(8):887–894

Veerapathran A, Dakshinamoorthy G, Gnanasekar M, Reddy MV, Kalyanasundaram R (2009) Evaluation of *Wuchereria bancrofti* GST as a vaccine candidate for lymphatic filariasis. PLoS Negl Trop Dis 3(6):e457

Wang SH, Zheng HJ, Dissanayake S, Cheng WF, Tao ZH, Lin SZ, Piessens WF (1997) Evaluation of recombinant chitinase and SXP1 antigens as antimicrofilarial vaccines'. Am J Trop Med Hyg 56:474–481

Weiss WM, Winch PJ, Burnham G (2009) Factors associated with missed vaccination during mass immunization campaigns. J Health Popul Nutr 27(3):358–367

Wenk P, Mössinger J (1991) Recovery of adult stages and microfilaraemia after low dose inoculation of third stage larvae of *Litomosoides carinii* in *Sigmodon hispidus*. J Helminthol 65(3):219–225

WHO Weekly, Epidemiol Rec (2006) Informal consultation on preventing disability from lymphatic filariasis, WHO, Geneva, August 2006. Wkly Epidemiol Rec 81(40):373–383 English, French

Williams SA (1999) Deep within the filarial genome: progress of the filarial genome project. Parasitol Today 15(6):219–224

Wong MM, Fredericks HJ, Ramachandran CP (1969) Studies on immunization against *Brugia malayi* infection in the rhesus monkey. Bull World Health Organ 40:493–501

Wuhrer M, Rickhoff S, Dennis RD, Lochnit G, Soboslay PT, Baumeister S, Geyer R (2000) Phosphocholine-containing zwitterionic glycosphingolipids of adult *Onchocerca volvulus* as highly conserved antigenic structures of parasitic nematodes. J Biochem 348:417–423

Yates JA, Higashi GI (1985) *Brugia malayi*: vaccination of jirds with 60cobalt-attenuated infective stage larvae protects against homologous challenge. Am J Trop Med Hyg 34:1132–1137

Zinkernagel RM, Doherty PC (1979) MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction-specificity, function, and responsiveness. Adv Immunol 27:51–177

6 Recombinant Filarial, *Wolbachia* Antigens and their Role in the Immunopathogenesis of Human Lymphatic Filariasis

Kirthika Sreenivas, Kamalakannan Vijayan, and Rangarajan Badri Narayanan

Abstract

Lymphatic filarial infection prevails in the tropical region and is a serious health problem in India. This is mainly because of the absence of any effective drugs against larval and adult parasites. Therefore, there is a necessity for an alternative strategy to eradicate these parasites. Further, these parasites and their products interfere with effective molecules of innate and adaptive defense responses of the host. This interaction induces modulation in the levels of molecules, and they serve as markers of infection and attributes to the development of the disease. The identification of immune evasion genes from filarial genome project aided in deducing the pathway that leads to chronic disease from infection. This chapter highlights the various mechanisms underlying filarial infection and further characterization of filarial antigens resulted in exploiting them as an effective therapeutic tool against filariasis. Various studies have shown the possible strategies and have identified key molecules for immunological research.

6.1 Introduction

Mosquitoes are estimated to cause 7, 25,000 human deaths worldwide every year by transmitting a variety of diseases, especially in the underdeveloped countries. Lymphatic filariasis or elephantiasis, a serious public health problem in India, is one such parasitic disease that is transmitted by mosquitoes to humans (Kulkarni et al.

K. Sreenivas · K. Vijayan · R. B. Narayanan (✉)
Department of Biotechnology, Sri Venkateswara College of Engineering (SVCE), Irunkattukottai, Sriperumbudur, India
e-mail: rbn@svce.ac.in

© Springer Nature Singapore Pte Ltd. 2018
B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_6

2014; Witt and Ottesen 2001). This neglected tropical disease is caused by lymph-dwelling nematodes *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* which also harbor endosymbiont bacteria, *Wolbachia* sp.

The concept of mass drug administration (MDA) is to approach every individual in the target community and administer an annual single dose of anti-filarial drugs (DEC or DEC+ albendazole). A high coverage of more than 85% in endemic areas for 5 years is required to achieve interruption of transmission and elimination of the disease in India. Even though the drug distribution in the country is more than 85%, people who actually consume the drugs widely vary on the geographical and socio-cultural bases. The major challenge is drug delivery in urban areas and the low priority given to lymphatic filariasis (Agarwal and Shashindran 2006). However, people harboring filarial infections are likely to experience side effects as a consequence of the interaction between the drug and the parasite, particularly if microfilariae (*mf*) counts are high (Sabesan et al. 2010). In others, the symptoms are usually nonspecific and self-limiting. This might be due to the fact that the current drugs are ineffective against the larval (L3) stage and adult parasites and also the occurrence of nausea, dizziness, and other adverse reactions during parasite killing.

The emergence of drug resistance in parasites has led to an alternative approach called anti-*Wolbachia* therapy, i.e., killing of *Wolbachia* to control the disease transmission. The presence of *Wolbachia* is necessary for worm survival. The approach of administering antibiotics (tetracyclines like doxycycline) to kill *Wolbachia* resulted in better depletion than MDA drug combinations. But, tetracycline affected female adult worms and caused male-biased sex ratio, and so killing *Wolbachia* might not be sufficient, and also the use of doxycycline is less suitable for treatment in children and pregnant women, and also its longer treatment time makes it less suitable with MDA strategy (Hoerauf 2008). There are no effective preventive measures, and so the total control of this disease relies on drug treatments. Thus, this strongly necessitates for an alternative strategy of developing an effective microfilaricidal and macrofilaricidal agents.

The presence of *mf* with increased number of parasites and suppressed host immune response is one extreme of the disease, and the other extreme includes the absence of *mf* and few or no parasites with strong host immune response. Considering the dual presentation of the disease, an effective treatment strategy has to be designed to offer protective immunity to host, thus eliminating this debilitating disease.

Thus, all the signs and symptoms of the disease are an outcome of the interaction of parasites and host immune system. So, in order to offer a better protection strategy, knowledge on the cellular and molecular basis of the disease pathogenesis is indispensable. This review spotlights on the interaction of filarial parasite antigens and wolbachial antigens with the circulating host immune cells, and knowledge on this would help in deciphering the role of these individual antigens in filarial pathogenesis, thereby leading to the discovery of appropriate therapeutic intervention for this disease.

6.2 Filarial Antigens in Immunomodulation

Among several other human pathogens, filarial parasites have a long lifespan inside the human host, i.e., adult worms can live up to 7 years or more and their offspring microfilariae for up to 1 year in the bloodstream. This is possible mainly because of the various immune evasion strategies adopted by the parasites. Helminth parasites have large genomes (~10^8 bp) which are likely to encode a spectrum of products that block or divert the host immune response. The immune evasion genes have been identified using various approaches which include a database of expressed sequence tag (EST) sequences (exceeding 22,000) and in silico analysis of the filarial database. The rate of gene discovery in large parasite genomes is rapidly accelerating, due to the success of EST projects which perform large-scale sequencing of cDNAs from multiple life cycle stages (Williams et al. 2000).

6.2.1 Immune Response Elicited through Secretory Molecules of Filarial Nematodes

Few filarial gene products have been implicated as important components that influence host immunological profile, and it includes adult surface-associated protein, microfilarial surface, and secreted proteins, larval proteins, and cytokine/cytokine receptor homologs (Maizels et al. 2001a, b). Characterization of these proteins would have an impact on filarial research and further help to elucidate the pathways that lead to chronic disease from infection.

Host immune response to filarial parasites and their products triggers a complex network of both innate and adaptive immune cells, and the differential immune response in different individuals is based on their immune status. Therefore the filarial-infected individuals are placed in three groups. The first group includes a group of asymptomatic resistant individuals with protective Th1 immunity even upon exposure to infective larvae as a result of their residence in endemic area to lymphatic filariasis. Hence the name “endemic normals” (EN).Next in degree of severity are asymptomatic subjects with microfilaremia and are termed as “microfilaremics” (MF). Many individuals in this category have T lymphocytes which are specifically unresponsive to filarial antigens and exhibit Th2 immune response. The last group of individuals is “chronic patients” (CP), and they exhibit a range of symptoms like filarial fever, retrograde lymphangitis, lymphadenitis, and inflammation of the lymphatics of the male genitalia (epididymitis, thickening of the spermatic cords, transient hydrocele, or testicular swelling).They show a mixed immune response (Nanduri and Kazura 1989). Characterization of the immune response in filarial-infected individuals would favor the discovery of the pathway leading to filarial pathogenesis. Further, this acts as a reference to correlate filarial antigen-specific immune response and its association with disease progression.

Filarial parasites produce and release excretory-secretory (ES) products that have the potential to play a role in immune evasion and immune regulation. The proposed mechanisms of these putative ES-derived immune modulators, such as

proteases (Tamashiro et al. 1987), protease inhibitors (Lustigman et al. 1992; Yenbutr and Scott 1995), and antioxidant proteins (Cookson et al. 1992; Lu et al. 1998; Tang 1994), neutralize or interfere with the effector molecules of the innate and adaptive defense responses to maintain infection and parasite survival (Harnett and Parkhouse 1995). Since these parasites are long-lived within the host, the parasites modulate host immune reactions to allow their persistence within immunocompetent hosts.

Modulations in the levels of molecules related to immune response have been attributed as a cause of development of chronic disease in human filariasis. Elevated levels of serological indicators such as IgG4 and IgE antibodies are associated with lymphatic filarial infection. Different IgG subclass profiles of different filarial subjects correspond to the clinical severity of the infection (Dafa'alla et al. 1992). In addition, there is also a close association of antibody levels and type of immune response (Th1/Th2), and so determining antibody levels to filarial antigens at each stage of filarial infection is important to understand the nature of the immune response to filarial antigens.

Filarial antigens exhibited differential reactivity at antibody levels at different stages of infection. In a study by Joseph et al. (2012), pro-inflammatory molecular fraction, F6 identified from human filarial parasite *Brugia malayi*, induced *F6*-reactive IgG1, IgG2, and IgG3 in chronic symptomatic patients, but the most prominent increase was found in IgG2. This increased IgG2 could be attributed to F6 molecules that were accessible to host largely in the chronic stage of infection during which the dying or dead parasites also produce lymphatic pathology. Another important protein from all important parasitic stages, namely, Bm-iPGM, also demonstrated filarial-specific reactivity at IgG levels (Singh et al. 2014). Sahu et al. (2008) reported filaria-specific IgA to be an immunological window for the sex-related differences in susceptibility to infection observed in human filariasis. The antigen-specific changes in antibody levels could be used as a marker in filarial-specific diagnosis.

Filarial parasites besides changing antibody response also modulate host cellular response. Upregulation of Th1 cytokines (IFN-γ, TNF-α, GM-CSF, IL-1α, and IL-8) but not Th2 cytokines (IL-4, IL-5, IL-6, IL-10, and IL-13) to *Brugia malayi* larvae (L_3) suggests that the primary immune response to the live infective stage of the parasite (L_3) is not predominantly Th2 in nature but rather dominated by a pro-inflammatory response. Thus, live L_3 of filarial parasites induce early activation of Th1 cells in naive individuals and serve as the basis for understanding the pathogenesis of infection (Babu and Nutman 2003).This was further supported by a study where extracts of all the life stages of the parasite were shown to stimulate pro-inflammatory response (increased cytokines like IL-1β, IL-6, and TNF-α and high NO levels) and anti-inflammatory (IL-10) cytokines in both Raw (mouse monocytes cell line) and THP-1 (human monocyte cells) cells. Among all extracts, microfilariae (mf) elicited strong pro-inflammatory cytokine response followed by L3 and adult worms (Ad), and Ad was a strong stimulator of IL-10 release (Shiv kumar verma et al. 2011). In order to understand cellular immune response among filarial patients, a study by Mohanty et al. (2007) employed infected (MF) and uninfected (UN) individuals where a differential immune response with MFs shows a strong Th2 bias and

EN showing a Th1 bias upon stimulation of *B. malayi* adult low molecular weight IgG4-reactive antigens present in Fr4 and Fr5. Further to elucidate the molecular transcriptional events that control differential cytokine responses (Th1/Th2) among filarial patients, PBMCs from infected (INF) and uninfected (UN) were stimulated with *B. malayi* antigen (BmA). INF individuals were able to mount Th2 responses, not because of increased GATA-3 expression but indirectly through the downregulation of Th1-inducing SOCS-1, SOCS-5, and SOCS-7 genes (Babu et al. 2005a).

The T-cell and B-cell responses are regulated by activation of integrins and chemokine receptors. Recruitment of T cells and B cells to the site of infection, therefore, represents an important step in the pathogenesis of lymphedema. Therefore, expression of CXC and CC chemokine receptors was assessed on the surface of T cells and of B cells. An augmented expression of CCR9 and diminished expression of CXCR1 and CXCR3 by T and B cells were examined in chronic patients, and not in MF and UN individuals. These findings reveal a significant correlation between the expression of CCR9 and the grade of lymphedema in CP individuals. This highlights the possible participation of the chemokine network in the pathogenesis of lymphedema (Babu et al. 2005b). In addition to this, the filarial disease-specific cellular response was assessed upon BmA induction in filarial patients. Expression of the Th17 family of cytokines IL-17A, IL-17F, IL-21, and IL-23 along with Th1-type cytokines IFN-gamma and TNF-alpha was also significantly upregulated by BmA stimulation in lymphedema patients (Babu et al. 2009). Human CD4 (+) T-cell subpopulation from chronic lymphatic filariasis patients co-expressed IL-9 and IL-10, but not IL-4 (Anuradha et al. 2014). These observations are recorded in symptomatic and amicrofilaremics (CP) and hence mark an important role in the pathogenesis of the disease. To understand the natural history of filarial infection in early childhood, Achary et al. (2013) studied the influence of maternal filarial infection with the development of immunity in children. The study revealed the presence or absence of anti-sheath antibodies in association with cytokines that skewed the filarial-specific immunity to either Th1 or Th2 responses in neonates.

Platelets are the first cell to come into action upon antigen encounter, but there are not many reports on platelet interaction with filarial antigens. In this context, platelets were isolated from infected and uninfected patients, and its functions were assessed. BmA inhibited collagen-stimulated platelet aggregation by elevated NO production. Diminished NO accompanied by platelet aggregation in *E. coli*-exposed platelets and collagen-stimulated platelets without any antigen exposure suggests this effect to be filarial antigen-specific, and this effect is observed as a result of exposure to both mf and adult parasite antigens. Filarial parasite antigen can suppress platelet activation in vitro (Krushna et al. 2010). Hence the role of blood platelets needs to be investigated further in the immunopathogenesis of human lymphatic and this being pursued to characterize the recombinant filarial antigen-specific platelet responses.

Thus antigens from different stages of the parasite interacted with immune cells to produce a differential (Th1 /Th2) response, thus modulating the host immune status. This helps in the thorough understanding of the nature of the antigen based on their immune response and could be exploited for different purposes like diagnosis, vaccine design, etc.

6.2.2 Regulatory Cytokines

The role of suppressive cytokines such as IL-10 and TGF-β has been shown to involve in host immune regulation during filarial infections. Increased levels of IL-10, along with suppressed expression of Th1 (such as IFN-γ) and Th2 (such as IL-5) cytokines, were induced upon filarial antigen stimulation in asymptomatic, infected patients but not in invidiuals with chronic pathology (Mahanty et al. 1996; Nutman et al. 1987). Further blockade of IL-10 effect could partially reverse the impaired proliferation and Th1 differentiation of PBMC in infected individuals (King et al. 1992). Immunomodulation during filarial infections involves CTLA-4 and PD-1(programmed cell death 1) (Babu et al. 2006). Blocking of inflammatory bystander responses in infected patients involves CTLA-4 and PD-1 (King et al. 1993). A link between T_{regs} and hyporesponsive state was demonstrated by Babu et al. (2009) to show suppressive Th1 and Th2 cytokine production by regulatory T cells specifically from microfilaremic individuals. Further, the T_{reg} deficiency was limited to filarial-infected chronic pathology by showing suppressed Foxp3 expression by PBMC upon filarial antigen stimulation (King et al. 1993). In addition to this, MF individuals produced TGF-β and suppressed T-cell proliferation upon filarial antigen stimulation and thus evoke an anti-inflammatory response. Thus IL-10 and TGF-β plays a major role in contributing filarial infection-induced hyporesponsiveness.

6.2.3 Circulating Mediators Specific to Filarial Immune Response

Filarial antigens in blood circulation bind to antibodies and result in immune complexes. Circulating immune complexes (CICs) have been identified as a characteristic feature of human lymphatic filariasis. Asymptomatic, filarial-infected patients exhibited increased levels of CICs. Accumulated complexes activate complement pathway to induce an inflammatory response and contribute to disease progression. Functional efficiency of the classical and mannose-binding lectin pathways of the complement system was observed in filarial-infected individuals compared with chronic patients and EN. Increased pro-inflammatory cytokines and neutrophil granular proteins by polyethylene glycol-precipitated CICs from asymptomatic, infected individuals and chronic patients activated granulocytes (Senbagavalli et al. 2011). Binding C1q to a calcium-binding protein from *B. malayi* calreticulin (BmCRT, a constitutively expressed protein of *B. malayi)* blocks the complement activation of the host, and further enables the parasite establishment within the host (Yadav et al. 2014). Thus host complement system is being altered by different parasite molecules.

Levels of certain mediators such as acute-phase proteins and microbial translocation products possess clinical significance in inflammatory diseases. Hence assessment of circulating microbial translocation products (lipopolysaccharide and LPS-binding protein) and acute-phase proteins (haptoglobin and serum amyloid protein-A) together with inflammatory cytokines (IL-1β, IL-12, and TNF-α) in plasma of filarial-infected individuals would help to identify dysregulated host

inflammatory tissue damage (Anuradha et al. 2012). Tissue damage correlates with the levels of MMPs (matrix metalloproteinases) and TIMPs (tissue inhibitors of matrix metalloproteinases), and their ratio associated with tissue fibrosis is one of the clinical symptoms observed in chronic patients. Increased ratios of MMP1/TIMP4 and MMP8/TIMP4 along with increased pro-fibrotic cytokine (IL-5, IL-13, and TGF-β) levels are indicators of filarial lymphatic pathology (Anuradha et al. 2012).

Plasma endothelin-1 (ET-1) and TNF receptor (type I and tII) levels were observed to be higher in hydrocele/elephantiasis patients when compared to other groups and suggested a role for ET-1 in the development of hydrocele and elephantiasis. The higher frequency of ET-1 (Ala288Ala) genotype was identified as a contributing factor in the pathogenesis of hydrocele by facilitating the accumulation of plasma proteins and fluid in the tunica vaginalis, and higher frequency of the Met196Met genotype of TNFR-II observed in elephantiasis patients could be a predisposing factor for the development of elephantiasis (Das et al. 1996; Satapathy et al. 2006).

Assessment of these circulating molecules aided in identifying their role in filarial pathogenesis and thus could serve as a prognostic marker for filarial-driven morbidity.

6.2.4 Human Lymphatic Filarial Protease Inhibitors

Pathogens produce homologs of host molecules that are involved in immune signaling and diverting the host immune response. Two of the most extensively characterized parasite-derived cytokine homologs are *B. malayi* MIF-1 and *B. malayi* MIF-2. They are macrophage migration inhibitory factor (MIF) exhibiting significant homology to a human cytokine that functions to alter the behavior of human cells (Pastrana et al. 1998; Zang et al. 2002). It was shown to alter the levels of certain cytokines produced by antigen-presenting cells, creating an immunological environment that supports parasite survival. Another potential reason for manipulation of the cytokine profile may be to obtain host-derived factors necessary for parasite development. In addition to MIF, *Brugia* also encodes ligands from TGF-β superfamily, i.e., Bm-tgh-1 and Bm-tgh-2. These homologs possess 28–42% amino acid identity with human proteins. Besides ligands of TGF-β superfamily, helminths also express type-1 TGF-β receptors (Gomez-Escobar et al. 1997).

A class of conserved molecules that play a vital role in immune modulation include protease inhibitors. The best-characterized protease inhibitors of helminths are cystatins and serpins. *Brugia malayi* cystatin (Bm-CPI-2) intrudes the host antigen processing and presentation processes (Klotz et al. 2011) and thus dampens the antigen-dependent immune reactions. Besides, *B. malayi* microfilariae produce a serpin (Bm-SPN-2) that inhibits the proteases released by the neutrophils and thus escapes the host's first line of defense (Manoury et al. 2001). Bm33, a surface protein of the parasite *B. malayi*, is constitutively expressed by the parasite and was characterized as Bmaspin. Further, Bmaspin was classed to inhibit an array of aspartic proteases such as pepsin, renin, cathepsin D, and cathepsin E (Nagampalli et al. 2014). The antigenic nature of this protein was also assessed by in vitro studies. Recombinant Bm33 was produced by Krushna et al. (2009) using bacterial expression system, and

in vitro studies using PBMCs from infected (MF, CP) and uninfected (EN) yielded interesting results. rBm33 induced predominant IgG4 response high in MF compared to CP and EN, and this correlated with the rBm33-induced unsustained CD4+ T-cell activation in filarial patients (MF) suggesting the contribution of rBm33 to the filarial pathogenesis. Further, to investigate rBm33-specific cellular response, an in vitro study using THP-1 cells was initiated by Sreenivas et al. (2012), and it resulted in rBm33-specific enhancement of monocyte activation by enhanced phagocytosis and increased pro-inflammatory cytokine expression. Subsequently, human PBMCs were used to identify the role of rBm33 in apoptosis. The rBm33 did not induce apoptosis of human lymphocytes and monocytes. However, the exact molecular mechanism underlying rBm33-induced immune response is not known yet, and hence studies are underway to know the mechanism of rBm33-mediated inflammatory response.

Filarial nematodes spend maximum lifespan within the host compared to any other parasites. This opens up an opportunity for filarial parasites or its derived products to interact with a variety of host immune cells. Filarial nematodes along with their endosymbiont *Wolbachia* secrete a bunch of molecules that cause immunomodulation in the host. They further downregulate the activity of host cells, thus rendering them hyporesponsive. This state of hyporesponsiveness might lead to the development of the chronic stage upon continuous antigenic exposure. Among the important issues relating to parasite-host interactions, there is a lacuna in apprehending the mechanisms leading to induction and maintenance of the immune response that accommodates chronic, long-term filarial infections. Our understanding of the mechanisms would help to design a molecule that can target the major pathway responsible for disease progression.

6.3 Mechanisms Underlying Filarial Antigen–Induced Immune Response

The host inflammatory responses are seen as a consequence of parasites and their secreted antigens (Ags), *Wolbachia*, and secondary bacterial or fungal infections (Figueredo-Silva et al. 2002; Taylor et al. 2005). The molecular signaling mechanisms underlying could be mediated in multiple ways. A wide range of molecular patterns expressed on pathogens, commonly known as pathogen-associated molecular patterns (PAMPs), is recognized first by innate immune cells to elicit an immune response. It includes the following.

6.3.1 Toll-Like Receptors (TLRs)

6.3.1.1 TLRs and Immune Responses

TLRs are important initiators of immune responses through their ability to recognize a variety of microbial products, but if they are unregulated, TLR-dependent pro-inflammatory cascades can also escalate to cause severe pathology within the host. The involvement of ten pathogen recognition receptors (PRRs) specifically

TLRs in immune defense mechanisms has enriched our understanding of the connection between innate and adaptive immune response. Upon TLR activation by their cognate ligands, an innate immune response is triggered via gene transcription of NF-κB leading to the production of pro-inflammatory cytokines and chemokines which ultimately cause an adaptive response.

TLR2, TLR4, and TLR6 have been identified to primarily mediate *Wolbachia*-induced inflammatory response. Further, downregulation of TLRs expression and function on monocytes, B and T cells was identified as one of the key mechanisms in patent filarial infections (Babu et al. 2005a). TLR ligands triggered various pathways that contribute to chronic infection. Increased activation of the NF-κB pathway in chronic individuals was routed via TLR2 and TLR9 ligands involving ERK1/2 and p38 MAP kinase activation. Similarly, TLR2 and 5 ligands induced the excessive production of VEGF-A (vascular endothelial growth factor-A) and Ang-1(angiopoietin-1) in the peripheral blood mononuclear cells of individuals with chronic pathology compared with those of asymptomatic, infected individuals. Thus, it has been identified that TLR2 ligand mediates angiogenic growth factor production through MAPK and NF-κB signaling in chronic pathology patients (Babu et al. 2011).

Lymphatic filariasis with chronic pathology is characterized by TLR-dependent pro-inflammatory cytokine production and suppressed the immune response. Investigations have shown that filarial antigens contribute to suppression of normal T-cell proliferation through induction of apoptosis of human monocytes and are mediated through TLR4 (Das et al. 2014). Further, the interaction of human monocytes with *Wolbachia* HSP60 triggered the TNF receptor pathway via NF-κB resulting in increased IL-6 and TNF-α level. Recently, microfilarial protein (MfP) from *W. bancrofti* was identified as the new ligand of TLR4 triggering NF-κB activation resulting in the secretion of pro-inflammatory cytokines (Mukherjee et al. 2017). We had reported for the first time that in addition to apoptosis, recombinant *Wolbachia* HSP60 antigen also induces molecular senescence in human monocytes (Kamalakannan et al. 2015). TLR engagement and activation might also be responsible for many other immune features that are characteristic of parasitic infections including polyclonal lymphocyte activation and autoantibody formation.

6.3.1.2 APCs and TLRs

Monocyte dysfunction observed in patent filarial infection is identified as one of the most important components in the immune network that suppresses the Th1 type of response to filarial antigens, thereby creating a suppressive environment for the parasite to thrive (Sasisekhar et al. 2005). Also, we have shown that *Wolbachia* antigens (HSP60 and WSP) induced apoptosis in monocytes (Kamalakannan et al. 2012; Kalyanaraman et al. 2014). Further, Shiny et al. (2012) have shown that *Wolbachia* HSP60-induced expression of CTLA4 and CD25 together with elevated IL-10 and TGF-β in patients indicates that *Wolbachia* HSP60 modulated the immune responses by reducing T-cell activation in filarial patients. Furthermore, *Wolbachia* surface protein (WSP) that readily comes in contact with the host immune system upon its release from filarial worm had also been shown to suppress the T-cell activation in filarial patients (Shiny et al. 2012). Antigen-presenting cells also express

other receptors in addition to TLRs during filarial infection. Specifically, increased expression of receptors such as MRC-1 (mannose receptor C-type 1), MGL (macrophage galactose-type C-lectin), and CCL18 (chemokine ligand 18) with altered levels of Nos2, Arg-1, and resistin was observed in monocytes derived from asymptomatic filarial patients (Babu et al. 2009).

Similarly, the status of other effector cells during filarial infection has been reported. Upon exposure to filarial parasites, NK cell numbers decrease, and this operates through caspase-dependent apoptosis mechanism and type 1 and type 2 cytokine secretions. NK cells arbitrate to the outcome of filarial infection by altering the milieu of T-cell subset differentiation pathways (Babu et al. 2007). Platelets are another effector cells in inflammation and least investigated with respect to lymphatic filarial infections. Platelets act as a bridge between innate and adaptive immune response, and a detailed understanding on filarial and wolbachial antigen-induced platelet function would provide interesting insights on this disease. For the first time, a study on platelet response upon *Wolbachia* antigen exposure showed activation of platelets by rWSP and was mediated through TLRs to elicit an inflammatory response in asymptomatic, amicrofilaremic individuals (Haripriya et al. 2017 Unpublished).

An effector cell such as mast cells was also modulated during filarial infection. A filarial antigen such as ES-62 forms complex with TLR4 to block the key signal transduction events and NF-κB activation to cause FcγRI inhibition (Goodridge et al. 2005). These data provide insight into the central role TLR dysregulation plays in APC function and suggest strategies for overcoming this dysregulation. Compromised TLR expression and function on APCs could certainly explain the diminished immune responses, and this state indeed makes the host vulnerable to infection.

6.3.2 Nod–Like Receptors (NLRs)

There are intracellular PRRs such as Nod1, Nod2, and NALP3. These are grouped as NLRs and are known to be involved in the NF-κB, caspase-dependent pathway activation, and regulation of inflammatory responses (Inohara and Nunez 2003). Filarial crude antigen, namely, BmA, significantly induced the expression of Nod1 and Nod2 but not NALP3.Increased gene expression of Nod1 and Nod 2 in an antigen-specific manner in CP patients emphasizes their role in inflammatory response-associated tissue damage in lymphatics. Altogether, NLRs appear to play a crucial role in skewing the cytokine profile in lymphatic pathology (Babu et al. 2009). However, further studies would address the correlation of NLR expression and lymphatic pathology.

6.3.3 C–Type Lectin Receptors (CLRs)

Helminth glycans were identified as the conserved molecular pattern for *Brugia malayi*, as well as the free-living nematode *Caenorhabditis elegans* antigens induced a Th2 response in vivo (Figueiredo et al. 2010). Predominantly DCs were shown to

signal through CLRs to drive Th2-biased immune responses. There are evidences indicating the involvement of other PRRs for filarial infections which could extend beyond TLR family to include CLRs (van Liempt et al. 2007).

6.4 Filarial Antigens in Diagnosis

The ultimate result of understanding the immunopathogenesis of filarial infections and characterization of filarial antigens is to develop a suitable therapeutic intervention for lymphatic filariasis. First and foremost is the diagnosis. Filarial patients mostly remain asymptomatic in the acute stage, and this makes the diagnosis the need of the hour. Traditional methods of diagnosis such as blood smears, serologic tests, and DNA PCR were not useful in case of low microfilaremia infection. Other clinical tests include detection of high IgE levels and lymphoscintigraphy. The adult worm could be detected in fluids drawn from swollen areas or serous collections. X-ray tests can show calcified adult worms in lymphatics; ultrasonography identifies the "filarial dance" (Hotez et al. 2007; Lahariya and Tomar 2011; Molyneux and Zagaria 2002. Early diagnosis of filarial infections was made based on sero-markers (IgM to initially IgG1 and IgG2, which changes to IgG4) (Adjobimey and Hoerauf 2010). Serological tests are the tests of choice for early markers of infection, but would not be suitable for individuals who are asymptomatic. An overview of various methods developed for filarial diagnosis is presented below (Table 6.1):

Though there are lots of developments in the diagnostic approach and several drug combinations that are effective in controlling the filarial infection, nevertheless there is a need for a vaccine to control and eradicate this disease completely. The pursuit of the anti-filarial vaccine is complicated by the phenomenon of immunosuppression, immunologically induced pathology, and strict primate host specificity of target human filarids *W. bancrofti* and *B. malayi*. Targeted vaccine approaches might help in blocking parasite morphogenesis, migration, and reproduction or in targeting an enzyme highly essential for the physiological function of the parasite.

However, characterized recombinant filarial antigens offer several advantages in terms of filarial research. It accelerated the diagnosis process and further provided avenues for filarial vaccine development. Screening a suitable vaccine candidate among an array of filarial and *Wolbachia* proteins from various stages of the parasites is made possible only by recombinant filarial antigens.

6.5 Conclusion

Human lymphatic filarial research has several setbacks like low availability of parasite material and problems that may arise with the use of crude antigen for diagnosis and vaccination purposes. But, with the advent of genetic engineering approaches, it has been possible to produce well-characterized recombinant proteins of the filarial parasite. An interesting feature of the filarial parasites is the presence of an endosymbiont *Wolbachia*. Antigens from *Wolbachia* were also produced and

Table 6.1 Various approaches for filarial diagnosis

S. no.	Antibody-based diagnosis	Antigens used	Sensitivity	Pros/Cons	References
1	*Brugia* rapid immunochromatography –Elisa and in the form of "dip stick"	BmR1	100% for *Brugia malayi*	Use of recombinant antigens/antibodies helped to overcome the problem of parasite material scarcity	Rahmah et al. (2003), Raharnan et al. (2007) Lammie et al. (2004), Weil et al. (2011) Alli et al. (2001), Moss et al. (2011), Chanteau et al. (1994), Harinath (1984), Harinath and Reddy (1997) Jawaharlal et al. (2013), Pandey et al. (2011), and Weil et al. (2011) Pandiaraja et al. (2010), Madhumati et al. (2010), Noordin et al. (2007) Itoh et al. (1998), Bhunia et al. (2002), Weil et al. (1997), Simonsen et al. (2004), Weil et al. (2013), Ansel Vishal et al. (2014)
		Bm14	91–96% for *Wuchereria bancrofti and Brugia malayi*		
		WbSXP	91% for *Wuchereria bancrofti*		
		BmSXP	95% sensitive for *Wuchereria bancrofti*		
2	WB rapid	WbSXP and BmSXP	96–99% for *Wuchereria bancrofti*		
3	Test for pan filariasis	BmRl and BmSXP	98% for Brugian sp.		
	Clinical testing	*Detection*	*Sensitivity*	*Pros/cons*	
1	Seva FilaChek	Immune complexed antigen, filarial-specific IgG4 antibody detection	80% for antibody detection and 88% for antigen detection	Applicable only for microfilariae-negative cases; difficulty in distinguishing past and present infection	
2	"OnSite" filariasis IgG/IgM rapid test, Bm14 and Bm33 from *Brugia malayi*	IgG and IgtM levels	*Wuchereria bancrofti* and *Brugia malayi*		
	Antigen-based diagnosis	*Detection*	*Sensitivity*	*Pros/cons*	
1	Og4C3-ELISA	Detects filarial-specific antibodies in day blood sample	*Wuchereria bancrofti*	Quantitative and definitive results	
2	Circulating filarial antigen detection test (CFA)/immunochromatographic test (ICT) – Dip-stick assay microscopic slide-based assay			Qualitative and indeterminate results	
3	Detection using mf stage antigens (rWbShp-1, Bml4 and rWbSXP-1)			Stage-specific detection	
4	Alere filariasis test strip			Sensitive for field survey	
5	RT PCR using DNA probes	DNA from blood sample		Not cost-effective	

characterized for immunological investigations. These filarial and *Wolbachia* derived antigens have been utilized to understand the in vitro response of immune cells like T cells, macrophages and platelets. However, an understanding of the immunopathologic and protective role of B- and T-cell responses to specific parasite antigens is critical for the development of a vaccine to control or eradicate the infection in endemic areas. Further, characterization of recombinant filarial antigens and deciphering their immunomodulatory role could also provide new avenues to treat other allergic and inflammatory diseases.

References

Achary KG, Mandal NN, Mishra S, Sarangi SS, Kar SK, Satapathy AK, Bal MS (2013) Maternal filarial infection: association of anti-sheath antibody responses with plasma levels of IFN-γ and IL-10. Parasitology 140:598–503

Adjobimey T, Hoerauf A (2010) Induction of immunoglobulin G4 in human Filariasis: an Indicator of Immunoregulation. Ann Trop Med Parasitol 104:55–64

Agrawal V, Sashindran V (2006) Lymphatic Filariasis in India : problems, challenges and new initiatives. Med J Armed Forces India 62:359–362

Alli R, Kulkarni S, Reddy MVR, Harinath BC (2001) Evaluation of SEVAFILACHEK immunoassays and rapid ICT-Filariasis: test for detection of Bancroftian Filariasis. Ind J Clin Biochem 16:207–210

Ansel Vishal L, Nazeer Y, Ravishankaran R, Mahalakshmi N, Kaliraj P (2014) Evaluation of rapid blood sample collection in the detection of circulating filarial antigens for epidemiological survey by rWbSXP-1 capture assay. PLoS One 9(7):e102260

Anuradha R, George PJ, Pavan Kumar N, Fay MP, Kumaraswami V, Nutman TB, Babu S (2012) Circulating microbial products and acute phase proteins as markers of pathogenesis in lymphatic filarial disease. PLoS Pathog 8:e1002749

Anuradha R, George PJ, Chandrasekaran V (2014) Interleukin 1 (IL-1) and IL-23-mediated expansion of filarial antigen-specific Th17 and Th22 cells in filarial lymphedema. Clin Vaccine Immunol 21:960–965

Babu S, Nutman TB (2003) Proinflammatory cytokines dominate the early immune response to filarial parasites. J Immunol 171:6723–6732

Babu S, Blauvelt CP, Kumaraswami V, Nutman TB (2005a) Diminished expression and function of TLR in lymphatic filariasis: a novel mechanism of immune dysregulation. J Immunol 175:1170–1176

Babu S, Kumaraswami V, Nutman TB (2005b) Transcriptional control of impaired Th1 responses in patent lymphatic filariasis by T-box expressed in T cells and suppressor of cytokine signaling genes. Infect Immun 73:3394–3401

Babu S, Blauvelt CP, Kumaraswami V, Nutman TB (2006) Diminished T cell TLR expression and function modulates the immune response in human filarial infection. J Immunol 176:3885–3889

Babu S, Blauvelt CP, Nutman TB (2007) Filarial parasites induce NK cell activation, type 1 and type 2 cytokine secretion, and subsequent apoptotic cell death. J Immunol 179:2445–2456

Babu S, Bhat SQ, Pavan Kumar N, Lipira AB, Kumar S, Karthik C, Kumaraswami V, Nutman TB (2009) Filarial lymphedema is characterized by antigen-specific Th1 and Th17 proinflammatory responses and a lack of regulatory T cells. PLoS Negl Trop Dis 3:e420

Babu S, Anuradha R, Kumar NP, George PJ, Kumaraswami V, Nutman TB (2011) Filarial lymphatic pathology reflects augmented toll-like receptor-mediated, mitogen-activated protein kinase-mediated Proinflammatory cytokine production. Infect Immun 79(11):4600–4608

Bhunia B, Kulkarni S, Reddy MVR, Harinath BC (2002) Diagnostic evaluation of circulating filarial antigen assay using sera samples with and without acid-heat treatment. Biomed Res 13(1):39–42

Chanteau S, Glaziou P, Moulia-Pelat JP, Plichart C, Luquiaud P, Cartel JL (1994) Low positive predictive value of anti-Brugia malayi IgG and IgG4 serology for the diagnosis of *Wuchereria Bancrofti*. Trans R Soc Trop Med Hyg 88:661–662

Cookson E, Blaxter ML, Selkirk ME (1992) Identification of the major soluble cuticular protein of lymphatic filarial nematode parasites (gp29) as a secretory homolog of glutathione peroxidase. Proc Natl Acad Sci USA 89:5837–5841

Dafa'alla TH, Ghalib HW, Abdelmageed A, Williams JF (1992) The profile of IgG and IgG subclasses of onchocerciasis patients. Clin Exp Immunol 88:258–263

Das BK, Sahoo PK, Ravindran B (1996) A role for tumour necrosis factor-alpha in acute lymphatic filariasis. Parasite Immunol 18:421–424

Das MA, Panda SK, Pradhan AK, Prusty BK, Satapathy AK, Ravindran B (2014) Filarial antigens mediate apoptosis of human monocytes through toll-like receptor 4. J Infect Dis 210:1133–1144

Figueiredo CA, Barreto ML, Rodrigues LC, Cooper PJ, Silva NB, Amorim LD, Alcantara-Neves NM (2010) Chronic intestinal helminth infections are associated with immune hyporesponsiveness and induction of a regulatory network. Infect Immun 78:3160–3167

Figueredo-Silva J, Noroes J, Cedenho A, Dreyer G (2002) The histopathology of bancroftian filariasis revisited: the role of the adult worm in the lymphatic-vessel disease. Ann Trop Med Parasitol 96:531–541

Gomez-Escobar N, van den Biggelaar A, Maizels R (1997) A member of the TGF-β receptor gene family in the parasitic nematode Brugia. Gene 199:101–109

Goodridge HS, Marshall FA, Else KJ, Houston KM, Egan C, Al-Riyami L, Liew FY, Harnett W, Harnett MM (2005) Immunomodulation via novel use of TLR4 by the filarial nematode phosphorylcholine containing secreted product ES-62. J Immunol 174:284–293

Harinath BC (1984) Immunodiagnosis of bancroftian filariasis– problems and progress. J Biosci 6:691–699

Harinath BC, Reddy MVR (1997) Diagnosis and immunomonitoring in successful management of bancroftian filariasis. J Parasit Dis 21:41–51

Harnett W, Parkhouse RME (1995) The nature and function of parasitic nematode surface and excretory-secretory antigens. In: Perspectives in nematode physiology and biochemistry, pp 207–242

Hoerauf (2008) Filariasis: new drugs and new opportunities for lymphatic filariasis and onchocerciasis. Curr Opin Infect Dis 21:673–681

Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Sachs SE, Sachs JD, Savioli L (2007) Control of neglected tropical diseases. N Engl J Med 357:1018–1027

Inohara N, Nunez G (2003) NODs: intracellular proteins involved in inflammation and apoptosis. Nat Rev Immunol 3:371–382

Itoh M, Gunawardena N, Xu-Guang Q, Weerasooriya M, Kimura E (1998) The use of whole blood absorbed on filter paper to detect *Wuchereria bancrofti* circulating antigen. Trans R Soc Trop Med Hyg 92:513–515

Jawaharlal JP, Ravishankaran R, Shridharan RN, Lawrence AV, Karande AA, Perumal K (2013) Evaluation of Brugia malayi sheath protein (Shp-1) as a diagnostic antigen for human lymphatic filariasis. Diagn Microbiol Infect Dis 78:249–254

Joseph SK, Verma SK, Sahoo MK, Sharma A, Srivastava M, Reddy MVR, Murthy PK (2012) IgG subclass responses to a proinflammatory fraction of *Brugia malayi* in human filariasis. Indian J Med Res 135:650–655

Kalyanaraman H, Vijayan K, Sreenivas K, Babu S, Narayanan RB (2014) Recombinant Wolbachia surface protein from Brugia Malayi modulates human monocyte functions in vitro. Int Trends Immun 2:53–61

Kamalakannan V, Kirthika S, Haripriya K, Babu S, Narayanan RB (2012) *Wolbachia* heat shock protein 60 induces proinflammatory cytokines and apoptosis in monocytes in vitro. Microbes Infect 14:610–618

Kamalakannan V, Shiny A, Babu S, Narayanan RB (2015) Autophagy protects monocytes from Wolbachia heat shock protein 60–induced apoptosis and senescence. PLoS Negl Trop Dis 9(4):e0003675

King CL, Kumaraswami V, Poindexter RW, Kumari S, Jayaraman K, Alling DW, Ottesen EA, Nutman TB (1992) Immunologic tolerance in lymphatic filariasis. Diminished parasite-specific T and B lymphocyte precursor frequency in the microfilaremic state. J Clin Invest 89:1403–1410

King CL, Mahanty S, Kumaraswami V, Abrams JS, Regunathan J, Jayaraman K, Ottesen EA, Nutman TB (1993) Cytokine control of parasite-specific anergy in human lymphatic filariasis. Preferential induction of a regulatory T-helper type 2 lymphocyte subset. J Clin Invest 92:1667–1673

Klotz C, Ziegler T, Danilowicz-Luebert E, Hartmann S (2011) Cystatins of parasitic organisms. Adv Exp Med Biol 712:208–221

Krushna NS, Shiny C, Dharanya S, Sindhu A, Aishwarya S, Narayanan RB (2009) Immunolocalization and serum antibody responses to *Brugia malayi* pepsin inhibitor homolog (Bm-33). Microbiol Immunol 53:173–183

Krushna NS, Shiny C, Verma P, Nithya D, Basker P, Elango S, Babu S, Narayanan RB (2010) *Wuchereria bancrofti*: diminished platelet activation in filarial patients. Exp Parasitol 125:114–123

Kulkarni P, Kumar R, Rajegowda RM, Channabasappa HG, Ashok NC (2014) MDA program against lymphatic filariasis: are we on the path to success? Experience from Uttara Kannada District. Karnataka Int J Med Public Health 4:243–246

Lahariya C, Tomar SS (2011) How endemic countries can accelerate lymphatic Filariasis elimination? An analytic review to identify strategic and programmatic interventions. J Vector Borne Dis 48:1–6

Lammie PJ, Weil G, Noordin R, Kaliraj P, Steel C, Goodman D, Lakshmikanthan VB, Ottesen E (2004) Recombinant antigen-based antibody assays for the diagnosis and surveillance of lymphatic filariasis – a multicentre trial. Filaria J 3:9

Lu W, Egerton GL, Bianco AE, Williams SA (1998) Thioredoxin peroxidase from Onchocerca volvulus: a major hydrogen peroxide detoxifying enzyme in filarial parasites. Mol Biochem Parasitol 91:221–235

Lustigman S, Brotman B, Huima T, Prince AM, McKerrow JH (1992) Molecular cloning and characterization of onchocystatin, a cysteine protease inhibitor of *Onchocerca volvulus*. J Biol Chem 267:17339–17346

Madhumati J, Pradiba D, Prince PR, Jeyaprita PJ, Rao DN, Kaliraj P (2010) Crucial epitopes of Wuchereria bancrofti abundant larval transcript recognized in natural infections. Eur J Clin Microbiol Infect Dis 29:1481–1486

Mahanty S, Mollis SN, Ravichandran M, Abrams JS, Kumaraswami V, Jayaraman K, Ottesen EA, Nutman TB (1996) High levels of spontaneous and parasite antigen-driven interleukin-10 production are associated with antigen-specific hyporesponsiveness in human lymphatic filariasis. J Infect Dis 173:769–773

Maizels RM, Blaxter ML, Scott AL (2001a) Immunological genomics of *Brugia malayi:* filarial genes implicated in immune evasion and protective immunity. Parasite Immunol 23:27–44

Maizels RM, Gomez-Escobar N, Gregory WF, Murray J, Zang X (2001b) Immune evasion genes from filarial nematodes. Int J Parasitol 31:889–898

Manoury B, Gregory WF, Maizels RM, Watts C (2001) Bm-CPI-2, a cystatin homolog secreted by the filarial parasite Brugia malayi, inhibits class II MHC restricted antigen processing. Curr Biol 11:447–451

Mohanty BP, Lahari R, Bhattacharya S, Kar S (2007) Brugia malayi adult low molecular weight IgG4-reactive antigens induce differential cytokine response in lymphocyte of endemic normal and asymptomatic microfilariae carriers in vitro. J Clin Immunol 27:397–308

Molyneux DH, Zagaria N (2002) Lymphatic filariasis elimination: progress in global program development. Ann Trop Med Parasitol 96:S15–S40

Moss DM, Priest JW, Boyd A, Weinkopff T, Kucerova Z, Beach MJ, Lammie PJ (2011) Multiplex bead assay for serum samples from children in Haiti enrolled in a drug study for the treatment of lymphatic Filariasis. Am J Trop Med Hyg 85:229–237

Mukherjee S, Mukherjee S, Maiti TK, Bhattacharya S, Sinha Babu SP (2017) A novel ligand of toll- like receptor 4 from the sheath of Wuchereria bancrofti microfilaria induces a proinflammatory response in macrophages. J Infect Dis 215:954–965

Nagampalli RSK, Gunasekaran K, Narayanan RB, Peters A, Bhaskaran R (2014) A structural biology approach to understand human lymphatic filarial infection. PLoS Negl Trop Dis 8(2):e2662

Nanduri J, Kazura JW (1989) Clinical and laboratory aspects of filariasis. Clin Microbiol Rev 2:39

Noordin R, Itoh M, Kimura E, Abdul Rahman R, Ravindran B, Mahmud R, Supali T, Weerasooriya M (2007) Multicentre evaluation of two new rapid IgG4 tests (WB rapid and pan LF rapid) for detection of lymphatic Filariasis. Filaria J 6:9

Nutman TB, Kumaraswami V, Pao L, Narayanan PR, Ottesen EA (1987) An analysis of in vitro B cell immune responsiveness in human lymphatic filariasis. J Immunol 138:3954–3959

Pandey V, Madhumathi J, Karande AA, Kaliraj P (2011) Antigen detection assay with parasite specific monoclonal antibodies for diagnosis of lymphatic filariasis. Clin Chim Acta 412:1867–1873

Pandiaraja P, Arunkumar C, Hoti SL, Rao DN, Kaliraj P (2010) Evaluation of synthetic peptides of WbSXP-1 for the diagnosis of human lymphatic Filariasis. Diagn Microbiol Infect Dis 68:410–415

Pastrana DV, Raghavan N, FitzGerald P, Eisinger SW, Metz C, Bucala R, Schleimer RP, Bickel C, Scott AL (1998) Filarial nematode parasites secrete a homologue of the human cytokine macrophage migration inhibitory factor. Infect Immun 66:5955–5963

Rahaman RA, Hwen-Yee C, Noordin R (2007) Pan LF-ELISA using BmR1 and BmSXP recombinant antigens for detection of lymphatic Filariasis. Filaria J 6:10

Rahmah N, Shenoy RK, Nutman TB, Weiss N, Gilmour K, Maizels RM, Yazdanbakhsh M, Sartono E (2003) Multicentre laboratory evaluation of Brugia rapid dipstick test for detection of Brugian Filariasis. Tropical Med Int Health 8:895–900

Sabesan S, Vanamail P, Raju K, Jambulingam P (2010) Lymphatic filariasis in India: epidemiology and control measures. J Postgrad Med 56:232–238

Sahu BR, Mohanty MC, Sahoo PK, Satapathy AK, Ravindran B (2008) Protective immunity in human filariasis: a role for parasite-specific IgA responses. J Infect Dis 198:434–443

Sasisekhar B, Aparna M, Augustin DJ, Kaliraj P, Kar SK, Nutman TB, Narayanan RB (2005) Diminished monocyte function in microfilaremic patients with lymphatic filariasis and its relationship to altered lymphoproliferative responses. Infect Immun 73:3385–3393

Satapathy AK, Sartono E, Sahoo PK, Dentener MA, Michael E, Yazdanbakhsh M, Ravindran B (2006) Human bancroftian filariasis: immunological markers of morbidity and infection. Microbes Infect 8:2414–2423

Senbagavalli P, Anuradha R, Ramanathan VD, Kumaraswami V, Nutman TB, Babu S (2011) Heightened measures of immune complex and complement function and immune complex-mediated granulocyte activation in human lymphatic filariasis. Am J Trop Med Hyg 85:89–96

Shiny C, Krushna NS, Haripriya K, Babu S, Elango S, Manokaran G, Narayanan RB (2012) Recombinant Wolbachia surface protein (WSP)-induced T cell responses in *Wuchereria bancrofti* infections. Parasitolres 110:787–797

Simonsen PE, Magesa SM, Dunyo SK, Malecela-Lazaro MN, Michael E (2004) The effect of single dose ivermectin alone or in combination with albendazole on *Wuchereria bancrofti* infection in primary school children in Tanzania. Trans R Soc Trop Med Hyg 98:462–472

Singh PK, Kushwaha S, Rana AK, Misra-Bhattacharya S (2014) Cofactor independent phosphoglycerate mutase of Brugia malayi induces a mixed Th1/Th2 type immune response and inhibits larval development in the host. Biomed Res Int 2014:1. https://doi.org/10.1155/2014/590281

Sreenivas K, Vijayan K, Babu S, Narayanan RB (2012) Recombinant *Brugia malayi* pepsin inhibitor (rBm33) induced monocyte function and absence of apoptotic cell death: an *in vitro* study. Microb Pathog 53:19–27

Tamashiro WK, Rao M, Scott AL (1987) Proteolytic cleavage of IgG and other protein substrates by *Dirofilaria immitis* microfilarial enzymes. J Parasitol 73:149–154

Tang L, Ou X, Henkle-Dührsen K, Selkirk ME (1994) Extracellular and cytoplasmic CuZn superoxide dismutases from Brugia lymphatic filarial nematode parasites. Infect Immun 62:961–967

Taylor MD, LeGoff L, Harris A, Malone E, Allen JE, Maizels RM (2005) Removal of regulatory T cell activity reverses hyporesponsiveness and leads to filarial parasite clearance in vivo. J Immunol 174:4924–4933

Van Liempt E, van Vliet SJ, Engering A, García Vallejo JJ, Bank CM, Sanchez-Hernandez M, van Kooyk Y, van Die I (2007) Schistosoma mansoni soluble egg antigens are internalized by human dendritic cells through multiple C-type lectins and suppress TLR-induced dendritic cell activation. Mol Immunol 44:2605–2615

Verma SK, Kushwaha V, Dubey V, Saxena K, Sharma A, Murthy PK (2011) Inflammatory mediator release by *Brugia malayi* from macrophages of susceptible host *Mastomys coucha* and THP-1 and RAW 264.7 cell lines. Asian Pac J Trop Med 4:92–96

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

Weil GJ, Curtis KC, Fischer PU, Won KY, Lammie PJ, Joseph H, Melrose WD, Brattig NW (2011) A multicenter evaluation of a new antibody test kit for lymphatic filariasis employing recombinant Brugia malayi antigen Bm-14. Acta Trop 120(Suppl 1):S19–S22

Weil GJ, Curtis KC, Fakoli L, Fischer K, Gankpala L, Lammie PJ, Majewski AC, Pelletreau S, Won KY, Bolay FK, Fischer PU (2013) Laboratory and field evaluation of a new rapid test for detecting *Wuchereria bancrofti* antigen in human blood. Am J Trop Med Hyg 89:11–15

Williams SA, Lizotte-Waniewski MR, Foster J, Guiliano D, Daub J, Scott AL, Slatko B, Blaxter ML (2000) The filarial genome project: analysis of the nuclear, mitochondrial and endosymbiont genomes of *Brugia malayi*. Int J Parasitol 30:411–419

Witt C, Ottesen EA (2001) Lymphatic filariasis: an infection of childhood. Tropical Med Int Health 6(8):582–606

Yadav S, Gupta S, Selvaraj C, Doharey PK, Verma A, Singh SK, Saxena JK (2014) *In Silico* and *In Vitro* studies on the protein-protein interactions between *Brugia malayi* immunomodulatory protein Calreticulin and human C1q. PLoS One 9:e106413

Yenbutr P, Scott AL (1995) Molecular cloning of a serine proteinase inhibitor from *Brugia malayi*. Infect Immun 63:1745–1753

Zang X, Taylor P, Wang JM, Meyer DJ, Scott AL, Walkinshaw MD, Maizels RM (2002) Homologues of human macrophage migration inhibitory factor from a parasitic nematode. Gene cloning, protein activity, and crystal structure. J Biol Chem 277:44261–44267

7 Transmission Potential of *Wuchereria bancrofti* by *Culex quinquefasciatus* in Malaysia and Its Global Significance

Indra Vythilingam

Abstract

Filariasis caused by three parasites, namely, *Brugia malayi*, *B. timori* and *Wuchereria bancrofti*, is of immense public health importance in Asia. Although this disease is in the process of elimination, we are now faced with a daunting challenge of increased susceptibility of *Culex quinquefasciatus* to foreign strains of *W. bancrofti*. We now see cases of *W. bancrofti* in migrant workers coming into the country. International travel and cross-border migration have created a more complex situation that needs serious attention so as to progress unhindered towards elimination. This paper reviews the status of *W. bancrofti*-caused infection in Malaysia and the way forward.

7.1 Introduction

Lymphatic filariasis is caused by three filarial species, namely, *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* (WHO 2010). Of these 90% of the infection globally is caused by *W. bancrofti* (WHO 2013). In Malaysia filariasis was a public health problem in the 1960s and 1970s with *B. malayi* being the predominant species. With research and a special filariasis control programme, the Ministry of Health Malaysia was successful in reducing the number of filariasis cases in Malaysia.

The first reference to filariasis in Malaysia was by Daniels (1908) who found three microfilaria carriers among 100 patients at the Kuala Lumpur Hospital. *Culex quinquefasciatus* was shown to be an efficient vector (Leicester 1908). Further work on filariasis started in 1934 when a filariasis survey was undertaken in the northern

I. Vythilingam (✉)
Parasitology Department, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

© Springer Nature Singapore Pte Ltd. 2018
B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_7

region of the country (Strahan and Norris 1934). Later it was shown that the northern region and Pahang (eastern region) were known to be endemic (Hodgkin 1937, 1938). *Mansonia* mosquitoes were implicated as vectors, and the filariasis was caused by *B. malayi*. It was then concluded that *B. malayi* was endemic in the low-lying riverine regions of the country, while *W. bancrofti* was seen only in immigrant Chinese and Tamil who had brought the infection from their countries (Poynton and Hodgkin 1938).

More studies were carried out in the 1950s, and it was found that long-tailed macaques were able to harbour *B. malayi*. In the 1970s active research was carried out by scientists from the Institute for Medical Research to determine the animal reservoirs of filariasis and the zoonotic transmission of *B. malayi* (Mak and Cheah 1973; Mak and Cheah 1974) and animal reservoir for rural strain of *W. bancrofti* (Ramachandran 1971).

Since 1983, the country had 17 teams working in the endemic areas, conducting house-to-house surveys at night to collect and examine blood. All positive cases were treated, and if the percentage positive was 5% or more, then all people in the village received mass drug administration of diethylcarbamazine citrate (DEC). Since 1988 the cases of filariasis have been decreasing in the country (Marzhuki et al. 1992).

A total of 267 filariasis cases were reported in 2012 showing a decreasing number of cases (31%) compared to the previous year (387 cases). In 2012, the prevalence rate of filariasis was 0.93 per 100,000 population. In 2011 and 2012, the number of cases had increased compared to 2010 due to increased detection of cases from survey activities in elimination programme (Annual Report Ministry of Health 2012).

In 2012, 155 cases (58%) were detected among the immigrants, and 112 cases (42%) were detected among locals. The predominant parasite species were *W. bancrofti* which contributed to 56%, followed by *B. malayi* (periodic) 35% and *B. malayi* (subperiodic) 9%. A total of 185,826 blood samples were taken and examined to detect microfilaremia (Annual Report Ministry of Health 2012). All cases of *W. bancrofti* were imported cases by migrant workers from Nepal, Myanmar, Bangladesh, Indonesia, India and the Philippines (Noordin et al. 2013). Unlike malaria, there is no screening of migrant workers at the port of entry. The Global Programme for the Elimination of Lymphatic Filariasis (GPELF) hopes to eliminate filariasis worldwide by 2020.

7.2 Vectors of Filariasis

In Malaysia, mosquitoes belonging to the genus *Mansonia* are responsible for the transmission of *B. malayi*. The six species so far implicated include *Mansonia bonneae*, *Ma. dives*, *Ma. uniformis*, *Ma. annulata* and *Ma. indiana*. The anopheline mosquito, *Anopheles donaldi*, has been incriminated as the vector of periodic form of *B. malayi* (Vythilingam et al. 1996). In Sabah, Malaysian Borneo, *An. balabacensis* was incriminated as the vector for *W. bancrofti* (Hii et al. 1985). Besides in Pulau Aur off the Southern State of Johor in peninsular Malaysia (2°26′57°N

104°31′30°E), *An. maculatus* has been incriminated as the vector of *W. bancrofti* (Cheong and Omar 1965). *Anopheles maculatus* is the predominant vector of malaria in peninsular Malaysia.

The *Mansonia* vectors are associated with a large number of aquatic plants in different habitats. They breed profusely in open swamps, ponds, rivers and canals associated with various types of plants such as *Eichornia*, *Salvinia* and *Pistia* spp. and also swamp forest with various plants such as rattan, *Dillenia*, *Eugenia*, herbs and arums. With these types of vegetation, it is generally very difficult to get rid of the breeding sites of the mosquitoes.

Mansonia mosquitoes are fierce biters and are attracted to a variety of hosts. Extensive studies on the vectors have been carried out in peninsular Malaysia and Malaysian Borneo (Chiang et al. 1984a, b; 1988; Chang et al. 1988, 1991; Chiang 1991).

7.3 *W. bancrofti* and Its Potential Transmission in Malaysia

Looking back at history, it is seen that *W. bancrofti* was introduced into the country in the early 1930s by immigrants. Currently, cases of filariasis are at very low numbers, and the country is moving towards elimination. However, migrant population are ceaselessly bringing the *W. bancrofti* into the country.

Studies carried out by Ramachandran et al. (1964) in an aborigine village about 19 km away from the city of Kuala Lumpur demonstrated that *W. bancrofti* was present among the aborigine population. Experimental studies conducted with various strains of *Cx. quinquefasciatus* suspected as *W. bancrofti* carrier had revealed that all strains were capable of supporting development of microfilariae to the infective stage (Thomas and Ramachandran 1970).

In 2005, studies on the susceptibility status of *Cx. quinquefasciatus* to Myanmar strain of *W. bancrofti* found that the L_3 stage of the worm developed in the *Culex* mosquito in 12 days (Vythilingam et al. 2005). This shows that the local strain of *Cx. quinquefasciatus* is susceptible to *W. bancrofti* and thus reenforces the previous findings of Thomas and Ramachandran mentioned above. Thus, it can be said that there is a potential for *W. bancrofti* to be transmitted in urban areas of Malaysia. However, according to McCarroll et al. (2000), insecticide-resistant strain of *Cx. quinquefasciatus* in their laboratory study did not produce filarial larvae, while 76% of the susceptible strain was positive. Studies have shown that most field strains of *Cx. quinquefasciatus* are resistant to insecticides (Nazni et al. 2005; Low et al. 2013).This could perhaps hamper the development of the filarial parasite in the mosquitoes.

7.4 Control of Filariasis

In Malaysia, vector control was not instituted for filariasis. Cases of filariasis were treated with DEC, and Mass Drug Administration (MDA) was carried out in villages where the infection rate was more than 5%. In areas where *Anopheles* were

vectors of filariasis, vector control measures used for malaria vectors would have also controlled the filariasis vectors. However, in areas where *Mansonia* species were vectors, development of the areas removed the breeding sites.

7.5 Challenges and Global Significance

Asia contributes about 59% of the active cases of filariasis, and more than 70% of the cases occur in the Indian subcontinent of India, Sri Lanka, Nepal, Bangladesh and Maldives (Manguin et al. 2010). Migrant workers from these countries are employed by countries in Southeast Asia, and the probabilities of the parasite being brought with them are great. Besides, Indonesia is a huge country where LF is endemic in all provinces, and 119 million people were reported to be at risk of infections caused by *B. malayi*, *W. bancrofti* and/or *B. timori* (Noordin et al. 2013). It has been reported that the endemic foci still exist in urban, rural and remote areas (Sudomo et al. 2010). It must also be noted that many from Indonesia migrate to work in neighbouring countries. It has also been suggested that Southeast Asia may be the home of this parasite and may have been disseminated to other continents from here (Hawking 1976). Thus, Malaysia is vulnerable to the establishment of *W. bancrofti* both in urban and rural areas since migrant workers work in the construction sites in urban areas and in plantations in the rural areas.

Filariasis has always been a neglected disease of the poor, and a person gets infected during childhood and only shows the symptoms during later stages in life. Although MDA has been successful in reducing the number of microfilariae (*mf*) cases, with cheap international travel and cross-border migration, the parasites will be easily brought into the countries, and with suitable vectors, it is possible for new transmission to take place. Studies have also shown that with MDA there is a reduction in the prevalence of filariasis. However, when MDA is stopped, the cases slowly start to increase because of the few that did not undergo treatment and harboured the microfilaria, and since vector control was not carried out, it led to an increase (Cartel et al. 1992). Thus multiple rounds of MDA in many countries did not achieve the predicted interruption in transmission since vector control was not implemented (Bockarie et al. 2009). A good example is Solomon Island which was successful in the elimination of filariasis using vector control (Bockarie et al. 2009).

In studies carried out in periodic *B. malayi* areas, it was shown that DEC normal treatment regimen (6 mg/kg body weight) or single-dose treatment was able to reduce the prevalence of microfilaria (Hakim et al. 1995). However, the infective bites per month in the vector *An. donaldi* were higher after treatment, though not significantly different before and after treatment (Vythilingam et al. 1996). This shows that perhaps not all people received treatment due to inaccessibility of the terrain. Thus, there is a clear evidence that MDA alone will be not be successful.

In East Africa, by combining laboratory and published field data, it has been estimated that a low-density carrier (bancroftian filariasis) could serve as a source for 0 to 15 L_3 per year in *Cx. quinquefasciatus* (McGreevy et al. 1982). They have shown that a proportion of the carriers continue to circulate microfilaria at low

levels after treatment and these carriers serve as reservoir of infection for mosquitoes (McGreevy et al. 1982). It is very difficult to maintain surveillance system once it has been declared that the disease has been eliminated. It needs a lot of commitment from government bodies to have a proper surveillance after elimination. In the case of filariasis, the situation is worse since the disease does not cause mortality and thus people who get infected will be forgotten and will only know when they have reached the incurable stage. By this time they would have passed the infection to the mosquitoes triggering silent transmission (Vythilingam 2012).

The greatest barrier to the effective control of *Cx. quinquefasciatus* is the lack of appropriate tools for sustained interruption of breeding in multiple polluted breeding sites and the resistant status of the vector to conventional insecticides as mentioned previously.

7.6 Conclusion

Although countries are gearing towards elimination of filariasis, a combination of tools should be in place to control the disease and the vectors. It must be remembered that it is not easy to eliminate the vectors and also their behaviour keeps changing. There has to be commitment from various governments and the political will to continue to provide funding for research on filariasis and its vectors. The environment is changing all the time, and with increase in temperature compared to decades ago, perhaps a smaller number of mosquitoes may be more efficient in disseminating the parasites since time taken for the development of the infective stage is reduced. This is a global challenge since parasites and vectors do not respect borders.

Acknowledgement The author would like to thank the Head of Department of Parasitology, University of Malaya, for his continuous support.

References

Bockarie MJ, Pedersen EM, White GB, Michael E (2009) Role of vector control in the global program to eliminate lymphatic filariasis. Annu Rev Entomol 54:469–487

Cartel JL, Nguyen NL, Moulia-Pelat JP, Plichart R, Martin PM, Spiegel A (1992) Mass chemoprophylaxis of lymphatic filariasis with a single dose of ivermectin in a Polynesian community with a high *Wuchereria bancrofti* infection rate. Trans Roy Soc Trop Med Hyg 86:537–540

Chang M, Jute N, Lah J (1988) Breeding site productivity of the swamp forest mosquitoes *Mansonia bonneae* and *Mansonia dives* in Sarawak, East Malaysia. Med Vet Entomol 2:19–28

Chang MS, Chan KL, Ho BC, Hawley WA (1991) Comparative transmission potential three *Mansonia* spp. (Diptera: Culicidae) for filariasis in Sarawak, Malaysia. Bull Entomol Res 81:437–444

Cheong W, Omar A (1965) *Anopheles maculatus*, a new vector of *Wuchereria bancrofti* in Malaysia (Pulau Aur) and a potential vector on mainland Malaya. Med J Malaya 20:74–75

Chiang GL (1991) Update on distribution and bionomics of *Mansonia* vectors. World Health Organization FIL/EC/91/WP.25, pp 1–8

Chiang GL, Cheong WH, Samarawickrema WA, Sulaiman I, Yap HH (1984a) Species composition, seasonal abundance and filarial infections of *Mansonia* in two ecotypes in peninsular Malaysia. Trop Biomed 1:41–47

Chiang GL, Samarawickrema WA, Cheong WH, Sulaiman I, Yap HH (1984b) Biting activity, age composition and survivorship of *Mansonia* in two ecotypes in peninsular Malaysia. Trop Biomed 1:115–120

Chiang GL, Loong KP, Mahadevan S, Eng KL (1988) A study on the dispersal, survival and gonotrophic cycle estimates of *Mansonia uniformi*s in an open swamp ecotype. Southeast Asian J Trop Med Public Health 19:271–292

Daniels CW (1908) Animal parasites in man and some of the lower animals in Malaya. Studies Inst Med Res F.M.S. 3, pp 1–13

Hakim SL, Vythilingam I, Marzukhi MI, Mak JW (1995) Single-dose diethylcarbamazine in the control of periodic brugian filariasis in peninsular Malaysia. Trans Roy Soc Trop Med Hyg 89:686–689

Hawking F (1976) The distribution of human filariasis throughout the world. Part II Asia Trop Dis Bull 73:967

Hii J, Kan S, Pereira M, Parmar S, Campos R, Chan M (1985) Bancroftian filariasis and malaria in island and hinterland populations in Sabah, Malaysia. Trop Geog Med 37:93–101

Hodgkin E (1937) Annual report of the Institute for Medical Research, Kuala Lumpur

Hodgkin E (1938) Annual report of the Institute for Medical Research, Kuala Lumpur

Leicester GF (1908) Notes on the pathogenicity of certain mosquitoes with respect to *Filaria nocturna* and malignant tertian malaria. Stud Inst Med Res Federated Malay States 3:267

Low V, Chen C, Lee HL, Lim P, Leong C, Sofian–Azirun M. (2013) Current susceptibility status of Malaysian *Culex quinquefasciatus* (Diptera: Culicidae) against DDT, propoxur, malathion, and permethrin. J Med Entomol 50:103–111

Mak JW, Cheah WC (1973) Annual report. Institute for Medical Research, Kuala Lumpur

Mak JW, Cheah WC (1974) Annual report. Institute for Medical Research, Kuala Lumpur

Manguin S, Bangs M, Pothikasikorn J, Chareonviriyaphap T (2010) Review on global co-transmission of human *Plasmodium* species and *Wuchereria bancrofti* by *Anopheles* mosquitoes. Infect Genetic Evol 10:159–177

Marzhuki M, Tham A, Poovaneswari S (1992) Current status of filariasis in Malaysia. Southeast Asian J Trop Med Public Health 24:10–14

Mccarroll L, Paton M, Karunaratne S, Jayasuryia H, Kalpage K, Hemingway J (2000) Insecticides and mosquito-borne disease: insecticide resistance in mosquitoes can also interfere with developing parasites. Nature (London) 407:961–962

Mcgreevy P, Kolstrup N, Tao J, Mcgreevy M, Marshall TDC (1982) Ingestion and development of *Wuchereria bancrofti* in *Culex quinquefasciatus*, *Anopheles gambiae* and *Aedes aegypti* after feeding on humans with varying densities of microfilariae in Tanzania. Trans Roy Soc Trop Med Hyg 76:288–296

Nazni WA, Lee HL, Azahari A (2005) Adult and larval insecticide susceptibility status of Culex quinquefasciatus (Say) mosquitoes in Kuala Lumpur Malaysia. Trop Biomed 22:63–68

Noordin R, Shenoy RK, Lim BH, Ramachandran CP (2013) Filarial worms in Southeast Asia. In: Lim AL, Vythilingam I (eds) Parasites and their vectors: a special focus on Southeast Asia. Springer, London, pp 33–56

Poynton J, Hodgkin E (1938) Endemic filariasis in the federated Malay states. Bull No 1 Inst Med Res 67

Ramachandran C (1971) Annual report. Institute for Medical Research, Kuala Lumpur

Ramachandran C, Hoo C, Bin O (1964) Filariasis among aborigines and Malays living close to Kuala Lumpur. Med J Malaysia 18:193–200

Strahan JH, Norris VH (1934) Notes on the incidence of filariasis in province Wellesley north. Malayan Med J 9:44–47

Sudomo M, Chayabejara S, Duong S, Hernandez L, Wu WP, Bergquist R (2010) Elimination of lymphatic filariasis in Southeast Asia. Adv Parasitol 72:205–233

Thomas V, Ramachandran C (1970) Selection of *Culex pipiens fatigans* for vector ability to the rural strain of *Wuchereria bancrofti*-a preliminary report. Med J Malaya 24:196–199

Vythilingam I (2012) *Plasmodium knowlesi* and *Wuchereria bancrofti*: their vectors and challenges for the future. Front Syst Biol 3:115

Vythilingam I, Hakim SL, Chan ST, Mak JW (1996) *Anopheles donaldi* incriminated as a vector of periodic *Brugia malayi* in Grik, Perak, Malaysia. Southeast Asian J Trop Med Public Health 27:637–641

Vythilingam I, Huat TC, Ahmad NW (2005) Research note transmission potential of *Wuchereria bancrofti* by *Culex quinquefasciatus* in urban areas of Malaysia. Trop Biomed 22:83–85

World Health Organisation (2010) Regional strategic plan for elimination of lymphatic filariasis 2010–2015. WHO Regional Office Southeast Asia, pp 1–27

World Health Organisation (2013) Lymphatic filariasis, fact sheet No 102

8 Genetic Diversity, Molecular Markers, and Population Genetics of Human Lymphatic Filarial Parasites

S. L. Hoti and R. Dhamodharan

Abstract

One of the most disfiguring diseases is lymphatic filariasis (LF) which is a major public health problem in tropical world. The disease is caused majorly by *Wuchereria bancrofti* and to smaller extent by *Brugia malayi* and *B. timori*. It is transmitted by mosquito vectors belonging to genera *Culex*, *Anopheles*, *Aedes*, and/or *Mansonia*. Currently, LF is targeted for elimination with mass drug administration of a combination of diethylcarbamazine/ivermectin and albendazole as a tool. Several rounds of MDA have already been administered in endemic communities, and questions have arisen pertaining to the continuance of parasite prevalence in some areas despite repeated rounds of MDA. This could be due to variations in the parasite strains that may not be responding to the anti-filarial drug administered. These variations could be the result of various factors such as geographic isolation, infra-population or refugia, environmental factors, and drug pressure. The long-term administration of the drug in the elimination program itself might have led to this phenomenon, or wide geographic distribution spanning continents might have affected variation. Investigating genetic variations among these variants may reveal the differential response to the anti-filarial drugs, and such studies are important for devising the drug administration strategies. In summary, there is a need to understand the genetic variation among the

S. L. Hoti (✉)
Regional Medical Research Centre (ICMR), Belgaum, Karnataka, India

Vector Control Research Centre (ICMR), Puducherry, India

R. Dhamodharan
Vector Control Research Centre (ICMR), Puducherry, India

Behavioral and Population Ecology Lab, Department of Biological Sciences, Seoul National University, Seoul, South Korea
e-mail: rbdhamu@snu.ac.kr

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_8

parasite populations in different LF endemic areas for which there is a need to develop appropriate markers. This review discusses the biological, physiological, and genetic variations among LF parasites.

8.1 Introduction

Lymphatic filariasis (LF) is considered as a neglected tropical disease caused by mosquito-borne nematode parasites, viz., *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori* (Routh and Bhowmik 1994). It affects around 120 million people in over 73 countries (WHO, 2014), and *W. bancrofti* is responsible for ~90% cases of LF (WHO 1992, 2002). The infection and disease caused by these worms are commonly termed as bancroftian filariasis, brugian filariasis, and timorian filariasis, respectively. The parasites are transmitted among humans by the bites of female mosquitoes belonging to genera *Culex*, *Anopheles*, *Aedes*, and/or *Mansonia* (Table 8.1). These mosquitoes act as vectors of specific filarial parasites by developing their larval stages and carrying the infective stage to human hosts (Manson-Bahr and Bell 1995; Simonsen 2003).

Filariasis is widely distributed in the tropical and subtropical regions of Africa, Asia, Northern South America, Western Pacific, and Eastern Mediterranean region. Approximately 70% of infections occur in India, Nigeria, Bangladesh, and Indonesia, and more than 1 billion people worldwide are at risk of acquiring LF infection (Molyneux and Zagaria 2002). Of these, 95% are infected with *W. bancrofti* and the remainder with *B. malayi* and *B. timor*i (WHO 1995). A population of 44 million is affected with various clinical forms of the disease, while 76 million carry the parasites with silent clinical damages of lymphatics and renal systems (Ottesen et al. 1997; Melrose 2002; Remme et al. 2002; Hotez et al. 2006). Reports until the year 2006 showed that an estimated 1.254 billion people in 83 endemic countries and territories are at the risk of infection of LF. The WHO Southeast Asian region has the highest proportion of cases (64%), followed by the African region (32%). The European region remains free from LF (WHO 2007, 2014, http://www.who.int/wer).

In India, data available until the year 2000 showed that up to 27.09 million microfilaremic and 20.83 million symptomatic filariasis cases existed, and about 429.32 million individuals were at the risk of infection (Sabesan et al. 2000). India alone accounts for 40% of the global burden of LF (Michael and Bundy 1997), and at least one-third of the people affected with the disease live in India. Bancroftian filariasis caused by *W. bancrofti* accounts for about 98% of the national burden, and brugian filariasis by *B. Malayi* is responsible for the remaining 2% which is distributed mainly in Odisha and Kerala. The recent report showed that an estimated 554.2 million people are at risk of LF infection in India (WHO 2007).

The clinical and immunological outcomes of infection and disease vary from individuals living in different endemic regions due to genetic polymorphisms, environmental factors, or inherent innate immunity (Tisch et al. 2001; Hise et al. 2003;

Table 8.1 Important discoveries and events related to disease, parasite life cycle, pathogenesis, and genetic diversity of human lymphatic filariasis *W. bancrofti*

S. no.	Name of the scientist/project	Year	Discovery/event
1	William Prout	1849	Chyluria
2	Jean-Nicholas Demarquay	1863	Mf of *W. bancrofti* (hydrocele fluid)
3	Otto Henry Wucherer	1868	Mf of *W. bancrofti* (urine)
4	Timothy Lewis	1872	Mf of *W. bancrofti* (blood)
5	Joseph Bancroft	1876	Adult female of *W. bancrofti*
6	Thomas Spencer Cobbold	1877	Adult worm of *W. bancrofti*
7	Timothy Lewis	1877	Adult worm of *W. bancrofti*
8	Patrick Manson	1877	Life cycle of *W. bancrofti*
9	Bourn	1888	Adult female of *W. bancrofti*
10	Sibthorpe and Bourne	1888	Adult male of *W. bancrofti*
11	Bancroft	1889	The mode of transmission
12	George Carmichael Low	1900	Mf in the mouthparts of mosquitoes
13	Low	1900	The mode of transmission
14	Lichtenstein and Brug	1927	*Filaria malayi* (*Brugia malayi*)
15	Poynton and Hodgkin	1939	Zoonotic filariasis
16	Hewitt et al.	1947	DEC as filaricidal compound
17	David and Edeson	1965	*B. Timori*
18	Buckley	1958	*B. malayi* – Nomenclature
19	WHO	1997	MDA
20	WHO	2000	GPELF
21	Aboobaker and Blaxter	2003	RNAi in *B. malayi*
22	Foster et al.	2005	Complete genome of *Bm-Wolbachia*
23	Ghedin et al.	2007	Mitochondrial genome of *B. malayi*
24	Daub et al.	2008	Complete Mt. genome of *B. malayi*
25	Filarial genome project	2009	*B. malayi* whole genome sequencing
26	McNulty et al. (2012)	2012	Mitochondrial genome of *Wuchereria bancrofti*
27	*Wuchereria bancrofti* WGS project	2016	*Wuchereria bancrofti* whole genome shotgun sequencing project

Choi et al. 2001). The clustering of filarial infection within the household (Ottesen et al. 1981) showed the evidence of genetic polymorphism in different outcomes of the disease.

8.2 Strains Based on Periodicity of Microfilariae

Filarial parasites, both *W. bancrofti* and *B. malayi*, occur as different physiological strains in different geographical regions of the world (Sasa 1974). In most areas (Asia, Africa, Malaysia, the Philippines, Papua New Guinea), the periodicity of *W. bancrofti* is nocturnal, i.e., presence of microfilariae (mf) in peripheral circulation during night time (Sasa and Tanaka 1974; Shriram et al. 2002; Pichon and Treuil 2004; Bockarie

et al. 2009). In some areas it is diurnally sub-periodic (South Pacific, Andaman & Nicobar Islands) or nocturnally sub-periodic (Thailand), where *mf* are present continuously in the peripheral blood, where the concentrations are higher than the average during the day and midnight, respectively (Kalra 1974; Pothikasikorn et al. 2008). It is governed by a biological rhythm inherent in the *mf* of a particular strain but is influenced by the circadian rhythm of the specific mammalian host. Also, the periodicity is in agreement with the feeding behavior of the vector mosquito (Weerasooriya et al. 1998) enabling them to ingest the *mf* in large numbers by presenting themselves in high density in the peripheral blood during the biting period of the mosquito.

8.3 Diagnosis and Genotyping of the Disease

Filariasis can be diagnosed by detection of *mf* through microscopic examination of blood smear or membrane filtration of the blood, taken during the time of circulation of *mf* depending upon its periodicity. However, the method of diagnosis based on finger prick thick blood smear test for *mf* has low sensitivity and is costly and not well accepted by either community or program personnel (Weil et al. 1997; Das et al. 2005). As an alternative and, more importantly, as a replacement for the night blood collection, more rapid immunological tests such as monoclonal antibody (Og4C3)-based ELISA or immuno-chromatographic test (ICT) are in use. In an attempt to detect antigens in day blood samples collected on filter paper, through Og4C3 test, Itoh et al. (1998) could get high sensitivity and specificity in the immune reaction. The ICT test kit (Weil and Ramzy 2007), though costly, is claimed to be specific and sensitive and could provide on-the-spot results (Weil et al. 1997; Pani et al. 2000). Detection of IgG4 antibodies using recombinant antigen also has been proved sensitive for the detection of filarial infection (Rahmah et al. 2003; Supali et al. 2004; Weil et al. 2011; Athisaya Mary et al. 2011). Apart from these, molecular tests based on DNA (PCR, PCR-RFLP, RT-PCR, and DNA sequencing) are also in place for detection of active infection both in human and mosquito vectors (Abbasi et al. 1996; Williams et al. 1996; Hoti et al. 2008; Vasuki et al. 2003, 2012). Molecular tests for diagnosis and species differentiation were mainly targeted HhaI repeat (McReynolds et al. 1986; Rao et al. 2006; Triteeraprapab et al. 2001), SspI repeat (Fischer et al. 1999; McCarthy et al. 1996; Williams et al. 1996; Zhong et al. 1996), glutathione peroxidase, cytochrome oxidase I and internal transcribed spacer-1 (Fischer et al. 2002; Nuchprayoon et al. 2005; Thanomsub et al. 2000), cofactor-independent phosphoglycerate mutase isoform-1, and abundant larval transcript-2 genes (Fong et al. 2013; Sakthidevi et al. 2010; Dhamodharan et al. 2012).

8.4 Treatment and Drug Resistance

Currently, diethylcarbamazine (DEC) or combination with ivermectin (IVM) and albendazole (ALB) is used to target the *mf*, to reduce or interrupt transmission, and these drugs have low macrofilaricidal activity (Ottesen et al. 1997; Ottesen et al. 1999;

Plaisier et al. 2000; Meyrowitsch et al. 2004). Interruption of transmission is expected to be achieved by mass annual drug administration (MDA) of DEC to entire communities at risk of infection when community drug consumption rates are adequate. Recently, treatment with doxycycline to kill the symbiotic bacterium of filarial parasite has also drawn attention of many workers (Hoerauf et al. 1999, 2008; Smith and Rajan 2000; Casiraghi et al. 2002; Rao 2005; Taylor et al. 2005). An earlier trial of doxycycline against *Wolbachia* of *W. bancrofti* has shown complete clearance of *mf* and significant level of reduction in adult worm activity (Taylor et al. 2005). In India, recently the National Task Force for Elimination of Lymphatic Filariasis (NTF-ELF) decided to modify its existing strategy of MDA using DEC alone to DEC + albendazole co-administration based on the recommendation of the Indian Council of Medical Research (ICMR). MDA with DEC or its combination with other microfilaricides has its own limitation as there are instances of recurrences of microfilaremia, possibly due to reproduction from surviving female adult worms (Fernando et al. 2011). There is also a possibility of resurgence of infection and the emergence of drug-resistant strains after MDA due to the strong selective pressure on parasites (Grant 1994; Anderson and Jaenike 1997; Schwab et al. 2007). Though it was not possible to assess resistance to DEC, resistance to IVM has been reported in animal filarial parasites as well as in the human filarial parasite, *Onchocerca volvulus* (Prichard 1990; Awadzi et al. 2004). Resistance to ALB has been seen in many parasitic nematodes of animals, and many workers demonstrated the possibility of ALB resistance in *W. bancrofti* too due to phenylalanine to tyrosine mutation at 200th and 167th positions, responsible for resistance existing in *W. bancrofti*, and also developed allele-specific PCR assays (Prichard 2007; Schwab et al. 2005, 2007; Hoti et al. 2009) for screening resistance alleles in treated parasite populations.

8.5 Molecular Biology and Genomics of Filariasis/Genetic Variation

The development of molecular methods and techniques has led to the advancement in isolation, purification, and characterization of nucleic acids and proteins from filarial parasites. Many structural and functional genes from the genome of *B. malayi* and *W. bancrofti* and their endosymbiont, *Wolbachia*, have been characterized and used for phylogenetic analysis (Williams et al. 2000). The entire mitochondrial genome of *B. malayi* (13.67 Kb) and *W. bancrofti* (13.63 Kb) (Ghedin et al. 2007; McNulty et al. 2012) and endosymbiont genome of *Wolbachia* from *B. malayi* (~1 Mb) and *W. bancrofti* (~1 Mb) (Foster et al. 2005; http://www.ncbi.nlm.nih.gov/genome/11274?project_id=199733) have also been characterized. The draft genome of *B. malayi* (93.65 Mb) (Ghedin et al. 2007) and whole genome short gun sequences of *W. bancrofti* (81.5 Mb) (McNulty et al. 2012, http://www.ncbi.nlm.nih.gov/Traces/wgs/?val=ADBV01#contigs) have been released into the public domain. The sequence data for the genomes of major human filarial pathogens, *W. bancrofti*, *O. volvulus*, and *Loa loa*, have been made publically available (http://

www.broadinstitute.org/annotation/genome/filarial_worms/MultiHome.html). The genome annotation and analysis are essential for the determination of the complete sequence of the chromosomal genome, identification of the coding genes, prediction of function of structural/functional genes, and investigation of genetic variation in the genome, population structure, and gene polymorphism in relation to drug resistance and other selective forces. Genomics of filariasis is mainly focused on identifying new drug/vaccine targets, diagnostic development and biological basis of drug resistance, antigenic diversity, infectivity and pathology, and gene polymorphism (Unnasch and Williams 2000; Blaxter et al. 1999).

8.6 *Wolbachia* Endosymbiont of Filarial Parasites

Wolbachia pipientis is a bacterial endosymbiont, maternally transmitted and associated with arthropods and filarial nematodes (Werren 1997). Most of the filarial parasites, including human parasites such as *W. bancrofti*, *B. malayi*, and *O. volvulus*, are infected with the intracellular *Wolbachia* (Sironi et al. 1995; Bandi et al. 1998; Taylor et al. 2000). The genus *Wolbachia* of filarial nematode and arthropods was identified based on DNA sequence data (Henkle-Duhrsen et al. 1998). *Wolbachia* plays an important role in the biology of the host and in the immunopathology of filariasis (Brattig et al. 2000; Taylor 2002; Keiser et al. 2002). The phylogenetic analysis of *Wolbachia* endosymbiont of the lymphatic filarial parasite showed that their phylogeny agrees with the phylogenetic structure of their host-parasite such as *B. malayi*, *W. bancrofti*, *O. volvulus*, etc., which indicates that they have evolutionary significance on parasite-endosymbiont association (Taylor et al. 1999; Casiraghi et al. 2004, 2005; Foster et al. 2005; Ferri et al. 2011; Sharma et al. 2013). Many genes of *Wolbachia* of human lymphatic filarial parasite have been characterized to focus on *Wolbachia*-based control strategies of filariasis (Brattig et al. 2000; Hoerauf et al. 2000; Taylor 2000; Dhamodharan et al. 2011; Sharma et al. 2013; Slatko et al. 2014; Shahab et al. 2014).

8.7 The Nuclear Genome

The draft genome size of *B. malayi* is 100 Mb and encodes about ~20,000 genes (Ghedin et al. 2004; Parra et al. 2007; Scott et al. 2013). It consists of five pairs of chromosomes (four autosomal pairs and one sex determination pair), and the genome is rich in adenosine and thymidine (75% AT). *Brugia* genome consists of a single 322 bp repetitive sequence, the HhaI repeat which occupies 10–12% of the genome. This HhaI repeat is genus-specific and exists in around 30,000 copies per haploid chromosome set, organized in tandem arrays (Mcreynolds et al. 1986). The draft genome of *W. bancrofti* (81.5 Mb) (McNulty et al. 2012) is yet to be annotated (http://www.ncbi.nlm.nih.gov/sra/?term=SRP000772).

8.8 Mitochondrial Genome Diversity

The complete genome of *B. malayi* mitochondria was characterized by Ghedin et al. (2007). Recently, the complete mitochondrial genome of *W. bancrofti* was sequenced (McNulty et al. 2012), and the genetic variation among the mitochondrial genome of three *W. bancrofti* strains from India, West Africa, and Papua New Guinea is analyzed (Ramesh et al. 2012). The Mt. genomes of nematodes are usually smaller than other metazoans, and the size varies from ~13.6 to 26 kb. Most of the nematode Mt. genomes contain 12 protein-coding genes, 22 trn genes, and 2 rrn genes, and they usually lack an atp8 gene. The arrangements of nematode Mt. genes are more variable and AT-rich (Okimoto et al. 1992; Keddie et al. 1998; Lavrov and Brown 2001). High mutation rate and maternal inheritance make mitochondrial genome a good source of molecular marker for studying population genetic structure (Avise et al. 1994; Wallace 1999). Cytochrome oxidase subunit 1 (COI) has been used as a taxonomic and population genetic marker to analyze the genetic diversity of *W. bancrofti* strains (de Souza et al. 2014).

8.9 Genetic Variation and Molecular Markers in LF

8.9.1 Genetic Diversity of Human Lymphatic Filarial Parasites

Nematode parasite populations are genetically heterogeneous (Nadler 1987, 1990), and genetic variation in parasitic nematodes is a prerequisite for a genetic response to a selection pressure. Proper identification of genetic variation in parasitic nematodes is essential to understand the genetic structure (e.g., effective population size, heterozygosity) or as epidemiological markers. Molecular methods have proven to be useful for assessing both inter- and intra-genetic variation in parasite populations (Nadler 1990; Grant 1994; Gasser and Newton 2000).

The analysis of genetic diversity and heterogeneity of filarial parasites is essential for understanding the phylogeny, the evolutionary history, and the emergence of drug-resistant phenotypes after treatment with antifilarial drugs (Hoti et al. 2008; Dhamodharan et al. 2008; Hoti et al. 2003. The presence of polymorphism in parasites can be identified from biological (e.g., morphology, infectivity, and periodicity), biochemical (e.g., enzymatic and drug sensitive/resistance), immunological (e.g., difference in immune response due to antigenic/antibody diversity), or molecular biological (e.g., variation in DNA and proteins sequence that leads to functional differences) characteristics. Genetic diversity of parasitic nematodes has been studied by analyzing the variability using molecular markers such as isozymes, mitochondrial DNA (mt DNA), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), microsatellites, and internal transcribed spacers (ITS) of ribosomal DNA. All these markers have been used in different combination to characterize the genetic diversity of nematodes.

8.9.2 Morphological and Behavioral Variations in Filarial Parasites

Almost all the developmental stages of the filarial parasites show differences in morphological features, with which they can be differentiated up to the species level. Since *W. bancrofti* and *B. malayi* coexist in many places (Raina et al. 1990; Rajendran et al. 1997), their identification to species level is very important in diagnosis as well as epidemiological surveys. The size of adult *W. bancrofti* worm is larger than the adult *B. malayi*. Adult male worms of *B. malayi* also show morphological variation due to their posterior cuticular structure (Maizels and Kurniawan-Atmadja 2002). Apart from differences in body size, cephalic space, and distance from head to nerve ring, the *mf* of *B. malayi* and *W. bancrofti* can be distinguished, very clearly, by the presence of caudal nuclei at the tip of the tail of the former which is not seen in the latter. The infective L3 stage of these species can be differentiated by examining their caudal papillae. There are three caudal papillae, two lateral and one terminal. All the three caudal papillae are distinctly protruding in *W. bancrofti* as compared to *B. malayi*. The lateral papillae of *B. malayi*, under electron microscopy, show a gutter-like indentation around their bases, and this character is absent in *W. bancrofti* (Zaman and Narayanan 1986).

Behavioral variations occur between these two species, in terms of *mf* periodicity and localization of the adult worms in the mammalian hosts. Microfilaria periodicity, which has been discussed in an earlier section, leads to strain variations, which ultimately decide the vector adaptation (Thorpe 1896; Basu 1958; Kalra 1974; Russel et al. 1975). Regarding adult worm localization, *W. bancrofti* adults are most commonly localized both in the lymphatics of limbs and the intrascrotal lymphatic vessels as against *B. malayi* worms, which are found mostly localized in the lymphatic system of limbs (Dreyer et al. 2006; Partono, 1987).

8.9.3 Biochemical Variations

Biochemical properties, structures, and roles of parasite proteins/proteases vary due to variation in amino acid sequence. Identification of biochemical variation is essential for the development of suitable diagnostic markers and vaccine and drug targets for the control of parasitic diseases. Though isoenzymes (or allozymes) are the essential biochemical markers and have been used for analyzing genetic variation in *Ascaris*, *Anisakis simplex*, and many nematodes (Leslie et al. 1982; Anderson et al. 1998), till now no such studies are reported for lymphatic filarial parasites, probably due to the scarcity of parasite materials. However, there are strong indications of interspecific as well as stage-wise intraspecific biochemical variations in filarial parasites (Maizels and Kurniawan-Atmadja 2002). Protein variations among species could be the reason for the enhancement of specificity of certain diagnostic filarial antigens such as *Bm*-SXP-1 and *Wb*-SXP-1, when the same species molecules are used (Rao et al. 2000; Pandiaraja et al. 2010).

8.9.4 Immunological Variations

Antigenic diversity and polymorphism in parasite populations are evolutionary strategies to escape from the host immune response (Maizels 2009). Filarial parasite antigenic surface binding to human antibody varies (Ravindran et al. 1994; Maizels and Kurniawan-Atmadja 2002), and this variation has direct effect on the recognition of immune system, immunity, and parasite immune evasion mechanisms because the survival of parasite is associated with the immune response stimulated in the host (Piessens et al. 1980). The outcome of in vitro analysis with sera samples collected from *mf* carriers also showed that *mf* could express a variety of surface antigens (Ravindran et al. 1994). The variations in *mf* sheath proteins (SHPs) which are composed of the tightly cross-linked set of repeat-rich proteins, with some carbohydrate structures, were reported (Hirzmann et al. 1995; Zahner et al. 1995). And variants of SHP-1, SHP-2, and SHP-3 are the key proteins identified, but the level of host immune response due to these variations is yet to be reported. Many studies have already reported the diversity of genes coding for immune immunogenic proteins in filarial parasites (Choi et al. 2001; Hise et al. 2003; Jaoko et al. 2007; Hoti et al. 2007).

8.9.5 Molecular Variations

Analysis of polymorphism in coding sequences of structural and functional genes, regulatory elements (promoter and enhancer sequences), RNA elements, and introns of protein-coding genes have received more attention because of their potential in treatment and diagnosis. DNA sequence-based assays have become easier to use and more efficient at screening for nucleotide sequence-based polymorphisms. Polymorphism in abundant larval transcript-2, cysteine protease inhibitor-2, and sheath proteins (SHPs) has been reported from filarial parasites *B. malayi* and *W. bancrofti* (Gregory et al. 1997; Gomez-Escobar et al. 2002; Maizels and Kurniawan-Atmadja 2002). Molecular variations of filarial antigens have also been reported from *B. malayi* cDNA clones sequenced under Filarial Genome Project (Maizels et al. 2001). Hoti et al. (2007) have studied polymorphism of lipid binding/transport molecules (gp15/400 allergen gene) of *W. bancrofti* from different regions of India and found that the parasite populations from different geographical locations, viz., Thanjavur and Tiruvannamalai (two districts in Tamil Nadu), are heterogeneous with three to five genotypes, and influence of drug pressure is associated with the gene diversity of the populations studied.

8.9.6 Molecular Markers/Methods for Analyzing Genetic Variation of Filarial Parasites

Currently, only a few molecular markers are available for filarial parasitic nematodes, which include RAPD, AFLP, RFLP, and rDNA. Molecular markers serve as effective tools for analyzing inter- and intraspecific genetic variations and

phylogenetic relationships (Williams et al. 1990; Cameron et al. 1988; Patra et al. 2007; Thangadurai et al. 2006; Dhamodharan et al. 2008; McNulty et al. 2013; Small et al. 2014).

8.9.6.1 Random Amplification of Polymorphic DNA (RAPD)

Random amplification of polymorphic DNA (RAPD) is an effective marker to differentiate the parasite species, as well as to determine the gene polymorphism within the genus. Nucleic acid sequence variation due to point mutations, inversions, deletions, and additions will determine the number and size of the RAPD bands. This technique is very rapid and simple and does not depend on previous knowledge of the target DNA sequences. It is mainly used for phylogenetic inference among closely related species through distance and parsimony analyses (Morgan et al. 1993; Sire et al. 2001).

Several studies have reported the use of the RAPD markers in analyzing the genetic diversity of *W. bancrofti* populations. The analysis of genetic polymorphism by RAPD markers in few studies with the selective population (from LF endemic areas) from India has revealed different levels of inter- and intra-genetic diversity of *W. bancrofti* populations (Pradeep Kumar et al. 2002; Patra et al. 2007). Thangadurai et al. (2006) used RAPD marker for inferring phylogeography of *W. bancrofti* in India and found it to be complex with high genetic divergence and varying gene flow between populations. Genetic heterogeneity of *W. bancrofti* populations at spatially hierarchical levels in south India (Hoti et al. 2008) showed the high degree of variability associated with human populations. Phylogenetic analysis of *W. bancrofti* microfilariae isolated from dry blood smears collected from microfilaria carriers residing in villages under MDA and selective chemotherapy with DEC was also analyzed using RAPD markers (Bisht et al. 2006). Dhamodharan et al. (2011) attempted to elucidate the influence of anti-filarial chemotherapy strategies on the genetic structure of *W. bancrofti* populations using RAPD marker-based population genetic analysis. Also, these investigators have used RAPD marker for differentiating periodic and sub-periodic *W. bancrofti* (Das et al. 2011). Analysis of genetic diversity of diurnally sub-periodic *W. bancrofti* in the Andaman and Nicobar Islands using RAPD marker showed that the parasites transmitted by *Cx. quinquefasciatus* and *Ochlerotatus* (*Aedes*) *niveus* from Car Nicobar Island and from neighboring islands, respectively, are phylogenetically distinct (Dhamodharan et al. 2008). Nuchprayoon et al. (2007) developed RAPD assay to differentiate a nocturnally subperiodic Thai strain from nocturnally periodic Myanmar strains of *W. bancrofti*.

8.9.6.2 Restriction Fragment Length Polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) is used both as diagnostic and phylogenetic marker (Gasser et al. 1996; Thanomsub et al. 2000; Nuchprayoon et al. 2003a, b) in nematode parasites. It is achieved by digesting either the genomic DNA directly or PCR amplified fragments (PCR-RFLP) with a suitable restriction enzyme followed by generating RFLP profile using gel electrophoresis (Nuchprayoon 2009). Bhandari et al. (2005) used RFLP of the internal transcribed spacer (ITS) region of the ribosomal RNA to study the intraspecific variation in *W. bancrofti*

isolates collected from different endemic zones but found that they were genetically highly similar. Nuchprayoon et al. (2005) used PCR-RFLP for the digestion of the ITS1 product with the restriction endonuclease Ase I, and based on the genetic diversity, they differentiated species-level identification of filarial parasites such as *W. bancrofti*, *B. malayi*, *B. pahangi*, *Dirofilaria immitis*, and *D. repens*.

8.9.6.3 Amplified Fragment Length Polymorphism (AFLP)

Amplified fragment length polymorphism (AFLP) is a powerful molecular marker, and this technique also doesn't require prior sequence data. It is a combination of RFLP and RAPDs, and the basic methodology includes digestion of genomic DNA using two different restriction enzymes (a rare and a frequent cutter), ligation of adapters to the digested fragments, and PCR amplification with primers homologous to the adapters plus one to four additional random bases at its 3′ end. The subset of digested DNA fragments amplified will give distinct bands that can be analyzed on a polyacrylamide gel. Patra et al. (2007) have identified suitable AFLP marker for genotyping filarial parasite, *W. bancrofti*, collected from India.

8.9.6.4 Microsatellite Markers

The hypervariable repeat length of microsatellites is used to study DNA polymorphisms among related organisms (Makova et al. 2000). Microsatellite markers are ideal for studying genetic diversity (Rosenthal et al. 2002) because it is highly polymorphic, selectively neutral, distributed throughout the genome, reproducible, and expressed co-dominantly. Microsatellites have been characterized in animal parasitic nematodes such as *Strongyloides ratti*, *Trichinella spiralis*, and *Haemonchus contortus* (Zarlenga et al. 1996; Fisher and Viney 1996; Hoekstra et al. 1997), in potato cyst nematode *Globodera* spp. (Thiery and Mugniery 2000), and in *B. malayi* (Tautz 1989). Underwood et al. (2000) have studied microsatellite-based polymorphism among *B. malayi* isolates from Indonesia and Malaysia. Currently, no existing report on microsatellite markers for *W. bancrofti* is available and is yet to be studied/developed.

8.9.6.5 Genetic Markers Based on DNA Sequencing

Mitochondrial markers are more suitable for investigating the population genetics of nematodes in inter- and intra-genetic variations, differentiating sibling species, because they are maternally inherited and have higher substitution rates than nuclear genes (Anderson et al. 1998). Many studies were reported already on the phylogenetic analysis of nematode populations based on mtDNA markers (Tarrant et al. 1992). Ribosomal DNA (rDNA) markers have also been used extensively for studying genetic variation within and between species of nematodes (Dame et al. 1991; Nadler 1992). Internal transcribed spacers (ITS) of rDNA as genetic markers for phylogenetic and phylogeographical identification of nematode species, including filarial parasites, have been reported recently (Zhu and Gasser 1998). Ribosomal RNA (rRNA) such as 16S rRNA (for Wolbachia) and 18 s rRNA (for nematode) have also proven to be used as molecular marker for species identification and phylogenetic analysis (Gogarten et al. 1996; Jain et al. 1999). Recently Bhandari et al.

(2005) studied the polymorphism of the 18S rRNA gene in *W. bancrofti* parasites collected from three different zones in India which showed that these parasites were genetically similar. Fong et al. (2013) phylogenetically differentiated the *B. malayi* isolated from Northeast Borneo Island and Thailand using ITS1 nucleotide sequences (Fong et al. 2013).

The polymorphism of gp15/400 of *W. bancrofti*, a polyprotein allergen (NPA), was investigated through sequencing a single repeat subunit of this 10-mer gene from 35 isolates of *W. bancrofti* collected from different geographic locations of India (Hoti et al. 2007). The repeat subunit was found to be highly conserved in all the isolates with only two nucleotide changes at positions 285 (A-G) and 336 (C-T), which are synonymous. This gene is multifunction in nature ranging from lipid binding and transportation to elicitation of elevated levels of IgE and Th2 type of immune response in the infected host. It is also reported to be involved in the transportation of arachidonic acid and metabolites, which are known to be the mode of action of anti-filarial drug, diethylcarbamazine. It is a good target for the development of anti-filarial drugs and immunomodulation and immunochemotherapy.

8.10 Phylogeny of Filarial Parasites

The phylum Nematoda has a large number of parasitic groups, and all the filarial parasites belonging to this phylum were grouped under the family Onchocercidae and the subfamily Onchocercinae. Molecular phylogenetic analysis using rDNA and mtDNA showed that the human lymphatic filarial parasite, *W. bancrofti*, is more closely related to *B. malayi* than *B. pahangi*, the animal filarial parasite, and *O. volvulus*, the human cutaneous filarial parasite (Casiraghi et al. 2001). Phylogenetic tree constructed recently by aligning complete mtDNA of nematodes available from online databases also clearly indicates this phylogenetic position of filarial parasites.

In a recent study, the existence of parallelism between the evolution of the polyprotein genes and small subunit (SSU) rDNA genes of parasitic nematodes has been observed. This was also evidenced by the phylogenetic tree constructed using the nucleotide sequence of the polyprotein gene of mammalian parasitic nematodes. Recently mitochondrial cytochrome oxidase 1 sequence-based population genetics of the *W. bancrofti* in Papua New Guinea with respect to posttreatment (Small et al. 2013) showed that parasite diversity was similar among people residing within the same village and clustered within transmission zones.

8.11 Phylogeography of *W. bancrofti*

Genetic analysis of *W. bancrofti* populations collected from 71 *mf* carriers from different geoclimatic regions of India through RAPD profiles showed that the parasite populations are comprised of at least five genotypes (Thangadurai et al. 2006). Generally, parasite populations from urban areas are genetically heterogeneous exhibiting two

to four different genotypes, possibly due to the convergence of infected individuals from different areas for trade and employment and consequent intermixing of parasite populations (Hoti et al. 2008). In their study, analysis of parasite populations collected from 31 *mf* carriers residing in Pondicherry and surrounding villages showed that *W. bancrofti* populations of rural area are highly homogenous comprising one or two genotypes, compared to the urban populations, which showed high genetic divergence and varying levels of gene flow between populations of different areas. The diversity of parasites in relation to different age groups has also been investigated, and the results showed that the parasite heterogeneity increases with age of the carrier, possibly due to acquiring of new infections at different time points in life (Hoti et al. 2008).

All these studies on the phylogeography of *W. bancrofti* populations in India have been indicative of the following:

a. High genetic divergence and gene flow between populations.
b. The route of entry of the parasite into Indian subcontinent possibly appeared to be from an ancient origin from the countries of the Southeast Asian archipelago, through the eastern coastal line of the southern peninsula.
c. The Western Ghats would have played a major role in the selection process by geographic isolation leading to the genetic drift between the two strains on its western and eastern side. Also, the chemotherapeutical pressure would have contributed to the high genetic heterogeneity of the populations in the eastern side of the country (Thangadurai et al. 2006).

8.12 Conclusion

Parasite populations respond to selective pressures like drug pressure and are genetically heterogeneous. Understanding the genetic variability and diversity of genes and genome within and between populations of the filarial parasite is essential for appropriate treatment schedules and designing control programs. Currently, molecular markers have been successfully used in analyzing the population genetic structure of filarial parasites in India on a limited scale. Such efforts need to be undertaken on national and further on a global scale.

Acknowledgment Author Ramasamy Dhamodharan is recipient of BK21 Plus Postdoctoral Research Fellowship, South Korea.

References

Abbasi I, Hamburger J, Githure J, Ochola JJ, Agure R, Koech DK, Ramzy R, Gad A, Williams SA (1996) Detection of *Wuchereria bancrofti* DNA in patients' sputum by the polymerase chain reaction. Trans R Soc Trop Med Hyg 90:531–532

Anderson TJ, Jaenike J (1997) Host specificity, evolutionary relationships and macrogeographic differentiation among Ascaris populations from humans and pigs. Parasitology 115(Pt 3):325–342

Anderson TJ, Blouin MS, Beech RN (1998) Population biology of parasitic nematodes: applications of genetic markers. Adv Parasitol 41:219–283

Athisaya Mary K, Hoti SL, Krishnamoorthy K, Das PK, Rahmah N (2011) Detection of filarial specific IgG4 antibodies in individuals residing in endemic areas using panLFRAPID test card. J Parasit Dis 35:77–79

Avise JC, Nelson WS, Sibley CG (1994) DNA sequence support for a close phylogenetic relationship between some storks and new world vultures. Proc Natl Acad Sci USA 91(11):5173–5177

Awadzi K, Boakye DA, Edwards G, Opoku NO, Attah SK, Osei-Atweneboana MY, Lazdins-Helds JK, Ardrey AE, Addy ET et al (2004) An investigation of persistent microfilaridermias despite multiple treatments with ivermectin, in two onchocerciasis-endemic foci in Ghana. Ann Trop Med Parasitol 98:231–249

Bandi C, Anderson TJ, Genchi C, Blaxter ML (1998) Phylogeny of Wolbachia in filarial nematodes. Proc Biol Sci 265(1413):2407–2413

Basu PC (1958) A note on malaria and filariasis in Andaman and Nicobar. Bull Nat Soc India Mal Mosq Dis 6:193

Bhandari Y, Dabir P, Nandhakumar K, Dayananda KM, Shouche YS, Reddy MV (2005) Analysis of polymorphism of 18S rRNA gene in *Wuchereria bancrofti* microfilariae. Microbiol Immunol 49(10):909–914

Bisht R, Hoti SL, Thangadurai R, Das PK (2006) Isolation of *Wuchereria bancrofti* microfilariae from archived stained blood slides for use in genetic studies and amplification of parasite and endosymbiont genes. Acta Trop 99(1):1–5

Blaxter M, Aslett M, Guiliano D, Daub J (1999) Parasitic helminth genomics. Filarial Genome Project. Parasitology 118(Suppl):S39–S51

Bockarie MJ, Pedersen EM, White GB, Michael E (2009) Role of vector control in the global program to eliminate lymphatic filariasis. Annu Rev Entomol 54:469–487

Brattig NW, Rathjens U, Ernst M, Geisinger F, Renz A, Tischendorf FW (2000) Lipopolysaccharide-like molecules derived from Wolbachia endobacteria of the filaria Onchocerca volvulus are candidate mediators in the sequence of inflammatory and antiinflammatory responses of human monocytes. Microbes Infect 2(10):1147–1157

Cameron ML, Levy P, Nutman T, Vanamala CR, Narayanan PR, Rajan TV (1988) Use of restriction fragment length polymorphisms (RFLPs) to distinguish between nematodes of pathogenic significance. Parasitology 96(Pt 2):381–390

Casiraghi M, Anderson TJ, Bandi C, Bazzocchi C, Genchi C (2001) A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of Wolbachia endosymbionts. Parasitology 122(Pt 1):93–103

Casiraghi M, McCall JW, Simoncini L, Kramer LH, Sacchi L, Genchi C, Werren JH, Bandi C (2002) Tetracycline treatment and sex-ratio distortion: a role for Wolbachia in the moulting of filarial nematodes? Int J Parasitol 32(12):1457–1468

Casiraghi M, Bain O, Guerrero R, Martin C, Pocacqua V, Gardner SL, Franceschi A, Bandi C (2004) Mapping the presence of Wolbachia pipientis on the phylogeny of filarial nematodes: evidence for symbiont loss during evolution. Int J Parasitol 34:191–203

Casiraghi M, Bordenstein SR, Baldo L, Lo N, Beninati T, Wernegreen JJ, Werren JH, Bandi C (2005) Phylogeny of Wolbachia pipientis based on gltA, groEL and ftsZ gene sequences: clustering of arthropod and nematode symbionts in the F supergroup, and evidence for further diversity in the Wolbachia tree. Microbiology 151:4015–4022

Choi EH, Zimmerman PA, Foster CB, Zhu S, Kumaraswami V, Nutman TB, Chanock SJ (2001) Genetic polymorphisms in molecules of innate immunity and susceptibility to infection with *Wuchereria bancrofti* in South India. Genes Immun 2(5):248–253

Dame JB, Yowell CA, Courtney CH, Lindgren WG (1991) Cloning and characterization of the ribosomal RNA gene repeat from Ostertagia ostertagi. Mol Biochem Parasitol 45(2):275–280

Das D, Kumar S, Sahoo PK, Dash AP (2005) A survey of bancroftian filariasis for microfilariae & circulating antigenaemia in two villages of Madhya Pradesh. Indian J Med Res 121:771–775

Das MK, Dhamodharan R, Hoti SL, Dash AP (2011) Molecular differentiation of nocturnally periodic and diurnally sub-periodic *Wuchereria bancrofti* by Randomly Amplified Polymorphic DNA (RAPD). World J Microbiol Biotechnol 27:1525–1530

de Souza DK, Osei-Poku J, Blum J, Baidoo H, Brown CA, Lawson BW, Wilson MD, Bockarie MJ, Boakye DA (2014) The epidemiology of lymphatic filariasis in Ghana, explained by the possible existence of two strains of Wuchereria bancrofti. Pan Afr Med J 17:133. https://doi.org/10.11604/pamj.2014.17.133.3370

Dhamodharan R, Das MK, Hoti SL, Das PK, Dash AP (2008) Genetic variability of diurnally subperiodic *Wuchereria bancrofti* in Nicobarese tribe of Nicobar group of islands, Andaman and Nicobar Islands, India. Parasitol Res 103(1):59–66

Dhamodharan R, Hoti S, Sivapragasam G, Das M (2011) Cloning and sequence analysis of partial genomic DNA coding for HtrA-type serine protease of Wolbachia from human lymphatic filarial parasite, *Wuchereria bancrofti*. Trop Parasitol 1:76–82

Dhamodharan R, Hoti SL, Sankari T (2012) Characterization of cofactor-independent phosphoglycerate mutase isoform-1 (Wb-iPGM) gene: a drug and diagnostic target from human lymphatic filarial parasite, *Wuchereria bancrofti*. Infect Genet Evol 12:957–965

Dreyer G, Addiss D, Williamson J, Noroes J (2006) Efficacy of co-administered diethylcarbamazine and albendazole against adult *Wuchereria bancrofti*. Trans R Soc Trop Med Hyg 100(12):1118–1125

Fernando SD, Rodrigo C, Rajapakse S (2011) Current evidence on the use of antifilarial agents in the management of bancroftian filariasis. J Trop Med:175941

Ferri E, Bain O, Barbuto M, Martin C, Lo N, Uni S, Landmann F, Baccei SG, Guerrero R et al (2011) New insights into the evolution of Wolbachia infections in filarial nematodes inferred from a large range of screened species. PLoS One 6:e20843

Fischer P, Liu X, Lizotte-Waniewski M, Kamal IH, Ramzy RM, Williams SA (1999) Development of a quantitative, competitive polymerase chain reaction--enzyme-linked immunosorbent assay for the detection of *Wuchereria bancrofti* DNA. Parasitol Res 85:176–183

Fischer P, Wibowo H, Pischke S, Ruckert P, Liebau E, Ismid IS, Supali T (2002) PCR-based detection and identification of the filarial parasite Brugia timori from Alor Island, Indonesia. Ann Trop Med Parasitol 96:809–821

Fisher MC, Viney ME (1996) Microsatellites of the parasitic nematode Strongyloides ratti. Mol Biochem Parasitol 80(2):221–224

Fong MY, Noordin R, Lau YL, Cheong FW, Yunus MH, Idris ZM (2013) Comparative analysis of ITS1 nucleotide sequence reveals distinct genetic difference between *Brugia malayi* from northeast Borneo and Thailand. Parasitology 140:39–45

Foster J, Ganatra M, Kamal I, Ware J, Makarova K, Ivanova N, Bhattacharyya A, Kapatral V, Kumar S, Posfai J, Vincze T, Ingram J, Moran L, Lapidus A, Omelchenko M, Kyrpides N, Ghedin E, Wang S, Goltsman E, Joukov V, Ostrovskaya O, Tsukerman K, Mazur M, Comb D, Koonin E, Slatko B (2005) The Wolbachia genome of *Brugia malayi*: endosymbiont evolution within a human pathogenic nematode. PLoS Biol 3(4):e121

Gasser RB, Newton SE (2000) Genomic and genetic research on bursate nematodes: significance, implications and prospects. Int J Parasitol 30(4):509–534

Gasser RB, LeGoff L, Petit G, Bain O (1996) Rapid delineation of closely-related filarial parasites using genetic markers in spacer rDNA. Acta Trop 62(3):143–150

Ghedin E, Wang S, Foster JM, Slatko BE (2004) First sequenced genome of a parasitic nematode. Trends Parasitol 20:151–153

Ghedin E, Wang S, Spiro D, Caler E, Zhao Q, Crabtree J, Allen JE, Delcher AL, Guiliano DB et al (2007) Draft genome of the filarial nematode parasite *Brugia malayi*. Science 317(5845):1756–1760

Gogarten JP, Olendzenski L, Hilario E, Simon C, Holsinger KE (1996) Dating the Cenancester of organisms. Science 274(5293):1750b–1751b

Gomez-Escobar N, Gregory WF, Britton C, Murray L, Corton C, Hall N, Daub J, Blaxter ML, Maizels RM (2002) Abundant larval transcript-1 and -2 genes from *Brugia malayi*: diversity of genomic environments but conservation of 5′ promoter sequences functional in Caenorhabditis elegans. Mol Biochem Parasitol 125(1–2):59–71

Grant WN (1994) Genetic variation in parasitic nematodes and its implications. Int J Parasitol 24(6):821–830

Gregory WF, Blaxter ML, Maizels RM (1997) Differentially expressed, abundant trans-spliced cDNAs from larval *Brugia malayi*. Mol Biochem Parasitol 87(1):85–95

Henkle-Duhrsen K, Eckelt VH, Wildenburg G, Blaxter M, Walter RD (1998) Gene structure, activity and localization of a catalase from intracellular bacteria in Onchocerca volvulus. Mol Biochem Parasitol 96(1–2):69–81

Hirzmann J, Schnaufer A, Hintz M, Conraths F, Stirm S, Zahner H, Hobom G (1995) Brugia spp. and Litomosoides carinii: identification of a covalently cross-linked microfilarial sheath matrix protein (shp2). Mol Biochem Parasitol 70:95–106

Hise AG, Hazlett FE, Bockarie MJ, Zimmerman PA, Tisch DJ, Kazura JW (2003) Polymorphisms of innate immunity genes and susceptibility to lymphatic filariasis. Genes Immun 4(7):524–527

Hoekstra R, Criado-Fornelio A, Fakkeldij J, Bergman J, Roos MH (1997) Microsatellites of the parasitic nematode Haemonchus contortus: polymorphism and linkage with a direct repeat. Mol Biochem Parasitol 89(1):97–107

Hoerauf A, Nissen-Pahle K, Schmetz C, Henkle-Duhrsen K, Blaxter ML, Buttner DW, Gallin MY, Al-Qaoud KM, Lucius R, Fleischer B (1999) Tetracycline therapy targets intracellular bacteria in the filarial nematode Litomosoides sigmodontis and results in filarial infertility. J Clin Invest 103(1):11–18

Hoerauf A, Volkmann L, Hamelmann C, Adjei O, Autenrieth IB, Fleischer B, Buttner DW (2000) Endosymbiotic bacteria in worms as targets for a novel chemotherapy in filariasis. Lancet 355(9211):1242–1243

Hoerauf A, Specht S, Buttner M, Pfarr K, Mand S, Fimmers R, Marfo-Debrekyei Y, Konadu P, Debrah AY et al (2008) Wolbachia endobacteria depletion by doxycycline as antifilarial therapy has macrofilaricidal activity in onchocerciasis: a randomized placebo-controlled study. Med Microbiol Immunol 197:295–311

Hotez P, Ottesen E, Fenwick A, Molyneux D (2006) The neglected tropical diseases: the ancient afflictions of stigma and poverty and the prospects for their control and elimination. Adv Exp Med Biol 582:23–33

Hoti SL, Subramaniyan K, Das PK (2003) Detection of codon for amino acid 200 in isotype 1 beta-tubulin gene of *Wuchereria bancrofti* isolates, implicated in resistance to benzimidazoles in other nematodes. Acta Trop 88(1):77–81

Hoti SL, Thangadurai R, Patra KP, Das PK (2007) Polymorphism of gp15/400 allergen gene of *Wuchereria bancrofti* from different regions of India endemic for lymphatic filariasis. Infect Genet Evol 7(2):213–218

Hoti SL, Thangadurai R, Dhamodharan R, Das PK (2008) Genetic heterogeneity of *Wuchereria bancrofti* populations at spatially hierarchical levels in Pondicherry and surrounding areas, South India. Infect Genet Evol 8(5):644–652

Hoti SL, Dhamodharan R, Subramaniyan K, Das PK (2009) An allele specific PCR assay for screening for drug resistance among *Wuchereria bancrofti* populations in India. Indian J Med Res 130:193–199

Itoh M, Gunawardena NK, Qiu XG, Weerasooriya MV, Kimura E (1998) The use of whole blood absorbed on filter paper to detect *Wuchereria bancrofti* circulating antigen. Trans R Soc Trop Med Hyg 92:513–515

Jain R, Rivera MC, Lake JA (1999) Horizontal gene transfer among genomes: the complexity hypothesis. Proc Natl Acad Sci USA 96(7):3801–3806

Jaoko WG, Michael E, Meyrowitsch DW, Estambale BB, Malecela MN, Simonsen PE (2007) Immunoepidemiology of *Wuchereria bancrofti* infection: parasite transmission intensity, filaria-specific antibodies, and host immunity in two east African communities. Infect Immun 75:5651–5662

Kalra NL (1974) Filariasis among aborigines of Andaman and Nicobar islands. J Commun Dis 6:40–56

Keddie EM, Higazi T, Unnasch TR (1998) The mitochondrial genome of Onchocerca volvulus: sequence, structure and phylogenetic analysis. Mol Biochem Parasitol 95(1):111–127

Keiser PB, Reynolds SM, Awadzi K, Ottesen EA, Taylor MJ, Nutman TB (2002) Bacterial endosymbionts of Onchocerca volvulus in the pathogenesis of posttreatment reactions. J Infect Dis 185(6):805–811

Lavrov DV, Brown WM (2001) Trichinella spiralis mtDNA: a nematode mitochondrial genome that encodes a putative ATP8 and normally structured tRNAS and has a gene arrangement relatable to those of coelomate metazoans. Genetics 157:621–637

Leslie JF, Cain GD, Meffe GK, Vrijenhoek RC (1982) Enzyme polymorphism in Ascaris suum (Nematoda). J Parasitol 68(4):576–587

Maizels RM, Kurniawan-Atmadja A (2002) Variation and polymorphism in helminth parasites. Parasitology 125(Suppl):S25–S37

Maizels RM, Gomez-Escobar N, Gregory WF, Murray J, Zang X (2001) Immune evasion genes from filarial nematodes. Int J Parasitol 31(9):889–898

Makova KD, Nekrutenko A, Baker RJ (2000) Evolution of microsatellite alleles in four species of mice (genus Apodemus). J Mol Evol 51(2):166–172

Manson-Bahr P EC, Bell DR (1995) Manson's tropical diseases, 20th edn. ELBS Pub, pp 350–355, 1425–1435

McCarthy JS, Zhong M, Gopinath R, Ottesen EA, Williams SA, Nutman TB (1996) Evaluation of a polymerase chain reaction-based assay for diagnosis of *Wuchereria bancrofti* infection. J Infect Dis 173(6):1510–1514

McNulty SN, Mullin AS, Vaughan JA, Tkach VV, Weil GJ, Fischer PU (2012) Comparing the mitochondrial genomes of Wolbachia-dependent and independent filarial nematode species. BMC Genomics 13:145

McNulty SN, Mitreva M, Weil GJ, Fischer PU (2013) Inter and intra-specific diversity of parasites that cause lymphatic filariasis. Infect Genet Evol 14:137–146

McReynolds LA, DeSimone SM, Williams SA (1986) Cloning and comparison of repeated DNA sequences from the human filarial parasite *Brugia malayi* and the animal parasite Brugia pahangi. Proc Natl Acad Sci USA 83(3):797–801

Melrose WD (2002) Lymphatic filariasis: new insights into an old disease. Int J Parasitol 32(8):947–960

Meyrowitsch DW, Simonsen PE, 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(11):627–634

Michael E, Bundy DA (1997) Global mapping of lymphatic filariasis. Parasitol Today 13(12):472–476

Molyneux DH, Zagaria N (2002) Lymphatic filariasis elimination: progress in global programme development. Ann Trop Med Parasitol 96(Suppl 2):S15–S40

Morgan UM, Constantine CC, Greene WK, Thompson RC (1993) RAPD (random amplified polymorphic DNA) analysis of Giardia DNA and correlation with isoenzyme data. Trans R Soc Trop Med Hyg 87(6):702–705

Nadler SA (1987) Biochemical and immunological systematics of some ascaridoid nematodes: genetic divergence between congeners. J Parasitol 73(4):811–816

Nadler SA (1990) Molecular approaches to studying helminth population genetics and phylogeny. Int J Parasitol 20(1):11–29

Nadler SA (1992) Phylogeny of some ascaridoid nematodes, inferred from comparison of 18S and 28S rRNA sequences. Mol Biol Evol 9(5):932–944

Nuchprayoon S (2009) DNA-based diagnosis of lymphatic filariasis. Southeast Asian J Trop Med Public Health 40:904–913

Nuchprayoon S, Sangprakarn S, Junpee A, Nithiuthai S, Chungpivat S, Poovorawan Y (2003a) Differentiation of *Brugia malayi* and Brugia pahangi by PCR-RFLP of ITS1 and ITS2. Southeast Asian J Trop Med Public Health 34(Suppl 2):67–73

Nuchprayoon S, Sanprasert V, Porksakorn C, Nuchprayoon I (2003b) Prevalence of bancroftian filariasis on the Thai-Myanmar border. Asian Pac J Allergy Immunol 21(3):179–188

Nuchprayoon S, Junpee A, Poovorawan Y, Scott AL (2005) Detection and differentiation of filarial parasites by universal primers and polymerase chain reaction-restriction fragment length polymorphism analysis. Am J Trop Med Hyg 73:895–900

Nuchprayoon S, Junpee A, Poovorawan Y (2007) Random amplified polymorphic DNA (RAPD) for differentiation between Thai and Myanmar strains of *Wuchereria bancrofti*. Filaria J 6:6

Okimoto R, Macfarlane JL, Clary DO, Wolstenholme DR (1992) The mitochondrial genomes of two nematodes, Caenorhabditis elegans and Ascaris suum. Genetics 130(3):471–498

Ottesen EA, Kumaraswami V, Paranjape R, Poindexter RW, Tripathy SP (1981) Naturally occurring blocking antibodies modulate immediate hypersensitivity responses in human filariasis. J Immunol 127(5):2014–2020

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

Ottesen EA, Ismail MM, Horton J (1999) The role of albendazole in programmes to eliminate lymphatic filariasis. Parasitol Today 15(9):382–386

Pandiaraja P, Murugan V, Hoti SL, Kaliraj P (2010) Molecular characterization of a truncated antigen (Wb14) of SXP-1 of *Wuchereria bancrofti* from four endemic regions in India. Exp Parasitol 125:236–243

Pani SP, Hoti SL, Elango A, Yuvaraj J, Lall R, Ramaiah KD (2000) Evaluation of the ICT whole blood antigen card test to detect infection due to nocturnally periodic *Wuchereria bancrofti* in South India. Tropical Med Int Health 5(5):359–363

Parra G et al (2007) CEGMA: a pipeline to accurately annotate core genes in eukaryotic genomes. Bioinformatics 23:1061–1067

Partono F (1987) The spectrum of disease in lymphatic filariasis. Ciba Found Symp 127:15–31

Patra KP, Ramu T, Hoti SL, Pragasam GS, Das PK (2007) Identification of a molecular marker for genotyping human lymphatic filarial nematode parasite *Wuchereria bancrofti*. Exp Parasitol 116(1):59–65

Pichon G, Treuil JP (2004) Genetic determinism of parasitic circadian periodicity and subperiodicity in human lymphatic filariasis. C R Biol 327:1087–1094

Piessens WF, McGreevy PB, Piessens PW, McGreevy M, Koiman I, Saroso JS, Dennis DT (1980) Immune responses in human infections with Brugia malayi: specific cellular unresponsiveness to filarial antigens. J Clin Invest 65:172–179. https://doi.org/10.1172/JCI109648

Plaisier AP, Stolk WA, van Oortmarssen GJ, Habbema JD (2000) Effectiveness of annual ivermectin treatment for *Wuchereria bancrofti* infection. Parasitol Today 16(7):298–302

Pothikasikorn J, Bangs MJ, Boonplueang R, Chareonviriyaphap T (2008) Susceptibility of various mosquitoes of Thailand to nocturnal subperiodic *Wuchereria bancrofti*. J Vector Ecol 33:313–320

Pradeep Kumar N, Patra KP, Hoti SL, Das PK (2002) Genetic variability of the human filarial parasite, *Wuchereria bancrofti* in South India. Acta Trop 82(1):67–76

Prichard RK (1990) Anthelmintic resistance in nematodes: extent, recent understanding and future directions for control and research. Int J Parasitol 20:515–523

Prichard RK (2007) Ivermectin resistance and overview of the consortium for anthelmintic resistance SNPs. Expert Opin Drug Discov 2:S41–S52

Rahmah N, Lim BH, Azian H, Ramelah TS, Rohana AR (2003) Short communication: use of a recombinant antigen-based ELISA to determine prevalence of brugian filariasis among Malaysian schoolchildren near Pasir mas, Kelantan-Thailand border. Tropical Med Int Health 8:158–163

Raina VK, Joshi MC, Singh S, Joshi RD, Bhattacharjee KK, Kumar A, Verghese T (1990) Epidemiology of *Brugia malayi* infection and its co-existence with *Wuchereria bancrofti* in and around Sillaberia PHC, district Midnapur, West Bengal. J Commun Dis 22:205–208

Rajendran G, Panicker KN, Krishnamoorthy K, Sabesan S, Snehlatha, Radhakrishnan R (1997) Current status of filariasis in Chavakad taluk, Trichur district, Kerala. J Commun Dis 29:333–343

Ramesh A, Small ST, Kloos ZA, Kazura JW, Nutman TB, Serre D, Zimmerman PA (2012) The complete mitochondrial genome sequence of the filarial nematode Wuchereria bancrofti from

three geographic isolates provides evidence of complex demographic history. Mol Biochem Parasitol 183:32–41. https://doi.org/10.1016/j.molbiopara.2012.01.004
Rao RU (2005) Endosymbiotic Wolbachia of parasitic filarial nematodes as drug targets. Indian J Med Res 122:199–204
Rao UR, Salinas G, Mehta K, Klei TR (2000) Identification and localization of glutathione S-transferase as a potential target enzyme in Brugia species. Parasitol Res 86(11):908–915
Rao RU, Atkinson LJ, Ramzy RM, Helmy H, Farid HA, Bockarie MJ, Susapu M, Laney SJ, Williams SA, Weil GJ (2006) A real-time PCR-based assay for detection of *Wuchereria bancrofti* DNA in blood and mosquitoes. Am J Trop Med Hyg 74(5):826–832
Ravindran B, Satapathy AK, Sahoo PK (1994) Bancroftian filariasis-differential reactivity of anti-sheath antibodies in microfilariae carriers. Parasite Immunol 16:321–323
Remme JH, Blas E, Chitsulo L, Desjeux PM, Engers HD, Kanyok TP, Kayondo JF, Kioy DW, Kumaraswami V, Lazdins JK, Nunn PP, Oduola A, Ridley RG, Toure YT, Zicker F, Morel CM (2002) Strategic emphases for tropical diseases research: a TDR perspective. Trends Microbiol 10(10):435–440
Rosenthal AN, Ryan A, Hopster D, Jacobs IJ (2002) Molecular evidence of a common clonal origin and subsequent divergent clonal evolution in vulval intraepithelial neoplasia, vulval squamous cell carcinoma and lymph node metastases. Int J Cancer 99(4):549–554
Routh HB, Bhowmik KR (1994) Filariasis. Dermatol Clin 12:719–727
Russel S, Das M, Rao C (1975) Filariasis in Andaman and Nicobar Islands I. survey findings. Nancowry, Teressa, Chowra, Car Nicobar and Port Blair. J Commun Dis 7:15–30
Sabesan S, Palaniyandi M, Das PK, Michael E (2000) Mapping of lymphatic filariasis in India. Ann Trop Med Parasitol 94(6):591–606
Sakthidevi M, Murugan V, Hoti SL, Kaliraj P (2010) Lymphatic filarial species differentiation using evolutionarily modified tandem repeats: generation of new genetic markers. Infect Genet Evol 10:591–594
Sasa M (1974) Anti-filariasis campaign: its history and future prospects. Prog Drug Res 18:259–268
Sasa M, Tanaka H (1974) A statistical method for comparison and classification of the microfilarial periodicity. Jpn J Exp Med 44(4):321–346
Schwab AE, Boakye DA, Kyelem D, Prichard RK (2005) Detection of benzimidazole resistance-associated mutations in the filarial nematode *Wuchereria bancrofti* and evidence for selection by albendazole and ivermectin combination treatment. Am J Trop Med Hyg 73(2):234–238
Schwab AE, Churcher TS, Schwab AJ, Basanez MG, Prichard RK (2007) An analysis of the population genetics of potential multi-drug resistance in *Wuchereria bancrofti* due to combination chemotherapy. Parasitology 134:1025–1040
Scott AL, Ghedin E, Nutman TB, McReynolds LA, Poole CB, Slatko BE, Foster JM (2013) Filarial and Wolbachia genomics. Parasite Immunol 34:121–129
Shahab M, Verma M, Pathak M, Mitra K, Misra-Bhattacharya S (2014) Cloning, expression and characterization of UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) from Wolbachia endosymbiont of human lymphatic filarial parasite *Brugia malayi*. PLoS One 9:e99884
Sharma R, Hoti SL, Vasuki V, Sankari T, Meena RL, Das PK (2013) Filamentation temperature-sensitive protein Z (FtsZ) of Wolbachia, endosymbiont of *Wuchereria bancrofti*: a potential target for anti-filarial chemotherapy. Acta Trop 125:330–338
Shriram AN, Murhekar MV, Ramaiah KD, Sehgal SC (2002) Prevalence of diurnally subperiodic bancroftian filariasis among the Nicobarese in Andaman and Nicobar Islands, India: effect of age and gender. Tropical Med Int Health 7:949–954
Simonsen PE (2003) Filariasis. In: Cook GC, Zumla A (eds) Manson's tropical diseases, 21st edn. W. B. Saunders, London, pp 1489–1526
Sire C, Langand J, Barral V, Theron A (2001) Parasite (Schistosoma mansoni) and host (Biomphalaria glabrata) genetic diversity: population structure in a fragmented landscape. Parasitology 122(Pt 5):545–554
Sironi M, Bandi C, Sacchi L, Di Sacco B, Damiani G, Genchi C (1995) Molecular evidence for a close relative of the arthropod endosymbiont Wolbachia in a filarial worm. Mol Biochem Parasitol 74(2):223–227

Slatko BE, Luck AN, Dobson SL, Foster JM (2014) Wolbachia endosymbionts and human disease control. Mol Biochem Parasitol 195:88–95

Small ST, Ramesh A, Bun K, Reimer L, Thomsen E, Baea M, Bockarie MJ, Siba P, Kazura JW et al (2013) Population genetics of the filarial worm *Wuchereria bancrofti* in a post-treatment region of Papua New Guinea: insights into diversity and life history. PLoS Negl Trop Dis 7:e2308

Small ST, Tisch DJ, Zimmerman PA (2014) Molecular epidemiology, phylogeny and evolution of the filarial nematode *Wuchereria bancrofti*. Infect Genet Evol 28:33–43

Smith HL, Rajan TV (2000) Tetracycline inhibits development of the infective-stage larvae of filarial nematodes in vitro. Exp Parasitol 95(4):265–270

Supali T, Rahmah N, Djuardi Y, Sartono E, Ruckert P, Fischer P (2004) Detection of filaria-specific IgG4 antibodies using Brugia rapid test in individuals from an area highly endemic for Brugia timori. Acta Trop 90:255–261

Tarrant CA, Blouin MS, Yowell CA, Dame JB (1992) Suitability of mitochondrial DNA for assaying interindividual genetic variation in small helminths. J Parasitol 78(2):374–378

Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res 17(16):6463–6471

Taylor MJ (2000) Wolbachia bacteria of filarial nematodes in the pathogenesis of disease and as a target for control. Trans R Soc Trop Med Hyg 94(6):596–598

Taylor MJ (2002) A new insight into the pathogenesis of filarial disease. Curr Mol Med 2(3):299–302

Taylor MJ, Bilo K, Cross HF, Archer JP, Underwood AP (1999) 16S rDNA phylogeny and ultrastructural characterization of Wolbachia intracellular bacteria of the filarial nematodes *Brugia malayi*, B. Pahangi, and *Wuchereria bancrofti*. Exp Parasitol 91(4):356–361

Taylor MJ, Bandi C, Hoerauf AM, Lazdins J (2000) Wolbachia bacteria of filarial nematodes: a target for control? Parasitol Today 16(5):179–180

Taylor MD, LeGoff L, Harris A, Malone E, Allen JE, Maizels RM (2005) Removal of regulatory T cell activity reverses hyporesponsiveness and leads to filarial parasite clearance in vivo. J Immunol 174(8):4924–4933

Thangadurai R, Hoti SL, Kumar NP, Das PK (2006) Phylogeography of human lymphatic filarial parasite, *Wuchereria bancrofti* in India. Acta Trop 98(3):297–304

Thanomsub BW, Chansiri K, Sarataphan N, Phantana S (2000) Differential diagnosis of human lymphatic filariasis using PCR-RFLP. Mol Cell Probes 14(1):41–46

Thiery M, Mugniery D (2000) Microsatellite loci in the phytoparasitic nematode Globodera. Genome 43(1):160–165

Thorpe V (1896) Filaria sanguinis hominis in the South Sea islands, with photomicrographs from Tonga & the friendly islands. Br Med J 2:922–924

Tisch DJ, Hazlett FE, Kastens W, Alpers MP, Bockarie MJ, Kazura JW (2001) Ecologic and biologic determinants of filarial antigenemia in bancroftian filariasis in Papua New Guinea. J Infect Dis 184(7):898–904

Triteeraprapab S, Karnjanopas K, Porksakorn C, Sai-Ngam A, Yentakam S, Loymak S (2001) Lymphatic filariasis caused by *Brugia malayi* in an endemic area of Narathiwat Province, southern of Thailand. J Med Assoc Thail 84(Suppl 1):S182–S188

Underwood AP, Supali T, Wu Y, Bianco AE (2000) Two microsatellite loci from Brugia malayi show polymorphisms among isolates from Indonesia and Malaysia. Mol Biochem Parasitol 106:299–302

Unnasch TR, Williams SA (2000) The genomes of Onchocerca volvulus. Int J Parasitol 30:543–552

Vasuki V, Hoti SL, Sadanandane C, Jambulingam P (2003) A simple and rapid DNA extraction method for the detection of *Wuchereria bancrofti* infection in the vector mosquito, Culex quinquefasciatus by Ssp I PCR assay. Acta Trop 86(1):109–114

Vasuki V, Subramanian S, Hoti SL, Jambulingam P (2012) Use of a simple DNA extraction method for high-throughput detection of filarial parasite *Wuchereria bancrofti* in the vector mosquitoes. Parasitol Res 111:2479–2481

Wallace DC (1999) Mitochondrial diseases in man and mouse. Science 283(5407):1482–1488

Weerasooriya MV, Mudalige MP, Gunawardena NK, Kimura E, Samarawickrema WA (1998) Microfilarial periodicity of *Wuchereria bancrofti* and man landing periodicity of the vector Culex quinquefasciatus say in Matara, Sri Lanka. Ceylon Med J 43:78–83

Weil GJ, Ramzy RM (2007) Diagnostic tools for filariasis elimination programs. Trends Parasitol 23:78–82

Weil GJ, Lammie PJ, Weiss N (1997) The ICT Filariasis test: a rapid-format antigen test for diagnosis of bancroftian filariasis. Parasitol Today 13(10):401–404

Weil GJ, Curtis KC, Fischer PU, Won KY, Lammie PJ, Joseph H, Melrose WD, Brattig NW (2011) A multicenter evaluation of a new antibody test kit for lymphatic filariasis employing recombinant *Brugia malayi* antigen Bm-14. Acta Trop 120(Suppl 1):S19–S22

Werren JH (1997) Biology of Wolbachia. Annu Rev Entomol 42:587–609

WHO (1992) Lymphatic filariasis, the disease and its control. Fifth report of the WHO expert committee on filariasis. Tech Rep Ser 821:1–31

WHO (1995) World health report 1995. Bridging the gaps, Geneva

WHO (2002) Annual report on lymphatic filariasis 2001. Global programme to eliminate lymphatic filariasis. WHO/CDS/CPE/CEE/2002 28:1–8

WHO (2007) Global programme to eliminate lymphatic filariasis. Wkly Epidemiol Rec 82:361–380

WHO (2014) Global programme to eliminate lymphatic filariasis. Wkly Epidemiol Rec 89:409–420

Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Williams SA, Nicolas L, Lizotte-Waniewski M, Plichart C, Luquiaud P, Nguyen LN, Moulia-Pelat JP (1996) A polymerase chain reaction assay for the detection of *Wuchereria bancrofti* in blood samples from French Polynesia. Trans R Soc Trop Med Hyg 90:384–387

Williams SA, Lizotte-Waniewski MR, Foster J, Guiliano D, Daub J, Scott AL, Slatko B, Blaxter ML (2000) The filarial genome project: analysis of the nuclear, mitochondrial and endosymbiont genomes of *Brugia malayi*. Int J Parasitol 30(4):411–419

Zahner J, Hobom G, Stirm S (1995) The microfilarial sheath and its proteins. Parasitology today 11: 116–120

Zaman V, Narayanan E (1986) Differentiation of infective larvae of *Brugia malayi* and *Wuchereria bancrofti* by scanning electron microscopy. Ann Trop Med Parasitol 80:333–338

Zarlenga DS, Aschenbrenner RA, Lichtenfels JR (1996) Variations in microsatellite sequences provide evidence for population differences and multiple ribosomal gene repeats within Trichinella pseudospiralis. J Parasitol 82(4):534–538

Zhong M, McCarthy J, Bierwert L, Lizotte-Waniewski M, Chanteau S, Nutman TB, Ottesen EA, Williams SA (1996) A polymerase chain reaction assay for detection of the parasite *Wuchereria bancrofti* in human blood samples. Am J Trop Med Hyg 54:357–363

Zhu XQ, Gasser RB (1998) Single-strand conformation polymorphism (SSCP)-based mutation scanning approaches to fingerprint sequence variation in ribosomal DNA of ascaridoid nematodes. Electrophoresis 19:1366–1373

9 Transmission Dynamics of Diurnally Subperiodic Lymphatic Filariasis in the Andaman and Nicobar Islands

A. N. Shriram, K. Krishnamoorthy, and P. Vijayachari

Abstract

Diurnally subperiodic filariasis (DspWb) is confined to the Nicobar district of Andaman and Nicobar archipelago, India. Point studies have suggested that this type of lymphatic filariasis (LF) is prevalent in the Nancowry group of an archipelago with *Downsiomyia nivea* as the vector. In-depth studies were undertaken to quantify the transmission dynamics of LF in Nicobar district keeping in perspective its control and assessment of interventions, hitherto not known. Parameters that reflect the dynamics and intensity of transmission, i.e., the annual biting rate (ABR), annual infective biting rate (AIBR), annual transmission index (ATI), risk of infection index (RII), and annual transmission potential (ATP), were estimated. These estimates indicated that a person received 21,851 bites (ABR) of which 107 had harbored infective stage (L_3) larvae (AIBR). Each person stood at threat of getting a probable number of 22 L_3 larvae per year. Host efficiency index of *Do. nivea* showed that >40% of the microfilariae ingested developed into L_3 stages. ATP indicated perennial transmission. The sequential monthly transmission potential indicated that the force of transmission was high during summer. Persistent transmission of DspWb in the characteristic sylvatic ecology was noticeable with a high risk for transmission during summer. Although personal protection measures are the method of choice for risk reduction, in view of the adult behavior of the vector mosquito and larval ecology, conventional larvicidal measures are not applicable. Alternate methods of reducing the parasite load in the community are discussed in this chapter.

A. N. Shriram (✉) · P. Vijayachari
Department of Health Research, Ministry of Health & Family Welfare, Government of India, ICMR-Regional Medical Research Centre, Port Blair, India
e-mail: shriraman@icmr.org.in

K. Krishnamoorthy
Department of Health Research, Ministry of Health & Family Welfare, Government of India, ICMR-Vector Control Research Centre, Puducherry, India

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_9

9.1 Introduction

Wuchereria bancrofti, the causative agent, is disseminated by the preeminently polluted water breeding *Culex quinquefasciatus*, while *Brugia* filariasis is spread by the Mansonoid mosquitoes which breed in water bodies infested with hydrophytes. Dual infection of *W. bancrofti* and *Brugia* parasites has also been recognized in demarcated neighborhoods in Indonesia (Manguin et al. 2010).

Physiological deviations in *Wuchereria* and *Brugia* have implications in the epidemiology. Three physiological types in *Wuchereria* and one in *Brugia* are distinguishable, founded on the biological rhythm of microfilariae (*mf*) circulating in the peripheral human blood. The principal variant is the nocturnally periodic *W. bancrofti* (NpWb), dispersed in parts world over through the tropical and subtropical regions, where the *mf* appears during night in peripheral blood, which is spread by *Cx. quinquefasciatus* (a ubiquitous mosquito, preferring to breed in a wide range of polluted water bodies). The diurnally subperiodic *W. bancrofti* (DspWb), a second variant, is confined to the archipelago of southwestern Pacific and the Nicobars in the Bay of Bengal, where the *mf* is present in the peripheral blood throughout but appears in high numbers during the day. The nocturnally subperiodic *W. bancrofti* (NspWb), the third variant, has been described in south Asia, to be completely distributed alongside forested tracts adjoining the Thailand-Myanmar border, northern Vietnam, Sabah (Malaysian Borneo), and the Philippines (Harinasuta et al. 1970; Mak 1981; Meyrowitsch et al. 1998; Pothikasikorn et al. 2008). In the two subperiodic forms, the microfilariae (*mf*) circulate in the peripheral blood throughout the 24 h day with maximal counts in the late afternoon and early dusk hours (18.00–20.00 h) (Gould et al. 1982). Subsequently, Tewari and coworkers (1952) demonstrated that the counts of *mf* in the peripheral blood of humans were vastly inconsistent, with maximum at 18.00 h and minimum between 03.00 and 06.00 h. Whereas, in the third, i.e., the nocturnally periodic *B. malayi* (NpBm), the *mf* appears only during night in the circulating peripheral blood. This form is present in an isolated principal focus alongside central coastline in Kerala, and minor foci in six other states, toward the east coast in the peninsular India. These forms or in other words physiological races are transmitted by diverse vector mosquitoes. The *mf* periodicity of each of the physiological races corresponds to 24 h periodicity in the blood-feeding activity of the respective main vector mosquito. This occurrence is a physiological adaptation to the nocturnal biting activity of the principal vector mosquito.

Certain mosquito species of the genus Anopheles in the countryside or *Culex quinquefasciatus* in metropolitan cities are responsible for transmitting the NpWb (Buck 1991). While certain species of *Aedes*, *Downsiomyia*, and/or *Ochlerotatus* are the vectors of NspWb and DspWb, ubiquitous in the southeast Asia and western Pacific region. In India, both the NpWb and DspWb forms of lymphatic filariasis (LF) are established. The former, transmitted by *Cx. quinquefasciatus*, is widely prevalent in 20 states and union territories, while the latter form transmitted by *Ochlerotatus niveus* is limited to the remotely located Nancowry archipelago of the union territory of Andaman and Nicobar in India (Wilcock 1942; Basu 1958).

Consequent to the taxonomic changes, *Ochlerotatus* (*Finlaya*) *niveus* is presently known as *Downsiomyia nivea* (Reinert et al. 2004). This mosquito species and selected species of the group have been incriminated in the transmission of NspWb in Thailand (Gould et al. 1982). Information on transmission dynamics is central to formulate a focused strategy for the containment of vector mosquito or interrupt man-vector contact. Vector competency may perhaps afford cues on the levels of *mf* prevalence, which would enable to decide on the termination of the mass drug administration (MDA). In information generated on the indicators of transmission dynamics and force of transmission of filariasis, viz., annual biting rate (ABR), annual infective biting rate (AIBR), annual transmission index (ATI), risk of infection index (RII), and annual transmission potential (ATP) of *Do*. *nivea*, the vector of DspWb are analyzed and presented in this chapter.

9.2 Andaman and Nicobar Archipelago: Topography and Climate

The A and N archipelago (6–14^0 N lat. and 92–94°E long.) constitute three districts, viz., north, middle, and south Andaman and Nicobar, which comprises approximately 570 islands, which is inhabited by a population of 379,944 (2011 census). The Andaman district encamps the northward, while the Nicobar district positions the southward archipelago, respectively. The Nancowry archipelago in the Nicobar district (8.5–9.5°N lat. and 93–94°E long.) encompasses seven distantly situated islands, viz., Chowra, Teressa, Bompoka, Katchal, Kamorta, Nancowry, and Trinket. These seven islands are inhabited by 10,488 people (2011 census), predominantly by the indigenous Nicobarese race, who are at the threat of acquiring the DspWb infection.

The tremors beneath the ocean, which triggered the tsunami on the 26th of December 2004, produced widespread destruction in the Andaman and Nicobar archipelago, categorized as seismic zone 5, indicative of high level of threat perception toward tremors. The degree of disarrangement in the environment produced through tsunami is substantial from the post-tsunami reports. Approach level of seawater, i.e., exceeding normal tide/mean sea level, ranged between 1.4 and 7.0 meters in certain places. Besides the seawater incursion distance from the coast is extended between 100 and 250 meters (Ramanamurthy et al. 2005). Greater portion of the northern Andaman archipelago were elevated, whereas subsidence of landform between 1 and 1.5 meters was observed in South Andaman, viz., Port Blair and Ross Island (Bilham et al. 2005). The damage to humans and property holdings was maximal in Nicobar and lesser in Andaman district. This catastrophe dislocated the residence of the native Nicobarese from their traditional homestead, the Nicobarese hut (huts raised on wooden stilts), located on the coast and also in the dense jungles interspersed with coconut and areca nut groves. Reestablishment and restoration of the Nicobar group of islands were undertaken by the Andaman and Nicobar administration. Permanent shelters were constructed for rehabilitating Nicobarese tribal community. Currently, the native Nicobarese community resides in permanent

shelters. The other rehabilitation measures include provision of facilities like water, sanitation, electricity, restoration of approach roads, etc. Currently, each human dwelling/permanent shelter has at least 2–3 plastic tanks (commonly known as *Sintex* tanks), with a holding capacity of 500–1000 liters of water. The water meant for various domestic purposes is stored in these tanks, in addition to metal and plastic drums.

The mean minimum temperatures were extended between 22.97 °C (January) and 25.44 °C (March), while the mean maximum temperature was between 28.31 °C (January) and 32.36 °C (March). The relative humidity (RH) was high and reached between 72.9% in January and 87.0% during November. Both southwest and northeast monsoons result in substantial precipitation between May and November. During the other months, precipitation is normally less, with driest month being February. The precipitation fluctuated between 32.7 mm (March) and 351.1 mm (May). The earth is pervious with coral and sandy texture that instantly absorbs the rainfall and hardly renders any water standing. The key habitats that support the breeding of *Do. nivea* are the tree holes in the forested tracts of Nancowry archipelago (Tewari et al. 1995).

9.3 Transmission Dynamics of DspWb Vectored by *Downsiomyia nivea* Prior to LF Elimination Program

In-depth investigations were undertaken on transmission dynamics of DspWb through 12 monthly observations covering different periods during 1999–2000 (Shriram et al. 2008). The biting density (number of females/man-hour) of *Do. nivea* was found to range between 2.0 (December) and 8.1 (February), implying that biting was perennial with seasonal variations.

The mean number of L_3 (infective larva) per dissected mosquito is extended between 0 and 1.75 at different points of the year. During the period of study, the quantum of vector mosquitoes biting a person in 1 year (annual biting rate – ABR) was observed to be 21,851, of which 107 mosquitoes had harbored infective stage larvae-L_3 (annual infective biting rate – AIBR). Annual transmission potential (ATP), which conveys the quantum of exposure to the L_3 (infective stage) by a person in 1 year, was assessed to be 169. This suggested that 169 infective stage larvae were available with an estimated 107 vector mosquitoes, to be transmitted to a person.

It has been propounded by De Meillon et al. (1967) that a fraction of 0.414 infective stage larvae is likely to be deposited on the skin of the human during the act of blood feeding. Considering this analogy, the number of infective stage larvae expected to be released from the mosquito would be 70 per year. Different microclimatic elements have a far-reaching effect on the persistence of the infective stage larvae dropped on the skin (Lindsay et al. 1984). Experimental infection studies involving cats and *Brugia pahangi* have established that merely 32% of the dropped infective larvae could pierce through the host skin (Lindsay et al. 1984; Ewert and Ho 1967). Adapting these inferences to the progression of transmission of infective

stage larvae from the mosquito vector toward human host for *W. bancrofti*, the quantity of infective stage larvae that could have been deposited on the host skin and those could have penetrated into the human body may perhaps be 22.

The ABR or the overall number of *Do. nivea* biting a single individual per annum was predicted to be 21,851. Estimation of AIBR as 107 indicated that a person remained at the threat of acquiring 107 infective bites. The estimated measure of infective stages to which a person was exposed during the study period was 169. Investigations undertaken on the transmission dynamics of DspWb vectored by *Ae. polynesiensis* in Samoa, a persistent endemic focus, in the south Pacific islands indicated high estimates of the ABR (150268), the AIBR (968), and the ATP (3433). The abundance of *Do. nivea* coupled with infective stage larvae in the vector mosquito in the investigations undertaken in the Nancowry Island was about 1/seventh of that observed in *Ae. polynesiensis*. However, the total infective stage larvae available with the vector mosquito to infect human host were about 1/20th. Consequently, the setting in Nancowry Island had a relatively high microfilariae load (11.83%) (Shriram et al. 2002) and low transmission indices in comparison to the situation in Samoa with comparatively low (5.6%) parasitemia levels and high transmission indices (Samarawickrema et al. 1987). This may perhaps be due to superior vector competency of *Ae. polynesiensis* in comparison to *Do. nivea*. Information on vector competency is not available for *Ae. polynesiensis*, which limits confirmation.

The risk of infection intensity (RII) was 0.02332 in the Nancowry Island. While the monthly biting rate (MBR) was observed to be considerably different among the different time periods, the maximum MBR was noticed during the monsoon months (May–October). A comparable tendency in monthly transmission potential (MTP) was perceived. This means that MBR is an important element of the MTP. The threat of acquiring infective stage larvae through the bites of vector mosquitoes was existent right through the year, with the exception of March, July, and August. Transmission of infective stage larvae was detected to be truncated during the monsoon period (May–October) in comparison to summer (February–April) and cool (November–January) seasons of the time. The transmission potential was comparatively high during summertime. Overall, the rise and fall in the transmission potential was consistent to the MBR of the vector mosquito. The host efficiency index of *Do. nivea* was computed to be 0.43. This implies that slightly above 40% of the *mf* ingested may perhaps be sustained by *Do. nivea* toward developing into infective stage larvae. This competency of *Do. nivea* ranged between 0 and 0.88 during different time periods.

Field research with respect to transmission dynamics indicated that the RII was lower. However, the ATP was observed to be above threshold limits described for *Cx. quinquefasciatus*, vector of NpWb (Ramaiah et al. 1992). Host efficiency index for *Do. nivea* indicated that over 40% of the *mf* ingested developed to infective stage larvae. This proportion was comparatively higher than that observed in *Cx. quinquefasciatus* (0.329 and 2.830), meaning that *Do. nivea* showed higher efficiency in sustaining and supporting the development of the parasite. Therefore, transmission of DspWB with *Do. nivea*-*W. bancrofti* vector-parasite combination is expected to be persistent in the absence of intervention measure.

9.4 Strategy for LF Elimination

A program was initiated to realize one of the goals of National Health Policy of India (Ministry of Health and Family Welfare, GOI 2002) to eliminate LF by 2015. Currently, the National program is realigning in consonance with the Global Programme to Eliminate LF (GPELF) by 2020. The fundamental plan is to dispense mass single dose of DEC in combination with albendazole (alb) to all the eligible persons every year for a minimum period of 4–5 years. Currently, 255 districts covering 20 states and union territories are under the ambit of the elimination program. In the Andaman and Nicobar archipelago, the program for LF elimination originated in 2004 by the Directorate of Health Services under the Andaman and Nicobar administration, indicating that DspWb LF would also attain eliminable levels. Ten rounds of MDA have been administered in the Nancowry Island, endemic for DspWb along with other islands endemic for NpWb. During the initial four rounds of MDA, DEC alone was used. Subsequently, DEC was co-administered with alb.

9.5 Vector Parasite Combinations Characterize Transmission Dynamics and Impact LF Elimination Programs

Control of LF is based on density-dependent constraints in the vector mosquitoes. The GPELF approach is centered on MDA with DEC + alb and either DEC or ivermectin to decrease the *mf* load in the community to below a threshold level, in order to interrupt transmission by the intermediate hosts. The justification for backing this approach is based on the research findings on vector-parasite combinations that are fundamental in assessing the vector competency in *mf* uptake and transmitting infective stages at diminishing parasitemia load in the community (Gyapong et al. 2005).

These vector-parasite groupings are illustrated under the density-dependent processes of "Facilitation" and "Limitation" (Brengues and Bain 1972). Facilitation is the process where, less than a definite threshold level of microfilariae, commonly referred to as Webber's critical point (Pichon 2002), the transmission of infective stages by anopheline vectors gets interrupted (Pichon et al. 1974; Southgate and Bryan 1992; Webber 1991). Whereas, limitation signifies a process wherein even at low *mf* levels there is steady transmission, as evidenced in *culicines* (Subramanian et al. 1998; Duerr et al. 2005). While, in "Proportionality," there remains a continual proportion of *mf* ingested by the vector during the act of blood feeding which progresses to the development of infective stage larvae, this phenomenon demonstrates a linear relationship between uptake of *mf* and the development of infective stage larvae. Limitation and Facilitation in vectors cause deviations from this linear relationship (Duerr et al. 2005).

Natural series of changes in "Limitation" are associated with the fact that one affects the other, implying that the number of parasites per mosquito cannot increase for unspecified span of time. The association between *mf* intake and L_3 yield is

linear at the onset and approaches an equilibrium or could decline with increasing *mf* intake, due to extra mortality of vectors resulted by ingestion of too many *mf* (Duerr et al. 2005; Dietz 1982). In Limitation, there is a maximal threshold lower than which the limited process is "overefficient" and above which it is "underefficient" (Duerr et al. 2005). Therefore, in limitation, elimination of LF is considerably weakened by changing transmission thresholds toward reduced values, necessitating impetus for additional control strategies.

9.6 Conclusions

Infection prevalence is on the decline due to mass drug administration (MDA) of antihelminthic drugs coordinated by the Directorate of Health Services, Andaman Nicobar administration under the *aegis* of the NVBDCP (Shriram et al. 2014b). This single-dose administration of antihelminthic drugs targets *mf* in the bloodstream and consequently impedes transmission to mosquitoes and has implication on the parasite load in mosquito vector. Except one island, all other islands in the Nancowry have recorded *mf* prevalence >1%, indicating persistence of infection. Elsewhere, spatiotemporal clustering and persistence of microfilaremics have been reported in settings where the infection is transmitted by *Aedes polynesiensis* (Joseph et al. 2011). In such settings, the receding transmission levels could pose challenge to the control measures (Snow et al. 2006). Pre-MDA observations in *Do. nivea* have indicated the operation of density-dependent mortality of vectors or parasite loss or a combination of both, implying comparable level of parasite regulation in the vector mosquito which has parallel effect on the transmission threshold (Shriram et al. 2014a). Thus, the consideration of *Aedes* with *Culex* for deducing the critical level of antigen positive for arriving at decisions on cessation of mass drug administration (MDA) is justifiable. However, in order to reach the goal of elimination, numerous challenges need to be addressed. Post-MDA observations on the infection prevalence (Shriram et al. 2014b) provide insights to maximize the control measures with alternate or supplementary control strategies, as additional control pressure to accelerate the elimination process of DspWB from the Andaman and Nicobar archipelago. Prevailing situation in Nancowry archipelago provides a unique opportunity to demonstrate the administration of DEC fortified salt approach for eradicating the lone foci of diurnally subperiodic bancroftian filariasis from the country (Shriram et al. 2011).

References

Basu PC (1958) A note on malaria and filariasis in Andaman and Nicobar. Bull Nat Soc India for Mal and other Mosq Borne Dis 6:193–206

Bilham R, Engdahl ER, Feldl N, Satyabala SP (2005) Partial and complete rupture of the Indo-Andaman plate boundary 1847-2004. Seism Res Lett 76:299–311

Brengues J, Bain O (1972) Passages de microfilaires vers l'hémocèle du vecteur, dans les couples *Wuchereria bancrofti – Anopheles gambiae*, *W. bancrofti – Aedes aegypti* et Setaria

labiatopapillosa – *A. aegypti*. Cahiers ORSTOM, Série d'Entomolgie Mèdicale et Parasitologie 10, 235–249

Buck A (1991) Filariasis. In: Strickland TG (ed) Hunter's tropical medicine, vol 1, 7th edn. W.B. Saunders Company, Baltimore, 153 p

De Meillon B, Hayashi S, Sebastian A (1967) Infection and reinfection of *Culex pipiens fatigans* with *Wuchereria bancrofti* and the loss of mature larvae in blood feeding. Bull World Health Organ 36:91–100

Dietz K (1982) The population dynamics of onchocerciasis. In: Anderson RM (ed) The population dynamics of infectious diseases: theory and applications. Chapman & Hall, London, pp 209–241

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

Ewert A, Ho BC (1967) The fate of *Brugia pahangi* larvae immediately after feeding by infective vector mosquitoes. Trans R Soc Trop Med Hyg 61:659–662

Gould D, Bailey CL, Vongpradist S (1982) Implication of forest mosquitoes in the transmission of *W. bancrofti* in Thailand. Mosq News 42:560–564

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

Harinasuta C, Sucharit S, Deesin T, Surathin K, Vutikes S (1970) Bancroftian filariasis in Thailand, a new endemic area. Southeast Asian J Trop Med Health 1:233–245

Joseph H, Moloney J, Maiava F, McClintock S, Lammie P, Melrose W (2011) First evidence of spatial clustering of lymphatic filariasis in an Aedes polynesiensis endemic area. Acta Trop 120S:39–47

Lindsay SW, Denham DA, Mc Greevy PB (1984) The effect of humidity on the transmission of *Brugia pahangi* infective larvae to mammalian hosts by *Aedes aegypti*. Trans R Soc Trop Med Hyg 78:19–22

Mak JW (1981) Filariasis in Southeast Asia. Ann Acad Med 10:112–119

Manguin S, Bangs MJ, Pothikasikorn J, Chareonviriyaphap T (2010) Review on global co-transmission of human Plasmodium species and *Wuchereria bancrofti* by *Anopheles* mosquitoes. Infect Genet Evol 10(2):159–177

Meyrowitsch DW, Nguyen DT, Hoang TH, Nguyen TD, Michael E (1998) A review of the present status of lymphatic filariasis in Vietnam. Acta Trop 70:335–347

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(Suppl 2):S143–S152

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

Pothikasikorn J, Bangs MJ, Boonplueang R, Chareonviriyaphap T (2008) Susceptibility of various mosquitoes in Thailand to nocturnal subperiodic *Wuchereria bancrofti*. J Vector Ecol 33:313–320

Ramaiah KD, Das PK, Arunachalam N, Rajavel AR, 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

Ramanamurthy MV, Sundaramoorthy S, Pari Y, Ranga Rao V, Mishra P, Bhat M, Usha T, Venkatesan R, Subramanian BR (2005) Inundation of sea water in Andaman and Nicobar Islands and part of Tamil Nadu coast during 2004 Sumatra tsunami. Curr Sci 88:1736–1740

Reinert JF, Harbach RE, Kitching IJ (2004) Phylogeny and classification of Aedini (Diptera: Culicidae) based on morphological characters of all life stages. Zool J Linn Soc 142:289–368

Samarawickrema WA, Kimura E, Spears GFS, Penaia L, Sone F, Paulson GS et al (1987) Distribution of vectors, transmission indices and microfilaria rates of sub-periodic *Wuchereria bancrofti* in relation to village ecotypes in Samoa. Trans R Soc Trop Med Hyg 81:129–135

Shriram AN, Murhekar MV, Ramaiah KD, Sehgal SC (2002) Prevalence of diurnally subperiodic bancroftian filariasis among the Nicobarese in Andaman and Nicobar Islands, India: effect of age and gender. Tropical Med Int Health 7:949–954

Shriram AN, Krishnamoorthy K, Sehgal SC (2008) Transmission dynamics of diurnally subperiodic lymphatic filariasis transmitted by *Ochlerotatus* (Finlaya) *niveus* in the Andaman & Nicobar Islands. Indian J Med Res 127:37–43

Shriram AN, Krishnamoorthy K, Saha BP, Roy A, Kumaraswami V, Shah WA, Jambulingam P, Vijayachari P (2011) Diurnally sub periodic filariasis in India-prospects of elimination: precept to action? Parasitol Res 109(5):1–8

Shriram AN, Krishnamoorthy K, Vanamail P (2014a) Survival of diurnally sub periodic *Wuchereria bancrofti* in *Downsiomyia nivea* (Diptera: Culicidae): a density dependent factor from Andaman & Nicobar Islands. Indian J Med Res 139:167–173

Shriram AN, Krishnamoorthy K, Sivan A, Saha BP, Kumaraswami V, Vijayachari P (2014b) Impact of MDA and the prospects of elimination of the lone focus of diurnally sub periodic lymphatic filariasis in Nicobar Islands, India. Acta Trop 133:93–97. https://doi.org/10.1016/j.actatropica.2014.02.004

Snow LC, Bockarie MJ, Michael E (2006) Transmission dynamics of lymphatic filariasis: vector-specific density dependence in the development of *Wuchereria bancrofti* infective larvae in mosquitoes. Med Vet Entomol 20:261–272

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

Subramanian S, Krishnamoorthy K, Ramaiah KD, Habbema JDF, Das PK, 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

Tewari SC, Hiriyan JH, Reuben R (1995) Epidemiology of sub periodic *W. bancrofti* infection in the Nicobar Islands, India. Trans Roy Soc Trop Med Hyg 89:163–166

Webber RH (1991) Can Anopheline-transmitted filariasis be eradicated? J Trop Med Hyg 94:241–244

Wilcock C (1942) Medical organization and diseases of Andaman and Nicobar Islands. Abst Trop Dis Bull 41:703–708

Success Story and Challenges Faced to Achieve 'Elimination of Lymphatic Filariasis' Status in Tamil Nadu

10

S. Elango

Abstract

Tamil Nadu in South India is pioneer in Public Health Programme Innovations. Its health status indicators are better than other states in India. Lymphatic filariasis elimination (ELF) strategies initiated and piloted in Tamil Nadu. Thirteen districts and few wards in three corporations including Chennai are endemic for LF. Tamil Nadu government has many models in prevention and control of VBDs with innovative measures like constituting a state-level advisory committee, establishment of the Institute of Vector Control and Zoonoses (IVCZ) at Hozur, developing web-based surveillance system for vectors and VBDs (EDISIS), and conducting more than 10 MDAs for ELF. Tamil Nadu is the only state which has recognized lymphoedema as a disability and granted disability pension of Rs1000/PM for grade III and IV LF cases besides morbidity management. The public health department had faced a lot of challenges, but all of them had been overcome with the success of elimination of LF well ahead of other states in India in 2004 itself.

10.1 Introduction

Lymphatic filariasis is a scourge of civilization for thousands of years. It is a highly debilitating and stigmatizing disease caused by thread-like parasitic worms known as *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori*. While only *W. bancrofti* and *B. malayi* prevail in India (former being predominant), *B. timori* exists in Indonesian islands. They live exclusively in humans for 4 to 6 years producing millions of microfilariae (mf) that circulate in the blood and are picked up by a hoard of

S. Elango (✉)
Department of Community Medicine, Trichy SRM Medical College Hospital & Research Center, Tiruchirapalli, TN, India

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_10

mosquito species depending on geographical distribution of a specific parasite but mainly by the *Culex quinquefasciatus* mosquitoes in the Indian subcontinent and are transmitted to healthy humans. These worms lodge in lymphatic channels causing blocks in the lymph flow and oedema (Elango 2012).

The disease was recorded in India as early as sixth century BC by the famous Indian physician Sushruta who described about it in his book *Sushruta Samhita* (Bhasker et al. 2000). In seventh century AD, Madhava-kara described the signs and symptoms of the disease in his treatise, *Madhava Nidana*, which hold true even today. In 1709, Clarke called the elephantoid legs in Cochin as 'Malabar legs'. The discovery of microfilaria (mf) in the peripheral blood smear was first made by Lewis in 1872 in Calcutta (now Kolkata).

Lymphatic filariasis is the leading cause of permanent and long-term disability worldwide; the parasitic infection imposes a severe physical and socioeconomic burden in 81 countries in the world. The disease has achieved historical importance because it has been neglected at all levels. Since symptoms are not acute and painless, hence patients neglect the disease. The patients come to the hospital very late; therefore the health-care provider could also neglect this. The governments neglect the disease because the politicians give least importance to a disease that only disables and does not kill. The NGOs neglect the condition because they do not get any fund from the funding agencies and do not get any publicity. Thus lymphatic filariasis tops the list of the neglected tropical diseases (NTDs) in a true sense.

10.2 Burden of Lymphatic Filariasis

10.2.1 Global and SEA Region Burden

The World Health Organization (WHO) estimated that 1.1 billion people live in high-risk areas. Of these 120 million people are already infected with LF, and 76 million have suffered from damaged lymphatic and renal systems (WHO 2007). About 22 million children below 15 years of age are infected with the disease (Ottesen 2000). It is estimated that there are about 700 million people living in the endemic areas in South East Asia region constituting about 64% of the global burden with 60 million persons harbouring microfilaraemia and suffering from clinical manifestation, which constitute about half of the global figure. *Culex quinquefasciatus* is the principle vector in continental Asia for *W. bancrofti*, Mansonia species transmit *B. malayi* in peninsular India (Kerala State) and other countries in south Asian region, Some of the Anopheles species have also been implicated as also those of Aedes (*Ochlerotatus niveus*) in island situations in the southeast Asian region.

10.2.2 LF Burden in India

LF is endemic all over India except Jammu and Kashmir, Himachal Pradesh, Punjab, Haryana, Delhi, Rajasthan, Nagaland, Manipur, Tripura, Meghalaya, Sikkim, Arunachal Pradesh, Mizoram, and Dadra and Nagar Haveli. Lymphatic filariasis is highly endemic in UP, Bihar, Jharkhand, Andhra Pradesh, Telangana, Odisha, Tamil Nadu, Kerala, and Gujarat. An estimate of 600 million people are at risk of lymphatic filariasis in 250 endemic districts in 20 states/union territories in India. Morbidity survey (2012) shows 8 lakh cases of lymphoedema and 4 lakh cases of hydrocele. The *mf* survey report of 205 districts reveals the Mf rate as 0.45%.

10.2.3 LF Burden in Tamil Nadu

Lymphatic filariasis (LF) survey conducted in all the districts of Tamil Nadu found that coastal districts are endemic for filariasis. The topography, temperature, and humidity as well as mosquitogenic conditions are conducive for the breeding of the vectors in these areas. Therefore, the LF cases are mostly reported in these districts and adjacent districts in the eastern side than in the hilly western and central zones of Tamil Nadu. Thirteen districts are endemic, and almost all the blocks are reporting cases. These districts are Kanchipuram, Tiruvallur, Nagapattinam, Vellore, Thiruvarur, Thanjavur, Tiruvannamalai, Villupuram, Cuddalore, Tiruchirappalli, Perambalur, Pudukkottai, and Kanyakumari. In addition, 64 wards in Chennai Corporation and few primary health centres (PHCs) in Tirunelveli, Thoothukudi, Viruthu Nagar, Madurai, Krishnagiri, and Karur districts have also been identified as endemic to LF. Thus, in total, 187 blocks, 7250 villages, 763 primary health centres, 4207 health sub-centres, 243 town panchayats, 71 municipalities, and some wards in 3 municipal corporations of Chennai, Tiruchirappalli, and Vellore are considered endemically affected, with a total of 31 million population at risk of infection with lymphatic filariasis.

10.3 LF Control and Elimination Initiatives

The strategy of filarial control was initiated as a first filarial project in 8 villages in Odisha from 1949 to 1954. Based on the results, the National Filarial Control Programme (NFCP) was launched in India in 1955. In Tamil Nadu, diethylcarbamazine (DEC) Health Salt programme was carried out in Kiliyur village in Villupuram district. The DEC-fortified salt was distributed through active and passive vendors in 1989. The *mf* rate was brought down to 0.16% in 1992 and 0 in 1994. The study had proved it as a low-cost technology in breaking the transmission of LF. It was extended to Kanyakumari district in 1995. Unfortunately the programme was forced to wind up in 2001 because of operational problems.

In 1997, the World Health Assembly resolution 50.29 encouraged member states to eliminate lymphatic filariasis as a public health problem and launched its Global Programme to Eliminate Lymphatic Filariasis (GPELF). In Tamil Nadu, under NFCP, 25 control units (CU) and 44 filaria night clinics (FNC) were started with headquarters at Chengalpattu, Chidambaram, Vellore, Kumbakonam, and Nagercoil districts. Line-listing and mapping of LF cases have been carried out from 2006 onward, and every year it was updated. The total number of cases listed was 52022, of which 40572 were lymphoedema and 11450 cases were hydrocele.

Tamil Nadu is the pioneer in mass drug administration (MDA), launched in Cuddalore district in 1996, for the first time in the country. Later on, MDA was extended in other filarial endemic districts in Tamil Nadu from 1998 onward. The Government of India funded programme was launched in 2004. In the pilot project, only a single drug, DEC alone, was administered to the people under the programme. The combined DEC plus albendazole was given in six districts from 2001 onward, and the double-drug administration under MDA programme was continued in the subsequent years also. In 2005 and 2006, MDA was not done in Tamil Nadu because of some operational reasons, and in 2013 MDA was stopped since the goal of ELF was already achieved well ahead of the target year, 2015. Validation process was initiated through transmission assessment survey (TAS) in 2012.

10.4 Landmark Achievements in Tamil Nadu

The Tamil Nadu Government has issued a government order G.O.(D) No.1367 dated 8.10.1998, a milestone as far as vector-borne diseases are concerned, constituting a state-level advisory committee to submit a report to the government on the measures to be taken for the abatement of mosquito nuisance and prevention and control of vector-borne diseases (VBDs) in the state. The convener of the committee was Dr. N.C. Appavoo, the then Additional Director of Malaria and Filaria, while Prof. A.V. Sadanand, the then Chief Entomologist at the directorate, was the Chairman, ably joined by Thiru K. Gajapathi, the then Chief Entomologist of Chennai Corporation. The committee had submitted its report in September 1999 with many constructive recommendations.

Tamil Nadu is the only state which established with the state budget a specialized institute in 1987 at Hosur in Dharmapuri district known as Institute of Vector Control and Zoonotic (IVCZ) diseases. The objectives of the institute are training, research epidemic and entomological investigation, surveillance, and production of diagnostics. It is also a nodal agency for disseminating knowledge about plague. Under the elimination of lymphatic filariasis (ELF) programme, the IVCZ is a partner in task force.

In 2007, when the author was the Additional Director of public health and preventive medicine (Malaria and Filaria), he developed an effective disease

surveillance information system (EDSIS) which is a web-based monitoring system that includes as its main components the vector density and the incidence of VBDs. This system worked to facilitate the department in obtaining prompt and accurate information on disease occurrence from various reporting units throughout the state such as the hospitals, PHCs, and local bodies. Being a web-based system, the process of data collection, compilation, and collation was faster, making the information from various nooks and corners of the state instantly available on the Internet for reference and execution for updates at the point of origin thereby facilitating effective surveillance. User interface is simple and pro forma-based so that novice users can handle the system without any difficulties. Daily and weekly surveillance data are collected from the reporting units.

10.5 MDA in Tamil Nadu

The MDA programme with special inbuilt strategies was launched in the endemic districts. Information, education, and communication (IEC), drug distribution, and management of adverse events followed by the drug consumption were thrust areas in the MDA. Political leaders and decision-making top level officials' commitment and their participation in the programme are a great advantage in accomplishing a huge task like NPELF. Ministers, elected representatives like MPs (Members of Parliament), MLAs (Members of Legislative Assembly), and Collectors had set an example by consuming the antifilarial tablets in front of the public and media to infuse confidence in people for effectively implementing the programme. The patients suffering from lymphoedema themselves came forward as living victims to motivate the public to consume the drugs during the MDAs. The MDA was implemented throughout the state in a single day as a pulse. Task force committees and rapid response teams (RRTs) were formed at the state level, district level, and block level to supervise and monitor the MDA programme. Micro plan developed at various levels with effective monitoring and execution was the key for the success of the programme in Tamil Nadu.

10.6 Legal Tools

Tamil Nadu enacted and enforced certain acts like (1) Tamil Nadu Public Health Act 1939, (2) Tamil Nadu Panchayat Act 1994, and (3) Tamil Nadu Local Board Act 1988, which are considered unique and legal weapons to prevent breeding of the vectors of various VBDs. Provisions were made in these acts to inspect any premises and prosecute and fine the defaulter-resident people who were responsible for creating mosquitogenic conditions, thereby accentuating likelihood of intensive transmission of various diseases including lymphatic filariasis.

10.7 Coordination

Tamil Nadu is fortunate in having the ICMR Centre for Research in Medical Entomology at Madurai (CRME) and Vector Control Research Centre at Puducherry (VCRC), both of which have been very supportive to the health department with their several path-breaking research programmes (Tyagi 2015, Sabesan et al. 2000, Ramaiaha et al. 2000), incorporating strong translational value as well handing over their findings and products to the government for facilitating implementation of the LF elimination programme. Tamil Nadu government, on their part, permitted the public health department to extend full administrative and technical support for their research. The Directorate of Public Health and Preventive Medicine supported the two ICMR Centres to carry out research activities on lymphatic filariasis in Villupuram and Cuddalore districts, like the use of expanded polystyrene (EPS) beads, modification of cess pits, and use of pyrethroid impregnated curtains at Tirukoilur (cf. Tyagi 2015).

10.8 Care of the LF Patients

Morbidity management for lymphoedema cases with heat therapy, compression therapy, and interferential therapy is offered in the filarial clinics. Patients get relieved of the pain, and there is considerable reduction in the swelling. Patients were trained for self-care practice by specially trained team in the endemic districts. Free morbidity management kits for self-care practice and food hygiene were provided to the patients free of cost. Hydrocelectomy was also carried out in taluk hospitals, district hospitals, medical college hospitals, and also some private hospitals. The patients were given remuneration to compensate their wages during the period of hospitalization.

10.9 Cash Benefit to Grade III and IV Lymphoedema Cases

Financial assistance in the form of cash benefit to the tune of ₹ 1000 (one thousand) is given to the Grade III and IV lymphoedema cases equating the disability at par to other orthopedically/physically challenged people getting their pension. This initiative was an achievement of the author who (as the Director of the Department of Public Health and Preventive Medicine) proposed it to the government. Tamil Nadu is the only government in India extending this ‘LF disability pension’ for the lymphoedema cases from the state budget.

10.10 Leaders of ELF in Tamil Nadu

Some of the leaders who had served and contributed enormously for the elimination of lymphatic filariasis (ELF) in Tamil Nadu are, among others, namely, Dr. V. K. Kabali, Dr. A. Ramalingeshwara Rao, Dr. E. S. Ragavendra, Dr. N. C. Appavoo, Dr. S. Elango, Prof. A. P. Sadanand (Chief Entomologist (CE)), Thiru Ramadas (CE), Thiru Sarangapani (CE), Thiru Nagurpichai (CE), Thiru Murugesan (CE), Thiru Madhavan (CE), Thiru Srither, Dr. Kesavan (Filaria Officer), and Thiru Gajapathy (CE, Chennai Corporation).

10.11 Challenges Faced in Implementation

10.11.1 Rejection, Refusal, and Insults to the Field Workers

During the implementation of night blood surveys in the 1980s, the author went along with the field staff to take night blood samples after 10 PM. There was rejection, refusal, and abuse of the workers stating as 'night vampires' – coming for sucking the blood. In spite of such hurdles and insults, the field staff worked hard to ensure effective prevention and control of lymphatic filariasis under the ELF programme in Tamil Nadu.

10.11.2 Urban Areas

The public health department had a separate wing for malaria and filariasis control under the charge of an Additional Director (Malaria and Filaria) and a Joint Director, ably assisted by a team of senior entomologists at the headquarters, as well as the zones and the districts. This is the strength of the public health department government of Tamil Nadu who have succeeded in eliminating the disease. The World Bank (WB) and National Vector Borne Diseases Control Programme (NVBDCP) teams who visited Kerala in 2006 during the outbreak of Chikungunya have openly lauded the efforts of Tamil Nadu's public health department in tackling the vector-borne diseases. Nevertheless, public health department in Tamil Nadu is facing an upcoming challenge in the urban local bodies like corporation and municipalities since the department has to fight out disease outbreaks from the rural budget.

10.11.3 Insecticides and Larvicides

Besides vector resistance the work force faces insecticide toxicity in the long run. There is environmental pollution by indiscriminate use of the insecticides. Pesticides used in the agricultural sector are also a big challenge to the ecosystem causing vector resistance which includes the LF vectors.

10.11.4 Drug Contamination and Quality

During 2004 the MDA programme was stopped because five deaths were reported in Perambalur and Tiruchirappalli districts. High-level investigations were carried out including Central Bureau of Investigation (CBI) enquiry, and drug contamination was suspected. It was a very big challenge to overcome since such serious adverse events triggered strong criticisms from political circles making the hard-working officials as scapegoat for the loss of life and giving jolt to the implementation of the ELF programme. Another challenge was to ensure quality of drug under MDA since in one instance 3 million DEC tablets supplied for the MDA were sent back due to poor quality.

10.11.5 Approval and Maintenance of Equipment and Insecticides

Some of the insecticides and equipment acquired had poor quality and/or performance and posed a great challenge before outright rejection. Hasty and unsolicited purchase of some low-quality insecticides and equipment as well as machine by certain local bodies posed another challenge which was overcome by stringent adherence to the rules of purchase and storage before deploying it in the ELF programme.

10.12 Conclusion

In spite of various different constraints, Tamil Nadu was able to achieve ELF well ahead of other states in India considering the targeted year of ELF mentioned in the National Health Policy 2002 being 2015! There are no new cases of filariasis, and the *mf* rate of less than 1% was achieved as early as 2004 (*mf* 0.04). Microfilaria rate in Tamil Nadu in 2014 was 0.11% compared to the national average of 0.44% (NVBDCP 2015). Tamil Nadu has stopped MDA from 2014 since the transmission assessment survey (TAS) had asserted no active transmission in Tamil Nadu. However, drug distribution was carried out in a couple of blocks in Tiruvannamalai and Cuddalore districts in 2015. The MDA had brought about the following benefits in Tamil Nadu:

1. It has strengthened the health system by setting a national example of good governance in having reduced the *mf* rate far below the cutoff level (1%), much earlier than the country as a whole.
2. It collaterally reduced the burden of soil transmitted helminths (STH), and thereby anaemia among the children had come down.
3. The gap between the health-care provider and the community was minimized.

10.13 Recommendations

To sustain the benefits of ELF in Tamil Nadu at the national level, the following recommendations are offered:

1. Some of the recommendations in the report of the state level Technology Advisory Committee 1999 are yet to be implemented. For example, the teaching of medical entomology is being carried out to the undergraduate MBBS students in government medical colleges by the entomologists from the public health department. They are also members of the teaching faculty in the medical colleges. But posts of entomologists in private medical colleges are not being included. Therefore, in view of emerging and re-emerging vector-borne diseases, the private medical colleges need be instructed to have an entomologist post as a faculty in the department of community medicine to teach medical entomology and also to carry out surveys and research in vector-borne diseases.

   The author, as a senate member of the Tamil Nadu Dr. MGR Medical University, has made an agenda for recruiting an entomologist in the department of community medicine, and a favourable resolution was passed in 2013. The same type of resolution may be adopted in other medical and public health universities in India. Medical Council of India (MCI) should move forward in this direction in view of the increasing burden of VBDs in India.
2. All local bodies should have an entomologist's post like the Chief Entomologist, Senior Entomologist, and Junior Entomologist, to plan and implement the vector control measures and also national programmes.
3. All that equipment and insecticides should get certification from the competent national and state level experts in the public health department.
4. Adequate funds should be provided for research activities to be carried out in the medical colleges on vectors and vector-borne diseases. ICMR institutes may be allotted 2 to 5 medical colleges including private medical colleges for this purpose.
5. Every state should have its own institute for research on vectors and VBDs in the same way as Tamil Nadu has the Institute of Vector Control and Zoonosis (IVCZ).
6. Separate cess may be levied/ fined against people who are found responsible for vector breeding. This fund should be used only for control of VBDs in the state.
7. Comprehensive Public Health Act and Rules may be enacted and passed in the respective Assembly. Public health officers and entomologists may be empowered to enforce the act and rules. The act shall cover the area demarcation, zones, house plans, drainages, cesspools, and sewage tanks in the local area, etc.
8. All construction plans in the local area should be passed through the vector control officer and health officer in the local area.

References

Elango S (2012) Mass double drug administration is the key for elimination of LF in Tamil Nadu, India. ISO2-need of understanding the NTDs, Department of Microbiology and Research Cell, CMCH&RC

Ottesen EA (2000) Editorial: the global programme to eliminate lymphatic filariasis. Tropical Med Int Health 5(9):591–594. https://doi.org/10.1046/j.1365-3156.2000.00620.x

Ramaiaha KD, Das PK, Michael E, Guyatt HL (2000) The Economic burden of lymphatic filariasis in India. Parasitology Today 16(6):251–253

Sabesan S, Palaniyandi M, Das PK, Michael E (2000) Mapping of lymphatic filariasis in India. Ann Trop Med Parasitol 94(6):591–606

Tyagi BK (2015) The Tirukoilur Project. Centre for Research in Medical Entomology, Madurai, p 291

11 Prevalence of Lymphatic Filariasis in the Northeastern States of India, with Particular Reference to Assam and Prospects of Elimination

A. M. Khan, P. Dutta, S. A. Khan, and J. Mahanta

Abstract

India is committed for the elimination of lymphatic filariasis (ELF) by 2020. Efforts were made to achieve this goal across the country during the last 10 years or so, and data thus generated reflect both the success and weaknesses of the ELF programme. India is contributing highest load of Lymphatic Filariasis (LF) infection to the global scenario, thus facing a massive challenge to make ELF programme a success. In this endeavour, the implementation of single annual dose of diethylcarbamazine citrate (DEC) and albendazole (Alb), in more than 250 endemic districts of the country for 8–10 years in a row, speaks volumes about the daunting commitment of the Government of India towards this programme. Apart from ensuring consistency in delivery of drugs for LF, the mapping of the areas which are still not covered under mass drug administration (MDA) is also a challenge for the programme managers. Data on endemicity of the LF are lacking specially from northeastern states of India.

In the northeastern region, which comprises eight states, only Assam is known as the endemic state for Lymphatic Filariasis (Anonymous, 1991) and, therefore, mass drug administration for ELF is being implemented only in this state. Of the 35 districts of Assam state, at least seven are endemic for LF and received MDA. Though, after several rounds of MDA, declining trend of microfilaria (*mf*) rate had been noticed in many districts of Assam, however, few districts still continued to report *mf* rate higher than the cutoff level of 1%. Unlike many parts of the country, LF is not uniformly distributed in the endemic districts of Assam. The tea garden worker population reports high microfilaria carriers, while the non-tea garden population records significantly low *mf* rate. As a result a district may report *mf* rate less than 1% and not qualify for MDA, whereas the population

A. M. Khan (✉) · P. Dutta · S. A. Khan · J. Mahanta
Division of Entomology and Filariasis, ICMR-Regional Medical Research Centre, Northeastern Region, Dibrugarh, Assam, India

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_11

concentrated in the tea garden areas of the same district, which is sizeable in number, depicts *mf* rate as high as 5% even after several rounds of MDA (Khan et al. 2015). Therefore, ELF programme in northeast India needs a special attention to cover this vulnerable population with MDA for the success of the ELF.

11.1 Introduction

The causative agent of Lymphatic Filariasis (LF), Wuchereria bancrofti is the predominating species in Assam although *Brugia malayi* has been also reported in the Chabua tea estate of Dibrugarh, Assam. Documentary information about the presence of filariasis in tea gardens of Assam is available as early as the1940s (Rao 1942). In Bokakhat and Chabua, lymphatic filariasis caused by *W. bancrofti* and *B. malayi* was recorded during 1956–57 (Basu et al. 1957). Sasa (1976) published a detailed global survey on filariasis, indicating LF endemicity in Assam. Subsequently, a survey was conducted in Achabam Tea Estate, Dibrugarh, during 1982 by Assam Medical College and Hospital Dibrugarh and reported >16% microfilaria (mf) rate (*unpublished document*). The National Institute for Communicable Diseases (NICD), Delhi, also took cognizance of the above observations and conducted surveys during 1993 in Lakhipur village and adjoining tea estates in Southern Assam, reporting the presence of LF (Raina et al. 1993). Since 1995, ICMR- Regional Medical Research Centre, Dibrugarh, had been doing intensive surveys in different parts of Assam covering more than 10,000 populations (Dutta et al. 1995; Khan et al. 1998; Prakash et al. 1998; Mahanta et al. 1998; Khan et al. 1999a, b, c; Khan et al. 2004, Khan and Mahanta 2005; Khan et al. 2015). The literature retrieved on LF prevalence in the country's northeastern region reveals only the State of Assam as endemic for LF except reports from Sikkim (Singh et al. 2010) where *W. bancrofti* is prevalent in a village population.

The situation of LF in Assam is a little different from other states as prevalence of LF is not uniformly distributed among the districts especially in Upper Assam where plenty of tea estates are present and report high prevalence of LF. Therefore, the problem of LF distribution has to be tackled in the context of local situations and operational feasibility of the ELF programme. It is understood that on the national level, such stray foci bear enormous significance before proclaiming elimination of LF from the country.

11.2 Material and Methodology

11.2.1 Parasitological Survey

The authors and their team of ICMR-RMRC, Dibrugarh, have made comprehensive surveys during 1995–2015 on prevalence of LF in the State of Assam and two districts in the state of Tripura.

Fig 11.1 Prevalence of lymphatic filariasis across India during 1995

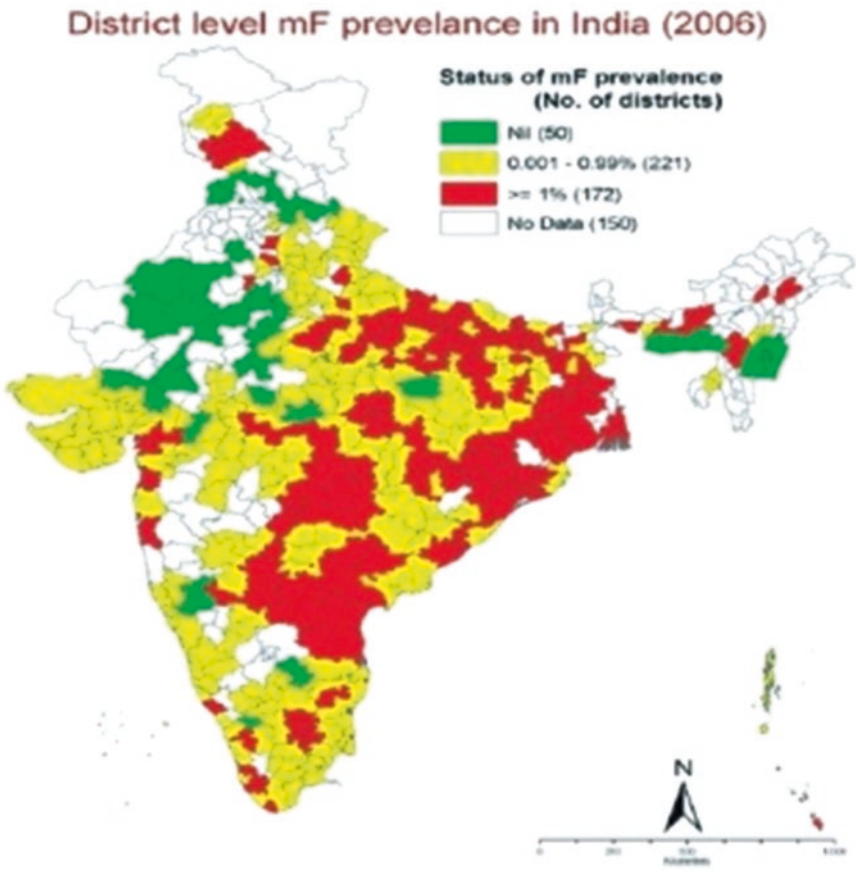


Fig 11.2 Prevalence of lymphatic filariasis across India during 2006

Selected tea garden worker's population and indigenous local population were surveyed between 20:00 h and 00:00 h by taking 20 mm^3 of finger-pricked blood and making thick smears on glass micro slides in house-to-house visits. Population coverage ranged from 15 to 35%. Both male and female subjects 2 years and above were screened. Thick blood smears were dried, de-haemoglobinized and fixed in methanol. After staining the blood smears in 10% Giemsa stain, microscopic observation was done. Blood smears found positive for microfilaria were identified using morphological characters and total number of the *mf* were enumerated (Fig. 11.5). The ICT kit was also selectively used in the LF survey. Microfilaria surveys have been done following ethical approval of Institutional Ethics Committee of ICMR-Regional Medical Research Centre, Dibrugarh. Also the information, education and communication (IEC) activities were done during each survey, and informed consent/assent has been taken from each participant included in the surveys.

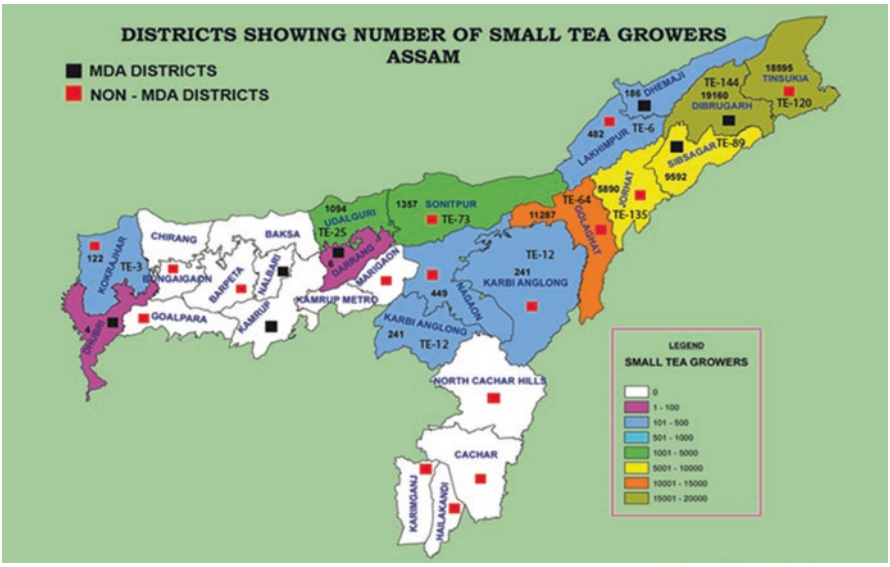


Fig. 11.3 Map of Assam showing distribution of tea estates and small tea growers. MDA and non-MDA districts are also shown

Fig. 11.4 (**a**) LF survey areas conducted by ICMR-RMRC, Dibrugarh (Assam), during 1995–2005; (**b** and **c**) Swirling Brahmaputra river which needed to be crossed over for accessing remote locations in Assam during LF surveys. (Source: A.M. Khan; Figs. 11.3b and c are the images of survey team including the team leader (Dr. A. M. Khan, author) proceeding to remote locations for survey work. The team consented for publication of images at Fig. 11.3b and c)

11.2.2 Entomological Survey

Entomological surveys were carried out in indoors during dawn hours for resting vector mosquitoes. In survey, manually operated aspirators, suction tubes and flash lights were used. Only *Culex quinquefasciatus*, the vector of LF, was collected (Fig. 11.6). Among the collected mosquitoes, females were identified and selected for dissection. Dissected mosquitoes were examined under microscope for presence of larval stages of the parasite. Several mosquitoes were found positive for different larval forms of the parasite (L_1, L_2 and L_3). Vector infection rate and infectivity rate were calculated for each survey using standard procedure (Khan et al. 2015).

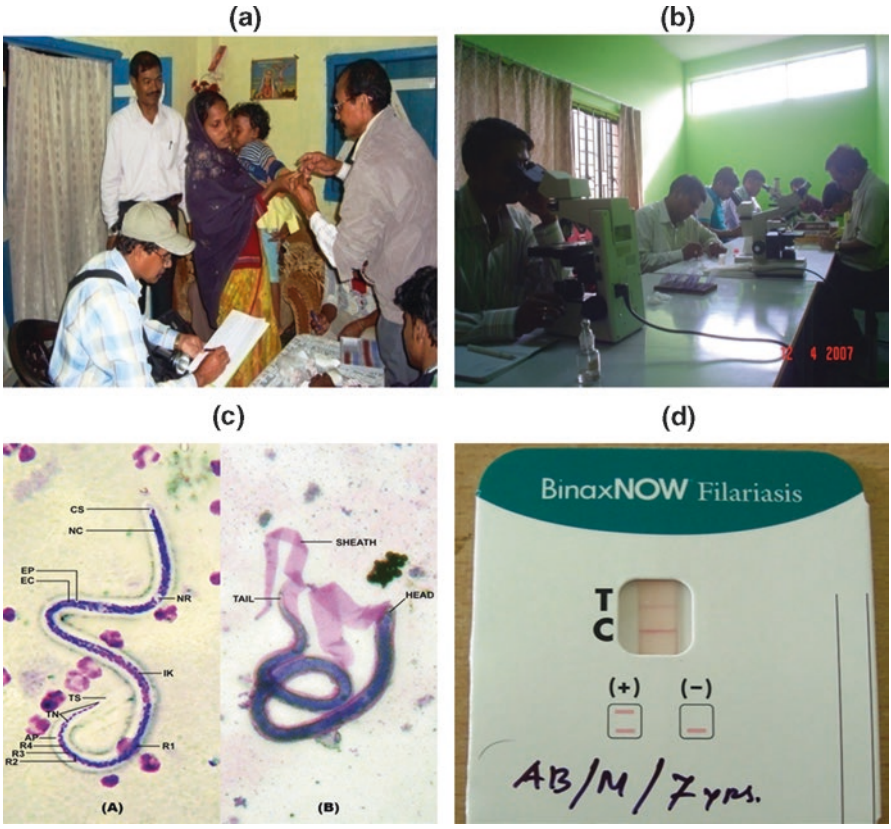


Fig. 11.5 (**a**–**b**) Community-aided surveys for microfilariae parasite. (*Wuchereria bancrofti*) during the late evening hours; (**c**) parasite in human blood and (**d**) ICT-aided diagnosis. (Source: A.M. Khan; Consents were obtained from the participants for publication of the images at Fig. 11.5**a** and **b**)

Fig. 11.6 The difficult forested terrains and human living conditions where entomological collections were routinely carried out with the help of local communities. (Source: A.M. Khan; Fig. 11.6: Consents were taken from the participants for publication of the above images)

11.3 Results

11.3.1 Parasitological Survey

LF survey locations, topography in tea garden areas (Nahartoli Tea Estate, Fig. 11.7), and status of microfilaria rate have been shown in Table 11.1.
Locations that were surveyed for microfilaria carriers include the following places:

1. Nahartoli TE (Dibrugarh, Upper Assam)
2. Achabam TE (Dibrugarh, Upper Assam)
3. Bokel TE (Dibrugarh Upper Assam)
4. Majgaon village (Dibrugarh Upper Assam)
5. Namrup TE (Dibrugarh Upper Assam)
6. Kalugaon village (Sibsagar, Upper Assam)
7. Bokakhat TE (Golaghat, Central Assam)
8. Hathikholi TE (Golaghat, Central Assam)
9. Koilamari TE (North Lakhimpur, North Bank of Brahmaputra)
10. Sonabheel TE (Sonitpur, Central Assam)
11. Birjhora TE (Bongaigaon, Lower Assam)
12. Sualkuchi Town (Kamrup, Lower Assam) and Binakandi TE (Cachar, Southern Assam)

Fig. 11.7 Tea garden topography and physiographic where microfilariae surveys were made

Table 11.1 Survey locations and microfilaria prevalence. (*Wuchereria bancrofti*) in the State of Assam

Site code	Males surveyed	Females surveyed	Total population surveyed	*mf* + ve	*mf* rate
1	664	795	1459	121	8.29
2	541	564	1105	74	6.7
3	379	400	779	92	11.81
4	397	424	821	9	1.1
5	403	443	846	22	2.6
6	325	331	656	31	4.73
7	271	175	446	8	1.79
8	647	466	1113	11	0.99
9	208	218	426	2	0.47
10	495	476	971	3	0.31
11	414	360	774	2	0.26
12	588	578	1166	0	0

Analysis of findings clearly indicated that most of the tea gardens of Assam were endemic for lymphatic filariasis, and microfilariae (*Wuchereria bancrofti*) occurred abundantly in the tea garden population, albeit the LF prevalence being generally low in the indigenous people. Microfilaria prevalence rate varied from 0.0 to 11.81%. While low microfilaria prevalence rate was recorded in tea gardens grown in foothill areas, appreciably high *mf* prevalence occurred in tea gardens located in plane areas (Khan et al. 1999c). Clinical manifestations included elephantiasis of the legs and hands as well as the presence of hydrocele (in males). Of these the most frequent manifestation was of course that of hydrocele. The rate of clinical manifestation varied between 0.99 and 5.4% which is considered pretty high for a largely nonendemic state. Both males and females were found affected by microfilariae, but adult males suffered more. The youngest microfilaria-positive child was only 4 years old. Clinical manifestation noted in the youngest subject was a 10-year-old male.

11.3.2 Survey in the State of Tripura

Out of the four districts (now eight) of the State of Tripura, two districts were surveyed for the presence of LF using ICT kit and Probability Proportional to Size (PPS) sampling techniques. We reported two clinical case of LF. However, we could not endorse presence of LF infection (microfilaria in human blood/infection in vector mosquito) in the surveyed districts.

11.3.3 Entomological Survey

In entomological collections and dissections, Culex quinquefasciatus was identified as the vector of lymphatic filariasis in the State of Assam. The man-hour density (MHD) of *Cx. quinquefasciatus* varied between 10.5 to 38.3. The vector density was found low

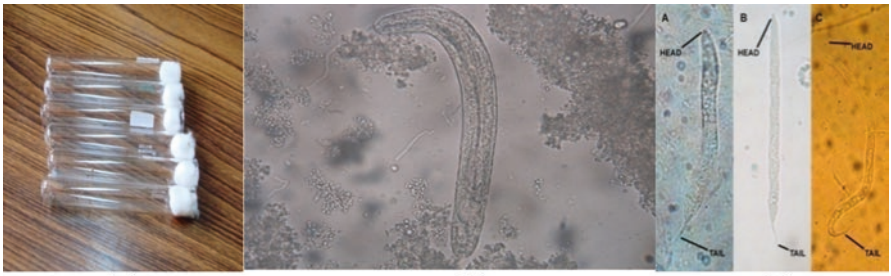


Fig. 11.8 (**a**) Sampled specimens of *Culex quinquefasciatus* and (**b–c**) larval stages of the parasite *Wuchereria bancrofti* isolated from the vector

in foothill tea gardens compared to that in plane areas. Presence of larval stages ($L_1/L_2/L_3$) of the parasite was recorded from *Culex quinquefasciatus* in many study areas (Fig. 11.8). However, vector infection rate, varying between 0 and 6.6%, was lesser in mosquitoes collected from foothill tea gardens compared to that in the planes.

11.4 Clinical Cases of LF

During parasitological surveys many clinical cases of LF were recorded. These infirmities mainly included elephantiasis of both legs. Elephantiasis of both limbs was observed although asymmetry was often noted in both sexes. The disease incapacitated severely inflicted patients as the enlarged foot could advance into the more debilitating form, the ADL (Fig. 11.9).

11.5 Chemotherapeutic Study

Surveys were carried out in tea garden settings in Assam during 2000–2006 for determining the effectiveness of diethylcarbamazine (DEC) (6 mg/kg body weight) through mass drug administration (MDA). The *mf* prevalence was significantly declined following six annual single-dose administration of DEC as depicted in the Fig. 11.10

After five annual rounds of DEC administration, nearly 90–95% *mf* clearance was noticed in the subject under study (Fig. 11.11).

Another significant observation of the effectiveness of DEC in clearing microfilariae in the endemic population was the reduction in geometrical mean intensity (GMI) achieved after mass DEC therapy when compared with pretreatment *mf* counts (Fig. 11.12).

Thus it became clear that DEC administration as an annual single dose, along with good compliance by the community, could bring down *mf* rate below 1% after five successful rounds of MDA.

Fig 11.9 (**a**–**g**) Various stages of asymmetrical developments of elephantoid legs in the affected population. (Source: A.M. Khan; Consents have been taken from the participants for publication of images at Fig. 11.9**b** and **d**)

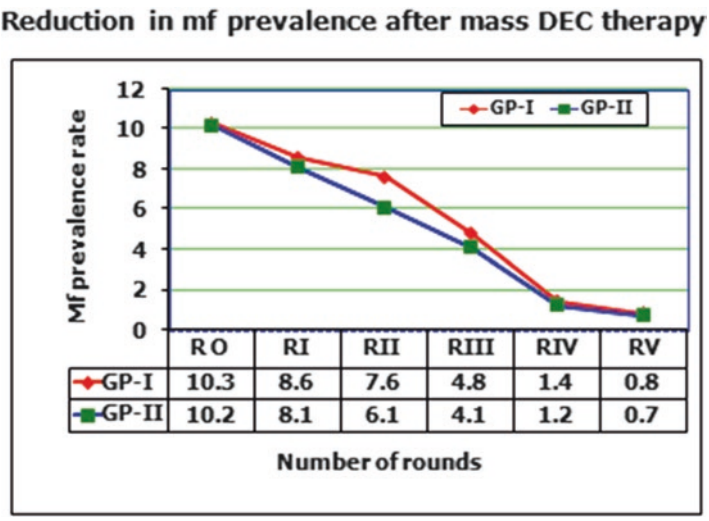


Fig. 11.10 Clearance of mf after five annual rounds of DEC administration

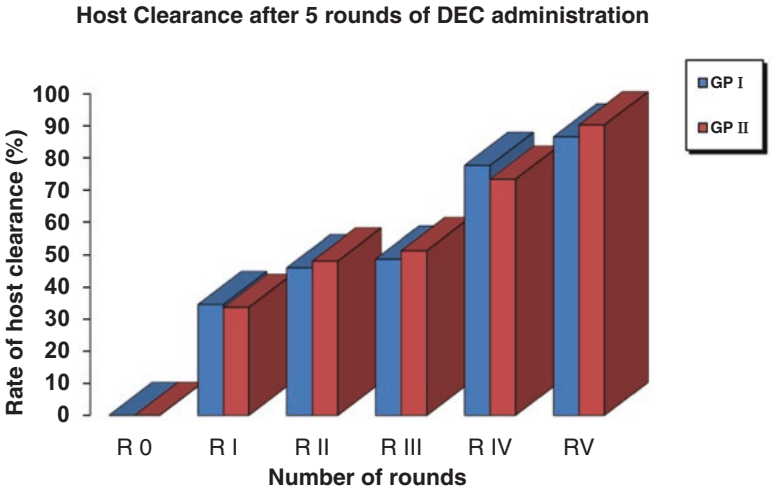


Fig. 11.11 More than 90% host clearance from microfilaria in the endemic population following DEC administration

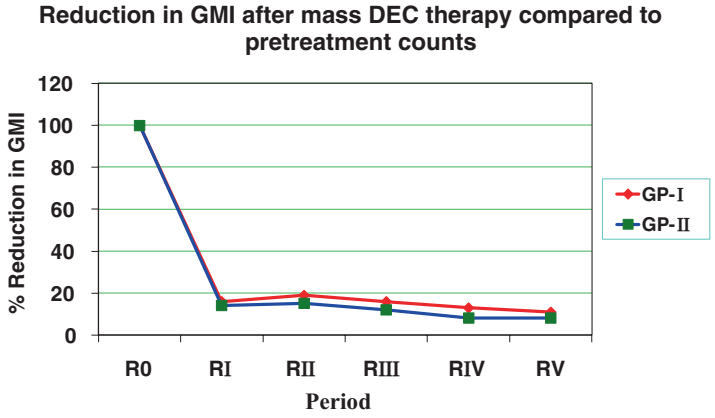


Fig. 11.12 Reduction in the GMI after annual DEC administration as compared to pretreatment counts

11.6 Mass Drug Administration

As per the Government of India policy, the ELF programme is being implemented once in a year generally in the month of September-November of each year. The implementation unit is the endemic district of the state. The first day is observed by house-to-house visit by the drug distributor where whole household is provided single dose of diethylcarbamazine citrate (DEC, 100 mg tablets 1/2/3 as per the age/

body weight of the participants) and albendazole (Alb, 400 mg). The following 2 days are observed as mop-up rounds to cover left-out population of the first day of drug distribution and ensure intake of drug.

Each year before MDA, the *mf* status is recorded by night blood survey to see the effectiveness of the MDA. After several rounds (five to six usually) of MDA assessment of antigenemia in children of 6–7 years of age are done in the implementation units reporting microfilaria rate below 1% in night blood surveys and if presence of antigenemia recorded was less than 1%, this outcome will finally initiate the WHO certification exercise (Fig. 11.13).

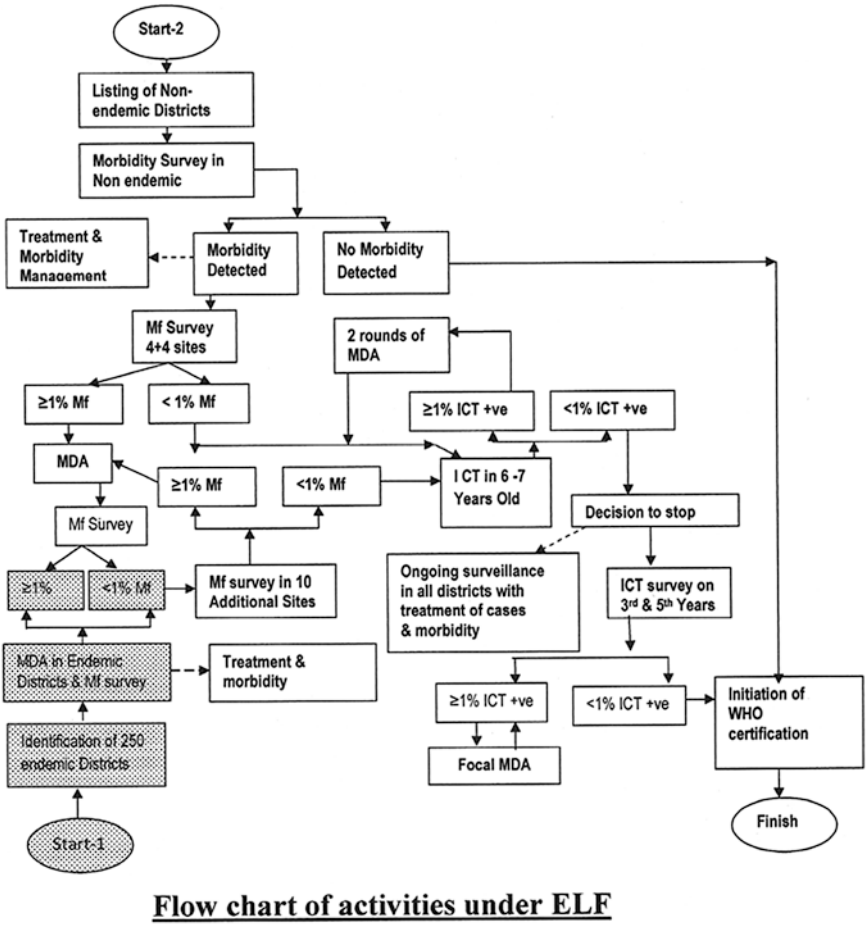


Fig. 11.13 Planned activities under the elimination of lymphatic filariasis in the northeastern states of India

11.7 Discussion

In the northeastern states of India, only Assam is endemic for lymphatic filariasis, though quite sporadically. Assam covers an area of about 78,438 sq. km and has a population of 31,169,272 (Government of India 2011 census data). The State is broadly divided into four regions, i.e. upper (eastern), central, lower (west and north) and southern Assam. Tea industries, which cover about 3.39% sq. km area in Assam, spread over the whole state and are one of the major sources of economy for the state. Tea estates, which were 848 in number till 1998, are now increased in numbers many times more with introduction of small tea growers, the latter largely as a result of turning of small farmers into tea growers because of encouragement and liberalization policy of Government of Assam (Anonymous 2004). Total quantum of workers in the State are more than 9.5 million of which 6.3% are engaged in tea industries (Anonymous 2004).

Globally, LF elimination goal is 2020 envisaged by World Health Organization (WHO). Many endemic countries including India are in the elimination programme globally (Ramaiah and Das 2004). Assam is one of the states of NE region of India where presences of bancroftian as well as brugian filariasis were recorded long back (Basu 1957; Sasa 1976). Since then, many surveys were conducted, and moderate to high prevalence of bancroftian filariasis were recorded (Dutta et al. 1995; Khan et al. 1999a, b, c; 2004). Because of the poor documentation, northeastern states and precisely Assam could not come into the limelight of the LF control programme until 2004. The possible reason could be due to limited resources of the state for night blood surveys. Secondly, it may be due to the fact that tea garden worker community is the focal point of LF, and health care of this population is under the respective tea estates of Assam apart from state health department. Therefore, the possibility of under-reporting of this disease might not be ruled out. Moreover, LF is mainly a disease of low socioeconomic people who often remain isolated from health-providing agencies due to various limitations and therefore, does not receive the needed limelight. Further the social stigma attached with the LF, particularly elephantiasis and hydrocele, makes the situation worse as far as revealing of the disease is concerned. In spite of the fact that morbidity and social stigma associated with this disease have paramount importance in public health, lymphatic filariasis still remains a neglected disease and is treated as an ailment of low priority.

In LF elimination, DEC as single annual dose (6 mg per kg body weight) alone or along with albendazole (400 mg) is given for five to six cycles across the endemic population globally (Bockarie et al. 2000; Ramaiah et al. 2000, 2002, 2003; Das et al. 2001a, b; Vanamail et al. 2005). Under this programme, administration of the antifilarial drug must be continued for a period of 4–6 years or even more with high drug coverage of eligible population (> 85%). However, poor coverage and compliance often give results below expectations. Initiative taken by the Government of Assam to join hands with other states of India in controlling or eliminating lymphatic filariasis is highly welcome, and administration of DEC in 4 of the 23 districts in 2004 and subsequently increasing the number of districts (seven) under mass drug administration (MDA) programme in 2005 and 2006 show commitment

of its programme managers. However, high MDA compliance by eligible population and sustenance of MDAs for the stipulated period are core issues behind the success of this important national programme especially in the State of Assam where difficult terrains and ecology preclude the pace of implementation.

Since prevalence of lymphatic filariasis is mainly confined among tea plantation workers, the MDA programme must be executed keeping a lot of local situations in mind to cover maximum population. Tea being an agricultural plantation crop and major revenue generator occupies a vital position in improving living standard of the people of the northeastern region and Assam in particular. Recently, liberalization policy and incentives provided to the small farmers by the Government of Assam for tea cultivation have changed the total scenario of the tea industry in the state. Presently a significant number of workers involved in tea cultivation are dispersed in different parts of the state, giving way to unintended spread and transmission of lymphatic filariasis in newer areas. Interestingly, the vector mosquito, *Cx quinquefasciatus* has been recorded from all those areas where these tea cultivators have settled for a living both in high ranges and plains. Transmission and propagation of LF in newer areas is a serious concern for the managers monitoring LF control. As a consequence the mass drug administration needs special attention for implementation in areas covered under tea cultivation. The success of LF elimination programme in Assam is dependent upon the support of the local community, tea industries, etc. besides of course all those who are governmentally entrusted with the responsibility directly or indirectly with the ELF programme. It would be ideal if the tea industry is taken into the MDA fold by integrating with their local and vital requirements. This will ensure dual gain for the tea industry as not only their workers will be healthy, on one hand, but their yield would also surge forward due to reduction in man days loss and sickness wages (Babu and Nayak 2003).

Acknowledgement We sincerely thanks to the director general of Indian Council of Medical Research, New Delhi, and director of ICMR-RMRC, Dibrugarh, for permission, encouragement and financial support for carrying out this study. We also acknowledge support and encouragement received from the director and programme managers, National Vector Borne Disease Control Programme, Delhi. This study would not have been possible without active and consistent support from the staff of the Division of Entomology and Filariasis, ICMR-Regional Medical Research Centre Dibrugarh, and we sincerely acknowledge contribution of my colleagues and supporting staff who have played pivotal role such as, Mr. CK Sarmah, technical officer; Mr. NK Baruah, laboratory technician; Mr. P. Doloi, laboratory technician; Mr. R. Doloi, laboratory technician; and the staff vehicle drivers who took the team to some of the most difficult and almost inaccessible survey areas endemic to lymphatic filariasis. We are also thankful to the director of Vector Control Research Centre, Puducherry, for sharing certain information and valuable suggestions. Support extended by district officials/PHCs/tea estate managers/doctors is also gratefully acknowledged.

References

Anonymous (1991) In health information of India –Population exposed to the risk of filariasis, Ministry of Health and Family Welfare, Govt. of India. pp 147–148

Anonymous (2004) Statistical hand book of Assam. Directorate of Economics and statistics, Govt. of Assam, Guwahati, India), p 116

Babu BV, Nayak AN (2003) Treatment costs and work time loss due to episodic adenolymphangitis in lymphatic filariasis patients in rural communities of Orissa, India. Trop Med Int Health 8(12):1102–1109 PubMed PMID: 14641845

Basu PC (1957) Filariasis in Assam state. Indian J Malariol 11(3):293–308

Bockarie MJ, Ibam E, Alexander ND, Hyun P, Dimber Z, Bockarie F, Alpers MP, Kazura JW (2000) Toward eliminating lymphatic filariasis in Papua New Guinea: impact of annual single-dose mass treatment on transmission of *Wuchereriabancrofti* in East Sepik Province. P N G Med J 43(3–4):172–182 PubMed PMID: 11939298

Das PK, Ramaiah KD, Augustin DJ, Kumar A (2001a) Towards elimination of lymphatic filariasis in India. Trends Parasitol 17(10):457–460 Review. PubMed PMID: 11587940

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

Dutta P, Gogoi BK, Chelleng PK, Bhattacharyya DR, Khan SA, Goswami BK, Mahanta J (1995) Filariasis in the labour population of a tea estate in upper Assam. Indian J Med Res 101:245–246

Khan AM, Dutta P, Khan SA, Baruah NK, Sharma CK, Mahanta J (1998) Long term effect of diethylcarbamazine citrate on microfilaraemia status in treated individuals. Indian J Med Res 108:134

Khan AM, Dutta P, Khan SA, Baruah NK, Sharma CK, Mahanta J (1999a) Bancroftianfilariasis in a weaving community of lower Assam. J Commun Dis 31:61

Khan AM, Dutta P, Khan SA, Baruah NK, Sharma CK, Mahanta J (1999b) Prevalence of bancroftianfilariasis in a foothill tea garden of upper Assam. J Commun Dis 31:145

Khan AM, Dutta P, Khan SA, Mohapatra PK, Baruah NK, Sharma CK, Mahanta J (1999c) Lymphatic filariasis in two distinct communities of upper Assam. J Commun Dis 31:101–106

Khan AM, Dutta P, Khan SA, Mahanta J (2004) A focus of lymphatic filariasis in a tea garden worker community of Central Assam. J Environ Biol 25(4):437–440

Khan AM, Dutta P, Sarmah CK, Baruah NK, Das S, Pathak AK, Sarmah P, Hussain ME, Mahanta J. Prevalence of lymphatic filariasis in a tea garden worker population of Dibrugarh (Assam), India after six rounds of mass drug administration. J Vector Borne Dis 2015 Dec;52(4):314–320. PubMed PMID: 26714512

Khan AM, Mahanta J (2005) Lymphatic Filariasis eradication programme. Curr Sci 88(9):1359–1360

Mahanta B, Dutta P, Handique H, Khan AM, Mahanta J (1998) Socio-cultural habits among tea garden population of Assam in relation to filariasis transmission. J Hum Ecol 9:187

Prakash A, Mohapatra PK, Das HK, Sharma RK, Mahanta J (1998) BancroftianFilariasis in Namrup Tea Estate, District Dibrugarh, Assam. Indian J Public Health 42:103–7,112

Raina VK, Tripathi VC, Das PB, Kumar A (1993) Declining trend of *Brugiamalayi* in district Cachar, Assam state, India. J Commun Dis 25:107–111

Ramaiah KD, Das PK (2004) Mass drug administration to eliminate lymphatic filariasis in India. Trends Parasitol 20(11):499–502 PubMed PMID: 15471698

Ramaiah KD, Das PK, Appavoo NC, Ramu K, Augustin DJ, Vinay Kumar KN, Chandrakala AV (2000) Aprogramme 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, Das PK, Vanamail P, Pani SP (2003) The impact of six rounds of single-dose mass administration diethylcarbamazine or ivermectin on transmission of Wuchereria bancrofti by Culex quinquefasciatus and its implications for lymphatic filariasis elimination programme. Trop Med Int Health 8:1082–1092

Ramaiah KD, Vanamail P, Pani SP, Yuvaraj J, 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(9):767–774

Rao SS (1942) Observations on Filariasis in Lakhipur and Binakandy tea-gardens (Cachar District, Lower Assam). Indian J Med Res 30:345–350

Sasa M (1976) Human filariasis, a global survey of epidemiology and control. Tokyo, University of Tokyo Press, p 819

Singh S, Bora D, Lal S. (2010) Lymphatic filariasis in East District, Sikkim. J Commun Dis 42(1):33–37. PubMed PMID: 22468549

Vanamail P, Ramaiah KD, Subramanian S, Pani SP, Yuvaraj J, Das PK (2005) Pattern of community compliance with spaced, single, 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

WHO (2005) Global programme to eliminate lymphatic Filariasis. Wkly Epidemiol Rec 23(80):201–212

An Overview of Lymphatic Filariasis Control in Puducherry, Union Territory, India

12

M. R. Bhagyasree

Abstract

Lymphatic filariasis control in Puducherry has offered many novelties, especially through environmental means of vector control. Various preliminary actions like shortlisting of potential health workers as well as volunteers, conducting orientation and training programmes for the volunteers, political participation and their commitment, advocacy, mobilization of resources and active social participation determine the fruitful execution of mass drug administration (MDA) programme. The precarious ELF 2015 goal might not have been achieved due to some issues, but the 2018 goal would be definitely achieved.

12.1 Introduction

Of all the known vector-borne human parasitic diseases, lymphatic filariasis definitely ranks among the frontliners due to intensity and eventuality of its impact as most debilitating and incapacitating neglected tropical disease in the world (Das et al. 2002; Das et al. 1992; Sabesan et al. 2000). History of lymphatic filariasis dates back to the period far before the nineteenth century when Patrick Manson, a British doctor serving in Taiwan, for the first time scientifically demonstrated, though incorrectly, an inseparable relationship of mosquitoes with lymphatic filariasis (Manson 1878, 1884; Manson-Bahr 1959). Later on, through Dr. Ronald Ross's epic discovery of the involvement of mosquitoes, in 1897, this indirect inference sowed the seed of the starting point of Ariadne's thread that would eventually lead to the exit from the labyrinth of human malaria and lay the foundations for scientifically based disease eradication through vector control (Ross 1923).

M. R. Bhagyasree (✉)
Vector Control Research Centre, Puducherry, India

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_12

The first ever filaria survey was carried out in Pondicherry in the year 1957 by a team deputed from Filariasis Training Centre, Ernakulum, now shifted to Calicut. The disease burden as given in the report was as follows:

- Population: 2, 58, 561.
- Mf rate in town: 10.3%.
- Infection rate: 3.1%.
- Infectivity rate: 1.4%.
- Disease rate: 2.7%.

12.2 Entomological Investigation

The report of the entomological indices shows intermediate to higher indices in 1988 compared to 1987 and 1986. The report of parasitological indices conducted in 1988 which was carried out by the control units was also gradually decreasing. The morbidity data for all the disease combined and for filaria cases were also being collected as supplementary information from all the hospitals and dispensaries operating under the jurisdiction of control units. Two control units were functioning in Pondicherry union territory, one in Puducherry and the other in Karaikal. The unit-wise details of assessment of the programme are as follows:

1. Pondicherry

   The average annual vector density was 69.5 which was of lower category and was less than that of 1987. The vector infectivity rate, *mf* rate and mean *mf* count were 0.0, 1.46 and 5.24, respectively, with the disease rate being 0.08.
2. Karaikal

   The average vector density was 45.9 which was lower than that of the previous year. Vector infectivity rate was 0.0 and the unit did not report the assessment survey.

12.3 Report of the Antiparasitic Measures

In order to supplement anti-larval with antiparasitic measures, the concept of clinics, i.e. detection-cum-treatment clinics, was introduced in the Fifth Five-Year Plan. Clinics consisted of the following:

(i) One filarial inspector.
(ii) One lab assistant.
(iii) One fieldworker.

Each team would cover 10,000 population. The team would go door to door for every house to detect and treat *mf* cases and diseased persons. The result was the performance of the clinic which was inadequate as very low priority was given by

the state government to this filarial control programme. Only 186 clinics were established by December 1988 against the requirement of 800 clinics.

12.4 Introduction of DEC-Medicated Salt

Diethylcarbamazine citrate (DEC)-medicated salt regimen was introduced in Karaikal in April 1982 and continued up to January 1986. In the beginning only 0.1% DEC content was introduced but later due to variation of DEC content in the samples and also most of the people used salt in crystal form, the content was increased to 0.2%. Results of the pre- and post-surveys showed 98% reduction in *mf* rate and 72% reduction in disease. The infection in mosquitoes was also eliminated completely. The DEC-medicated salt regimen was later taken up in Chidambaram district of Tamil Nadu in 1989. The results were also conducive to the government plans.

Though the programme has given rise to the reduction in filariasis prevalence in the executed study areas, it was found inadequate for the sustained control for eliminating LF.

The lack of coverage of vast majority of population living in rural areas was one of the major constrictions of the National Filariasis Control Programme, and the stratagem tailed for the detection of filarial parasites by night blood surveys was less sensitive, not cost-effective, and unspontaneous and has poor acceptability from the community (Das et al. 1995).

12.5 Integrated Vector Management

The observation was that the community participation contributes majorly for the success of the control programmes implemented against parasitic diseases. One of the strongest exponents of this has been the two ICMR institutes, viz. Vector Control Research Centre, Pondicherry, and Centre for Research in Medical Entomology, Madurai (TN) in South India. These institutes had played an important role in motivating and involving village communities to participate in various vector control activities under the project named "Filariasis Control Demonstration Project (FCDP)" for the control of malarial and filarial vectors.

The control method was that of integrated vector management (IVM) directed against mosquito larval breeding grounds. The IVM comprises not just the use of larvicides (here VCRC used malariol, fenthion and phenthoate) but also environmental measures such as filling, canalization (or channelization), desilting and deweeding, biological control with larvivorous fish and physical control including the closure of wells and the sealing of septic tanks and their vents (Rajagopalan and Das 1987). This strategy was demonstrated in Pondicherry for the control of bancroftian filariasis during 1980–1985. They used the following methods:

(i) *Biological control*: Use of *Bacillus sphaericus.*
(ii) *Source reduction*: Filling of breeding sites and promoting casuarina plantation.
(iii) *Chemical control*: Use of insecticide (fenthion) as well as few slow-release formulations.
(iv) *Legislation.*
(v) *Awareness programme.*
(vi) *Use of Public Works Department (inter-sectoral cooperation)*: For correcting defective drains.

The sole vector of filariasis in Puducherry is *Culex quinquefasciatus* and is by far the most common species encountered. In the monsoon season, about 45% of the biting mosquitoes originated from cesspits, >35% from drains and about 17% from wells. Interestingly, drains become the most prolific breeder (>90%) during post-monsoon period. The daily emergence of *Cx. quinquefasciatus* was estimated to be about 173,000 in November (during northeast monsoon) to over 9, 500, 000 in January (post-monsoon) with each person receiving some 88, 500 bites/year and more than 1000 bites from mosquitoes carrying microfilariae. In 1981 (pre-control) 8.4% of 24,946 people examined showed parasites in their blood (Rajagopalan and Das 1987).

12.5.1 Integrated Vector Management Implementation in Pondicherry

For the control of urban filariasis in Pondicherry, a new strategy called integrated vector management (IVM) was developed and implemented for 5 years (1981–85). This strategy is closely synonymous to that of integrated pest management in the field of agriculture. As a result of IVM implementation, there was reduction of *mf* rate from 10.3% to 6.35%, and mosquito biting rate (MBR) reduced from 25,561 to 1662 per year showing 93% reduction at the end of 5 years (Fig. 12.1). After 1985,

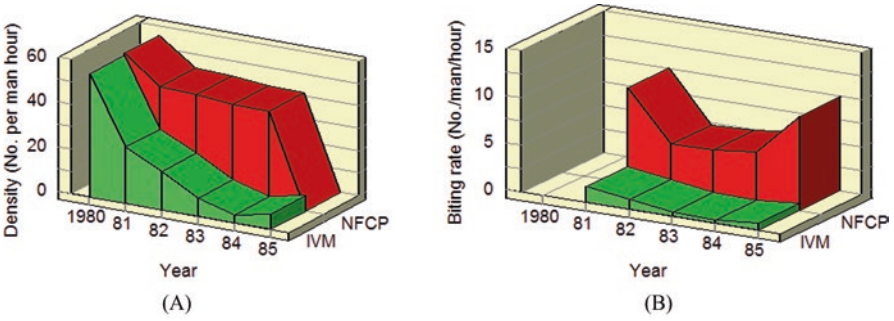


Fig. 12.1 (**a**) Resting density after IVM and (**b**) biting rate of vector mosquitoes after IVM in relation to NFCP

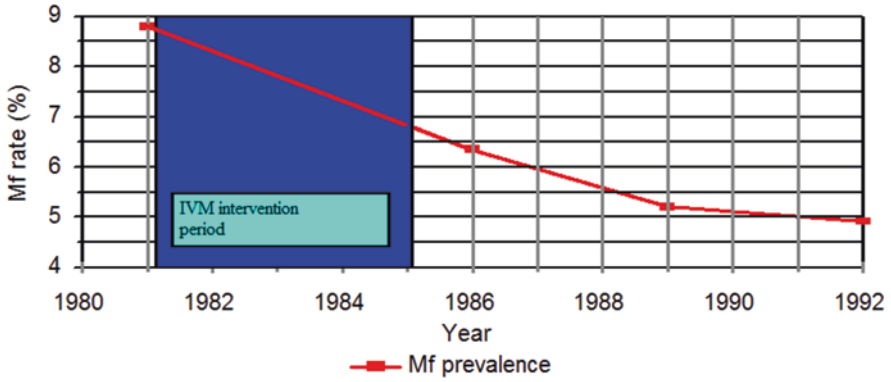


Fig. 12.2 Change in mf rate after IVM in Puducherry during 1980–1992

a jump to conventional strategy showed a rise in the vector density in both the areas (Das et al. 1992). Thus, IVM was best adopted for the successful reduction in the control of filarial vectors.

The IVM over the 5-year period reduced the mosquito biting rate to 6.34%, and only three new microfilaria carriers in the 0–5 years age group were detected in 1986. The infection rate in this age group had been reduced from 2.39% to 0.21% (Rajagopalan and Das 1987) (Fig. 12.2).

12.5.2 Integrated Vector Management Implementation in Kerala

Kerala is also one of the largest endemic places with 2.5 million people at risk of infection. The IVM programme in Kerala included the following:

I. Income-generating programme with vector control as by-product included:
   (i) Fish culture in ponds, as income-generating programme attracted the local community, helped eliminate vector breeding.
   (ii) Propagation of alternative green manure resulted in source reduction.
II. Community awareness:
   (i) Health education for enlisting community participation through different media.
III. Chemotherapy: Establishment of filariasis detection camp which created a self-reliant community for filariasis control.
IV. Community movement: "Shramadan" – a community action was conducted for clearing aquatic weeds to eliminate vector breeding.

After 5 years of IVM implementation, children of this region were free from infection and the results were sustainable.

12.6 Conclusion

India has indeed made a giant stride in controlling lymphatic filariasis, and the target to pronounce elimination by 2018, couple of years before that of the World Health Organization on global basis, seems a certain reality. This grand success is a result of a massive scientific, administrative and political determination providing timely extensive technical and financial support. This success will be yet another feather in the cap of the National Vector-Borne Diseases Control Programme (NVBDCP) after that of the elimination of dracunculiasis more than a decade ago.

Acknowledgement I would like to thank Dr. P. Jambulingam, director of Vector Control Research Centre, Puducherry, and Dr. B. K. Tyagi, director of CRME, Madurai, for the encouragement and permission to participate in the unique *Third National Conference of Lymphatic Filariasis: Elimination 2015, Mission Possible*, at Centre for Research in Medical Entomology Madurai (June 4–5, 2015). I am also grateful to Dr. S. Sabesan, Dr. A. Manomani and Dr. Aathisayamary for their generous help in motivating and assisting me to complete this article in time.

References

Das PK, Manoharan A, Subramanian S, Ramaiah KD, Pani SP, Rajavel AR, Rajagopalan PK (1992) Bancroftian filariasis in Pondicherry, South India: epidemiological impact of recovery of the vector population. Epidemiol Infect 108(3):483–493

Das PK, Manoharan A, Ramaiah KD, Balarajan K, Dhanda V (1995) Cost-analysis of blood surveys for the detection of microfilaria carriers in rural areas. National Medical Journal of India 8:143–144

Das PK, Pani SP, Krishnamoorthy K (2002) Prospects of elimination of lymphatic filariasis in India. ICMR Bull 32(5–6):1–13

Manson P (1878) On the development of Filaria sanguinis hominis and on the mosquito considered as a nurse. J Linn Soc (Zool) 14(14):304–311

Manson P (1884) The metamorphosis of Filaria sanguinis hominis in the mosquito. Trans Linn Soc London 3(2):367–388

Manson-Bahr P (1959) The story of filaria bancrofti; a critical review. I early history. J Trop Med Hyg 62(3):53–61

Rajagopalan PK, Das PK (1987) The Pondicherry project on integrated disease vector control (Filariasis Control Demonstration Project) 1981–1985. Vector Control Research Centre, Pondicherry

Ross R (1923) Memoirs with a full account of the great malaria problem and its solution. John Murray, London

Sabesan S, Palaniyandi M, Das PK, Michael E (2000) Mapping of lymphatic filariasis in India. Ann Trop Med Parasitol 94:591–606

13

Sporadic Incidence of Lymphatic Filariasis in Nonendemic State of Rajasthan and Control of the Vector (*Culex quinquefasciatus* Say, 1823), with Innovative Botanicals and a Possible Hypothesis on the Spread of 'Disease Endemism'

Arti Prasad and Girima Nagda

Abstract

Lymphatic filariasis is endemic in most of the states and union territories in the peninsular India, and Rajasthan in Western India, in spite of sporadic incidences, is still considered a nonendemic state. Ironically, microfilaraemics have been encountered now and then in recent times. The presence of sporadic incidences comprising both imported and indigenous cases has warranted a proper monitoring of emergence of positives especially in view of migration of labour forces from endemic neighbouring states of Uttar Pradesh and Gujarat, migration of cattle raisers in search of fodder, preponderance of the vector and likelihood of armed personnel and their associates who frequently move on duty or transfer amongst endemic and nonendemic states. The present chapter details out some of the startling sporadic incidences reported from Rajasthan and proposes for the first time a possible hypothesis for the spread of disease endemism. A succinct account of herbal products in various forms like repellents and larvicides, effective against vectors, such as *Cx quinquefasciatus*, is presented to tackle the problem of vector control.

A. Prasad (✉) · G. Nagda
Laboratory of Medical Arthropodology, Department of Zoology, Mohan Lal Sukhadia University, Udaipur, Rajasthan, India

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_13

13.1 Introduction

In Indian mainland, *Wuchereria bancrofti*, transmitted by *Culex quinquefasciatus* the ubiquitous vector of this disease, has been the predominant infection contributing to 99.4% of the problem in the country (Ottesen 1984; Witt and Ottesen 2001; WHO 1984; 1995, 2005; NVBDCP 2010). The disease exists endemically in 17 states and 6 union territories except northwestern states/UTs, namely, Delhi, Jammu and Kashmir, Punjab, Himachal Pradesh, Chandigarh, Haryana and Rajasthan, and the north-eastern states, namely, Arunachal Pradesh, Nagaland, Sikkim, Mizoram, Meghalaya, Tripura and Manipur, which are known to be earlier free from indigenously acquired filarial infection. A district-level microfilaria prevalence in India (2006) is shown in Fig. 13.1, clearly indicating nil microfilaraemia in Rajasthan State.

13.2 Lymphatic Filariasis in Rajasthan

Filariasis is a major public health problem in India, and the disease is endemic all over India except north-eastern states/UTs, namely, Jammu and Kashmir, Chandigarh, Himachal Pradesh, Rajasthan, Haryana, Punjab, Delhi and Uttaranchal, and north-eastern states, namely, Sikkim, Arunachal Pradesh, Mizoram, etc. However, migration of people from endemic to nonendemic zones has resulted in occurrence of cases occasionally in nonendemic areas (Jindal et al. 2014). There is a probability of spread of this parasitic disease in nonendemic areas also because of existence of its vector in such areas. Even though Rajasthan is a nonendemic state for the disease, sporadic cases have been diagnosed in various different parts of the State from time to time. This infrequent reporting in the State is of tremendous value to map out new foci in the country and/or low endemicity of a given area in the State. One of the major reasons is the absence of active surveillance of microfilaria prevalence in the populations living in the nonendemic state (like Rajasthan), albeit the mixing of the population with that of an endemic population of a state (like Gujarat). Sharma et al. (1977) has reported that 2.6% of the mosquitoes could be infected from an infected man's 40 cu. mm. of blood carrying one microfilaria. Moreover, the time interval from invasion of infective larvae to the development of clinical manifestation, known as 'clinical incubation period', spanning over 8–16 months, is too big to effectively follow-up for making any casual diagnosis (WHO 1984). Therefore, detecting a case of filariasis draws a concern for clinicians and microbiologists to have a high index of suspicion for patients migrating from endemic zones of filariasis and evaluate them with caution.

In border districts of a nonendemic state such as Rajasthan, it is indeed very difficult to determine a realistic quantum of *mf* carriers because only a small proportion of infected individuals exhibit clinical signs. The disease manifestations can be organized into two distinct types: (a) lymphatic filariasis caused by the parasite in the lymphatic system and (b) occult filariasis caused by an immune

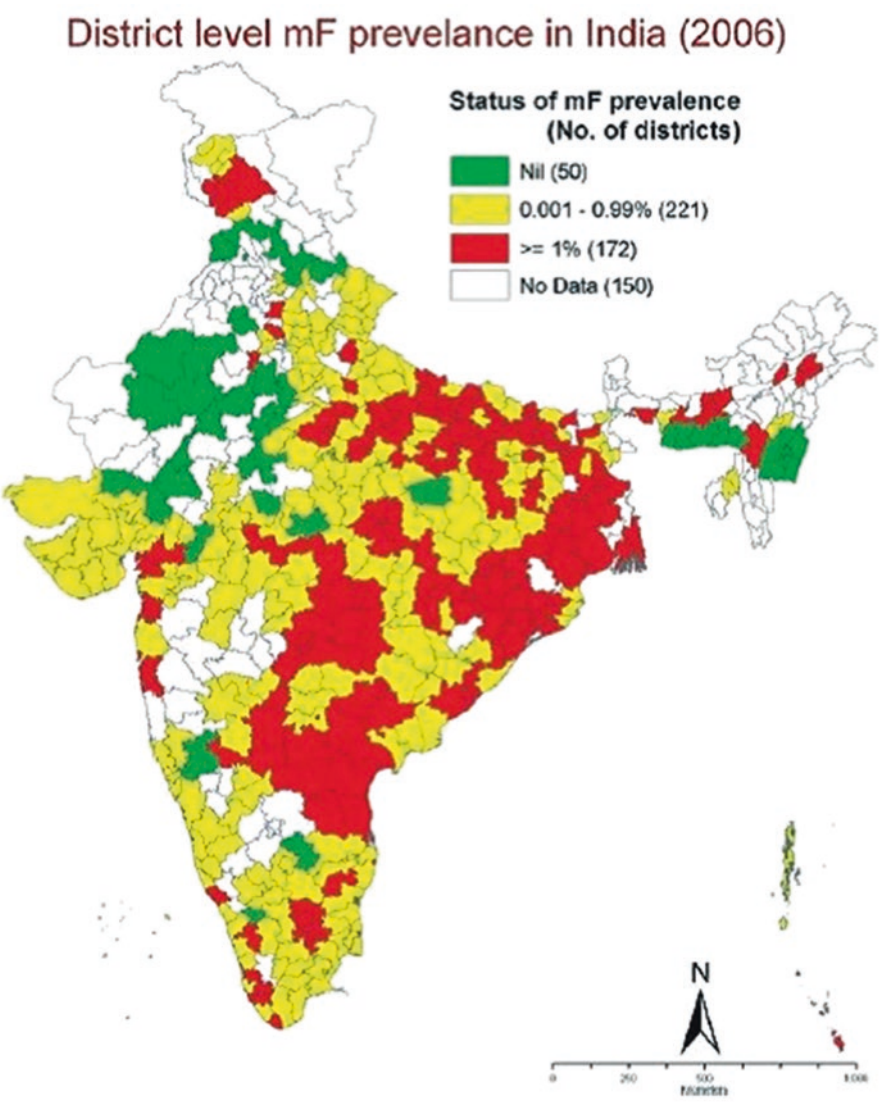


Fig. 13.1 District-level microfilaria (*mf*) prevalence in India (2006). (Source: Sabesan et al. 2010)

hyperresponsiveness of the human host (e.g. tropical pulmonary eosinophilia). There are four stages of lymphatic filariasis:

1. Asymptomatic amicrofilaraemia
2. Asymptomatic microfilaraemia
3. Stage of acute manifestations
4. Stage of chronic obstructive lesions

Filariasis most commonly presents as asymptomatic microfilaraemia, hydrocele, acute adenolymphangitis and chronic lymphatic disease. Asymptomatic patients can have some degree of subclinical manifestations including microscopic haematuria and/or proteinuria (Dreyer et al. 1992), dilated lymphatics and scrotal lymphangiectasia or pleural effusion WHO (1984).

A few case reports, some of which appeared to have picked up infection locally, are indicated below:

1. Singh et al. (2014) reported filariasis in a 21-year-old female patient in Udaipur (Rajasthan). The patient who attended at the outpatient department of M. B. Hospital presented history of high-grade fever for 25 days with nausea and vomiting for which she had consulted many physicians and was treated for malaria and enteric fever, which are endemic diseases in this area, without any relief in the symptoms. There was no significant past history of travel to endemic areas of filaria. On examination patient was found to be febrile (100.8 Fahrenheit). Her pulse, blood pressure and respiratory rate were within normal limits. Her general physical examination was normal. There was no oedema on any part of body, no lymphadenopathy and no hepatosplenomegaly, and her cardiorespiratory status was normal except tachycardia. Her haemogram revealed lymphocytosis and eosinophilia with deranged liver enzymes. Peripheral blood film showed microfilaria of *W. bancrofti*. Patient was treated with diethylcarbamazine 6 mg/kg body weight for 12 days, and patient became afebrile by fifth day and was discharged after a week from hospital. On follow-up patient was afebrile, and there was no microfilariae in peripheral blood.
2. Duggal et al. (2015) reported about a 26-year-old female patient, a housewife and resident of district Balia of Uttar Pradesh with history of frequent visits to her hometown (twice a year), who migrated to Jodhpur in 2010 and had presented with a pea-shaped swelling in the left elbow. She presented to the surgical OPD of the MDM Hospital on 27th November 2014 with the chief complaint of a painful swelling in left elbow joint for 10 days. The patient complained of anorexia, weight loss (7 kg) and lethargy for the last 3 years. However, there was no history of pruritus, urticaria or rashes nor also the history of cough, breathlessness, vomiting or diarrhoea. The patient does not have a known case of any chronic debilitating illness like asthma, diabetes or hypertension either. The tense and tender swelling measured 1.5 × 1.0 cm and occurred in the flexor aspect of the left elbow. On performing complete blood count (CBC), eosinophilia (12.90%) was seen with absolute eosinophil count of 950 per microlitre of blood. Fine needle aspiration cytology (FNAC) of the swelling revealed 1 ml clear, straw-colored fluid. On microscopic examination of Giemsa-stained smears prepared from the aspirated fluid, multiple, thin, coiled, threadlike structures were identified as *W. bancrofti*. The patient was prescribed with DEC (diethylcarbamazine) 100 mg TDS for 21 days to which the patient responded as shown by the absence of microfilariae in PBF prepared from night blood sample taken subsequently.

3. Parihar et al. (2015) reported a case of a 19-year-old girl from Basni locality of Jodhpur, Rajasthan, who presented in surgical OPD of the MDM Hospital associated with Dr. S.N. Medical College, Jodhpur, with the chief complaint of swollen lymph nodes in the right neck region for last 1 year with mild pain from last 10 days. The patient complained about headache and cold from the last 3 days. She did not have any history of breathlessness, sore throat, malaise, lethargy, vomit or weight loss nor suffered from any chronic illnesses like diabetes, asthma or hypertension. She had visited Uttar Pradesh during the past 3 years. On examination, she was found to be afebrile, but her right cervical lymph nodes were palpable, four in number, varying in size from pinhead to pea size, discrete, tender and non-matted. Complete blood count (CBC) revealed eosinophilia with eosinophil count ranging up to 1500 per cu. mm. of blood. Peripheral blood film (PBF) made from blood collected in EDTA vial did not show any parasite. Various tests were done to rule out many infections like tuberculosis, etc. A contrast-enhanced computerized tomography scan (CECT) was done to identify the nature and characteristics of enlarged lymph nodes. CECT scan showed several enlarged, homogenously enhancing, oval-shaped cervical lymph nodes in the right neck region. A non-enhancing, small and hypodense lesion with peripheral thick enhancing rim was seen in retropharyngeal space adjacent to adenoid (size 6 × 6 mm).

   Owing to eosinophilia, negative PBF findings, travel history to endemic belts of Uttar Pradesh and nocturnal periodicity of *Wuchereria bancrofti*, a night blood sample was collected again in EDTA vial at 01 a.m. Then, a wet mount was prepared from it. Live, motile microfilariae lashing the red blood cells were observed; the morphology of which was confirmed as *Wuchereria bancrofti* in thin blood smears. On confirmation of filarial infection, the treatment with 100 mg diethylcarbamazine, thrice a day for 21 days, was instituted, and the patient responded well to it.
4. Dr. B. K. Tyagi, officer in charge of the Desert Medicine Research Centre (ICMR), Jodhpur, while collaborating with major Dr. R. Srivastava, in charge of the Station Health Organization, Indian Army HQs, Jodhpur, during 1991–1993 (*cf.* DMRC Ann. Rep., 1993–1984), had made night blood surveys in BJS Colony and the Army residential unit along Banad Road, and, of a total of 2894 samples collected/slides made, 24 were found positive with *W. bancrofti*. All positive cases were treated with DEC. They also made fortnightly visits to the above areas to sample the vector, *Cx. quinquefasciatus*, during late evening hours and dissected 3051 female mosquitoes, but none was found parasitized with the filarial parasite. In view of some of the positive cases having never been out of Jodhpur, it was postulated that possibly active transmission could be occurring locally.

13.3 Vector Control with Potential Larvicidal Botanicals

Species of genera *Culex*, *Aedes*, *Mansonia* and *Anopheles* act as vector for filarial parasites in the world. *Culex quinquefasciatus* is the main vector for *W. bancrofti* transmission in India. While species of *Aedes*, *Mansonia* and *Anopheles* breed in rather fresh, stagnant or free-flowing waters, the vector, *Cx. quinquefasciatus*, breeds characteristically in polluted water such as sewage and sullage as well as in drain, cesspits, cement septic tanks, etc. However, in the absence of such type of water collections, they can breed in comparatively clean water collections also.

13.3.1 Aquatic Biology of Culex Quinquefasciatus

The genus *Culex* comprises medically several important species, such as *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. tarsalis*, *Cx. nigripalpus*, *Cx. tritaeniorhynchus*, *Cx. bitaeniorhynchus*, *Cx. pseudovishnui*, *Cx. restuans* and *Cx. salinarius*. In Indian context, of greatest importance are *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* of which the former mosquito species is in the first place responsible for transmitting bancroftian filariasis (*Wuchereria bancrofti*). These are brown-coloured mosquitoes of medium size. *Culex quinquefasciatus* Say (the southern house mosquito) is an important mosquito vector of nematodes that cause lymphatic filariasis as well as of viruses such as West Nile virus and St. Louis encephalitis virus. It is one of the species within the *Culex pipiens* species complex whose taxonomy is further complicated by the occurrence of viable species hybrids in many geographic areas. *Culex quinquefasciatus* generally rests with their entire body more or less close (prostrate posture) to the surface. The scales are narrow and not metallic coloured. There are no distinct patterns or markings on the body. The abdomen ends bluntly. Prespiracular and postspiracular areas are without bristles. Forelegs in males bear toothed claws. In females, the claws are there but simple. The tarsi in females have large pulvilli. Being a cosmopolitan species, it enjoys a distribution throughout tropical and temperate climates of the world. The ability of *Cx. quinquefasciatus* to take blood meals from birds, livestock and humans contributes to its ability to vector pathogens between species, including human being.

Culex quinquefasciatus breeds in storm sewer catch basins, street gutters, cesspools, open septic tanks and effluent from sewage disposal plants. Adults are active only at night. When disturbed, they can be easily spotted flying out of their hideouts in and around houses, outbuildings and other shelters. The adult has adapted to human living environment and thus is a highly anthropophilic and prefers to feed and rest indoors. The eggs are laid in rafts containing up to 150 eggs each, and the number depends on quality and quantity of blood meal taken. Within 24–48 h at the optimum temperature of 25–30 °C, the eggs hatch into the first instar larva which is the youngest stage. It further moults to subsequent instars each within 24–48 h at optimum temperature. There are a total of four instars in the larval stages all of which are voracious eaters feeding on everything of microscopic size by taking it into the buccal cavity aided by the instant vibration of its feeding brushes. They are

mainly bottom feeders but may feed from the surface also. At the end of its stage, the IV instar larva gives rise to a comma-shaped pupa, which lasts up to 24–48 h at optimal condition. The pupal stage is a very active stage; the pupae do not feed and respire through a pair of breathing trumpets. Finally, the pupa emerges into an adult mosquito from a longitudinal slit formed between the two trumpets. The entire cycle from egg to emergence of adult is completed in 10–14 days.

13.3.2 Aquatic Biology of Mansonia Species

Mansonoids are large-sized, yellowish brown to dark brown mosquitoes inflicting very painful bites. They readily enter the house and bite viciously. Some of *Mansonia* species such as *Ma. annulifera* and *Ma. uniformis* are vectors of brugian filariasis particularly prevalent in Kerala State, India. *Mansonia* mosquitoes are characterized by possessing postspiracular setae, but no prespiracular setae are found. They are in the habit of biting and resting both inside and outside the houses. They breed in the heavily vegetated pond water growing aquatic plants such as *Pistia* spp. etc. Larvae breathe by penetrating air-conducting plant tissues with their breathing tubes. Mostly the larvae spend their time submerged in the mosquito breeding habitats.

13.4 Experimental Larvicidal Botanicals

13.4.1 Repellents

Nothing ruins a good day in the sunlike spots of annoyingly itchy mosquito bites, and repellents, the substances that act locally or at a distance and deter an arthropod from flying to, landing on or biting human or animal skin, can help protect from the bites of an array of hematophagous arthropods including mosquitoes! The quest to prevent humans from the vicious and infective mosquito bites has fuelled decades of scientific research on mosquito behaviour and control. Many species in the plant kingdom synthesize a variety of secondary metabolites which play a vital role in defence of plants against insects/mosquitoes. Plants act as alternative source of mosquito repellent agents since they constitute a rich source of bioactive compounds/chemicals. Plant products can be used, either as an insecticide for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites, depending on the type of activity they possess. Products of secondary plant metabolism may be responsible for the chemical communication between plants and insects. Phytochemicals have been considered as potential natural insecticides and can be used for insect/mosquito management in integrated control. Phytochemicals obtained from plants are usually less environmentally harmful than synthetic chemicals, and it has renewed the interest in the research on phyto-compounds, considering them as an ecologically safe alternative for synthetic insecticides.

The mechanism and the factors involved in attracting mosquitoes to man are highly complex and not yet fully understood. Ideally an effective repellent must

exhibit an optimal degree of thermo- and photolability, still making it last reasonably long enough with its vapour concentration maintained at the skin surface. Even though quite some highly effective synthetic repellents are commercially available, nevertheless plants on planet Earth probably form the greatest source for varied groups of molecules arising out of an array of biotic and abiotic resources that can be given a serious thinking to develop effective antimosquito products (Tyagi and Shahi 2001; Tyagi 2002a, b; Tyagi and Shahi 2002; Tyagi 2003a, b; Shahi et al. 2000).

A large number of plants have been explored as potential sources of mosquito repellents, but obviously none of the plant-derived chemicals examined till date exhibit a broad spectrum of effectiveness and duration of either DEET (N,N-diethyl-3-methylbenzamide) or DEPA (N,N-diethyl phenylacetamide). A few plant products however do show a good amount of mosquito repellency, and efforts are underway to upscale their repellent activity through different mechanism. A large number of plants contain compounds which fall under several categories due to their chemical and interactive pathways, like feeding deterrents, repellents, toxins and growth regulators and help in preventing attack from phytophagous (plant-eating) insects. These compounds may be varyingly organized according to their active ingredients, target effectiveness and application formulation (Pichersky and Gershenzon 2002; Dam et al. 2000a, b).

13.4.1.1 Eucalyptus

Natural essential oils obtained from plants can be used to deter the mosquitoes that transmit malaria, filaria and a variety of encephalitides. Lemon eucalyptus oil is one of the safest and very effective essential oil for mosquito repellent. This natural plant oil contains p-menthane-diol, which has been proven to be more effective than any of the leading most potent chemical alternative, DEET (diethyltoluamide). Recently, nanotechnology has opened new vistas in using effectively the eucalyptus extracts (Sugumar et al. 2014). The repellent activity of *Eucalyptus* sp. oils (using coconut oil base) against the filariasis vector *Cx. quinquefasciatus* mosquito has been corroborated with three concentrations, 0%, 50% and 100% (v/v). The test oils showed excellent repellent action (93.37%) against *Cx. quinquefasciatus*, with the protection time up to 240 min.

13.4.1.2 Citronella

For times, immemorial citronella species has been known for its insect repellent activity. In many countries, citronella, derived from the lemony-scented citronella oil belonging to the grass plant *Cymbopogon nardus*, is even registered as an insect repellent. Studies have shown that citronella can act as an effective repellent, but when compared to other DEET-based products, it provides shorter complete protection time. In comparison with the DEET in which case only fragmented data are available on toxicity (0.01 juLmol of DEET per L of air was sufficient to prevent 90% of mosquitoes from landing on their targets), it was found that a 1000-fold higher concentration of citronellol (one of the active chemicals in citronella oil) was

required to achieve a similar effect as that of the DEET. Frequent reapplication of the repellent can partially compensate for this. Another set of tests have shown that their 10% lotion reduced mosquito bites by 84% during a 4-min test period, which was quite satisfactory when compared to DEET wherein 14% DEET reduced biting by 96% in the same test period. Citronella is said to be best effective against the dengue/chikungunya/Zika vector, *Aedes aegypti*, as merely 5% citronella oil provided 88% repellency during an average protection time of 1.9-h to 2-h exposure.

Citronella plants, commonly called as lemongrass (family: Poaceae), examples of which such as *Cymbopogon citratus*, *C. nardus*, *C. schoenanthus*, *C. winterianus* and *C. jwarancusa*, with their essential oils and extracts, are common ingredients of plant-based mosquito repellents. Encapsulated citronella oil nano-emulsion is prepared by homogenization of 2.5% surfactant and 100% glycerol, to create stable droplets which increases the retention of the oil and slows down release rates. There are needs for further exploration (Tyagi et al. 1998).

13.4.1.3 Neem

Neem (*Azadirachta indica*) is widely appreciated globally for its manifold agricultural applications. Dried neem leaves, owing to their repellency against pests of various kinds, were in use for protecting stored food grains in many communities world over for centuries. Neem kernels, leaves and bark as well as their smoke were all known to repel hematophagous insects including mosquitoes. Several field studies from India have shown very high efficacy of neem-based preparations (Sharma et al. 1993). Unfortunately, it could not be yet exploited fully for its unmatched and wonderful repellency characteristics, albeit considered as a natural alternative to DEET.

13.4.1.4 Moringa

Moringa oleifera Lam. (commonly called as drumstick tree, benzolive tree, horseradish tree, saijhan, sajna, kelor, moonga, marango, mlonge, mulangay, nebeday or ben oil tree), indigenous to northwestern India, which is also spread almost all over the peninsular part of the country, is the most widely cultivated species of the family Moringaceae. In the West, *Moringa* is used to flocculate contaminants and purify drinking water with its powdered seeds. The plant extracts exhibit properties, viz. ovipositional, larvicidal, repellents, deterrents and insect growth regulators. Extract of *M. oleifera* seed has shown a great potential as larvicidal and pupicidal agent against mosquitoes.

Methanol leaf extracts of *M. oleifera* exhibit excellent repellent action against *Aedes aegypti* and *Ae. Albopictus* mosquitoes. The seeds of *M. oleifera* possess antimicrobial properties (Ali et al. 2004), with ovicidal and larvicidal effects on *Ae. aegypti* L. (Paulo et al. 2009). The toxicity and growth regulatory effects were varyingly assessed against several important vector mosquito species such as *Anopheles gambiae* (Njom et al. 2011) and *Aedes aegypti* (Paulo et al. 2009; Prasad and Sharma 2014).

13.5 Possible Hypotheses for the Spread of 'Disease Endemism' of Lymphatic Filariasis from the Neighbouring Endemic States

Rajasthan is a nonendemic zone for human lymphatic filariasis; therefore, occurrence of LF cases in the State, albeit infrequent, is a serious concern to policymakers, clinicians and health managers of the State since this is seen as a tip of a prospective serious problem (Fig. 13.2).

Three possibilities are worth considering:

(i) Migration of labour populations from the adjoining or neighbouring endemic state like Gujarat who can be mf carriers (Jindal et al., 2014)
(ii) Frequent travel of nonendemic population to states which are endemic for lymphatic filariasis so that such people could get infection through infective bites of the vector and on return to their native in Rajasthan develop as the patient later on
(iii) Though yet to be demonstrated, a remote but explainable possibility that the local *Cx. quinquefasciatus* population getting infected from the above LF patients and transmitting, however abysmally at present, the infection to indigenous population

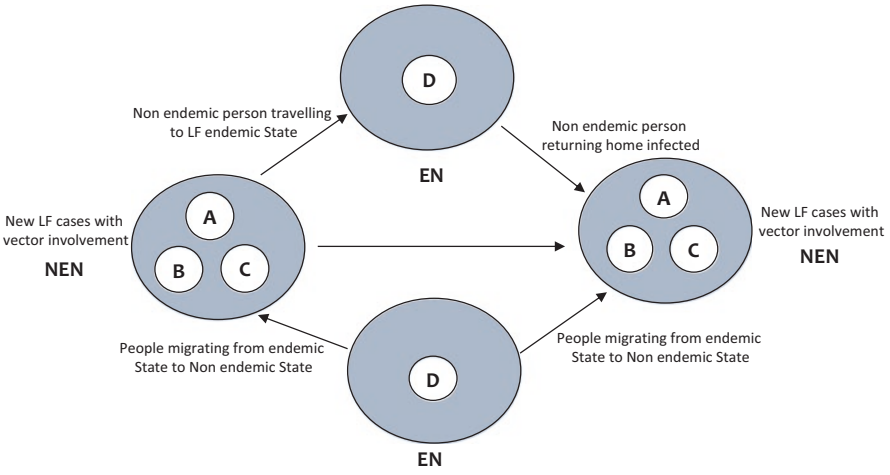


Fig. 13.2 Hypotheses of spread of 'lymphatic filariasis disease endemism' to a nonendemic state from neighbouring/endemic states. (Source: Dr. B.K. Tyagi, unpublished); *A* = nonendemic population; *B* = nonendemic infected population; *C* = vector *Cx. quinquefasciatus*; *D* = endemic population; *NEN* = nonendemic state; *EN* = endemic state)

13.6 Discussion

The human lymphatic filariasis, which is ranked as the most neglected tropical disease, in India accounts for almost 40% of the global burden. The disease is eradicable, with the medication of diethylcarbamazine (DEC) or ivermectin through mass drug administration (MDA), along with albendazole (Alb) or alone. Notwithstanding the above possible links to elimination of microfilaraemia, the most important task will always be a foolproof monitoring/surveillance of the residual infection particularly in vector and the risk of nonendemic states becoming endemic due to multiple reasons. Vector control is an integral part to entire control programme and will be able to sustain the benefits of the mass drug administration. Application of plant extracts has become a potent alternative in sustainable vector control strategy due to their less toxic, easy availability and non-persistent nature. In the last five decades, synthetic insecticides have been mainly used to control insect pests, though not without certain serious drawbacks (Omena et al. 2007; Govindrajan et al. 2008a, b). Botanicals like neem, eucalyptus, moringa, citronella, etc. can prove to be excellent sources for eradication of the vectors. A continuous and regular surveillance is required to check the sporadic incidences of LF in nonendemic states like Rajasthan. Phytochemicals being novel in their approach besides non-toxic to nontarget organisms and nonhazardous to environment are always preferable. Phytochemicals can be applicable to various different stages of life cycle of the mosquito. In aquatic stages, the different phytochemicals can be used as either a larvicides to exact mortality or hormone-regulating compounds affecting the process of ecdysis or moulting. The plant-based chemicals are highly applicable as repellents against adults of vectors, such as that of lymphatic filariasis. Since the filariasis parasite does not cause explosive epidemics of disease and a large number of infective mosquito bites are required to produce a patent case, the plant-based repellents can always keep the mosquito biting abysmally low (Rajagopalan et al. 1977); thus, the transmission of infection could be reduced or prevented by keeping the vector away through source reduction and personal protection measures as demonstrated in Pondicherry (now Puducherry) (Subramanian et al. 1989) and Shartalai in Kerala (Sabesan et al. 2010). India is targeting 2018 for declaring the nation free from LF, but this could be realized when apparently nonendemic states under influence of transmission from neighbouring endemic states are also carefully screened.

References

Ali GH, El-Taweel GE, Ali MA (2004) The cytotoxicity and antimicrobial efficiency of Moringa oleifera seeds extracts. Int J Environ Stud 61:699–708

Dam PK, Ramanath T, Yadav SP et al (2000a) Use of ethnomedicinal practices in the Thar Desert, northwestern India. J Econ Taxon Bot 24:303–318

Dam PK, Yadav SP, Tyagi BK (2000b) Thar Marukschetrameinaushadhi-uktavanaspatiyan: ekpurvalokana (in Hindi) [The medicinal plants of Thar Desert region: a review]. ICMR Patrika 14(8):61–65

Dreyer G, Ottesen EA, Galdino E et al (1992) Renal abnormalities in microfilaremic patients with bancroftianfilariasis. Am J Trop Med Hyg 46:745–751

Duggal S, Khatri PK, Parihar RS, Chandora A, Dhoundyal R, Deval M, Choudhary T (2015) Unusual presentation of Filariasis in a tertiary care hospital in Western Rajasthan: a case report. Int J Curr Microbiol Appl Sci 4(1):685–689

Govindarajan M, Jebanesan A, Pushpanathan T, Samodurai K (2008a) Studies on effect of Acalyphaindica L. (Euphorbiaceae) leaf extracts on the malarial vector, Anopheles stephensi Liston (Diptera: Culicidae). Parasitol Res 103:691–695

Govindarajan M, Jebanesan A, Pushpanathan T (2008b) Larvicidal and ovicidal activity of Cassia fistula Linn. leaf extract against filarial and malarial vector mosquitoes. Parasitol Res 102:289–292

Jindal A, Sukheeja D, Midya M (2014) Cervical lymphadenopathy in a child-An unusual presentation of filariasis. Int J Sci Res Publ 4(8):2250–3153

NJOM, Victor Stephen and EZE, Chukwuma Simon (2011) Toxicity and life expectancy effects of Moringaoleifera seed extracts on the larvae of Anopheles gambiae. Ani Res Int 8(2):1388–1391

NVBDCP (2010) National Vector Borne Disease Control Programme, Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India. Website: Available from http://nvbdcp.gov.in/filariasis-new.html. [cited on 2010 Jan 13]

Omena MC, de Navarro DMAF, de Paula JE, Luna JS, Ferreira de Lima MR, Sant'Ana AEG (2007) Larvicidal activities against Aedesaegypti of some Brazilian medicinal plants. Biores Technol 98:2549–2556

Ottesen EA (1984) Immunological aspects of lymphatic filariasis and onchocerciasis in man. Trans R Soc Trop Med Hyg 78:9–18

Parihar RS, Duggal S, Khatri PK, Rathore SS, Meena S, Dhoundyal R (2015) Atypical presentation of cervical lymphadenopathy in a case of lymphatic filariasis. Int J Sci Res Publ 5(6):1–4

Paulo MPF, Ana FUC, Davi FF, Nara GC et al (2009) Larvicidal activity of the water extract of Moringa oleifera seeds against Aedesaegypti and its toxicity upon laboratory animals. Ann Acad Bras Cienc 81(2):207–216

Pichersky E, Gershenzon J (2002) The formation and function of plant volatiles: perfumes for pollinator attraction and defense. Curr Opin Plant Biol 5:237–243

Prasad A, Sharma E (2014) Phytotoxicological assessment of Moringaoleifera Lam. against larvae of important human malaria vector Anopheles stephensi Liston (Insecta:Diptera: Culicidae). Int J Innov Appl Stud 7(4):1633–1641

Rajagopalan PK, Kazmi SJ, Mani TR (1977) Some aspects of transmission of Wuchereriabancrofti and ecology of the vector Culexpipiensfatigans in Pondicherry. Indian J Med Res 66:200–215

Sabesan S, Vanamail P, Raju K, Jambulingam P (2010) Lymphatic filariasis in India: epidemiology and control measures. J Postgrad Med 56:232–238

Shahi AK, Tyagi BK, Chaudhary RC, Kaul BK (2000) Cymbopogonschoenanthus (L) Spreng. (Poaceae)-a versatile aromatic and medicinal plant of Indian Thar Desert. Flavours Fragr Assoc India J 1:3–33

Sharma VP, Ansari MA, Razdan RK (1993) Mosquito repellent action of neem (*Azadirachtaindica*) oil. J Am Mosq Control Assoc 9(3):359–360

Sharma SP, Das M, Rao CK (1977) Current estimates of filariasis problem in India. J Commun Dis 9:111–116

Singh DP, Mahur H, Jhirwal KS, Ameta M, Mudghal DS (2014) Filariasisin Southern Rajasthan. A case report. Int J Curr Res 6(12):11055–11056

Subramanian S, Pani SP, Das PK, Rajagopalan PK (1989) Bancroftianfilariasis in Pondicherry, South India: 2, epidemiological evaluation of the effect of vector control. Epidemiol Infect 103:693–702

Sugumar S, Clarke SK, Niramala MJ et al (2014) Nanoemulsion of eucalyptus oil and its larvicidalacitivities against Culexquinquefasciatus. Bull Entomol Res 104(3):393–402

Tyagi BK (2002a) Future of phytochemicals against vector mosquitoes. In: Paper on Abstract VI AZRA Congress on Advance in applied zoological researches for food PRODUCTION and environmental safety, Cuttack, 19–21 December 2002

Tyagi BK (2002b) Future of phytochemicals against vector mosquitoes. J Appl Zool Res 14:101–106
Tyagi BK (2003a) A review of plant based materials active against vector mosquitoes with particular emphasis on repellent potential of Cymbopogon (Poaceae). J Econ Taxon Bot 28:489–493
Tyagi BK (2003b) Cymbopogon essential oils: their chemistry and mosquito repellent activities. Paper abstract national SYMPOSIUM on biochemical science health & environmental aspects, Agra, pp 140–142
Tyagi BK, Shahi AK (2001) Evaluation of repellent potential of two Cymbopogon species (Poaceae), endemic to the Thar Desert (India), against major vector mosquitoes. International conference globalization of Indian essential oils, Agra, 21–23 September 2001
Tyagi BK, Shahi AK (2002) Genus Cymbopogon (Poaceae) – a potent group of mosquito repellent bearing characteristics. Paper Abstract XVI National Congress on Parasitology, Bareilly, 31 Oct, 2 Nov 2002, p 91
Tyagi BK, Shahi AK, Kaul BL (1998) Evaluation of repellent activities of Cymbopogon essential oils against mosquito vectors of Malaria, Filariasis and Dengue Fever in India. Phytomedicine 5(4):324–329
WHO (1984) Filariasis. Report of the Fourth Expert Committee. Technical Report series No. 702
WHO (World Health Organization) (1995) Bridging the gaps. World Health Report. WHO, Geneva
WHO (2005) Sixth meeting of the Technical Advisory Group on the Global Elimination of Lymphatic Filariasis, Geneva, Switzerland. Wkly Epidemiol Rec 80:401–408
Witt C, Ottesen EA (2001) Lymphatic filariasis: an infection of childhood. Trop Med Intl Health 6(8):582–606

14

Integrated Vector Control for the Elimination of Bancroftian Filariasis in the Villages of Tirukoilur, South India

I. P. Sunish, M. Kalimuthu, Ashok Kumar, R. Rajendran, A. Munirathinam, N. Nagaraj, N. Arunachalam, and B. K. Tyagi

Abstract

Integrated vector control (IVC) along with mass drug administration (MDA) was implemented in the filaria endemic villages of Tirukoilur, South India, for the elimination of bancroftian filariasis. The MDA was carried out by the state health machinery, including the 36 villages under study, which were clustered into three arms, viz. first arm had drug administration alone, while the other two arms had IVC with MDA. The latter two arms include (i) MDA + EPS (expanded polystyrene beads applied in stagnant water bodies) and (ii) MDA + EPS + PIC (pyrethroid-impregnated curtains were provided additionally to each household). The IVC was implemented by the community and was monitored by village volunteers. After 3 years of IVC + MDA, parameters of filarial infection like microfilaraemia, antigenaemia and transmission indices were found to decline in all the groups. Vector density showed a remarkable and significant reduction in

I. P. Sunish (✉)
Centre for Research in Medical Entomology (Indian Council of Medical Research), Madurai, Tamil Nadu, India

Regional Medical Research Centre (ICMR), Field Unit, Andaman & Nicobar Islands, India

M. Kalimuthu · A. Kumar · R. Rajendran · A. Munirathinam · N. Nagaraj · N. Arunachalam
Centre for Research in Medical Entomology (Indian Council of Medical Research), Madurai, Tamil Nadu, India

B. K. Tyagi
Centre for Research in Medical Entomology (Indian Council of Medical Research), Madurai, Tamil Nadu, India

Department of Environmental Biotechnology, Bharathidasan University, Tiruchirapalli, Tamil Nadu, India

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_14

both the IVC arms. During post-IVC period, transmission was nil in these arms. Higher reduction in vector density was observed in MDA + EPS + PIC arm as compared to arm without PIC. The paper discusses the key elements of IVC implementation through community mobilization in an area endemic to bancroftian filariasis.

14.1 Introduction

Lymphatic filariasis (LF) was recognized as a potentially eradicable disease in 1993, while the World Health Assembly (Resolution 50.29) in 1997 targeted LF for global elimination by 2020 (WHO 2000). This disease affects 120 million in low- and middle-income countries, and almost 1.4 billion people are exposed to the parasitic worm (WHO 2013). In this scenario, Global Programme to Eliminate Lymphatic Filariasis (GPELF) was launched by WHO, which aims to cease the transmission and alleviate suffering in affected persons. The programme is on the basis of annual MDA of single-dose albendazole combined with either ivermectin or diethylcarbamazine for 5 or more years (the estimated reproductive life span of adult worms), which is anticipated to break LF transmission from humans to mosquitoes. This is likely to reduce the infection prevalence below a threshold level, at which the transmission ceases to occur and remains sustainable. A standardized transmission assessment survey (TAS) is recommended to measure whether infection prevalence was below the threshold levels after intervention (WHO 2011).

Mass drug administration (MDA) comprising the use of DEC and albendazole has been found successful for LF elimination (Ottesen et al. 2008). Since inception, GPELF programme prevented or cured 96.71 million LF cases, in which 79.20 million were microfilaria carriers, and 18.73 million were hydrocele and 5.49 million lymphedema (Ramaiah and Ottesen 2014). However, MDA strategy has been facing various challenges, especially the non-attainability of expected population coverage, human migration and sustainability of the control programme due to financial and political limitations. LF elimination can be achieved, if vector control is integrated with MDA. This has been demonstrated in various settings in different parts of the globe (Webber 1979; Bockarie et al. 2009). The end points may be affected by the variations in the local endemicity, aggregation of infection and magnitude of acquired immunity. Vector control is effective, as the parasite transmission can be intervened. The parasite does not multiply in the mosquito vector and requires continuous exposure to infective bites of many vectors, in order to maintain infection in humans and cause new patent infection. The long-term sustainability of the vector control programme at community level has not been achieved.

An integrated vector control by a community-based approach, in addition to ongoing MDA, was carried out in the Tirukoilur revenue Block, Tamil Nadu, South India.

14.2 Materials and Methods

One hundred villages in Tirukoilur Block of Villupuram district, South India, which had a population of 147, 000, were surveyed for microfilaraemia (Mf) by random sampling, to cover 10% of the population in each village. Thirty-six villages were selected (Fig. 14.1) on the basis of microfilaraemia (*Mf*) prevalence and ecological scenario for implementation of IVC. Mass drug administration (MDA) was ongoing in all the villages as part of the national programme for LF elimination. These villages were divided into three, viz. one arm with MDA alone and the other two arms had IVC in addition to MDA. In the IVC arms, one arm used polystyrene beads (EPS) as the main IVC component, while in the other arm, deltamethrin-impregnated curtains (DIC) were installed in the households, in addition to EPS (Table 14.1). In the unused wells, fishes (mainly *Gambusia* sp.) were introduced to prevent vector breeding. These fishes multiplied in these wells and were later used to seed more water bodies by the local community, which prevented vector breeding further.

Before IVC initiation, the community was motivated to involve themselves in the programme through IEC meetings, school children rallies and village volunteers. The administrative heads of the villages and teachers of local schools were also solicited to participate in IVC. The householders were requested to clean the cesspits in their backyard, as these were the potential breeding source for *Culex quinquefasciatus*, the sole vector of LF parasite. Cesspits were modified by brick layers, and EPS were applied at 400 gm/m^2, in order to prevent vector breeding. Wire mesh was kept at the exit, to allow excess water to overflow and prevent the escape of beads. In the MDA + EPS + PIC arm, measurements of all the doors and windows

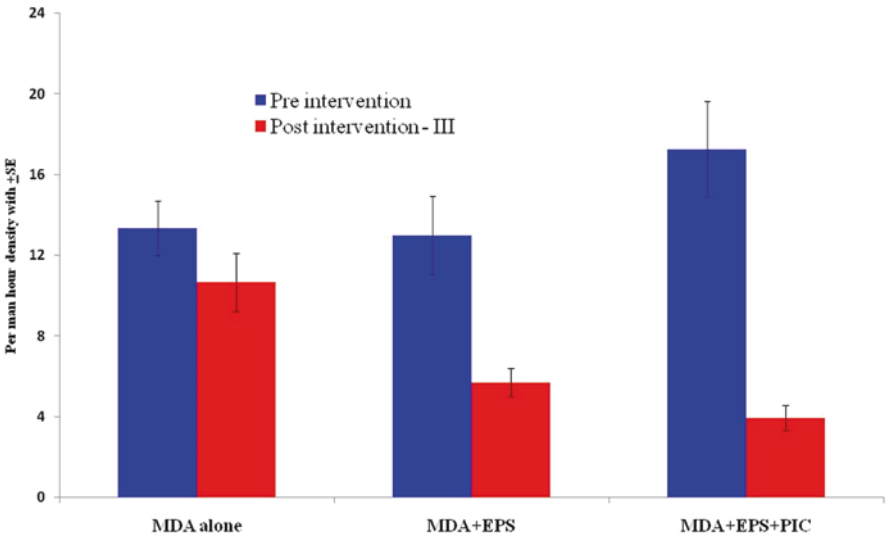


Fig. 14.1 Per man hour density of *Cx. quinquefasciatus* during the pre-IVC and 3-year post-IVC implementation

Table 14.1 Details of breeding habitats in the selected villages of three different intervention strategies

Intervention strategy	Number of villages	Population	No. of households	Cesspits		Unused wells		Drains	
				No.	Area m^2	No.	Area m^2	Length	Area m^2
MDA alone	12	26,128	4611	75	45	77	656	8713	4358
MDA + EPS	12	19,630	3510	160	95	74	372	6215	3167
MDA + EPS + PIC	12	19,519	3636	33	26	78	905	6775	3438

Table 14.2 Microfilaraemia prevalence among all ages in the 36 selected villages

Interventions	Pre-intervention		Post intervention III	
	No. screened	Mf rate (%)	No. screened	Mf rate (%)
MDA alone	3249	3.76	1828	0.60
MDA + EPS	2187	3.66	1687	0.89
MDA + EPS + PIC	2232	2.82	2049	0.29

of the households were made by women volunteers, belonging to the self-help group (SHG). Similarly, the eaves on the roof of houses were also measured. Polypropylene materials were procured and stitched by a local tailor as per the requirement. These were distributed to the householders for installation, after impregnating with deltamethrin @25 mg/m^2. The unused wells were cleaned, and fishes were introduced.

Two new concepts, *VCCom* (vector control through community; Sunish et al. 2014) and *Elephant Brigade* (Sunish et al. 2014), were coined and put innovatively into action for and by the volunteers involved in IVC implementation and subsequent monitoring. These volunteers were selected from each village and were involved in monitoring the IVC activities during the post-IVC periods. They were also involved in educating the villagers in the proper use/maintenance of EPS as well as PIC impregnated curtains. Every month, persistence of the insecticide on curtains was estimated by standard method. When the mosquito mortality reduced to <50%, the curtains were sprayed with the insecticide (6-month interval).

During the post-IVC periods, a parasitological survey in 36 villages was carried out every year by finger prick method, in order to determine the impact of IVC (Reuben et al. 2001). The details of microfilaraemics were submitted to the local health authorities for treatment and follow-up. Filarial antigenaemia was also tested by using the ICT card test, in the age group of 2–10 years (Table 14.2). The adult mosquitoes resting indoors were captured by a hand catch method, on a monthly basis. These mosquitoes collected were identified, and female *Cx. quinquefasciatus* were dissected to estimate different vector indices, viz. density, infection, infectivity and transmission intensity index (TII) (Rao et al. 1981; Sunish et al. 2003).

An informed consent was obtained from the participants (volunteers) for their involvement in the study and also from the parents/guardians of the children prior to testing for filarial antigenaemia. One-way analysis of variance (ANOVA) was used

to determine significant differences in various parameters. The analysis was carried out using statistical software, viz. SPSS ver.10.

14.3 Results

The community-based IVC implementation had a significant impact on transmission parameters, but the reduction in parasitaemia in the community did not demonstrate significant change, as compared to the villages without IVC.

After 3 years of IVC, the microfilaraemia prevalence (MFP) in MDA + EPS arm was 1.91% while 1.18% in MDA + EPS + PIC arm (Table 14.2). The MFP reductions were 75%–90% in the three intervention arms. The prevalence of antigenaemia in children ranged from 1.43% to 1.91% in the three arms after 3 years (Table 14.3), demonstrating a reduction of 70%–89%. The MFP reductions in MDA alone and in combination with IVC were comparable. However, after 3 years of IVC, there was higher reduction in antigenaemia prevalence in MDA + IVC arms than in MDA alone arm.

Prior to IVC initiation, the per man hour (PMH) density of the vector mosquito ranged from 12 to 17. During post-IVC periods, there was a drastic decline in the density of *Cx. quinquefasciatus* in both IVC strategy arms (Table 14.4, Fig. 14.1), and it was 56% and 77% in MDA + EPS and MDA + EPS + PIC arms, respectively. Infection and infectivity rates of *Cx. quinquefasciatus* were comparable among the three strategy arms. Vector infection in the two VC arms showed significant differences, as contrast to MDA alone arm, viz. MDA + EPS ($\chi 2 = 30.43$, $P < 0.005$) and

Table 14.3 Antigenaemia prevalence (AGP) among children

Intervention strategies	Pre-intervention (February 2010)		Post-intervention III (February to April 2013)	
	Number examined	AGP (%)	Number examined	AGP (%)
MDA alone	583	8.40	395	2.53
MDA + EPS	398	12.56	350	1.43
MDA + EPS + PIC	411	9.25	419	1.91

Table 14.4 Entomological parameters during pre- and post-intervention (indoor resting collection)

Intervention periods	Intervention arms	Per man hour density	Infection rate (%)	Infectivity rate (%)
Pre-intervention (2009–2010)	MDA alone	13.36 ± 1.35	0.38 ± 0.10	0.11 ± 0.04
	MDA + EPS	12.99 ± 1.97	0.54 ± 0.17	0.16 ± 0.06
	MDA + EPS + PIC	17.28 ± 2.37	0.18 ± 0.07	0.07 ± 0.03
Post-intervention III (2012–2013)	MDA alone	10.68 ± 1.43	0.25 ± 0.17	0.02 ± 0.06
	MDA + EPS	5.71 ± 0.71	0.45 ± 0.14	0.00 ± 0.00
	MDA + EPS + PIC	3.95 ± 0.61	0.25 ± 0.29	0.00 ± 0.00

MDA + EPS + PIC ($\chi 2 = 40.16$, $P < 0.005$). In MDA alone arm, infective mosquitoes (with L3 stage larvae) were observed throughout the post-intervention, while vector infectivity and TII in the two IVC arms were not recorded during the post-intervention period (Table 14.4).

There was an enhanced usage/application of vector control tool by the householders, after 3 years of programme implementation. Almost 35% and 15.2% of the cesspits had full covering of EPS beads in MDA + EPS and MDA + EPS + PIC arms, respectively, during post-intervention III. Almost 54.1% of unused wells had larvivorous fishes, which prevented vector breeding. After 3 years, even though there was irregular usage of curtains by a few householders, almost 63% of HHs were found to maintain the impregnated curtains flawlessly.

The awareness on LF disease and vector mosquito was greater in MDA + IVC arm than in MDA alone arm. Enhanced knowledge on vectors was observed in the arm where PIC was installed in the households, which could be due to more interaction of LF volunteers during curtain distribution.

14.4 Discussion

Integrated vector control in combination with mass drug administration has significantly reduced lymphatic filarial infection in many settings and/or countries (Das et al. 1992; Maxwell et al. 1999; Reuben et al. 2001; Sunish et al. 2007; Bockarie et al. 2009). Expanded polystyrene beads (EPS) are environment friendly and can remain on the water surface for longer duration (Curtis and Minjas 1985), and their application in pit latrines reduced the biting rates of *Cx. quinquefasciatus* by 99.7% in Zanzibar (Curtis and Maxwell 1997). In South India, when EPS were introduced in cesspits, filarial infection parameters were found to reduce significantly (Reuben et al. 2001). Resurgence of infection was also prevented with missed MDAs (Sunish et al. 2007). The role of IVC in reducing filarial transmission indices was also observed in the present study. There was a significant reduction in vector density in both the IVC arms (56% and 77% in MDA + EPS and MDA + EPS + PIC arms, respectively), while a mere 20% reduction was found in non-IVC arm. Declining the vector density significantly reduced the mosquito-biting nuisance and thus enhanced community awareness on the benefit of IVC. After the implementation of IVC, nil transmission was recorded in both the VC arms.

Deltamethrin-impregnated bednets have been widely distributed by various agencies in order to control malaria, both in African countries and also in other parts of the world, where malaria is endemic. In Nigeria, the transmission of LF parasite was found to decline significantly, solely by the use of long-lasting impregnated nets (Richards et al. 2013). The curtains installed on windows and eaves prevented the entry of other arthropods, which are vectors of disease pathogens (Wilson et al. 2014). In the present study, deltamethrin-impregnated curtains were employed to reduce the entry of vector mosquitoes to houses, which prevented man-vector contact significantly.

The vector mosquito of LF in our study area, and in the whole of Peninsular India, is highly endophagic. Hence, fixing deltamethrin-impregnated curtains in MDA + EPS + PIC arm had an added advantage of 20% in reducing vector density, than in villages without curtains. After the initial distribution of impregnated curtains, insecticide spray was used subsequently. This prevented laborious dislodging of the curtain for reimpregnation and installation.

In the VC integrated arm, the knowledge of the community that LF is transmitted by a mosquito bite was enhanced from 19.8% to 72.3%, after 3 years of programme implementation. Higher knowledge was recorded in the arm with PIC than in MDA + EPS arm, which could be due to frequent visits of LF volunteers to the households during the enquiry for curtain usage. The IVC tools, EPS and deltamethrin-impregnated curtains, played a crucial role in reducing LF transmission. These tools were accepted by the villagers and could be employed in areas where the disease still persist, in order to sustain the gains achieved through MDAs, implemented as a part of the LF elimination programme.

Acknowledgements This community-based approach was supported by the Bill and Melinda Gates Foundation through RCC-ELF grant. The study received approval from the institutional ethical committee. The authors express their thanks to the Director General, ICMR and to the staff of the Department of Public Health and Preventive Medicine, Tamil Nadu State, for their valuable cooperation. The village community of Tirukoilur revenue Block is also gratefully acknowledged for their cooperation and support. Acknowledgement is expressed to all the project staff of CRME Field Station at Tirukoilur, without whose support, this trial could not have been completed.

References

Bockarie MJ, Pedersen EM, White GB, Michael E (2009) Role of vector control in the global program to eliminate lymphatic filariasis. Annu Rev Entomol 54:469–487

Curtis CF, Maxwell CA (1997) Tanzania: control of *Culex*vectors of filariasis. Lancet 349(Suppl III):28

Curtis CF, Minjas J (1985) Expanded polystyrene beads for mosquito control. Parasitol Today 1:36

Das PK, Manoharan A, Subramanian S, Ramaiah KD, Pani SP, Rajavel AR, Rajagopalan PK (1992) Bancroftianfilariasis in Pondicherry, South India—epidemiological impact of recovery of the vector population. Epidemiol Infect 108:483–493

Maxwell CA, Mohammed K, Kisumku U, Curtis CF (1999) Can vector control play a useful supplementary role against bancroftianfilariasis? Bull World Health Organ 77:138–143

Ottesen EA, Hooper PJ, Bradley M, Biswas G (2008) The global programme to eliminate lymphatic filariasis: health impact after 8 years. PLoSNegl Trop Dis 2(10):e317

Ramaiah KD, Ottesen EA (2014) Progress and impact of 13 years of the global programme to eliminate lymphatic filariasis on reducing the burden of filarial disease. PLoSNegl Trop Dis 8(11):e3319. https://doi.org/10.1371/journal.pntd.0003319

Rao CK, Sundaram RM, Venkatanarayana M, Rao JS, Chandrasekharan A, Rao CK (1981) Epidemiological studies on bancroftianfilariasis in East Godavari district (Andhra Pradesh): entomological aspects. J Commun Dis 13:81–91

Reuben R, Rajendran R, Sunish IP, Mani TR, Tewari SC, Hiriyan J, Gajanana A (2001) Annual single dose diethylcarbamazine plus ivermectin for control of bancroftianfilariasis: comparative efficacy with and without vector control. Ann Trop Med Parasitol 95(4):361–368

Richards FO, Emukah E, Graves PM, Nkwocha O, Nwankwo L, Rakers L, Mosher A, Patterson A, Ozaki M, Nwoke BEB, Ukaga CN, Njoku C, Nwodu K, Obasi A, Miri ES (2013) Community-wide distribution of long-lasting insecticidal nets can halt transmission of lymphatic filariasis in southeastern Nigeria. Am J Trop Med Hyg 89(3):578–587

Sunish IP, Rajendran R, Mani TR, Munirathinam A, Tewari SC, Hiriyan J, Gajanana A, Reuben R, Satyanarayana K (2003) Transmission intensity index to monitor filariasis infection pressure in vectors for the evaluation of filariasis elimination programmes. Tropical Med Int Health 8(9):812–819

Sunish IP, Rajendran R, Mani TR, Munirathinam A, Dash AP, Tyagi BK (2007) Vector control complements mass drug administration against bancroftianfilariasis in Tirukoilur, India. Bull World Health Organ 85:138–145

Sunish IP, Rajendran R, Arunachalam N, Munirathnam A, Tyagi BK, Elango WGB (2014) Assessment of the vector control for augmentation of MDA to eliminate LF, South India. Annual report 2013–2014. Centre for Research in Medical Entomology, Madurai, p 46

Webber RH (1979) Eradication of *Wuchereriabancrofti* infection through vector control. Trans R Soc Trop Med Hyg 73:722–724

WHO (2000) Eliminate filariasis: attack poverty. The global alliance to eliminate lymphatic filariasis. In: Proceedings of the 1st Meet., Santiago de Compostela, Spain. 4–5 May. Doc. WHO/CDS/CPE/CEE/2000.5. Geneva: World Health Organ. 41 pp

WHO (2011) Monitoring and epidemiological assessment of mass drug administration in the global programme to eliminate lymphatic filariasis: a manual for national elimination programmes. World Health Organization, Geneva. http://apps.who.int/iris/bitstream/10665/44580/1/9789241501484_eng.pdf?ua=1

WHO (2013) Global programme to eliminate lymphatic filariasis: progress report for 2012. Releveepidemiologiquehebdomadaire/Section d'hygiene du Secretariat de la Societe des Nations. Weekly epidemiological record/Health Section of the Secretariat of the League of Nations 88(37):389–399

Wilson AL, Dhiman RC, Kitron U, Scott TW, van den Berg H, Lindsay SW (2014) Benefit of insecticide-treated nets, curtains and screening on vector borne diseases, excluding malaria: a systematic review and meta-analysis. PLoSNegl Trop Dis 8(10):e3228. https://doi.org/10.1371/journal.pntd.0003228

The Indian Lymphatic Filariasis Elimination Programme: The Success to Sustain

15

P. K. Srivastava, A. C. Dhariwal, and B. K. Tyagi

Abstract

India, being one of the signatories to the resolution of the World Health Organization in achieving global elimination of lymphatic filariasis by 2020, has in fact made phenomenal strides in getting rid of the scourge much earlier by 2018. In the process, while the nation has succeeded in reducing microfilaraemia rate below 1% in most states/UTs, it has actually eliminated the diseases in some others. Thus, India is clearly marching ahead to knock off the infection and offer a model to several endemic countries struggling to combat the crippling disease.

15.1 Introduction

The global burden of LF has a significant contribution of 42% from India alone. Therefore, India's role in realizing WHO-proclaimed Global Programme for Elimination of Lymphatic Filariasis (GPELF) is very significant (Dhariwal et al. 2015).

15.2 The Modus Operandi

India is a vast country with a fast-growing economy. The challenges to tackle LF in India are many, viz. extensive geographic limits of endemic areas from north to south and east to west, multicultural society, booming demography and climatic and

P. K. Srivastava · A. C. Dhariwal
National Vector Borne Disease Control Programme (NVBDCP), New Delhi, India

B. K. Tyagi (✉)
Centre for Research in Medical Entomology (ICMR), Madurai, TN, India
e-mail: abktyagi@gmail.com

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_15

Fig. 15.1 Launch of mass drug administration by (**a**) the Union Health Minister in 2005, (**b**) the Minister of State for Health in 2007, (**c**) Health Minister of Pondicherry and (**d**) Health Minister of Jharkhand. (*Source*: P.K. Srivastava/NVBDCP)

physiographic diversities as well as quantity and quality of response of the LF-endemic populations to mass drug administration (MDA) of the antifilarial diethylcarbamazine (DEC), with or without albendazole (Alb) (Fig. 15.1). Scattered dates of MDA and/or non-observance of the annual MDA rounds due to sudden and extreme climatic compulsions (such as cyclones or floods) or administrative reasons have caused the main challenges for the programme to be implemented synchronously across the country.

During the LF-elimination journey, there came along many moments of highs and lows, and many queries were raised. Documentedly India has indeed made a great leap forward in ridding its population from the scourge, coming close on the heels of its elimination from the country. Many of the queries and doubts about India's success in many states and union territories have since been resolved, and future course of action is being constantly reviewed dynamically to ensure target being achieved as rescheduled (i.e. year 2018), much earlier than the GPELF target of 2020. To understand India's success in reducing the disease burden all over the country, in general, and in some states and union territories, in particular, it is

considered opportune to revisit the various rather intricate processes and their impact vis-a-vis the magnitude and quantum of the problems associated with the disease.

15.3 India's National Programme for Elimination of Lymphatic Filariasis

Considering the fact that human lymphatic filariasis was an eradicable disease mainly through chemotherapy, the existing alliance met in a meeting in October 1998 to strengthen the novel ways to generate financial and other support to reach the first 200 million people at risk by the end of 2004. This obviously hastened the process of drafting a blueprint of action worldwide, and on May 4–5, 2000, the meeting of the "First Global Alliance to Eliminate Lymphatic Filariasis" was held at Santiago de Compostela, Spain, which by all accounts was a grand success. Taking cues from the worldwide resolve and the Indian progress in reducing the disease burden, the Union Health Secretary, Ministry of Health and Family Welfare, Government of India, who chaired the above meeting proclaimed that India would have to go a long way before declaring being free from the scourge during the next two decades. Thereafter, the peregrination started, and the significant contribution of pilot studies by NCDC and ICMR institutes like VCRC and CRME had amply helped in designing the concept and plan of implementation. The planning and commitment were charted out, and elimination by 2015 was envisaged in National Health Policy 2002 (Fig. 15.2). The national launch of elimination campaign was done at highest level, with national guidelines framed and disseminated.

15.4 Finding the Ariadne's Thread

Advocacy, social mobilization, sensitization, training and IEC/BCC activities were taken up to mobilize the service provider so as to cover approximately 600 million population (500 million eligible population required about 2 million human resource for drug administration). Thus, the states and UTs managed to mobilize and initiate a massive task of mass drug administration to the identified endemic population on periodic annual basis, as far as possible (Fig. 15.3a–d).

The mobilization campaign resulted in making the service provider reach the community even in remote places. The reported coverage rates improved from 73% in 2004 to 88% in 2015. However, the challenge to improve actual drug intake by community remained. This was improved in the southern, western and central states, but it remained a challenge in the eastern states. The success started to be visible after 2013 through the Transmission Assessment Survey (TAS) in various districts which resulted in stopping MDA and shrinking the LF map further.

Fig. 15.2 The planning and commitment envisaged in National Health Policy 2002. (*Source*: P.K. Srivastava/NVBDCP)

15.5 Breaking the Ice

By the end of 2015, the remainder population of about 190 million was planned to be covered under MDA subject to subsequent validation by the TAS. The achievements could be sustained, and Goa became the first state to show second successful validation through TAS. Analysis has narrowed down to 34 districts with around 90 million population above 1% mf rate at community level. This shows that 222 districts with about 520 million population have achieved an overall *mf* rate below 1% which is about 85% of target set initially.

15.6 The Road Ahead

India very much looks forward to eliminate the disease as envisaged in its National Programme for Elimination of Lymphatic Filariasis. The whole plan is highly meticulously and analytically prepared as at the moment of writing this note, 34 districts have been prioritized for the next mass drug administration (MDA) with National Deworming Day (NDD). The move is labelled as the first synchronized approach of this magnitude in the country (Fig. 15.4).

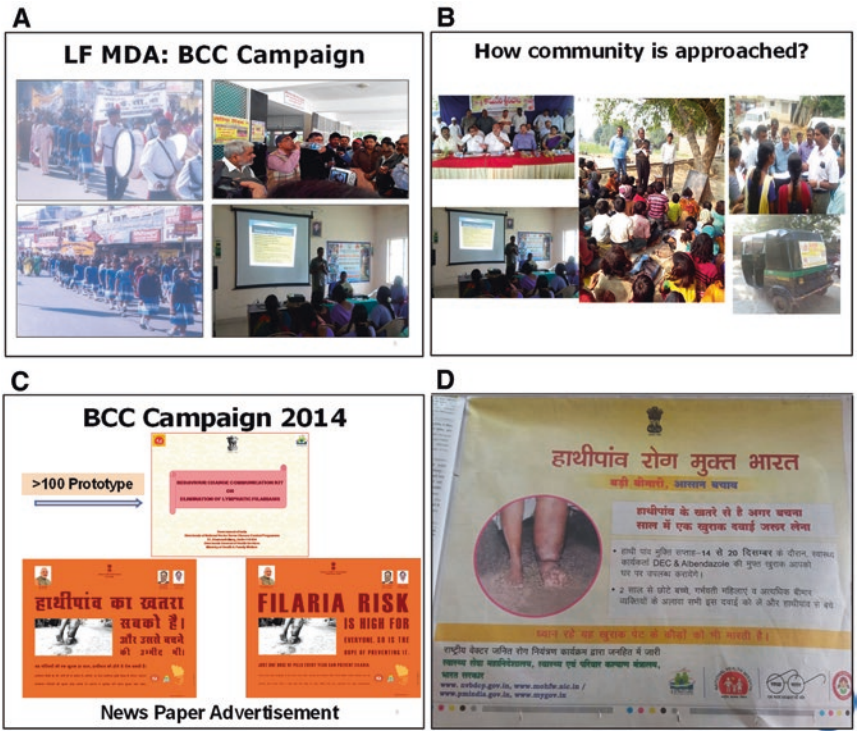


Fig. 15.3 (**a**–**d**) Massive mobilization of the NPELF in the states and UTs all over India. (*Source*: P.K. Srivastava/NVBDCP)

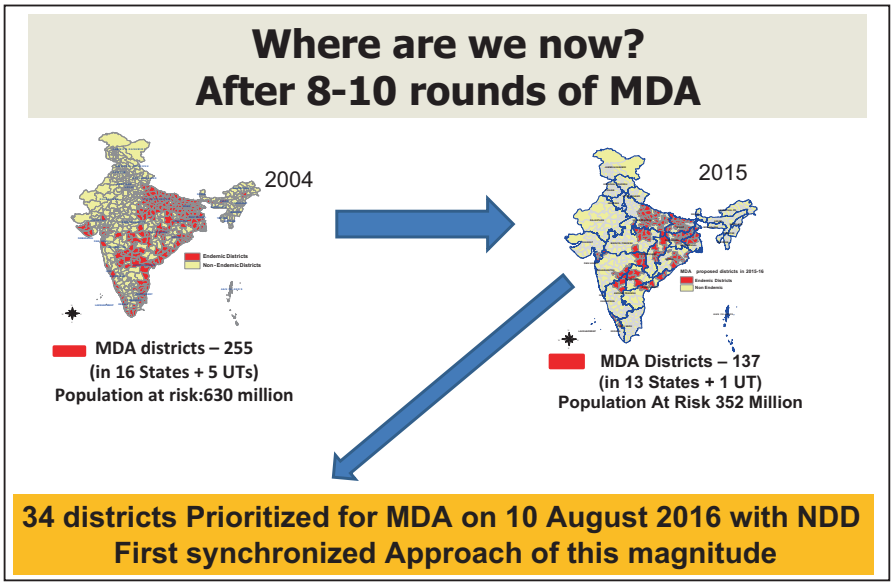


Fig. 15.4 Current LF scenario after 8–10 MDA rounds and future planning with a MDA planned for August 10, 2016. (*Source*: NVBDCP)

Reference

Dhariwal AC, Srivastava PK, Bhattacharjee J (2015) Elimination of lymphatic filariasis in India: an update. J Indian Med Assn 113(12):189–190

16 Bioecology, Insecticide Susceptibility and Management of *Culex quinquefasciatus* Say, 1823: A Major Vector of Lymphatic Filariasis in India

Reji Gopalakrishnan and Vijay Veer

Abstract

Culex quinquefasciatus is the most common mosquito species in urban and rural India, which is primarily responsible for biting nuisance. It is a major vector of lymphatic filariasis in India and is known to be associated with the transmission of Japanese encephalitis virus. It is a known vector of arboviral diseases such as West Nile fever and Rift Valley fever. *Cx. quinquefasciatus* belongs to *Cx. pipiens* species complex. It breeds on a variety of sites including wastewater drains, ditches, pools, paddy fields and water-filled containers. This species is reportedly resistant to insecticides such as DDT and malathion which makes chemical control of the vector population difficult. The present chapter reviews the taxonomy, distribution, biology, biting activity, vector competence, insecticide resistance and management aspects of this important vector species in India. The efficacy personal protective measures in reducing biting nuisance and disease transmission are discussed, along with the continued need for novel and more effective control strategies.

16.1 Introduction

Culex quinquefasciatus, well known ubiquitously as the southern house mosquito, is one of the most widely distributed mosquito species in the world. It assumes public health significance since it is the principal vector of the world's most grotesque and debilitating vector-borne diseases, i.e. lymphatic filariasis, and is also a known vector for Japanese encephalitis virus (JEV) and West Nile virus (WNV). About 120 million people are affected with lymphatic filariasis worldwide, and one-third

R. Gopalakrishnan (✉) · V. Veer
Defence Research Laboratory (Defence Research & Development Organisation), Tezpur, Assam, India

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_16

of the affected persons belong to India. The disease, which is mainly caused by a filarial nematode, *Wuchereria bancrofti,* is endemic in 17 states and 6 union territories of India with more than 550 million people at risk of infection (Chandel et al. 2013). Though the disease does not lead to fatalities, it causes severe morbidity and is a major health problem in India especially among people living in low socioeconomic conditions (Manimegalai and Sukanya 2014). WNV is a zoonotic disease wherein the virus is maintained in birds and mosquitoes, and humans are regarded as incidental hosts. The virus got introduced to the USA in 1999 and thereafter has expanded its geographical range to Canada, South America and Europe. Recent outbreaks reported from Assam and Kerala in India assumes significance due to the presence of highly competent vectors and amplifying hosts in the country (Sudeep et al. 2015). JEV is another viral disease where *Cx. quinquefasciatus* is incriminated as a vector in India. Under laboratory conditions, the mosquito species is able to transmit dengue, Ross River virus, reticuloendothelial virus and the protozoan *Hepatozoon breinli*.

Apart from its role as a disease vector, this species of mosquito is a common household pest in urban areas and creates nuisance due to its night biting activity. It is closely associated with human habitations and has a considerable adverse impact on outdoor activities.

16.2 Taxonomic Status

The first description of *Cx. quinquefasciatus* was by Thomas Say from a specimen from the Southern United States in 1823. *Culex quinquefasciatus* has been first designated as a subspecies of *Cx. pipiens* and named as *Cx. pipiens quinquefasciatus*. However, later studies revealed that *Cx. pipiens* and *Cx. quinquefasciatus* were two distinct sympatric mosquito species and differed from each other genetically, thus finally raising the mosquito nomenclature to the species level as *Cx. quinquefasciatus* Say, 1823 (Bhattacharya and Basu 2016). The synonyms of *Culex quinquefasciatus* include *Cx. pungens* Wiedemann, 1828; *Cx. fatigans* Wiedemann, 1828; *Cx. auestuans* Wiedemann, 1828; *Cx. acer* Walker, 1848; and *Cx. cingulatus* Doleschall, 1856 (Hill and Connelly 2015).

Since divergent physiological and behavioural traits occur without distinctive morphological differentiations, the *Cx. pipiens* complex is considered a controversial topic in mosquito taxonomy. The DV/D ratio and PCR amplification of acetylcholinesterase (Ace.2) gene sequences are the two methods currently used to differentiate *Cx. p. pipiens* and *Cx. p. quinquefasciatus*. The DV/D ratio refers to the relative overlap and measurement of the dorsal and ventral arms in male genitalia. DV/D ratios are less than 0.2 for *Cx. p. pipiens* and greater than 0.4 for *Cx. p. quinquefasciatus*. The DV/D ratio of 0.2–0.4 is for hybrids. Amplicons of different sizes specific for the two taxa are generated through PCR amplification of Ace.2 gene sequences (Diaz-Badillo et al. 2011).

16.3 Distribution and Abundance

As per recent studies, *Cx. quinquefasciatus* originated in Southeast Asia and then spread to the New World and Africa. Sailing vessels, military aircrafts and ships along with commercial air travel have aided in the global expansion of this species to the other regions of the world including New Zealand, Hawaiian Islands, the USA and islands of the Pacific and Indian oceans (Bhattacharya and Basu 2016). The species is usually found within the latitudes 36° N and 36° S and is present throughout the tropics and the lower latitudes of temperate regions. It is present in North America, South America, Australia, Asia, Africa, the Middle East, and New Zealand. In the Southern United States, it is the primary vector of St. Louis encephalitis virus (SLEV) and also transmits West Nile virus (WNV) (Hill and Connelly 2015).

Within India *Cx. quinquefasciatus* has been the dominant species in indoor resting collections from human habitations (88.44%) and cattle sheds (51.84%) in West Bengal, India.

16.4 Biology and Life Cycle

Adult *Cx. quinquefasciatus* is brown in colour with a body length of 3.96 to 4.25 mm. The head is light brown, while the proboscis, thorax, wings and tarsi are darker than the rest of the body. The antennae are of the same length or shorter than the proboscis. The thorax has narrow and curved scales, whereas the basal side of each abdominal tergite has pale, narrow, rounded bands. Eggs are laid in oval rafts loosely cemented together with 100 or more eggs in a raft. They normally hatch 24–30 h after oviposition. The larvae have a short and stout head, and the abdomen consists of eight segments, each segment having a unique setae pattern. The siphon on the dorsal side of the abdomen has multiple setae tufts. The barrel-shaped saddle is located on the ventral side of the abdomen with four long anal papillae in the posterior end. The pupae have a fused head and thorax (cephalothorax) and an abdomen. The breathing is through the trumpet, which is a tube that widens and becomes lighter in colour as it extends away from the body. The abdomen has eight segments (Hill and Connelly 2015).

As in the case of other mosquito species, temperature, nutrition and population density affect the development period from larva to adult. The development may be completed in a period of 7 days under optimal conditions of 30 °C. In India, *Cx. quinquefasciatus* may complete 2–3 gonotrophic cycles in a lifetime during summer and 4–8 cycles in the winter. During each gonotrophic cycle, a gravid female lays a single egg raft on the surface of a suitable water body. The egg rafts float on the water surface. The whitish eggs turn dark within a few hours of laying. Mosquito age, blood source and blood volume affect the number of eggs per raft although the mean number of eggs per raft is about 155 (Bhattacharya and Basu 2016; Subra 1981). Up to five rafts of eggs are laid by a single female in her lifetime (Hill and Connelly,

2015). In India, the percentage of egg hatching were 86.5 ± 7.3 and 91.5 ± 3.8 in Gorakhpur and Pune, whereas the mean number of eggs per raft were 169 ± 14.5 and 139.75 ± 15.96. The number of eggs/female life span was 676 ± 32 in Gorakhpur (Gokhale et al. 2013).

The duration of each stage in the life cycle at a place is dependent on temperature. Hatching usually occurs 1 day after egg-laying in tropical regions. The larvae feed on biotic material in the water and complete their development in 5 to 8 days at 30 °C. There are four larval instars. The larval stage lasts more in females (about 135 h) than in the males (about 118 h), whereas the reverse is true for the pupal stage. Both males and females can take sugar meals from plants, but the female seeks a blood meal after mating for its ova maturation. The adult females generally start host seeking within 48 h of emergence. Although females can feed on a range of hosts, those fed on birds lay a greater number of eggs than those fed on man, pointing out towards the fact that the mosquito is mainly a bird feeder. The species breeds mainly in surface waters rich in organic matter such as drains, ditches and cesspools. However, breeding is observed in a wide range of habitats including artificial containers, shallow ponds, streams, phytotelmata, wells and septic tanks (Bhattacharya and Basu 2016; Subra 1981; Hill and Connelly 2015), and the mosquito is able to breed in comparatively clean water in the absence of polluted water (Mishra 2014).

Studies on the midgut microbial community of *Culex quinquefasciatus* populations from India revealed the presence of 83 bacterial species belonging to 31 genera. Proteobacteria was the most dominant phylum (37 species), followed by Firmicutes (33 species) and Actinobacteria (13 species). The genus *Staphylococcus* was the largest genus represented by 11 species, whereas *Enterobacter* was the most prevalent genus (Chandel et al. 2013). Isolates belonging to 13 different genera were obtained in another study wherein the most abundant isolates were *Pseudomonas* sp., *Acinetobacter junii*, *Staphylococcus epidermis* and *Aeromonas culicicola* (Mourya et al. 2002). It was observed that individual mosquito harbours extremely diverse gut bacteria, and this variation in midgut microbiota may be one of the factors responsible for variation in vector competence within the population. The midgut bacteria are known to influence host physiology and may thus alter the response to various pathogens. Further investigations are needed to verify the potential role of the midgut bacteria in the transmission of diseases such as lymphatic filariasis and West Nile virus (Chandel et al. 2013).

16.5 Biting Activity and Host Preference

Cx. quinquefasciatus is predominant in urban areas, and its biting activity is observed in both outdoor and indoor environments. In addition to humans, this mosquito can feed on cattle, sheep, dogs, rabbits and amphibians. Feeding on humans and other mammals make it an important bridge vector, whereas feeding on birds makes them the ideal vector for avian pathogens like WNV and SLEV (Uttah et al. 2013). Studies on biting preference in Nigeria showed that the mosquito preferred to bite on the foot and ankle of human hosts (Oduola and Awe 2006). The mosquito causes discomfort in the night due to its biting nuisance, which may also lead to

allergic reactions in some individuals, besides of course man-hours lost. Generally, *Cx. quinquefasciatus* mosquitoes enter the households during the evening hours, feed during the night and leave in the morning. However, nowadays it is commonly observed in the urban areas that some these mosquitoes stay back in the households and bite even during the daytime. This is an interesting change in the mosquito's behaviour, which considerably increases the biting nuisance.

The biting behaviour of this mosquito assumes significance as its nocturnal biting cycle coincides with the periodicity of microfilariae, thereby intensifying the infection. The biting density in Mysore, India, increased from 1800 h and reached a peak during 2200–2300 h. Per man-hour density was the lowest (5.1) during June and January, while the highest (12.3) was during March (Gowda and Vijayan 1993).

In the tea agroecosystem of Assam in India, the mean biting density per hour varied from 0.8 (December) to 13.3 (March), whereas the mean monthly biting density was 5.2 per man-hour. Rainfall of the current month and mean monthly minimum temperature were significant factors affecting biting density. Indoor biting activity in the study sites showed two peaks during 1900–2000 and 2200–2300 h. The mean per man-hour density ranged 0.08–9.42 during the night, and March–May period was the season soaring the highest biting activity (Mahanta et al. 1999). In Goa, India, the highest biting activity was during 0300–0600 h (40.8%), whereas the lowest was during 2400–0300 h (16.4%). The peak mosquito activity was observed between 0500 and 0600 h both during pre- and post-monsoon seasons. The mosquito density during the post-monsoon season was higher in comparison to that during the pre-monsoon and monsoon seasons (Korgaonkar et al. 2012). This situation is compounded by the fact that the susceptibility of *Cx. quinquefasciatus* to pyrethroids such as permethrin and deltamethrin is relatively lower than those of the anophelines (Uttah et al. 2013). The peak outdoor biting in Nigeria was 18.00–20.00 h outdoors, which declined steadily to the lowest level during 03.00–04.00 h before rising again. Human density was the major factor influencing the biting behaviour of *Cx. quinquefasciatus* as per studies in Nigeria (Uttah et al. 2013).

The proportion of mosquitoes feeding on human blood (anthropophilic index) was 63.56% in West Bengal, India. This showed a high man-mosquito contact which is conducive for disease transmission (Azmi et al. 2015). The parous rate of host-seeking mosquitoes in Assam, India, ranged from 40.6% during August to 64.4% in January. The biting rhythms of parous and nulliparous mosquitoes were similar in the first half of the night, while the density of nulliparous mosquitoes is reduced earlier than that of the parous (Mahanta et al. 1999).

16.6 Role as a Disease Vector

Culex quinquefasciatus is a known vector of many diseases affecting humans as well as domestic and wild animals. It is a major vector of lymphatic filariasis (LF) and is also involved in the transmission of viruses such as West Nile virus (WNV), St. Louis encephalitis virus (SLEV) and Western equine encephalitis virus (WEEV) (Hill and Connelly 2015).

As per the estimates of the World Health Organization (WHO), 120 million people are infected with the filarial parasite, and further 1.3 billion people, which constitute 20% of the global population, are at risk of infection. About 40 million people are suffering from the long-term complications of the disease, which is prevalent in 81 tropical and subtropical countries. It is the second most common vector-borne parasitic disease after malaria, and one-third of the infected people live in India (Upadhyayula et al. 2012). *Wuchereria bancrofti* infections account for 95% of the lymphatic filariasis cases in India. About 473 million individuals are estimated to be at risk of filariasis infection in India, and with around 31 million microfilaraemics and 23 million symptomatic cases, it is a major public health problem in the country (Mishra 2014).

WNV infection in India is a self-limiting, nonfatal mild febrile illness but occasionally reported to cause encephalitis (Paramasivan et al. 2003), although in the USA it causes high mortality among infected people. Generally it causes mortality in horses and domestic and wild birds. West Nile virus was first isolated from the West Nile district of Uganda in 1937. The 1950s and 1970s witnessed outbreaks of the disease in Middle East, Europe and Africa. Unprecedented morbidity and mortality in humans, animals and birds due to WNV infections were reported from the USA during 1999–2000. Birds act as carriers and amplifying hosts for the virus which is spread to vertebrate hosts through ornithophilic mosquitoes. There is an incubation period of 2 weeks for the virus in the mosquito vector after which the vector is able to transmit the virus to a host. Under unfavourable conditions vertical transmission of the virus from infected female mosquitoes to their progeny might also take place (Gajanana 2002). In India, though hardly any death is associated with, the WNV is highly prevalent, and serologically confirmed cases of WNV infections were reported from Vellore and Kolar districts during 1977–1981. Clinically overt encephalitis cases and epidemics of febrile illness were observed in Buldhana, Marathwada and Khandesh districts of Maharashtra and Udaipur of Rajasthan. The presence of WNV neutralizing antibodies were detected in human sera collected from the Indian states of Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Gujarat, Madhya Pradesh, Orissa and Rajasthan (Paramasivan et al. 2003).

The first confirmation of Japanese encephalitis (JE) clinical case was from Vellore in 1955. Many major outbreaks of the disease were reported from Bihar, Uttar Pradesh, Assam, Manipur, Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu, Haryana, Kerala, West Bengal, Orissa, Goa and Pondicherry. The JE has now become endemic in many parts of the country especially in Southern India and parts of Uttar Pradesh (Singh et al. 2012). There were many recent outbreaks of the disease in Uttar Pradesh predominantly in Gorakhpur, where repeated epidemics were reported along with hundreds of deaths year after year especially during 2005. Currently about 1500 to 4000 cases are being reported every year and more than 590 million people in India live in JE-endemic regions (Tiwari et al. 2012). The infection is maintained in enzootic cycle involving pigs, birds and mosquitoes. Studies on transmission ability have shown that *Cx. quinquefasciatus* is capable of transmitting the virus to susceptible hosts (NIV 2016).

Birds, pigs and other animal hosts act as reservoirs, which maintain and amplify arboviruses transmitted by *Cx. quinquefasciatus*. Once infected, the mosquitoes carry the virus for life. Since the mosquito comes into contact with different

vertebrate hosts, it acts as an important urban bridge vector, which bridges different reservoir/amplifier hosts to humans. Due to its presence in the urban, peri-urban and rural areas, it also acts as a bridge between diverse ecological niches (Bhattacharya and Basu 2016). The JE virus was isolated from *Cx. quinquefasciatus* for the first time in Vietnam in 1974 (Nitatpattana et al. 2005). The JE is predominantly a disease affecting rural population but may spill over to the urban areas due to changes in ecological conditions. Since *Cx. quinquefasciatus* can bridge the rural-urban eco-epidemiological zones, it may emerge as a potential vector of urban Japanese encephalitis (Bhattacharya and Basu 2016).

The feeding behaviour may vary among different populations of the same species, and this can have an impact on disease transmission. In Southeast Asia, where *Cx. quinquefasciatus* feeds predominantly on humans, it is the principal vector of human lymphatic filariasis. However, in Hawaii where it feeds predominantly on birds, it is a vector of avian malaria (*Plasmodium relictum*) and avian pox. The mixed feeding patterns of the mosquitoes of *Cx. pipiens* complex lead to their playing important roles in transmission of human zoonotic pathogens with avian hosts (Farajollahi et al. 2011). *Culex quinquefasciatus* is a vector of Rift Valley fever virus, dog heartworm *Dirofilaria immitis* and other nematodes such as *Saurofilaria* sp. and *Oswaldofilaria* sp. (Bhattacharya and Basu 2016).

16.7 Insecticide Susceptibility

Widespread use of chemical insecticides for control of mosquitoes has led to the development of insecticide resistance in mosquitoes including *Cx. quinquefasciatus*. In India, the first observation on the development of DDT resistance in *Cx. quinquefasciatus* was in 1952 from a village near Delhi. This was followed by reports on resistance to DDT and dieldrin from Nagpur, Pune and Patna. Resistance to BHC was reported from India in the year 1954. Adult *Cx. quinquefasciatus* mosquitoes were observed to be highly resistant to DDT and malathion in Rajahmundry, Andhra Pradesh, whereas the larvae were highly susceptible to temephos and fenthion. An earlier study in the same town has shown that temephos 50% EC was not effective against culicine larvae even at four times higher dose (0.05%) than recommended (Mukhopadhyay et al. 2006; Patnaik et al. 1997).

High level of DDT resistance in *Cx. quinquefasciatus* with mortality ranging from 11.9 to 50.0% was reported from Assam, India. However, the mosquitoes were highly susceptible to deltamethrin in the study areas. Biochemical profiling of candidate detoxification enzyme systems from all of the study sites shows the presence of alpha-esterase, beta-esterase and GST elevation. Hence, it was evident that high DDT resistance was through metabolic sequestration or detoxification. Also, there was clear correlation between enzyme levels and resistance in the study areas. High levels of DDT resistance observed in these areas may be attributed to the high levels of GST activity in the mosquito populations studied (Sarkar et al. 2009).

In the filaria endemic districts (Chandauli and Varanasi) of Uttar Pradesh, India, *Cx. quinquefasciatus* was observed to be highly resistant to DDT and malathion the mortality being 28.33% and 27.5%, respectively. There was incipient resistance to

pyrethroids with mortality in the range of 95.83–98.33%. The mosquitoes in this area were regularly exposed to insecticides due to the application of DDT, malathion, deltamethrin, permethrin and temephos for the control of malaria, filariasis and Kala-azar vectors (Kumar et al. 2011).

Cx. quinquefasciatus resistance was documented from many parts of Africa. This includes resistance to pyrethroids in Tanzania, Benin and Côte d'Ivoire whereas resistance to organophosphorous, carbamates and DDT in Benin, Burkina Faso and Côte d'Ivoire. In Macha, Zambia, *Cx. quinquefasciatus* was highly resistant to DDT, and deltamethrin-treated long-lasting insecticidal net had some degree of resistance to pyrethroids and malathion. The common mechanism of DDT and pyrethroid resistance was based on the kdr L1014F allele present in the population (Norris and Norris 2011). The presence of resistances and/or resistance mechanisms to organophosporous (chlorpyrifos, temephos), organochlorine (dieldrin, DDT) and pyrethroid (permethrin) insecticides was reported from the Western Indian Ocean islands. The most common resistance mechanism to pyrethroids was the kdr^R mutation, which was prevalent through the whole region. This also confers resistance to DDT. Metabolic resistance due to an increased mixed function oxidase detoxification was also detected. Esterase (especially the $Ester^2$ allele) overexpression results in resistance to organophosphorous insecticides. This was widespread and present at high frequencies in the islands studied. Although less common, the ace-1^R mutation was also observed in the area. Resistance to dieldrin through the Rdl^R mutation was present in some areas (Pocquet et al. 2013).

Metabolic resistance or enzymatic detoxification and target site modification are the two main insecticide resistance mechanisms in mosquitoes. Cytochrome P450 oxidases, esterases and glutathione-S transferases are the major classes of enzymes involved in metabolic resistance. Oxidases play a major role in resistance to pyrethroids, whereas esterases confer resistance to organophosphorous insecticides. The $ester^2$ allele is found all over the world in *Cx. pipiens* populations (in both pipiens and *quinquefasciatus* subspecies). Resistance to DDT involves DDT dehydrochlorinase enzymes. The targets for neurotoxic insecticides includes the axonic voltage-gated sodium channels (Na-channels), the synaptic acetylcholinesterase (AChE1), encoded by the ace-1gene, and the synaptic γ-aminobutyric acid receptor (GABA receptor), encoded by the Rdl gene. The most common target modifications in *Cx. quinquefasciatus* are the L1014F mutation (kdr^R allele) in the voltage-gated sodium channel gene, conferring resistance to pyrethroids and DDT, the G119S ace-1mutation (ace-1^R allele), conferring resistance to OP and carbamates, and the A302S Rdl mutation (Rdl^R allele), conferring resistance to the OC dieldrin (Pocquet et al. 2013).

16.8 Management Strategies

Vector control remains as the only viable method to control vector-borne diseases in the absence of effective vaccines. Control of mosquito vectors has been largely dependent on synthetic insecticides such as DDT, organophosphorous, carbamate and synthetic pyrethroid insecticides. Concerns about the adverse effects on the

environment and toxicity to nontarget organisms have drawn considerable attention towards alternate control strategies. Another reason for the search for alternatives to commonly used insecticides is the development of resistance in mosquitoes and the consequent reduction in bioefficacy. Novel insecticides and formulations are being evaluated for efficacy against mosquitoes. These include insecticides with lower mammalian toxicity, plant extracts, microbial insecticides, etc. Personal protective measures such as the use of insect repellents, long-lasting insecticidal nets, repellent-treated clothing, etc. can play a major role in combating mosquito vectors. Also novel methods such as sterile insect technique are also being evaluated.

Personal protective measures against mosquitoes include the use of insect repellents on skin and clothing and the use of long-lasting insecticidal nets. The exposed parts of the body can be protected from mosquito bites by the application of DEET or any other suitable insect repellents such as the indigenously manufactured DEPA (N, N diethyl diphenyl acetamide). DEPA is an effective repellent, which can be used for protection from mosquitoes and other biting arthropods. Laboratory studies on the repellent efficacy have shown that DEPA 20% provided 5 h 30 min protection from mosquito bites (Mendki et al. 2015). Permethrin-impregnated clothing is recommended by WHO for effective protection from disease vectors. Impregnation of curtains with synthetic pyrethroids can control the mosquitoes in buildings since the mosquitoes generally rest on the curtains, thereby picking up a lethal dose of the insecticide. Long-lasting insecticidal nets are an effective method to control disease vectors and to get protection from painful bites of insects. These nets are used worldwide especially for the control of malaria, and they typically lasts for more than 20 washings, and retreatment of the insecticide is not required. Environmental management including the reduction in mosquito breeding sites by ensuring proper drainage and waste disposal is also helpful in controlling *Cx. quinquefasciatus* mosquitoes. Spatial or area repellents create a mosquito-free zone, thereby preventing man-mosquito contact. Currently, there is a renewed interest in these repellents as they can delay the onset of resistance to insecticides, provide protection in both indoor and outdoor and are effective against multiple species of disease vectors (Achee et al. 2012).

Another method known as the incompatible insect technique employs the bacterium *Wolbachia*. *Wolbachia* induces a form of embryonic lethality called cytoplasmic incompatibility, a sperm-egg incompatibility occurring when infected males mate either with uninfected females or with females infected with incompatible *Wolbachia* strains (Atyame et al. 2011). Genetic manipulation of the bacteria in the mosquito midgut has the potential to generate transgenic mosquitoes, which are refractory to disease transmission. Studies have shown that there are common representatives of the microbiota harboured by different mosquito species. These bacteria may be targeted for genetic manipulation so as to reduce the vectorial capacity of the host (Pidiyar et al. 2004). *Bacillus thuringiensis* var. *israelensis* (Bti), an aerobic spore-forming, entomopathogenic bacterium specific to dipterans, is regarded as the most promising microbial control agent against mosquitoes and black flies. It can be used alone or as a component in integrated vector control programme.

Under field conditions the efficacy of different *Bti* formulations lasted for 2–7 days against *Cx. quinquefasciatus* in polluted pools and drains. The efficacy of *B. sphaericus* formulations lasted for 1–4 weeks at 1–2 g/m^2 in polluted water habitats (Mittal 2003). Solvent extracts from the plants *Cleistanthus collinus*, *Leucas aspera*, *Hydrocotyle javanica*, *Murraya koenigii*, *Sphaeranthus indicus* and *Zanthoxylum limonella* were effective against the larvae of *Cx. quinquefasciatus* (Tennyson et al. 2012). Extracts of *Cymbopogon citratus* and *Abrus precatorius* were found to be the most effective larvicides among the plant extracts screened, with LC_{50} values of 24 and 30 mg/l. Plants such as *Vitex negundo*, *Cleome viscosa*, *Leucas aspera*, *Spermacoce hispida*, *Euphorbia thymifolia*, *Kaempferia galanga* and *Stachytarpheta jamaicensis* were also reported to be effective with LC_{50} less than 50 mg/l (Nazar et al. 2009).

16.9 Conclusion

Culex quinquefasciatus is a widely distributed mosquito species in urban and rural areas of the world. It is a vector of lymphatic filariasis and many arboviral diseases with millions of people potentially at risk of infections. In India it is important as the principal vector of filariasis which causes severe morbidity and socioeconomic constraints on affected people. Apart from the role as a disease vector, it is a biting nuisance in domestic and commercial environments. In the context of resistance development against commonly used insecticides, there is an urgent need to revise the control measures against this important pest of medical and veterinary importance. More importance needs to be given to the development of safe, effective and ecofriendly methods of pest control against *Cx. quinquefasciatus.*

References

Achee NL, Bangs MJ, Farlow R, Killeen GF, Lindsay S, Logan JG, Moore SJ, Rowland M, Sweeney K, Torr SJ, Zwiebel LJ, Grieco JP (2012) Spatial repellents: from discovery and development to evidence-based validation. Malar J 11:164

Atyame CM, Pasteur N, Dumas E, Tortosa P, Tantely ML, Pocquet N, Licciardi S, Bheecarry A, Zumbo B, Weill M, Duron O (2011) Cytoplasmic incompatibility as a means of controlling *Culexpipiensquinquefasciatus* mosquito in the islands of the South-Western Indian Ocean. PLoSNegl Trop Dis 5:e1440. https://doi.org/10.1371/journal.pntd.0001440

Azmi SA, Das S, Chatterjee S (2015) Seasonal prevalence and blood meal analysis of filarial vector *Culexquinquefasciatus* in coastal areas of Digha, West Bengal, India. J Vector Borne Dis 52(3):252–256

Bhattacharya S, Basu P (2016) The southern house mosquito, *Culex quinquefasciatus*: profile of a smart vector. J Entomol Zool Stud 4:73–81

Chandel K, Mendki MJ, Parikh RY, Kulkarni G, Tikar SN, Sukumaran D, Prakash S, Parashar BD, Shouche YS, Veer V (2013) Midgut microbial community of *Culex quinquefasciatus* mosquito populations from India PLoS One. 29;8(11):e80453. https://doi.org/10.1371/journal.pone.0080453. eCollection 2013

Diaz-Badillo A, Bolling BG, Perez-Ramirez G, Moore CG, Martinez-Munoz JP, Padilla-Viveros AA, Camacho-Nuez M, Diaz-Perez A, Beaty BJ, Munoz Mde L (2011) The distribution of poten-

tial West Nile virus vectors, *Culex pipiens pipiens* and *Culex pipiens quinquefasciatus* (Diptera: Culicidae), in Mexico City. Parasit Vectors. 9;4:70. https://doi.org/10.1186/1756-3305-4-70

Gajanana A (2002) West Nile virus epidemics: lessons for India. ICMR Bull 32:7 http://icmr.nic.in/bujuly02.pdf

Farajollahi A, Fonseca DM, Kramer LD, Marm Kilpatrick A (2011) "Bird biting" mosquitoes and human disease: a review of the role of *Culex pipiens* complex mosquitoes in epidemiology. Infect Genet Evol 11:1577–1585. https://doi.org/10.1016/j.meegid.2011.08.013

Gokhale MD, Paingankar MS, Sachin D, Dhaigude SD (2013) Comparison of biological attributes of *Culex quinquefasciatus* (Diptera: Culicidae) populations from India. ISRN Entomology 2013:1. https://doi.org/10.1155/2013/451592

Gowda NN, Vijayan VA (1993) Biting density, behavior and age distribution of Culexquinquefasciatus, say in Mysore City, India. Southeast Asian J Trop Med Public Health 24:152–156

Hill S, Connelly CR (2015) Southern house mosquito *Culex quinquefasciatus* say. https://edisifasufledu/pdffiles/IN/IN83700pdf. Accessed 15 Dec 2015

Korgaonkar NS, Kumar A, Yadav RS, Kabadi D, Dash AP (2012) Mosquito biting activity on humans & detection of *Plasmodium falciparum* infection in *Anopheles stephensi* in Goa, India. Indian J Med Res 135:120–126

Kumar K, Sharma AK, Kumar S, Patel S, Sarkar M, Chauhan LS (2011) Multiple insecticide resistance/susceptibility status of *Culexquinquefasciatus*, principal vector of bancroftianfilariasis from filaria endemic areas of northern India. Asian Pac J Trop Med 4:426–429. https://doi.org/10.1016/S1995-7645(11)60119-3

Mahanta B, Handique R, Dutta P, Narain K, Mahanta J (1999) Temporral variations in biting density and rhythm of *Culex quinquefasciatus* in tea afroecosystem of Assam, India. Southeast Asian J Trop Med Public Health 30:804–809

Manimegalai K, Sukanya S (2014) Biology of the filarial vector, Culex quinquefasciatus (Diptera: Culicidae). Int J Curr Micobiol App Sci 3:718–724

Mendki MJ, Singh AP, Tikar SN, Parashar BD, Veer V, Shukla SV, Prakash S (2015) Repellent activity of N, N-diethylphenylacetamide (DEPA) with essential oils against *Aedes aegypti*, vector of dengue and chikungunya. Int J Mosquito Res 5 2(3):17–20

Mishra C (2014) Culex Mosquito: vector of Filariasis. *Odisha Review.* http://odishagovin/e-magazine/Orissareview/2014/Sept-Oct/engpdf/96-99pdf. Accessed 12 Dec 2015

Mittal PK (2003) Biolarvicides in vector control: challenges and prospects. J Vect Borne Dis 40:20–32

Mourya DT, Gokhale MD, Pidiyar V, Barde PV, Patole M, Mishra AC, Shouche Y (2002) Study of the effect of the midgut bacterial flora of *Culex quinquefasciatus* on the susceptibility of mosquitoes to Japanese encephalitis virus. Acta Virol 46(4):257–260

Mukhopadhyay AK, Patnaik SK, Babu PS (2006) Susceptibility status of some culicine mosquitoes to insecticides in Rajahmundry town of Andhra Pradesh, India. J Vector Borne Dis 43:39–41

Nazar S, Ravikumar S, Williams GP, Ali SM, Suganthi P (2009) Screening of Indian coastal plant extracts for larvicidal activity of *Culex quinquefasciatus*. Indian J Sci Technol 2:24–27

Norris LC, Norris DE (2011) Insecticide resistance in *Culex quinquefasciatus* mosquitoes after the introduction of insecticide-treated bed nets in Macha, Zambia. J Vector Ecol 36(2):411–420. https://doi.org/10.1111/j.1948-7134.2011.00182.x

Nitatpattana N, Apiwathnasorn C, Barbazan P, Leemingsawat S, Yoksan S, Gonzalez JP (2005) First isolation of Japanese encephalitis from *Culex quinquefasciatus* in Thailand. Southeast Asian J Trop Med Public Health 36:875–878

NIV (2016) Japanese encephalitis. National Institute of Virology, Pune. http://www.icmrnicin/pinstitute/niv/japanese%20encephalitispdf. Accessed 10 Dec 2015

Oduola AO, Awe OO (2006) Behavioural biting preference of *Culexquinquefasciatus* in human host in Lagos metropolis Nigeria. J Vector Borne Dis 43:16–20

Paramasivan R, Mishra AC, Mourya DT (2003) West Nile virus: the Indian scenario. Indian J Med Res 118:101–108

Patnaik SK, Kanhekar LJ, Rao PK, Raina VK, Kumar A (1997) A study to evaluate the impact of different dose/frequency of temephos 50% EC for control of Cx. quinquefasciatus vector of bancroftianfilariasis. J Commun Dis 29:171–174

Pidiyar VJ, Jangid K, Patole MS, Shouche YS (2004) Studies on cultured and uncultured microbiota of wild *Culex quinquefasciatus* mosquito midgut based on 16s ribosomal RNA gene analysis. Am J Trop Med Hyg 70(6):597–603

Pocquet N, Milesi P, Makoundou P, Unal S, Zumbo B, Atyame C, Darriet F, Dehecq JS, Thiria J, Bheecarry A, Iyaloo DP, Weill M, Chandre F, Labbé P (2013). Multiple insecticide resistances in the disease vector *Culex p. quinquefasciatus* from western Indian Ocean. PLoS One. 21;8(10):e77855. https://doi.org/10.1371/journal.pone.0077855. eCollection 2013

Sarkar M, Bhattacharyya IK, Borkotoki A, Goswami D, Rabha B, Baruah I, Srivastava RB (2009) Insecticide resistance and detoxifying enzyme activity in the principal bancroftianfilariasis vector, *Culexquinquefasciatus*, in northeastern India. Med Vet Entomol J 23(2):122–131. https://doi.org/10.1111/j.1365-2915.2009.00805.x

Singh A, Saxena SK, Srivastava AK, Mathur A (2012) Japanese encephalitis: a persistent threat. Proc Natl Acad Sci Sect B Biol Sci 82(1):55–68. https://doi.org/10.1007/s40011-011-0005-x

Subra R (1981) Biology and control of *Culexpipiensquinquefasciatus*Say, 1823 (Diptera, Culicidae), with special reference to Africa. Insect Sc Appl 1(4):319–338

Sudeep AB, Mandar P, Ghodke YK, George RP, Gokhale MD (2015) Vector competence of two Indian populations of *Culex quinquefasciatus* (Diptera: Culicidae) mosquitoes to three West Nile virus strains. J Vector Borne Dis 52:185–192

Tennyson S, Ravindran KJ, Arivoli S (2012) Screening of twenty five plant extracts for larvicidal activity against *Culex quinquefasciatus*Say (Diptera: Culicidae). Asian Pac J Trop Biomed:S1130–S1134. https://doi.org/10.1016/S2221-1691(12)60372-4

Tiwari S, Singh RK, Tiwari R, Dhole TN (2012) Japanese encephalitis: a review of the Indian perspective. Braz J Infect Dis 16:564–573. https://doi.org/10.1016/j.bjid.2012.10.004

Upadhyayula SM, Mutheneni SR, Kadiri MR, Kumaraswamy S, Nagalla B (2012) A cohort study of lymphatic filariasis on socio economic conditions in Andhra Pradesh. IndiaPLoS One 7:e33779. https://doi.org/10.1371/journal.pone.0033779

Uttah EC, Wokem GN, Okonofua C (2013) The abundance and biting patterns of *Culex quinquefasciatus* say (Culicidae) in the coastal region of Nigeria. ISRN Zoology 2013:1. https://doi.org/10.1155/2013/640691

17

Ecology and Biology of *Culex quinquefasciatus* Say, 1823, in Two Physiographically Different Ecosystems with Special Reference to Human Lymphatic Filariasis in West Bengal, India

Sajal Bhattacharya and Probal Basu

Abstract

Lymphatic filariasis (LF) is a major cause of public health concern worldwide especially in a tropical country like India. About 1254 million people worldwide, inhabiting 83 countries including 554.2 million people in India, are at the threshold of the filarial infection. The nematode *Wuchereria bancrofti* accounts for an estimated 95 % of these infections worldwide. The other two nematodes *Brugia malayi* and *B. timori* are minor agents of filariasis. The periodic (nocturnal) form of *W. bancrofti* is prevalent in the Indian mainland transmitted by *Culex quinquefasciatus*, a member of *Culex pipiens* species complex. Recent studies have indicated that *Cx. pipiens* and *Cx. quinquefasciatus* are two distinct species. The complete genome sequencing studies of *Culex quinquefasciatus* reveal that the number of their protein-coding genes (18,883) is 22 % greater than that of *Aedes aegypti* and 52% greater than that of *Anopheles gambiae*. In addition to this, the species exhibit multiple gene family expansion which includes olfactory and gustatory receptors, salivary gland genes and genes associated with xenobiotic detoxification. The mosquito species is a nocturnal biter and has been predominantly recorded from human baits in Calcutta. *Culex quinquefasciatus* is considered as an opportunistic feeder in rural Bengal as it feeds on 26.45 % humans only, with mostly on ruminants (46.25 %), followed by pigs (14.19 %) and birds (6.45 %). This mosquito species is found in domestic collections of water, places like flooded open cement drains, flooded latrines, overflow water from roof-top tanks, kitchens as well as in ground pools, ditches and shallow wells. This species usually selects organically rich and polluted surface waters and artificial

S. Bhattacharya (✉) · P. Basu
Postgraduate Department of Zoology, Asutosh College (University of Calcutta),
92, S. P. Mukherjee Road, Kolkata 700026, India
e-mail: shailja_bhattacharya@cdri.res.in

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_17

containers for breeding. The post-monsoon season (September, October, November) records the highest per man-hour density of the vector, *Cx. quinquefasciatus*, followed by the monsoon (June, July, August) and winter (December, January, February). The summer months have the lowest per man-hour density of the vector population in the studied areas. The overall per man-hour density of *Cx. quinquefasciatus* in the urban area (72.23 %) is markedly higher than that of the rural areas (37.03 %). In West Bengal, urban areas were more endemic for bancroftian filariasis than the rural area. Urban developments such as road, fly-over, building, metro rail construction, etc. are the contributing factors in creating a complex mosquitogenic and filariogenic situations in urban areas. Global warming and globalization are likely to reshape the ecology of vector mosquitoes, especially ubiquitous *Cx. quinquefasciatus*. This will have wide-ranging consequences on the epidemiology of vector-borne diseases like lymphatic filariasis.

17.1 Introduction

Lymphatic filariasis (LF) is a major cause of public health concern worldwide especially in a tropical country like India. About 1254 million people worldwide, inhabiting 83 countries including 554.2 million people in India, are at the threshold of the filarial infection (WHO 2007). The causative agents of human lymphatic filariasis are *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. The nematode *Wuchereria bancrofti* accounts for an estimated 95 % of these infections worldwide (Dreyer et al. 2005). The periodic (nocturnal) form of *W. bancrofti* is prevalent in the Indian mainland where *Culex quinquefasciatus* is the principal vector of bancroftian filariasis (Das 1976; Bhattacharya 2009). *Aedes* (Finlaya) *niveus* is the vector of subperiodic *W. bancrofti* in Nicobar Islands (Tiwari et al. 1995). In the South Pacific, the vector is *Aedes polynesiensis* and other species of *Aedes* (Hati 2010). *Wuchereria bancrofti* is mainly prevalent in Africa, Southeast Asia and Papua New Guinea, whereas *Brugia malayi*, another human filarial nematode, is prevalent in Southeast Asia (WHO 1992). In India, *B. malayi* is transmitted by *Mansonia* mosquitoes, viz. *M. annulifera*, *M. uniformis* and *M. indiana* (Sabesan et al. 1992). *Brugia timori* is restricted to a few islands in Indonesia. India contributes about 40 % of the total global burden of filariasis and accounts for about 50 % of the people at risk of infection.

Wuchereria bancrofti is the predominant species accounting for about 98 % of the national burden of lymphatic filariasis (Das et al. 2002). Several arboviruses having public health importance, namely, West Nile virus (WNV) and Japanese encephalitis virus (JEV), have also been isolated from *Cx. quinquefasciatus* (Goddard et al. 2002; Thenmozhi et al. 2014). Therefore, it is also considered as a suspected potential vector of some arboviruses. *Culex quinquefasciatus* is a cosmopolitan mosquito species with a worldwide distribution, more frequent in tropical, subtropical and warmer temperate regions in association with human dwellings

(David et al. 2012; Gokhale et al. 2013). This species is one of the most abundant of Indian Culicinae and is found in all parts of the Indian region encompassing urban suburban and rural areas and is largely a domestic mosquito (Barraud 1934). Bancroftian filariasis is endemic in the state of West Bengal including Kolkata (erstwhile Calcutta) (Hati et al. 1989; Bhattacharya and Misra 1992). Keeping in view the above perspectives, an entomological survey was conducted in Kolkata and South 24 Parganas district during 2011–2014. The objective of this survey was to know the prevalence and population density of this mosquito species in two different ecological situations. An attempt has also been made to review certain aspects of ecology and biology of *Cx. quinquefasciatus* with special reference to bancroftian filariasis in the state of West Bengal.

17.2 Taxonomy

Culex quinquefasciatus is a member of globally distributed *Culex pipiens* species complex. Additionally, *Culex pipiens* species complex has many related species, ecotypes and hybrids which are situated in geographical introgression zones on multiple continents (Farajollahi et al. 2011). *Culex quinquefasciatus* was first described in 1823 by Thomas Say from a specimen collected along the Mississippi River in the Southern United States. At that time, a number of similar species around the world like *Culex fatigans* (Wiedemann 1828) from the Old World tropics were used synonymous to *Cx. quinquefasciatus* (Stone 1956; Belkin 1977). Females of *Culex pipiens* and *Cx. quinquefasciatus* are morphologically indistinguishable, and hybrid zones for the two species are well documented. Due to this, *Culex quinquefasciatus* has been considered and designated as a subspecies of *Cx. pipiens* with the name *Culex pipiens quinquefasciatus* (Barr 1957). Recent studies have indicated that *Cx. pipiens* and *Cx. quinquefasciatus* are two distinct sympatric mosquito populations (Cornel et al. 2003), and they also exhibit a distinct and clear genetic difference (Smith and Fonseca 2004) which led to the elevation of *Cx. quinquefasciatus* to a species status.

17.3 Genetics

Culex quinquefasciatus has been extensively used for considerable genetic research because of its long-standing taxonomic controversy and importance as a vector of human lymphatic filariasis (Fonseca et al. 2006). The species contains three metacentric chromosomes among which Chromosome 1 is considered the shortest, Chromosome 2 is intermediate in length and Chromosome 3 is the longest (McAbee et al. 2007). The complete genome-sequencing studies of *Culex quinquefasciatus* reveal that the number of their protein-coding genes (18,883) is 22 % greater than that of *Aedes aegypti* and 52 % greater than that of Anopheles gambiae. In addition to this, the species exhibit multiple gene family expansion which includes olfactory

and gustatory receptors, salivary gland genes and genes associated with xenobiotic detoxification (Arensburger et al. 2010).

17.4 Reproductive Biology

Gravid *Cx. quinquefasciatus* females lay averagely 155 eggs crafted in a boatlike raft during each gonotrophic (egg laying) cycle; the number of eggs depends on mosquito age, blood source and blood volume (Subra 1981). Egg rafts are laid on the surface of a suitable body of water selected using chemical cues derived from conspecific egg rafts (Laurence and Pickett 1985). Larval to adult development is dependent on temperature, nutrition and population density and can be as short as at 7 days under optimal conditions (30 °C) (Rueda et al. 1990). Females mate within 2–6 days of emerging and may begin to seek hosts within 48 h of emergence (Subra 1981). Since *Cx. quinquefasciatus* must acquire a blood meal for ova maturation and does not undergo a reproductive diapause, this species is active and reproduces year-round. In India, it may complete two to three gonotrophic cycles in a lifetime during the hotter season and four to eight cycles in the cooler season (Chandra et al. 1996). Age composition of the filarial vector mosquito *Culex quinquefasciatus* was determined by examining the number of ovariolar dilatations of 1200 adult females from Calcutta. Average duration of the gonotrophic cycle was 4.5 days. The daily survival rate and daily mortality rates of the natural population were 0.53, 0.87 and 13 %, respectively. The oldest mosquito sampled in the study area passed eight gonotrophic cycles in its lifetime (Chandra et al. 1996). Infection with *W. bancrofti* may disrupt the relationship between mosquito size and egg production during the first gonotrophic cycle of *Culex quinquefasciatus* such that fecundity is sometimes reduced (Lima et al. 2003).

17.5 Breeding

Culex quinquefasciatus is found in the water collected from the household situations and in places like flooded open cement drains, flooded latrines, overflow water from housetops with tanks, kitchens as well as in ground pools, ditches and shallow wells. This species usually selects organically rich and polluted surface waters and artificial containers for breeding (Weinstein et al. 1997). This species also breeds in shallow ponds, within streams, phytotelmata (Derraik 2005) and also in artificial habitats such as drains, wells, septic tanks and other small containers (Laird 1995).

While conducting a survey in metro rail construction sites in Kolkata of West Bengal, it was found that *Culex quinquefasciatus* mainly preferred polluted water for breeding in winter and post-winter months (Chaterjee et al. 1988). In North Central Nigeria, Africa, bancroftian filariasis is transmitted by *Culex quinquefasciatus* in urban and semirural areas where increased pollution of freshwater bodies and the introduction of pit latrines favour the breeding of the mosquito (Badaki 2010; Service 2012). A wide variety of sites mostly characterized by coloured foul water

Table 17.1 Categories of different habitats of *Cx. quinquefasciatus* (Data Sheet of The Invasive Species Compendium [ISC] 2015)

Category	Habitat	Presence
Freshwater	Irrigation channels	Secondary habitat
	Ponds	Secondary habitat
	Rivers/streams	Secondary habitat
	Buildings	Principal habitat
	Cultivated/agricultural land	Secondary habitat
	Disturbed areas	Principal habitat
Terrestrial-managed	Industrial/intensive livestock production systems	Principal habitat
	Managed grasslands (grazing systems)	Secondary habitat
	Rail/roadsides	Secondary habitat
	Urban/peri-urban areas	Principal habitat
	Natural forests	Secondary habitat
Terrestrial-natural/ seminatural	Rocky areas/lava flows	Secondary habitat
	Wetlands	Secondary habitat

with high nutrient values and low dissolved oxygen content, such as pumping and irrigation wells, canals, wastewater treatment ponds, sewage overflows, rain pools, rice paddy fields, fishponds, septic tanks, drains, cesspools, agricultural trenches, vegetable farms, etc., were preferred as breeding sites by *Cx. quinquefasciatus* (Matthys et al. 2006; Opoku et al. 2007). Categories of different habitats of *Cx. quinquefasciatus* have been shown in the Table 17.1.

17.6 Biting and Feeding

Culex quinquefasciatus has a predilection for urban environment and will feed on humans indoors as well as outdoors (Sirivanakarn 1976). The species, a nocturnal biter, was predominant among all mosquito fauna sampled from human baits in Calcutta and adjoining South and North 24 Parganas districts (Bhattacharya and Hati 1986; Bhattacharya and Santra 2005) and constitutes 97 % of the nocturnal man-biting mosquitoes of Calcutta (Mukhopadhyay and Hati 1978). In addition to human being, the female mosquito can efficiently bite amphibians (Lee et al. 1989), pigs, horses, cattle, sheep, dogs and rabbits (Holder et al. 1999). The annual transmission potential of *W. bancrofti* was calculated from the number of infective larvae found in the number of *Culex quinquefasciatus* expected to bite an individual exposed 24 h a

day for a year in Calcutta and in a rural area of Burdwan's Memari. In Calcutta, an average of 151 mosquitoes/night/person were collected; it was calculated that 55,028 mosquitoes could bite a person per year, including an average of 177 infective mosquitoes containing 319 infective larvae of *W. bancrofti*. At Memari an average of 284 mosquitoes/night/person were collected. The resulting calculation was that 103,718 mosquitoes could bite a person per year, including an average of 137 infective mosquitoes containing 223 infective larvae. The annual transmission potential was higher in the urban area than that in the rural area (Hati et al. 1989).

Culex quinquefasciatus is considered as an opportunistic feeder in rural Bengal as it feeds on 26.45 % humans, 46.25 % ruminants, 14.19 % pigs and 6.45 % birds (Bhattacharya et al. 1982). While in Southern India, this is a highly anthropophilic species, with up to 50–76 % feeding on humans (Reuben et al. 1992), attraction ratio of *Cx. quinquefasciatus* of man/cow was 101.1:1 in Burdwan district, West Bengal (Chakraborty et al. 1986).

17.7 Density of the Vector and the Filarial Infection

In Kolkata and adjoining suburbs, *Cx. quinquefasciatus* was found to be 62.35 % of the total mosquito populations belonging to 18 species and occupied the leading position (Bhattacharya and Santra 2005). Chandra et al. (2013) reported the corresponding figure of *Cx. quinquefasciatus* in Kolkata as 82.44 % and the overall per man-hour density, 27.56 %. Season-wise data show that the vector density was higher in the rainy season in both urban and rural areas, but in all the three meteorologically distinguishable seasons, density was higher in urban than rural areas. Urban and rural areas of West Bengal are endemic for bancroftian filariasis (Hati et al. 1989). Average man-hour density of *Cx. quinquefasciatus* was high (31.10), and a higher vector prevalence was seen in summer.

The infection and infectivity rates of the natural population of the vector were 2.3 and 0.28 %, respectively. About 9.2 % and 1.7 % of the human habitations were found to contain infected and infective *Cx. quinquefasciatus*. Both infection and infectivity rates were higher during the rainy season than other seasons (De and Chandra 1994). The causative parasite was identified as *W. bancrofti*, and *Cx. quinquefasciatus* was incriminated as the vector responsible. The human blood index (HBI) of human dwelling frequenting vector population was 70 %. Vector density, vector infection, infectivity rates and human blood index were all higher in the rainy season (Chandra et al. 2007). It was found that the biting density, natural infection and infectivity rates of *Cx. quinquefasciatus* were significantly higher in the third quadrant of the night (from midnight to 0300 AM) than at other times. This was true both in urban and rural environment (Chandra 1995). Average per man-hour density of *Cx. quinquefasciatus* was 9.0 (ranging from 7.0 to 11.3 in different months).

Availability of vector species in human habitations showed no seasonal ($p > 0.05$) variation as indicated by the prevalence in rainy season (37.8 %), winter (34.9 %) and summer (27.2 %). Out of 23 infected *Cx. quinquefasciatus*, a total of 13 (56.5 %), 6 (26.1 %) and 4 (17.4 %) were found to carry first- and second-stage

larvae of *W. bancrofti*, respectively. No third-stage larva was detected in the wild vector population. Overall vector infection rate was 2.7 %, ranging from 0 % to 4.7 % in different months. Seasonal vector infection rates were 3.0 %, 2.3 % and 2.5 % in rainy season, winter and summer, respectively, without any marked variation ($p > 0.05$). Vector infection rate and infectivity rate (2.7 %, 0 %) were lower in Bankura district, West Bengal, than Panna district, Madhya Pradesh (4.9 %; 1.0 %) (Rudra and Chandra 1998). Low vector infectivity rate (0 %) indicates that transmission cycle of filarial worm is not very active in the tribal villages. Probably they carry the infection from some highly endemic places outside where they used to go for work, indicating a correlation of higher infection among working age groups (Rudra and Chandra 1998). Man-hour density of vector species in the tribal areas is much lower than those of the nontribal areas studied in West Bengal (Rudra and Chandra 1998).

In West Bengal, infection and infectivity rates are generally peaking during the monsoon, or both in summer and monsoon, as was evident in Digha (Medinipur) and Katwa (Burdwan), but in Susunia (Bankura) these rates are found to be higher in summer season only (Das et al. 2003; Chandra et al. 2007; Pramanik and Chandra 2010). Experiments on *Culex quinquefasciatus* demonstrated that its density was found to be significantly lower ($p < 0.05$) in the rainy season than in the dry seasons, i.e. summer and winter, in different endemic areas of West Bengal (De and Chandra 1994; Chandra et al. 1993; Rozeboom et al. 1968). The peak time of filarial transmission in most endemic areas was observed to be the hot months of summer and rainy seasons. It is established by the highest infection and infectivity rates of the vector in nature, the shortest developmental period of the parasite in the vector (Chandra et al. 1997) and the highest transmission potential (Hati et al. 1989; Chandra and Rudra 2006). The rainy season provides optimum conditions to raise the vector efficiency index to its peak. This index is based on rapid parasitic development, proper nursing and low parasitic damage or death (Chandra et al. 1997). It has been observed that parasitic infection affects the host reproductive fitness or longevity. This could be initiated in very early infection before the parasite causes the burden on host specific resources (Hurd and Webb 1997).

Interestingly, laboratory work has shown that *Culex quinquefasciatus* is able to control the number of developing *W. bancrofti* (1–2/mosquito) during ingestion. This suggests that there must be a regulatory mechanism which causes the control of the number of infective larvae that can successfully mature in *Cx. quinquefasciatus* (Manyi et al. 2014). Thus further studies are necessary to understand the disease dynamics and the vector biology in the context of filarial infection and transmission.

17.8 Lymphatic Filariasis: West Bengal Scenario

According to the National Vector Borne Diseases Control Programme (2015), the average microfilaria infection rate (%) in West Bengal varied from 0.48 (2009) to 4.74 (2004) between 2004 and 2012, while the corresponding figure for India varied

from 0.37 % (2011) to 1.24 % (2004). In 1984, the attendance of new filarial cases in the 'Filaria outdoor clinic' of Calcutta School of Tropical Medicine (STM) was 2884 (Females 1581 and Males 1303). About 3000 new cases were detected every year in the said filariasis clinic (Bhattacharya and Hati 1986). In 2006, the corresponding figures of new cases were 727 (429 males and 298 females), while 828 male and 719 female old patients had attended the clinic.

It seems from the above data that the attendance of new filarial patients in outdoor clinic has decreased in recent years. However, these observations do not indicate the decreased level of transmission of the filarial infection in the areas from where the patients come to Calcutta STM clinic (Bhattacharya 2009). Chandra et al. (1993) reported that significantly more *Cx. quinquefasciatus* mosquitoes (> 55 %) attacked on the thighs, legs and feet of human volunteers than other sites on their body, whether the volunteers were indoors or outdoors, in rural or urban environments. Significantly more patients had clinical filariasis of the lower body parts than the upper parts. Hence, the vector's preferred biting sites and the anatomical sites most affected by clinical filariasis were found to be the lower body parts.

17.9 Materials and Methods

17.9.1 Mosquito Collection and Identification

Indoor resting mosquitoes were collected from human habitations including temporary hutments (Jhupries) in urban and suburban Kolkata and South 24 Parganas between 0600 and 0800 AM from December 2011 to November 2014. Mosquitoes were collected by using test tubes and battery operated torches. Mosquitoes were also collected from cattle sheds in South 24 Parganas. The total man-hours spent during the study period was 288 h, i.e. 96 h in each year. Mosquito species identification was based on Christophers (1933), Barraud (1934), Wattal and Kalra (1961), Hati (2010) and Das and Kaul (1998).

17.9.2 Results and Discussion

A total of 13,525 mosquitoes were collected during the present study from Kolkata (Table 17.2), belonging to 12 species of which *Culex quinquefasciatus* was the most predominant (72.23 %) followed by *Armigeres subalbatus* (6.96 %) and *Aedes aegypti* (6.57 %). Their respective proportion and ranking are given in Fig. 17.1.

Compared to Kolkata, of the 19,728 mosquitoes collected in South 24 Parganas (Table 17.3), belonging to 19 species, the percentage of *Cx. quinquefasciatus* was found to be 37.03 %, followed by *Cx. vishnui* (15.47 %) and *Ar. subalbatus* (9.42 %).

The proportion-wise ranking of each of the species sampled is shown in Fig. 17.2.

Most of the areas in South 24 Parganas are rural with partial urban situation bordering Kolkata along one side and mangrove ecosystem of Sundarbans on the other.

Table 17.2 Mosquito species recorded in Kolkata during 2011–2014

Mosquitoes	Numbers	Per man-hour (PMH)	Percentages (%)
Cx. quinquefasciatus	9769	33.9201	72.23
Cx. vishnui	211	0.7326	1.56
Cx. tritaeniorhynchus	101	0.3506	0.75
An. stephensi	214	0.7430	1.58
An. subpictus	428	1.4861	3.16
An. vagus	235	0.8159	1.74
An. annularis	65	0.2256	0.48
Ae. aegypti	889	3.0868	6.57
Ae. albopictus	454	1.5763	3.37
Ae. vittatus	159	0.5520	1.18
Ar. subalbatus	941	3.2673	6.96
Ma. indiana	59	0.2048	0.44
Total	**13,525**	**46.9618**	**100**

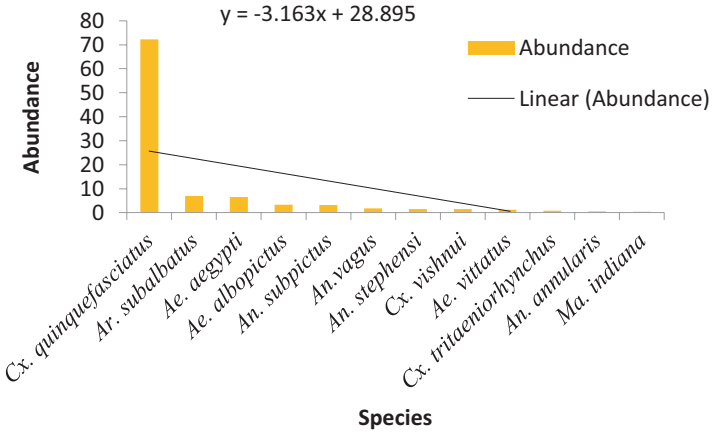


Fig. 17.1 Rank abundance curve of sampled mosquito species in Kolkata (Abundance expressed in %)

Anopheles sundaicus has been rediscovered in Sundarbans area after nearly two and a half decades (Bhattacharya et al. 2014). Density and diversity of mosquitoes were significantly greater in rural South 24 Parganas than the species discovered in urban Kolkata. However, per man-hour (PMH) density of *Cx. quinquefasciatus* was found to be the highest both in Kolkata and South 24 Parganas and occupied the first position among all the mosquito species collected. Highest density of *Cx. quinquefasciatus* was found in the post-monsoon season, while the lowest density was observed in the pre-monsoon season in both the studied area (Table 17.4).

This observation is different with that of Chandra et al. (2013). The PMH density of this species is significantly higher in urban areas in all the seasons barring pre-monsoon period in comparison to the rural areas. Analysis of the rank-abundance curve shows a more even distribution with slope (m = −1.085) of various species of

Table 17.3 Mosquito species recorded in South 24 Parganas during 2011–2014

Mosquitoes	Numbers	Per man-hour (Pmh)	Percentages (%)
Cx. quinquefasciatus	7305	25.3645	37.03
Cx. bitaeniorhynchus	1021	3.5451	5.17
Cx. vishnui	3052	10.5972	15.47
Cx. tritaeniorhynchus	1575	5.4687	7.98
Cx. pseudovishnui	464	1.6111	2.35
Cx. gelidus	79	0.2743	0.40
An. stephensi	176	0.6111	0.89
An. subpictus	385	1.3368	1.95
An. hyrchanus	49	0.1701	0.25
An. annularis	753	2.6145	3.82
An. culicifacies	535	1.8576	2.71
An. sundaicus	12	0.0416	0.06
An. vagus	201	0.6979	1.02
Ae. albopictus	1092	3.7916	5.54
Ae. aegypti	358	1.2430	1.81
Ae. vittatus	657	2.2812	3.33
Ar. subalbatus	1859	6.4548	9.42
Ma. annulifera	97	0.3368	0.49
Ma. indiana	58	0.2013	0.29
Total	**19,728**	**68.5**	**100**

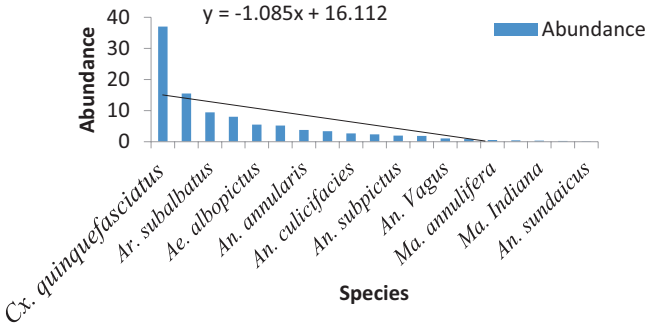


Fig. 17.2 Rank abundance curve of sampled mosquito species in South 24 Parganas (Abundance expressed in %)

mosquitoes in rural areas in comparison with urban area, where species dominance of *Cx. quinquefasciatus* over other species is clearly evident from the slope (m = −3.163) (Figs. 17.1 and 17.2).

In both the studied areas, the post-monsoon season (September, October, November) records the highest per man-hour density of the vector, *Cx. quinquefasciatus*, followed by the monsoon season (June, July, August) and winter season (December, January, February). The lowest population is recorded in both the areas in the pre-monsoon months of March, April and May. The high temperature during the hot and dry months of March, April and May possibly does not favour the

Table 17.4 *Cx. quinquefasciatus* recorded in rural and urban areas in different seasons

Seasons	Number of mosquitoes in rural area (South 24 Parganas)	Per man-hour density (PMH)	Percentage (%)	Number of mosquitoes in urban area (Kolkata)	Per man-hour density (PMH)	Percentage (%)
Pre-monsoon (March, April, May)	1005	13.757	13.8261	1201	16.680	12.2939
Monsoon (June, July, August)	2047	28.430	28.0219	2715	37.708	27.7919
Post-monsoon (September, October, November)	2815	39.097	38.5352	3956	59.944	40.4954
Winter (December, January, February)	1438	19.972	19.6851	1897	26.347	19.4185
	7305			**9769**		

significant growth of the vector population. This is why the summer months have the lowest per man-hour density of the vector population in the studied areas. During the rainy seasons, the heavy pour possibly flushes out the larval population; hence, the per man-hour density during the rainy months of June, July and August is found to be less compared to the post-monsoon season. The overall per man-hour density of *Cx. quinquefasciatus* in urban area (72.23 %) is markedly higher than that of the rural areas (37.03 %). This is indicative of the fact that the unsustainable urban development and lifestyle in Calcutta for the last three decades might have created mosquitogenic situations by producing various breeding sites conducive to the proliferation of *Cx. quinquefasciatus*. Moreover, this species is reported to breed in multiple outdoor and indoor containers (Thete and Shinde 2013). Containers were found more in the urban areas than the rural areas (unpublished data, Post-Graduate Department of Zoology, Asutosh College). In the absence of adequately piped water supply, water is stored in pots, tanks and cisterns. Household water storage coupled with rainwater increases the number of suitable mosquito breeding sites for container breeders (Bhattacharya 2012).

All these factors along with urban development such as road, flyover, building, metro rail construction, etc. contribute in creating a complex mosquitogenic and filariogenic situations in urban areas. In West Bengal, urban areas were more endemic for bancroftian filariasis than the rural area. People living in the urban areas were more exposed to infective filarial vectors and were more susceptible to filarial infections compared to those of the rural areas (Chandra et al. 1995). Average annual transmission potential of bancroftian filariasis as a whole, though fairly low, was significantly higher in the urban area (Kolkata) than that in the rural area

(Burdwan) (Hati et al. 1989). Therefore, more infective bites of vector mosquitoes are likely in the urban areas. People prefer to sleep outdoors and/or well-ventilated indoor conditions with open windows in the hot and humid weather in Kolkata especially in the pre- and post-monsoon months which increases the chances of more mosquito bites. Higher percentage and PMH density of *Cx. quinquefasciatu*s in Kolkata during the present study also reinforce the hypothesis of Hati et al. (1989) with reference to higher annual transmission potential of bancroftian filariasis in urban area.

In some studies, investigators have found some association between vector infection rate and the density of vector, *Cx. quinquefasciatus* (Rudra and Chandra 1998; Das et al. 2003; Chandra et al. 2007; Pramanik and Chandra 2010). It has been reported that in Taraba State, Nigeria, transmission of bancroftian infection occurs mainly during rainy season when mosquitoes are most abundant (Badaki 2010), while in Benue State, Nigeria, the vector potential was low during the wet season and high during the dry season (Manyi et al. 2014). Perennial vector infection has been reported in Kolkata, West Bengal (Chandra et al. 2013). Higher infectivity rate in the summer coinciding with higher man-hour density of vector mosquito has been reported in Bankura, West Bengal (Pramanik and Chandra 2010) (Table 17.2). However, higher density of this species differs in different areas of the state as well as in seasons and is not always related with higher infection and infectivity rate. Naturally, we are not in agreement to such a hypothesis regarding a causative association between higher vector density and vector infection and infectivity rate.

It seems that the higher number of vector mosquitoes in a given time is not important enough in developing effective transmission potential in an endemic area, while it is the relative prevalence of the number of infected mosquitoes in a meteorologically favourable conditions and the biting frequency of infective mosquitoes are more important factors. These aspects coupled with some immunological and social factors are possibly responsible in developing the tangible transmission potential and epidemiologically sustainable disease dynamics of bancroftian filariasis in any endemic area. The ecological factors like temperature and humidity play a significant role in mosquito-borne disease transmission including bancroftian filariasis. These two factors are not only important for the breeding, development and survival of the vector mosquito but also have a significant impact on the development of *Wuchereria* larvae in its vector (Nelson 1964). Climate change is one of the defining challenges of the twenty-first century. Global warming and globalization are likely to reshape the ecology of vector mosquitoes, especially the ubiquitous *Cx. quinquefasciatu*s. This will have wide-ranging consequences on the epidemiology of a vector-borne disease like lymphatic filariasis.

17.10 Conclusion

The World Health Organization (WHO) has called for the elimination of lymphatic filariasis (LF) by 2020. The prospects of global elimination of this disease will very much depend on its success in India since India harbours 40 % of the global disease

burden. India has shown a political commitment for LF elimination by becoming a signatory to the World Health Assembly (WHA) resolution.

In Indian perspectives, LF has been resolved to be eliminated by 2015 (now redated as 2018).The phenomenal progress in reducing disease burden in India is largely due to the extensive innovative research and administrative support. India is currently visualizing a possible elimination of LF in the near future, and therefore the retargeted year of 2018 has assumed even greater significance. Although there are many challenges ahead for LF elimination, a comprehensive programme encompassing mass drug administration (MDA) and integrated vector management (IVM) coupled with sustained administrative and scientific surveillance is required in achieving the target.

Acknowledgements Authors are grateful to Dr. D. K. Kar, principal of Asutosh College, and Prof. A. K. Hati, former director of Calcutta School of Tropical Medicine, for their kind suggestions and encouragement for this work. Thanks are due to Sandip Pal, faculty member, students of the Department of Zoology, Asutosh College and Ms. Swaha Bhattacharya, Department of Biotechnology, St. Xavier's College, Kolkata for their various assistance.

References

Arensburger P, Megy K, Waterhouse RM, Abrudan J, Amedeo P, Antelo B, Bartholomay L, Bidwell S, Caler E, Camara F, Campbell CL, Campbell KS, Casola C, Castro MT, Chandramouliswaran I, Chapman SB, Christley S, Costas J, Eisenstadt E, Feschotte C, Fraser-Liggett C, Guigo R, Haas B, Hammond M, Hansson BS, Hemingway J et al (2010) Sequencing of *Culex quinquefasciatus* establishes a platform for mosquito comparative genomics. Science (Washington) 330(6000):86–88

Badaki JA (2010) Parasitological and social aspects of lymphatic filariasis in Taraba State. Ph.D. thesis. Department of Zoology, University of Jos, Nigeria, p 137

Barr AR (1957) The distribution of *Culex p. pipiens* and *Cx. p. quinquefasciatus* in North America. Am J Trop Med Hyg 6(1):153–165

Barraud PJ (1934) The Fauna of British India, including Ceylone and Burmah, vol 5. Taylor & Francis, London, p 463

Belkin JN (1977) *Cx. quinquefasciatus* or *fatigans* for the tropical (southern) house mosquito (Diptera: Culicidae). Proc Entomol Soc Wash 79(1):45–52

Bhattacharya S (2009) Mosquito-borne diseases in India with special reference to malaria vectors and their control. J Asia Soc L1(2):15–34

Bhattacharya S (2012) Development and disease: interpretation of inter-relationship in the context of mosquito-borne maladies. Sci Cult 78(122):47–53

Bhattacharya S, Hati AK (1986) Man-*Culex quinquefasciatus* contact in relation to transmission dynamics of Bancroftian filariasis. In: Abstract book, 6th International congress of Parasitology held in Brisbane, Australia

Bhattacharya S, Misra B (1992) Population dynamics and vector potential of *Culex quinquefasciatus* in two different bioecological conditions. In: Abstract book, 5th International congress for infectious diseases, Nairobi, Kenya, p 48

Bhattacharya S, Santra SC (2005) Mosquito-borne diseases and vector diversity in Kolkata with special reference to malaria. In: Mukhopadhyay A, De AK (eds) Perspectives in environmental health- vector and water-borne diseases. Published by Originals, Delhi, pp 53–70

Bhattacharya S, Chakraborty S, Mitra AK, Ghosh KK, Das S, Chakraborty MS, Chatterjee A, Hati AK (1982) Blood meal analysis of certain species of mosquitoes of rural West Bengal. Bull Cal Sch Trop Med 30(2):43–45

Bhattacharya S, Chaudhuri P, Mandal AK, Majumdar S, Basu P (2014) Reappearance of *Anopheles sundaicus* at Sundarban coastal areas West Bengal. India J Appl Biosci 40(1):58–59

Chakraborty S, Choudhuri DK, Bhattacharya S, Hati AK (1986) Bait preference pattern of *Culex vishnui* mosquito in rural West Bengal. Bull Cal Sch Trop Med 34(1–4):15–17

Chandra G (1995) Short report: peak period of filarial transmission. Am J Trop Med Hyg 53(4):378–379

Chandra G, Rudra SK (2006) Comparative studies on man-biting population of filarial vector *Cx. quinquefasciatus* (Diptera: Culicidae) between tribal and non-tribal areas of Bankura district West Bengal India. Bul Pen Kesh 34:1–7

Chandra G, Banerjee A, Hati AK (1993) Seasonal prevalence of *Culex quinquefasciatus* in an urban and a rural area of West Bengal. Bull Cal Sch Trop Med 41:10–11

Chandra G, Majumdar G, Hati AK (1995) An alternative approach for assessing filarial endemicity. In: Hati AK(ed) Studies on some vectors of public health importance, Published by the Department of Medical Entomology, Calcutta School of Tropical Medicine, Govt. of West Bengal, pp 146–148

Chandra G, Seal B, Hati AK (1996) Age composition of the filarial vector *Culex quinquefasciatus* (Diptera: Culicidae) in Calcutta, India. Bull Entomol Res 86(3):223–226

Chandra G, Chatterjee SN, Banerjee BD, Majumdar G (1997) Effect of seasonal variations on the development of *Wuchereria* larvae in *Culex quinquefasciatus*. Basic Appl Biomed 5:21–24

Chandra G, Chatterjee SN, Das S, Sarkar N (2007) Lymphatic filariasis in the coastal areas of Digha, West Bengal, India. Trop Doct 37(3):136–139

Chandra G, Pramanik M, Mondal SK, Ghosh AK (2013) Comparative studies of different indices related to filarial vector of a rural and an urban area of West Bengal. India Trop Med Surg 1:104

Chatterjee KK, Biswas D, Chandra G, Bhattacharyya A, Ghosh A, Hati AK (1988) A longitudinal survey on mosquito larvae in relation to metro rail construction in Calcutta. Indian Med Gaz CXXII(2):56–59

Christophers SR (1933) The Fauna of British India including Ceylone and Burma, vol 4. Taylor and Francis, London, p 371

Cornel AJ, McAbee RD, Rasgon J, Stanich MA, Scott TW, Coetzee M (2003) Differences in extent of genetic introgression between sympatric *Culex pipiens* and *Culex quinquefasciatus* (Diptera: Culicidae) in California and South Africa. J Med Entomol 40(1):36–51

Das M (1976) Vectors of filariasis with special reference to India. J Com Dis 8:101–109

Das BP, Kaul SM (1998) Pictorial key to the common Indian species of *Aedes* (stegomyia) mosquitoes. J Com Dis 30:123–127

Das PK, Pani SP, Krishnamoorthy K (2002) Prospects of elimination of lymphatic filariasis in India. ICMR Bull 32(5&6):1–14

Das SK, Ghosh A, Behera MK, Chandra G (2003) Studies on vector of Bancroftian filariasis at Katwa. West Bengal J Parasitol Appl Anim Biol 12(1&2):1–7

Data Sheet of The Invasive Species Compendium (ISC) (Website**:** http://www.cabi.org/isc/datasheet/86848) Accessed on 30 Jan 2015

David DR, Ribeiro GS, Freitas RM (2012) Bionomics of *Culex quinquefasciatus* within urban areas of Rio de janeiro, south eastern Brazil. Rev Saude Publica 6(5):858–865

De SK, Chandra G (1994) Studies on the filariasis vector-*Culex quinquefasciatus* atKanchrapara, West Bengal, India. Indian J Med Res 99:255–258

Derraik JGB (2005) Mosquitoes breeding in phytotelmata in native forest in the wellington region, New Zealand. N Z J Ecol 29(2):185–191

Dreyer G, Addiss D, Noroes J (2005) Does longevity of adult *Wuchereria bancrofti* increase with decreasing intensity of parasitic transmission? insights from clinical observations. Trans R Soc Trop Med Hyg 99:883–892

Farajollahi A, Fonseca DM, Kramer LD, Kilpatrick AM (2011) "Bird biting" mosquitoes and human disease: a review of the role of *Culexpipiens* complex mosquitoes in epidemiology. Infect Genet Evol 11(7):1577–1585

Fonseca DM, Smith JL, Wilkerson RC, Fleischer RC (2006) Pathways of expansion and multiple introductions illustrated by large genetic differentiation among worldwide populations of the southern house mosquito. Am J Trop Med Hyg 74(2):284–289

Goddard LB, Roth AE, Reisen WK, Scott TW (2002) Vector competence of California mosquitoes for West Nile Virus. Emerg Infect Dis 8(12):1385–1391

Gokhale MD, Paingankar MS, Dhangude SD (2013) Comparison of biological attributes of *Culex quinquefasciatus* (Diptera: Culicidae) populations from India. ISRN Entemol 2013. Article ID 451592, 9 p

Hati AK (2010) Medical entomology. Allied Book Agency, Calcutta, pp 1–238

Hati AK, Chandra G, Bhattacharyya A, Biswas D, Chetterjee KK, Dwivedi HN (1989) Annual transmission potential of bancroftian filariasis in an urban and a rural area of West Bengal, India. Am J Trop Med Hyg 40(4):365–367

Holder P, Browne G, Bullians M (1999) The mosquitoes of New Zealand and their animal disease significance. Surveillance 26(4):12–15

Hurd H, Webb TJ (1997) The role of endocrinologically active substances in mediating changes in insect hosts and insect vectors. In: Beckage NE (ed) parasites: effects on host hormones and behavior. Chapman and Hall, London, pp 179–201

Laird M (1995) Background and findings of the 1993–94 New Zealand mosquito survey. N Z Entomol 18:77–90

Laurence BR, Pickett JA (1985) An oviposition attractant pheromone in *Culex quinquefasciatus* say (Diptera: Culicidae). Bull Entomol Res 75(2):283–290

Lee DJ, Hicks MM, Debenham ML, Griffiths M, Marks EN, Bryan JH, Russell RC (1989) The Culicidae of the Australasian region, vol 7. Australian Government Publishing Service, Canberra, p 281

Lima CA, Almeida WR, Hurd H, Albuquerque CMR (2003) Reproductive aspects of the mosquito *Culex quinquefasciatus* (Diptera: Culicidae) infected with *Wuchereria bancrofti* (Spirurida: Onchocercidae). Mem Inst Oswaldo Cruz, Rio de Janeiro 98(2):217–222

Manyi MM, Imandeh GN, Azua ET (2014) Vector potential of Anopheles and Culex species in the transmission of Bancroftian Filariasis in the localities of Markurdi, North Central Nigeria. JEZS 2(5):171–177

Matthys B, N'Goran EK, Koné M, Koudou BG, Vounatsou P, Cisse G et al (2006) Urban agricultural land use and characterization of mosquito larval habitats in a medium-sized town of Côte d'Ivoire. J Vector Ecol 31(2):319–333

McAbee RD, Christiansen JA, Cornel AJ (2007) A detailed larval salivary gland polytene chromosome photomap for *Culex quinquefasciatus* (Diptera: Culicidae) from Johannesburg, South Africa. J Med Entomol 44(2):229–237

Mukhopadhyay AK, Hati AK (1978) Man-biting activity of *Anopheles stephensi* in Calcutta. Bull Cal Sch Trop Med 26:5–7

Nelson CG (1964) Factors influencing the development and behavior of filarial nematodes in their arthropodan hosts. In: Angela ER (ed) Second symposium of the British Society for parasitology. Host-parasite relationships in invertebrate hosts, Taylor. Black-Well Scientific Publications, Oxford, pp 74–119

NVBDCP (2015.) http://nvbdcp.gov.in/fil-rate.html. Accessed 05 April 2015

Opoku AA, Ansa-Asare OD, Amoako J (2007) The occurrences and habitat characteristics of mosquitoes in Accra, Ghana. West Afr J Appl Ecol 11:81–86

Pramanik M, Chandra G (2010) Studies on seasonal fluctuation of different indices related to filarial vector, *Culex quinquefasciatus* around foothills of Susunia of West Bengal, India. Asian Pac J Trop Med 3(9):727–730

Reuben R, Thenmozhi V, Samuel P, Gajanana A, Mani T (1992) Mosquito blood feeding patterns as a factor in the epidemiology of Japanese encephalitis in southern India. Am J Trop Med Hyg 46(6):654–663

Rozeboom LE, Bhattacharya NC, Gillotra SK (1968) Observations of the transmission of filariasis in urban Calcutta. Am J Epidemiol 87(3):616–632

Rudra SK, Chandra G (1998) Bancroftian Filariasis in tribal population of Bankura District, West Bengal, India. Jpn J Trop Med Hyg 26(2):109–112

Rueda LM, Patel KJ, Axtell RC, Stinner RE (1990) Temperature-dependent development and survival rates of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). J Med Entomol 27(5):892–898

Sabesan S, Pradeepkumar N, Krishnamoorthy K, Panicker KN (1992) Seasonal abundance and biting behavior of *Mansonia annulifera, Mansonia uniformis* and *Mansonia indiana* and their relative role in the transmission of Malayan filariasis in Shertallai (Kerala State). Indian J Med Res 93:253

Say T (1823) Descriptions of dipterous insects of the United States. J Acad Nat Sci Phila 3:9–54

Service MW (2012) Medical entemology for students, 5th edn. Cambridge University Press, New York, p 303

Sirivanakarn S (1976) Medical entomology studies-III. A revision of the subgenus *Culex* in the oriental region (Diptera: Culicidae). Contrib Am Entomol Inst (Ann Arbor) 12(2):l–272

Smith JL, Fonseca DM (2004) Rapid assays for identification of members of the *Culex (Culex) pipiens* complex, their hybrids, and other sibling species (Diptera: Culicidae). Am J Trop Med Hyg 70(4):339–345

Stone A (1956) Corrections in the taxonomy and nomenclature of mosquitoes (Diptera: Culicidae). Proc Entomol Soc Wash 56(6):333–343

Subra R (1981) Biology and control of *Culex pipiens quinquefasciatus* say, 1823 (Diptera, Culicidae) with special reference to Africa. Insect Sci Appl 1(4):319–338

Thenmozhi V, Mariappan T, Krishnamoorthy R, Baskarn G, Krishnamoorthi R, Balaji T, Tyagi BK (2014) A first note on Japanese encephalitis virus isolation from *Culex quinquefasciatus* say in Northern West Bengal. Int J Mosq Res 1(1):1

Thete KD, Shinde LV (2013) Survey of container breeding mosquito larvae in Jalna city (M.S) India. Biological Forum An Int J 5(1):124–128

Tiwari SC, Hiriyan J, Reuben R (1995) Epidemiology of subperiodic *Wuchereria bancrofti* infection in Nicobar islands, India. Trans R Soc Trop Med Hyg 89:163

Wattal BL, Kalra NL (1961) Regionwise pictorial keys to the female Indian *Anopheles*. Bull Nath Soc Ind Malar Mosq Dis 9:85–138

Weinstein P, Laird M, Browne G (1997) Exotic and endemic mosquitos in New Zealand as potential arbovirus vectors. Occasional paper. Ministry of Health, Wellington

WHO (2007) Global programme to eliminate lymphatic filariasis. Wkly Epidemiol Rec 42(82):361–380

Wiedemann CRW (1828) AussereuropaischezweiflugeligeInsekten. Hamm 1:608 (Cited in http://entnemdept.ufl.edu/creatures/aquatic/southern_house_mosquito.htm. visited on 10.04.2015)

World Health Organization (1992) Lymphatic filariasis. Fifth report of the WHO expert committee onfilariasis, Geneva

18

Actinobacteria: A Promising Biocontrol Agent for Filariasis Vector, *Culex quinquefasciatus* Say, 1823 (Insecta: Diptera: Culicidae)

K. Rajesh and D. Dhanasekaran

Abstract

Filariasis is transmitted by mosquitoes, particularly *Culex quinquefasciatus*, which is the major carrier for *Wuchereria bancrofti* affecting more than 95% endemic population globally. Filarial vector *Cx. quinquefasciatus* breeds in waters polluted with organic debris such as aquatic plants, household waste and excreta. This species of filaria vector larvae are generally found in ditches, drainages, polluted water and sewage stagnant water. The main control tool against mosquito larvae is insecticidal treatment of chemicals and the *Bacillus thuringiensis* var. *israelensis* (slow release formulations), albeit associated with undesirable effects on human health and environment. Recently, biogenic nanoparticles have been proposed as highly effective larvicidals against mosquito vectors. Actinobacterial bioactive compounds may offer potentially effective mélange of larvicides and pupicides. This chapter describes histomorphological effects and in silico molecular mechanisms against the filarial vector, *Cx. quinquefasciatus.* It is emphasized that actinobacterial bioactive compounds are highly effective against *Cx. quinquefasciatus* and can be used as a promising biocontrol agent in the integrated vector management (IVM) in efforts for the elimination of lymphatic filariasis.

Keywords

Culex quinquefasciatus · *Streptomyces* · Larvicidal activity · Biocontrol · Molecular docking

K. Rajesh
Department of Microbiology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

D. Dhanasekaran (✉)
Department of Microbiology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

Department of Molecular, Cellular and Biomedical Sciences, University of New Hampshire, Durham, NH, USA

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_18

18.1 Introduction

Mosquitoes transmit several vector-borne diseases like malaria, filariasis, dengue, yellow fever and Japanese encephalitis causing millions of deaths every year. These vector-borne diseases have serious commercial impacts, including loss in commercial and labour outputs, particularly in countries with growing economy in tropical and subtropical climates (Fradin and Day 2002). Lymphatic filariasis is a serious public health problem in India, comprising one third of the infected population of the world (WHO 1997, Bernhard et al. 2003). A mosquito is associated with urbanization and towns with poor and inadequate drainage and sanitation. Under the unplanned and overstressed living conditions, the vector population increases rapidly. *Cx. quinquefasciatus* bites human beings and numerous animal hosts during night time. The mosquito normally rests indoors both before and after feeding, but it also finds shelter outdoors if disturbed (Service 2000).

At present, using fermentation, molecular characterization and combinatorial biosynthesis and high-throughput screening of new secondary metabolites related to existing pharmacophores, a great deal of progress has been made on the discovery of molecules from actinobacteria that are effective against *Cx. quinquefasciatus*. Actinobacteria are the Gram-positive, filamentous, high G+C content prokaryotic bacteria which are extensively distributed in the natural ecosystem all over the world. They are chiefly found in terrestrial habitats but are also widely dispersed in a variety of other habitats including compost, lake bottoms and river mud (Alexander 1977). Since actinobacteria have produced various economically important secondary metabolites of high marketable value, they continue to be regularly screened for novel bioactive metabolites. Actinobacterial bioactive compounds such as tetranectin, avermectins (Pampiglione et al. 1985), macrotetrolides (Zizka et al. 1989) and flavonoids (Rao et al. 1990) were found to be significantly toxic to mosquitoes. The extracellular secondary metabolites from the actinobacterial isolates had a strong larvicidal activity against *Culex* mosquitoes (Vijayan and Balaraman 1991). In the above context, this chapter outlines the general characteristics of actinobacterial bioactive compounds and their larvicidal, pupicidal and histomorphological effects. The *in silico* molecular mechanism against the filarial vector has to be explored and compared with the chemical insecticides for experimental validation.

18.2 Problems of Mosquitoes

Cx. quinquefasciatus is one of the important mosquitoes in vector-borne diseases. It is the most potential vector of *Wuchereria bancrofti* (Ramaiah et al. 2000). Among all types of habitat surveyed, larval index in cement canals (3.25%), cement tank (8.66%), sewage (3.5%) drains, sewage stagnant water or cesspools (7.33%) and ditches (2.6%) were found positive for the mosquito larvae. Our finding indicates that the mosquito, *Cx. quinquefasciatus*, clearly prefers sewage and polluted cement tank and cesspools/pits waters (Fig. 18.1).

Fig. 18.1 (**a**–**f**) Preferred breeding habitats of *Culex quinquefasciatus*, the filariasis vector

18.3 Control Measures

Vector control is a very effective means to reduce the overall disease burden, but in developing countries like India, it is yet to assume that significance due largely to the lack of general awareness and support among public besides socioeconomic reasons, growing crunch of resources and on the top of all development of resistance in the vector against the insecticides as being the main plank of the vector control. Larvicidal and adulticidal measures establish an important aspect of mosquito control programme. Yang et al. (2002) reported that the control of the mosquito larvae is frequently dependent on the use of residual organophosphates, pyrethroids and insect growth regulators. A noticeable technique used for the

control and management of mosquito-borne disease vectors is the applications of insecticides, and several synthetic chemicals have been established and working in field with significant accomplishment. However, it also triggered uninvited effects, including toxicity to nontarget organisms, and raised at the same time serious environmental and human well-being concerns (Lee et al. 2001). The toxicity problem, together with the growing incidence of insect resistance, has called attention to the requirement for new insecticides (Macedo et al. 1997) and for supplementary comprehensive investigations of naturally occurring insecticides (Ansari et al. 2000). These problems have emphasized the necessity for the development of novel approaches for careful mosquito control hitting either during their aquatic stages of development or when they are adults in aerial stage. Secondary metabolites originated from microbial sources may be potential alternative foundations of mosquito larval control agents.

18.4 Actinobacteria as a Biocontrol Agent for Filariasis Vector, *Culex quinquefasciatus*

Actinobacteria are the source of commercially important bioactive compounds. Okami (1952) screened for novel bioactive compounds antibiotics and other natural compounds from actinobacteria. Among actinobacteria, the members of the genus *Streptomyces* are considered as economically important because they constituted 50% of soil actinobacterial population and 75% of total bioactive molecules (Prabavathy et al. 2006). They remain to be prolific sources of secondary metabolites with various biological activities such as antibacterial (Dhanasekaran et al. 2005, 2009), antifungal (Dhanasekaran et al. 2008, Saha et al. 2012), herbicidal (Dhanasekaran et al. 2012a, Priyadharsini et al. 2017), antiparasitic (Dhanasekaran et al. 2012b), antitumor, anticancer agent (Lim et al. 2006), keratinolytic activity (Saha and Dhanasekaran 2010), probiotic potentials (Latha et al. 2016, 2017, Muthuselvam et al. 2016), plant symbiotic (Thajuddin et al. 2015) and mosquito larvicidal activity (Dhanasekaran et al. 2010, Rajesh et al. 2013a, b).

Actinobacteria occurrence and distribution are abundant in terrestrial soil and the marine sediments with diverse salt concentration (Grein and Meyers 1958). The bioactive compound productions from marine actinobacteria have yet to screen various bioprocess applications. Several species of actinobacteria needs to be screened extensively to determine for potential bioactive components (Fig. 18.2). Among the different mosquito biocontrol compounds obtained bacteria, cyanobacteria, fungi, algae and plants, actinobacteria have gained importance with innumerable advantages over the chemical insecticides. The biolarvicides are highly effective against mosquito larvae at very low doses and are completely safe to the nontarget organisms, as well as environment, man and the wildlife (Ranjani et al. 2016).

An intense search for insecticidal metabolites from actinobacteria began over 30 years ago following the discovery of the highly insecticidal, acaricidal and nematicidal properties from *Streptomyces avermitilis* (Burg et al. 1979, Putter et al. 1981). Afterwards, several secondary metabolites with insecticidal properties have

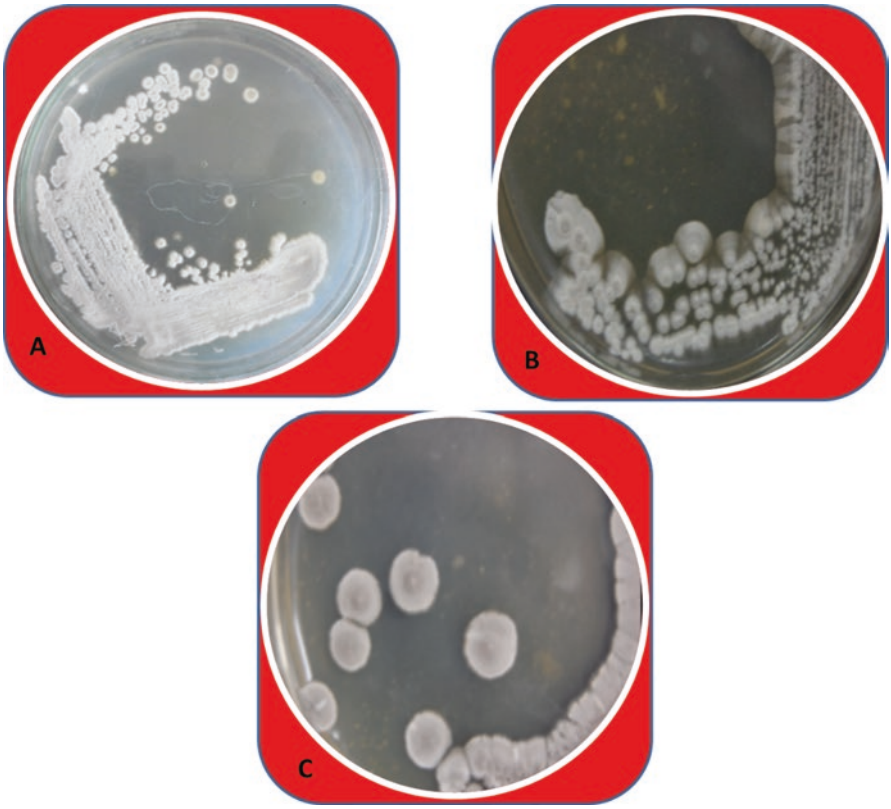


Fig. 18.2 Cultural morphology of actinobacteria isolates on starch casein agar plate

been discovered from actinobacteria. These and a few other insecticidal metabolites reported earlier are given below in Table 18.1. The larvicidal bioactive compounds such as avermectins, tetranectin, faerifungin and macrotetrolides isolated from actinobacteria, and their chemical structure is shown in Fig. 18.3.

18.4.1 Avermectins

The avermectins were discovered by Burg et al. (1979) in the fermentation broth of *Streptomyces avermitilis*. This actinomycete was originally isolated from a soil sample at the Kitasato Institute in Japan. Subsequent high performance liquid chromatographic analysis showed that the broth contained eight closely related compounds. The four major components were designated as Ala, A2a, Bla and B2a, and the four minor components were designated as Alb, A2b, Blb and B2b. These were later shown to exhibit broad insecticidal activity (Putter et al. 1981). Of these naturally occurring compounds, avermectin Bla was the most potent insecticide and therefore selected for development as an insecticide.

Table 18.1 Actinobacterial biolarvicidal metabolites against mosquito – *Ae. aegypti*, *An. stephensi*, *Cx. quinquefasciatus*, *Cx. gelidus* and *Cx. tritaeniorhynchus*

S. No.	Name of actinobacteria	Metabolites	Effective against mosquito species	Reference
1.	*Streptomyces aureus*	Tetranectin	*Ae. aegypti*	Oishi et al. (1970); Ando (1983)
2.	*Streptomyces prasinus*	Prasinons	*An. stephensi*	Box et al. (1973)
3.	*Streptomyces avermitilis*	Avermectins	*Cx. quinquefasciatus*	Burg et al. (1979) and Putter et al. (1981)
4.	*Streptomyces* sp.	Flavonoids	*Cx. quinquefasciatus*	Rao et al. (1990)
5.	*Streptomyces antibioticus*	Organophosphates	*Cx. quinquefasciatus*	Neumann and Peter (1987)
6.	*Streptomyces griseus*	Borrelidin	*Cx. quinquefasciatus*	Anonymous (1989)
7.	*Streptomyces* sp.	(2S,5R,6R)-2-hydroxy-3,5,6-trimethyloctan-4-one	*Cx. quinquefasciatus*	Deepika et al. (2012)
8.	*Streptomyces* VITSVK5 sp.	5-(2,4-dimethylbenzyl) pyrrolidin-2-one	*Cx. tritaeniorhynchus*	Saurav et al. (2013)
9.	*Streptomyces* sp.	Flavonoids	*Cx. quinquefasciatus*	Dhanasekaran and Thangaraj (2014)
10.	*Streptomyces albovinaceus*	Actiphenol	*Cx. quinquefasciatus*	Tanvir et al. (2014)
11.	*Saccharomonospora* spp., *Streptomyces roseiscleroticus* and *Streptomyces gedanensis*	–	*Cx. gelidus* and *Cx. tritaeniorhynchus*	Karthik et al. (2011)
12.	*Streptomyces* sp.	–	*Ae. aegypti*	Kekuda et al. (2010)
13.	*Streptomyces* sp. $KA_1$3-3	–	*Cx. quinquefasciatus*	Rajesh et al. (2013a, b)
14.	*Streptomyces minutiscleroticus* and *Streptomyces phaeoluteigriseus*	–	*Cx. quinquefasciatus*	Anwar et al. (2014)

18.4.2 Tetranectin

A macrotetrolide antibiotic, tetranectin, was isolated from the fermented broth of *Streptomyces aureus* (Ando 1983). The tetranectin acts to uncouple in oxidative phosphorylation. This antibiotic is the first pesticidal antibiotic ever commercialized. It has been in use in Japan as an agricultural miticide since 1973 (Ando 1983: Misato and Yoneyama, 1982).

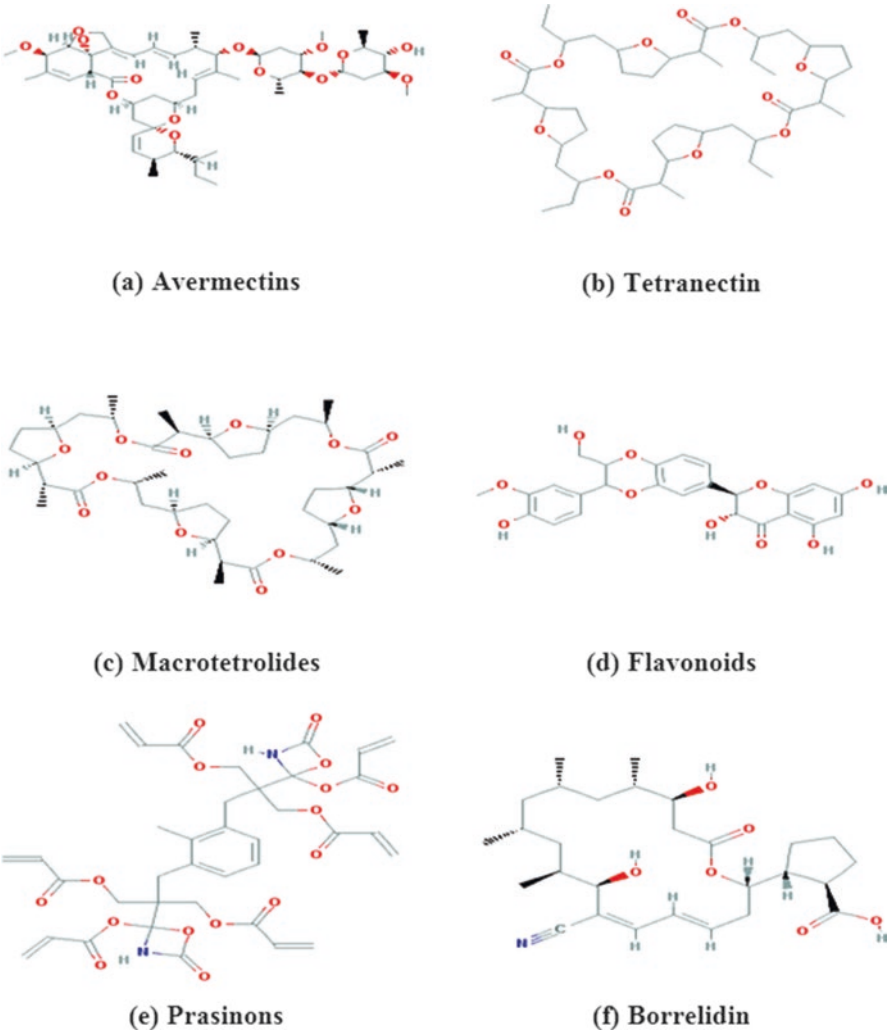


Fig. 18.3 Chemical structure of actinobacterial mosquito larvicidal metabolites

18.4.3 Faeriefungin

The mycelial biomass of *Streptomyces griseus* var. *autotrophicus* (ATCC 53668) grown in A-9 medium is the source of isolation of faeriefungin. Crystalline faeriefungin was stored in the dark at −20 °C for up to 3 months before use. It was mainly used as a mosquito larvicidal agent. Macrotetrolides (Zizka et al. 1989) and flavonoids (Rao et al. 1990) have been reported toxic to mosquitoes.

18.5 Mosquito Larvicidal Properties of Marine Actinobacteria

Mishra et al. (1987) screened a wide variety of actinobacterial isolates for insecticidal metabolites using mosquito larvae of different species. These isolates included 302 *Streptomyces* samples, yielding 502 novel actinobacteria including representatives of 18 genera and 28 unidentified aerobic actinobacteria. Metabolites were reported from 11 genera of actinobacteria such as *Streptomyces*, *Micromonospora*, *Actinomadura*, *Actinoplanes*, *Micropolyspora*, *Nocardiopsis*, *Streptosporangium*, *Oerskovia*, *Thermomonospora*, *Streptoverticillium* and *Chainia* and were found highly toxic to mosquito larvae.

Goodfellow (1983) and Hayes and Laws (1991) reviewed the literature on isolation of actinobacteria and suggested that only 10% of the actinobacteria could be isolated from nature and that there existed a vast capacity to synthesize many biologically active secondary metabolites (Lechevalier and Lechevalier 1967). Actinobacterial bioactive compounds are reported to be extremely toxic to mosquitoes, and until now they have low toxicity to nontarget organisms. Accordingly, the use of actinobacterial bioactive compounds may be a capable method for biological control of mosquitoes. The extracellular secondary metabolites from 94 actinobacteria screened for larvicidal activity against *Cx. quinquefasciatus* have been reported. The metabolites and new bioactive compounds extracted from marine actinobacteria are gaining importance as tools not only to human medicine or pharmaceuticals but also effective vector control agents (Annie Selva Sonia and Lipton 2012). Dhanasekaran et al. (2010) reported that mangrove sediment actinobacterial isolates of *Streptomyces* sp. SM13, *Streptosporangium* sp. SH15 and *Micropolyspora* sp. S22 showed larvicidal activity against *Anopheles* larvae. Actinobacterial derived bioactive against larvicidal activity of *Culex* larvae. The findings revealed 1000 ppm concentration of larvicide compounds derived from *Streptomyces* sp. KA13-3 showed 100% mortality, and *Streptomyces* sp. KA25-A showed 90% mortality (Rajesh et al. 2013a, b). An aculeximycin is a new actinobacterial antibiotic isolated and identified from *Streptosporangium albidum* culture broth, which exhibits strong larvicidal effect against mosquito larvae (Ikemoto et al. 1983).

18.6 Larvicidal and Pupicidal Activity of Actinobacteria Against *Cx. quinquefasciatus*

Based on the preliminary screening for mosquito larvicidal activity, an isolate RD 06 was found to be highly significant among 25 isolates, and therefore the isolate RD 06 was chosen for the taxonomic characterization. The isolate was mass propagated in starch casein broth. The production medium and growth condition were optimized using response surface methodology (RSM). The bioactive compound was extracted using ethyl acetate and purified by column chromatography. The compound was spectroscopically characterized and identified as 1,2-benzenedicarboxylic acid, diheptyl

ester using UV, FT-IR and GC-MS spectra. The compound 1, 2-Benzenedicarboxylic acid, diheptyl ester is used for evaluation of mosquitocidal properties.

We report that *Streptomyces* sp. RD 06 derived bioactive compound is found to have potential larvicidal and pupicidal activities against *Cx. quinquefasciatus*. The metabolites of the actinobacterial isolate revealed high mortality effect after 24 h of exposure at 1000 ppm on both larvae and pupae. The present results show that the crude extract of isolate RD 06 has LC_{50} value 154.13 ± 10.50 and LC_{90} value 642.84 ± 74.61 against the larvae of *Cx. quinquefasciatus*. The pupicidal activity of crude extract of the isolate showed $LC_{50} = 99.22 \pm 11.54$ and $LC_{90} = 591.84 \pm 55.41$. Results of upper confidential level (UCL), lower confidential level (LCL) and Chi squire (χ^2) values were significant at $P < 0.05$ levels (Table 18.2). The similar kind of report was recorded by Vijayakumar et al. (2010). Karthik et al. (2011) reported that the crude extracts of actinobacterial metabolites have potential for the development of novel and harmless control products for *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus* and *Cx. gelidus*.

18.7 Histomorphological Alteration by Larvicidal Compounds on *Cx. quinquefasciatus* Larvae

An assessment is made of the histomorphological alterations in midgut epithelial cells and anal gills in the third instar larvae of filarial vector, *Cx. quinquefasciatus*, following exposure to actinobacterial bioactive compound using a phase contrast microscopy (Fig. 18.4).

Histopathological data reveal differential toxic effects of actinobacterial metabolites bringing about indistinguishable damages to portions of the gastric caeca and single-layered epithelial cells (Ep). The periplasmic membrane (PM) was also damaged at 500 ppm concentration (Fig. 18.4, a and b), wherein the anal gills were surrounded by a thick permeable cuticle layer consisting of anal gill cells. Nevertheless, the undistinguished damaged cuticle was observed in treated larvae (c), leading to destroyed anal gill cells.

Ahmed et al. (2014) reported in case of *Pseudomonas frederiksbergensis* that the extract treatments of mosquito larvae resulted in a massive destruction of the larval midgut epithelial cells in *Cx. pipiens* larvae as compared to control larvae. This destruction of epithelium tissue might be the main reason of the observed cessation in feeding by 12 h post-treatment, because of septicaemia oxidative stress and finally death at 24 h post-treatment. *P. frederiksbergensis* toxic extract targets and causes the destruction of the integument as well as the gut epithelium and anal gills. It has been reported that the destruction of the epithelial cells lining the midgut of mosquito larvae is often associated with midgut paralysis. We also confirm similar observations in our study which may indicate high toxicity of *Streptomyces* sp. RD 06 against filarial vector mosquito in field conditions.

Table 18.2 Larvicidal and pupicidal activity of *Cx. quinquefasciatus*

Name of the activity	Concentration (mg/mL)	Mortality* ± SD	LC_{50} ± SE	(UCL–LCL)	LC_{90} ± SE	(UCL–LCL)	$\chi 2$ (df = 4)
Larvicidal	1000 ppm	100 ± 0.00	154.13 ± 10.50	(174.71–133.54)	642.84 ± 74.61	(789.07–496.61)	7.78
	500 ppm	82 ± 1.41					
	250 ppm	62 ± 1.82					
	125 ppm	42 ± 1.90					
Pupicidal	1000 ppm	100 ± 0.00	199.22 ± 11.54	(221.85–176.59)	591.84 ± 55.41	(700.46–83.22)	5.84
	500 ppm	85 ± 1.72					
	250 ppm	56 ± 1.74					
	125 ppm	28 ± 0.95					

LC_{50} lethal concentration that kills 50% of the exposed larvae, *LC_{90}* lethal concentration that kills 90% of the exposed larvae, *UCL* upper confidence limit, *LC* lower confidence limit, χ^2 *chi-square* df degree of freedom significant at $P < 0.05$ level

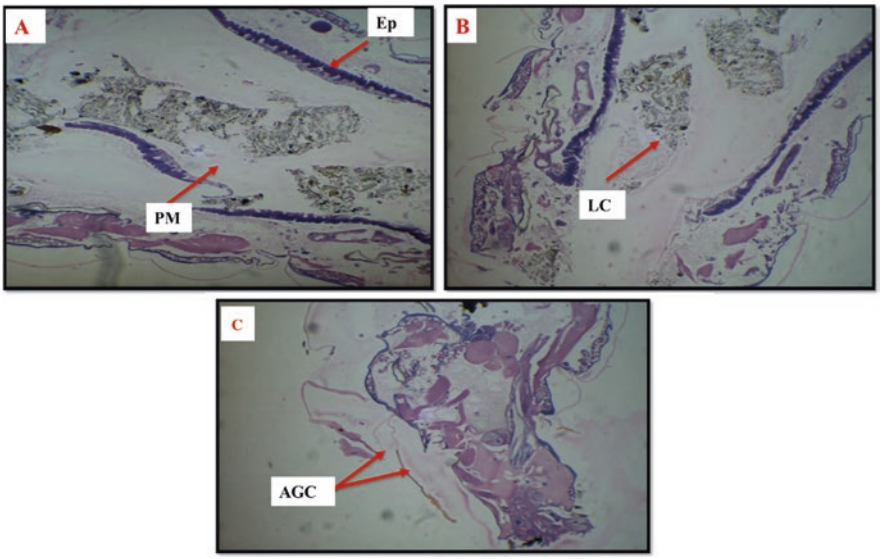


Fig. 18.4 (**a**) and (**b**) Longitudinal section in the posterior region of midgut of third larval instar of *Cx. quinquefasciatus* treated with LC_{50} of actinobacterial compound after 24 h epithelial cells (*Ep*), peritrophic membrane (*PM*) and (**c**) anal gill cell (*AGC*)

18.8 In Silico Molecular Mechanism of Actinobacterial Compound as a Growth Inhibitor

Docking studies were performed to understand the binding affinity of test compound, 1,2-benzenedicarboxylic acid, diheptyl ester with the target proteins derived from *Streptomyces* sp. RD 06. A group of target proteins was used for analysis. The group 1 consists of three protein structures from *Cx. quinquefasciatus* family. The corresponding PDB codes were 2C9K, 2LC2 and 3OGN. It is believed that malathion is one of the potential larvicidal agents used in the practice. Therefore, we have used malathion as a positive control molecule in the investigation. All the protein structures and the small molecule were energy minimized with the help of Gromos43a1 force field implemented in GROMACS 4.5.3 package (Hess et al. 2008). Subsequently, a docking study was initiated. It is clear from the table in reference that binding energy of 1,2-benzenedicarboxylic acid, diheptyl ester was significantly higher than control molecule, malathion, in all the investigated structures. For instance, the binding energy is in the order of 5–6 kcal/mol for the test compound, whereas it was only 4–5 kcal/mol for the control molecule. This signifies that larvicidal activity of test molecule is significantly good than control molecule. The docked complex structures of test and control molecule with the target proteins are shown in Figs. 18.5 and 18.6. The figures were generated with the help of LIGPLOT tool.

The docking score was evaluated for the three protein receptors of *Cx. quinquefasciatus*. The maximum docking score (6.37) was recorded in crystal structure of odorant-binding protein (3OGN), followed by a structure of the functional form of the mosquito

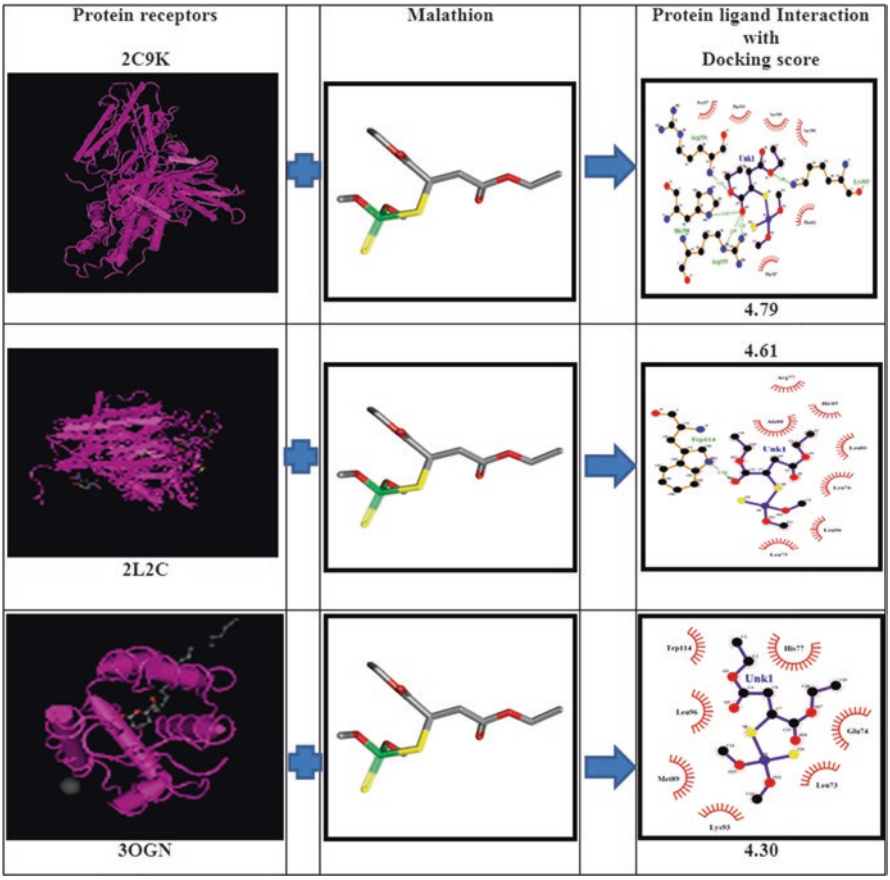


Fig. 18.5 Binding efficiency of synthetic insecticides on *Cx. quinquefasciatus*. Mosquito receptors: mosquito odorant-binding proteins (2C9K, 2L2C) and odorant-binding protein (3OGN)

larvicidal (2C9K), and NMR structure of mosquito odorant-binding protein was bound to mop (2L2C). The deactivation and degradation of the pheromone are due to the bioactive compound produced by *Cx. quinquefasciatus* mosquito system. The other protein-like structure of the functional form of the mosquito larvicidal (2C9K) function induced membrane pore formation on the mosquito which is responsible for insect toxicity and NMR structure of mosquito odorant-binding protein bound to mop (2L2C) playing a main role in inhibiting the hydrophobic signal peptide (24 residues) in the salivary gland of *Cx. quinquefasciatus*. In silico molecular docking of larvicidal compound reveals the chemical interaction of test compound with enzyme receptor of the filariasis vector mosquito. The interaction affinity of target protein and 1,2-benzenedicarboxylic acid, diheptyl ester derived from *Streptomyces* sp. RD 06 in docking score, besides the stability, about nature of the protein in mosquito host system. The bioactive compound showed significant inhibitory effect on the mosquito receptors, namely, odorant-binding protein (3OGN) from *Cx. quinquefasciatus*. The docking score of the bioactive compound from actinobacteria was higher than that of synthetic larvicidal agent (malathion).

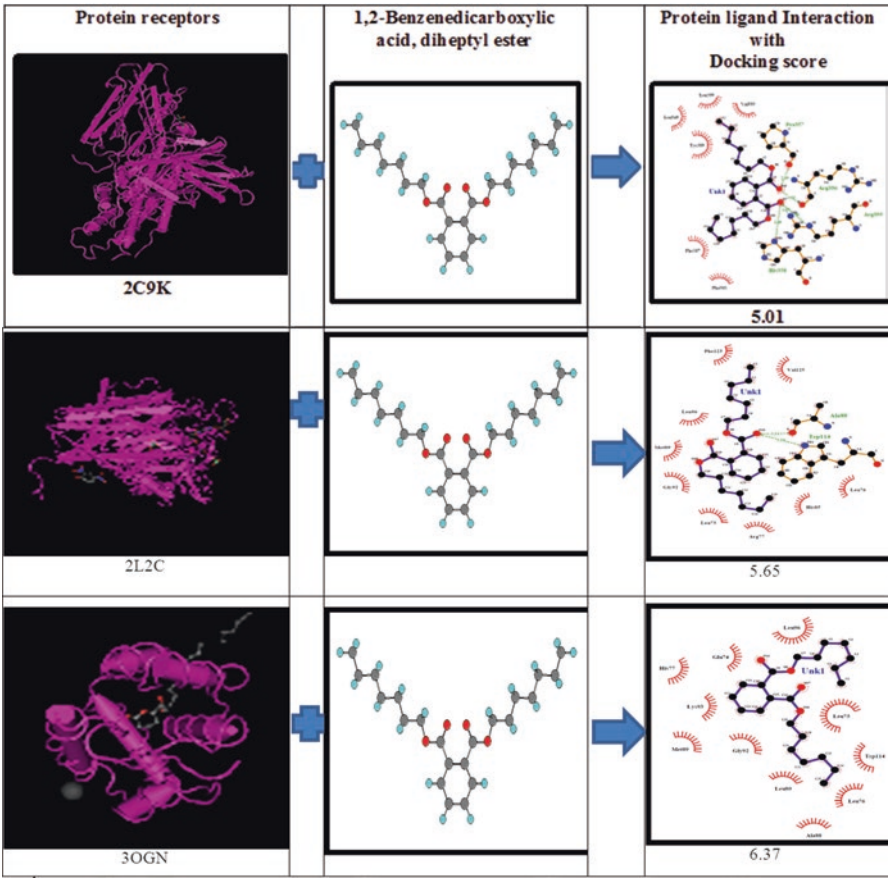


Fig. 18.6 Binding efficiency of larvicidal compound with *Cx. quinquefasciatus*. Mosquito receptor: mosquito odorant-binding proteins (2C9K, 2L2C) and odorant-binding protein (3OGN)

18.9 Larvicidal Activity of Actinobacterial-Derived Biogenic AgNPs Against *Cx. quinquefasciatus*

The nanoparticles are synthesized by physical, chemical and biological methods. There are several physical and chemical methods available to synthesis and characterization of nanoparticles. The physico-chemical methods produce toxic and hazardous chemicals. Hence, we are dependent on ecofriendly, low cost biological methods using the several microorganisms including bacteria, actinobacteria, fungi, mushroom, algae, diatoms, plants and insects (Kaushik et al. 2009). Marine actinobacteria are attractive to investigators since they are enhanced with bioactive compounds with inimitable biological properties.

The mechanism of actinobacterial-mediated nanoparticles which cause the death of the larvae could be the capability of the nanoparticles to enter through the mosquito larvae membrane. The biogenic nanosilver in the intracellular space can bind

to sulphur-containing proteins or to phosphorus-containing complexes like DNA, leading to the disintegration of some organelles and enzymes (Rai et al. 2009). The death effect of nanosilver on mosquito larvae could be permitted by the small size of the particles, which permits passage through the insect cuticle and into individual cells where they inhibit with moulting and other physiological processes (Table 18.3).

Rajesh et al. (2013a, b) reported that 5 mg/l concentration of biogenic nanosilver exhibited 100% mortality in early third instar larvae of *Cx. quinquefasciatus*. Thus, findings recommended LC_{50} value as 1.23 and 1.19 mg/L and the LC_{90} values as 2.97 and 4.93 mg/L for *Cx. quinquefasciatus*, individually. The findings of upper confidential level (UCL), lower confidential level (LCL) and chi squire (χ^2) values also advocated significant mosquitocidal activity.

Conclusively the biogenic nanosilver derived from *Streptomyces* has been categorically tested against the lymphatic filarial vector, *Cx. quinquefasciatus*, and it can be deduced from the results that by this approach a quick synthesis of nanoparticles would be accomplished for emerging a biological development for mosquito control. The nanoscience tools and mechanisms are fast becoming accessible to a growing scientific research, and a good set of data are becoming available for many agriculture pests in particular. However, since there is no significant detailed report on mosquito larvicidal activity using actinobacterial metabolites derived from nanosilver, thus, investigation will provide an altogether unexplored platform to biologists and attract the investigators to work further in mass production and field application of nanoparticles to diminish the vector population.

18.10 Conclusion

Culex quinquefasciatus is accountable for the insufferable biting nuisance and transmission of lymphatic filariasis (LF) disease. It causes serious well-being problems to humans and presents unsurmountable obstacles in the socioeconomic development of developing countries like India. For decades, synthetic chemical larvicides are continuously used for controlling mosquitoes in most parts of the world. The synthetic chemical methods produce toxic and hazardous chemicals. Hence, we need an ecofriendly, low-cost biological methods using the several microorganisms including bacteria, actinobacteria, fungi, mushroom, cyanobacteria and algae, and plants control the mosquitoes. Hence, a more efficient method to reduce the population of mosquitoes would be warranted. Mosquito-borne diseases are influencing the global economy now due to large-scale loss of human lives and precious working hours. We here present an ecofriendly approach for eradicating the egg, larva and pupa of the vector using actinobacterial metabolites. The actinobacteria derived metabolites could be useful as substitute for synthetic insecticides on our efforts to control field populations of *Cx. quinquefasciatus.* However, it must be first extended to extensive simulated laboratory and field trials for future course of action to recognize ideal biocontrol agents.

Table 18.3 Larvicidal effect of *Streptomyces* sp. GRD derived nanosilver against *Cx. quinquefasciatus*

Species	Concentration (mg/mL)	Mortality* ± SD	LC_{50} ± SE	(UCL−LCL)	LC_{90}±SE	(UCL−LCL)	$\chi 2$ (df = 4)
Cx. Quinquefasciatus	0.5 mg/L (0.5 ppm)	13 ± 2.00	1.23 ± 0.06	1.35−1.10	2.97 ± 0.23	3.42−2.53	15.2
	1 mg/L (1 ppm)	33 ± 1.82					
	2 mg/L (2 ppm)	75 ± 2.30					
	4 mg/L (4 ppm)	96 ± 1.60					
	5 mg/L (5 ppm)	100 ± 0.00					

Acknowledgement The author DD is grateful for the financial support to the ICMR's Vector Borne Diseases Science Forum (Project No. 2011-14670; File No. 5/8-7(312) V-2011/ECD-II dated 16.03.2012), University Grant Commission (UGC), New Delhi under Raman Fellowship for Post-Doctoral Research in the USA (F. No:5-29/2016(IC) Dt.10.02.2016) and the DST-FIST Programme (Ref. No.SR/FIST/LSI-013/2012 dated 13.08.2012). The author is also grateful to the Bharathidasan University, Tiruchirappalli, Tamil Nadu, for providing major facilities.

References

Ahmed AM, Abdel-Megeed AA, Alqahtani HM (2014) A novel mosquitocidal bacterium as a biocontrol agent in Saudi Arabia: II-A promising larvicide against *Culex pipiens* mosquito. Pak J Zool 46(3):773–782

Alexander M (1977) Introduction to soil microbiology, 2nd edn. Krieger Publishing Company, Malabar, p 467

Ando K (1983) How to discover new antibiotics for insecticidal use. In: Takahashi HY, Misato T, Matusunaka S (eds) Pesticide chemistry: human welfare and the environment, Natural products, vol 2. Pergamon Press, New York, pp 253–259

Annie Selva Sonia G, Lipton AP (2012) Pathogenicity and antibiotic susceptibility of *Vibrio* species isolated from the captive reared tropical marine ornamental blue damsel fish, *Pomacentrus caeruleus* (Quoy and Gaimard, 1825). Indian J Geomarine Sci 41(4):348–354

Anonymous (1989) New AB-006 antibiotic compound with fungicide and insecticide activity – obtained by *Streptomyces* sp. culture. Biotechnol Abst 8(8):63 (Europe Patent, No. 110791, 1988)

Ansari MA, Razdan RK, Tandon M, Vasudevan P (2000) Larvicidal and repellent actions of Dalbergia sissoo Roxb. (F. Leguminoseae) oil against mosquitoes. Bioresour Technol 73:207–211

Anwar S, Ali B, Qamar F, Sajid I (2014) Insecticidal activity of actinomycetes isolated from salt range Pakistan against mosquitoes and red flour beetle. Pak J Zool 46(1):83–92

Bernhard L, Bernhard P, Magnussen P (2003) Management of patients with Lymphoedema caused by filariasis in North-eastern Tanzania: alternative approaches. Physiotherapy 89:743–749

Box SJ, Cole M, Yecman GH (1973) Prasinons A and B: potent insecticides from *Streptomyces prasinus*. Appl Microbiol 26:699–704

Burg RW, Miller BM, Baker EE, Birubaum J, Currie S, Hartman R, Kong YL, Monagham RM, Olson G, Putter I, Tunac JB, Wallick H, Stapley EW, Oiwa R, hnura S (1979) Avermectins, new family of potent anthelmintic agents: producing organism and fermentation. Antimicrob Agents Chemther 15:361–378

Deepika TL, Kannabiran K, Khanna VG, Rajakumar G, Jayaseelan C, Santhoshkumar T, Rahuman AA (2012) Isolation and characterisation of acaricidal and larvicidal novel compound (2S, 5R, 6R)-2-hydroxy-3, 5, 6-trimethyloctan-4-one from *Streptomyces* sp. against blood-sucking parasites. Parasitol Res 111(3):1151–1163

Dhanasekaran D, Thangaraj R (2014) Microbial secondary metabolites are an alternative approaches against insect vector to prevent zoonotic diseases. Asian Pac J Trop Dis 4(4):253–261

Dhanasekaran D, Rajakumar G, Sivamani P, Selvamani S, Panneerselvam A, Thajuddin N (2005) Screening of salt pans actinomycetes for antibacterial agents. Internet J Microbiol 1(2):1–8

Dhanasekaran D, Paneerselvam A, Thajuddin N (2008) An antifungal compound: 4′ phenyl-1-napthyl -phenylacetamide from *Streptomyces* sp. DBTB16. Facta Universitatis Ser Med Biol 15(1):7–12

Dhanasekaran D, Panneerselvam A, Thajuddin N (2009) Distribution and ecobiology of antagonistic *Streptomyces* from agriculture and coastal soil of Tamil Nadu, India. J Cult Collect 6:10–20

Dhanasekaran D, Sakthi V, Thajuddin N, Panneerselvam A (2010) Preliminary evaluation of *Anopheles* mosquito larvicidal efficacy of mangrove actinobacteria. Int J Appl Biol Pharm Technol 1(2):374–381

Dhanasekaran D, Ambika K, Thajuddin N, Panneerselvam A (2012a) Allelopathic effect of actinobacterial isolates against selected weeds. Arch Phytopathol Plant Protect 45(5):505–521

Dhanasekaran D, Thajuddin N, Pannnerselvam A (2012b) Antiparasitic secondary metabolites from marine actinobacteria. In: Kim S-K (ed) Marine pharmacognosy: trends and applications. CRC press, Taylor & Francis Group, Boca Raton, pp 373–384

Fradin MS, Day JF (2002) Comparative efficacy of insect repellents against mosquitos' bites. N Engl J Med 347:13–18

Goodfellow M (1983) Ecology of actinomycetes. Annu Rev Microbiol 37:189–216

Grein A, Meyers SP (1958) Growth characteristics and antibiotic production of actinomycetes isolated from littoral sediments and material suspended in Seawater. J Bacterial 76:457–463

Hayes JB, Laws ER (1991) Handbook of pesticide toxicology, vol 3. Academic Press, San Diego/London, pp 1576–1578

Hess B, Kutzner C, Van Der Spoel D, Lindahl E (2008) GROMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation. J Chem Theory Comput 4(3):435–447

Ikemoto T, Katayama T, Shiraishi A, Haneishi T (1983) Aculeximycin, a new antibiotic from *Streptosporangium albidum*. II. Isolation, physicochemical and biological properties. J Antibiot 36(9):1097–1100

Karthik L, Gaurav K, Rao KB, Rajakumar G, Rahuman AA (2011) Larvicidal, repellent, and ovicidal activity of marine actinobacteria extracts against *Culex tritaeniorhynchus* and *Culex gelidus*. Parasitol Res 108(6):1447–1455

Kaushik NT, Snehit SMMS, Rasesh Y (2009) Parikh Biological synthesis of metallic nanoparticles. Nanomed Nanotechnol 10:1–6

Kekuda TP, Shobha KS, Onkarappa R (2010) Potent insecticidal activity of two *Streptomyces* species isolated from the soils of Western ghats of agumbe, Karnataka. J Nat Pharma 1(1):30–32

Latha S, Vinothini G, Calvin DJD, Dhanasekaran D (2016) *In vitro* probiotic profile based selection of indigenous actinobacterial probiont *Streptomyces* sp. JD9 for enhanced broiler production. J Biosci Bioeng 121(1):124–131

Latha S, Sivaranjani G, Dhanasekaran D (2017) Response surface methodology: A non-conventional statistical tool to maximize the throughput of *Streptomyces* species biomass and their bioactive metabolites. Crit Rev Microbiol. https://doi.org/10.1080/1040841X.2016.1271308

Lechevalier HA, Lechevalier MP (1967) Biology of actinomycetes. Annu Rev Microbiol 21:71–100

Lee SE, Kim JE, Lee HS (2001) Insecticide resistance in increasing interest. Agric Chem Biotechnol 44:105–112

Lim H, Kim MK, Lim Y, Cho YH, Lee CH (2006) Inhibition of cell-cycle progression in HeLa cells by HY52, a novel cyclin-dependent kinase inhibitor isolated from *Bauhinia forficata*. Cancer Lett 233(1):89–97

Macedo ME, Consoli RAGB, Grandi TSM, Anjos AMG, Oliveira AB, Mendes NM, Queiroz RO, Zani CL (1997) Screening of Asteraceae (Compositae) plant extracts for larvicidal activity against *Aedes fluviatilis* (Diptera: Culicidae). Mem Inst Oswaldo Cruz 92:565–570

Misato T, Yoneyama K (1982) Agricultural antibiotics. In: Subbarao NS (ed) Advances in agricultural microbiology. Oxford & IBH Publishing Co., New Delhi, pp 465–506

Mishra SK, Keller JE, Miller JR, Heisey RM, Nair MG, Putnam AR (1987) Insecticidal and nematicidal properties of microbial metabolites potential alternatives to synthetic pesticides. J Ind Microbiol 2:267–276

Muthuselvam R, Vinothini G, Palliyarai Thaiyammal S, Latha S, Arunachalam C, Dhanasekaran D, Archunan G (2016) The cell aggregating propensity of probiotic actinobacterial isolates: isolation and characterization of the aggregation inducing peptide pheromone. Biofouling 32(1):71–80

Neumann R, Peter HH (1987) Insecticidal organophosphates: nature made them first natural organophosphates isolated from *Streptomyces antibioticus* culture. Experientia 43:1235–1237

Oishi H, Sagawa T, Okutomi T, Suzuki K, Hayashi T, Sawada M, Ando K (1970) Insecticidal activity of macrotetrolide antibiotics. J Antibiot 23:105–106

Okami Y (1952) Utilization of nitrogen compounds by streptomycetaceae and its applications to classification, Japan. J Med Sci Biol 5:265–275

Pampiglione S, Majori G, Petrangeli G, Romi R (1985) Avermectins, MK-933 and MK-936, for mosquito control. Trans R Soc Trop Med 79:797–799

Prabavathy VR, Mathivanan N, Murugesan K (2006) Control of blast and sheath blight diseases of rice using antifungal metabolites produced by *Streptomyces* sp. PM5. Biol Conserv 39(3):313–319

Priyadharsini P, Dhanasekaran D, Gopinath PM, Ramanathan K, Shanthi V, Chandraleka S, Bhaskar B (2017) Spectroscopic identification and molecular modeling of Diethyl 7- hydroxytrideca - 2, 5, 8, 11- tetraenedioate - a herbicidal compound from *Streptomyces* sp. Arab J Sci Eng 42:2217–2227

Putter I, MacConnel JG, Preiser FA, Haidri A, Ristch SS, Dybas RA (1981) Avetmectins: novel insecticides, acaricides and nematicides from a soil microorganism. Experientia 37:963–964

Rai M, Yadav A, Gade A (2009) Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv 27:76–73

Rajesh K, Dhanasekaran D, Tyagi BK (2013a) Mosquito survey and larvicidal activity of activity of actinobacterial isolates against *Culex* larvae (Diptera: Culicidae). J Saudi Soc Agric Sci 14(2):166–122

Rajesh K, Pathmavathi KC, Ranjani A, Gopinath PM, Dhanasekaran D, Archunan G (2013b) Green synthesis, characterization and larvicidal activity of AgNPs against *Culex quinquefasciatus* and *Aedes aegypti* larvae. Am J Drug Discov Dev 3:245–253

Ramaiah KD, Das PK, Michael E, Guyatt H (2000) The economic burden of lymphatic filariasis in India. Parasitol Today 16(6):251–253

Ranjani A, Dhanasekaran D, Gopinath PM (2016) An introduction to actinobacteria. In: Dhanasekaran D, Jiang Y (eds) Actinobacteria – basics and biotechnological applications. InTech Publisher, Croatia, pp 3–37. https://doi.org/10.5772/62329

Rao KV, Chattopadhyay SK, Reddy GC (1990) Flavonoids with mosquito larval toxicity. J Agric Food Chem 38:1427–1430

Saha S, Dhanasekaran D (2010) Isolation and screening of keratinolytic actinobacteria form keratin waste dumped soil in Tiruchirappalli and Nammakkal, Tamil Nadu, India. Curr Res J Biol Sci 2(2):124–131

Saha S, Priyadharshini A, Dhanasekaran D, Thajuddin N, Chandraleka S, Chandramohan G, Panneerselvam A (2012) Preclinical evaluation and molecular docking of 4-phenyl-1-Napthyl phenyl acetamide (4P1NPA) from *Streptomyces* sp. DPTB16 as a potent antifungal compound. Comput Biol Med 42(5):542–547

Saurav K, Rajakumar G, Kannabiran K, Rahuman AA, Velayutham K, Elango G, Zahir AA (2013) Larvicidal activity of isolated compound 5-(2, 4-dimethylbenzyl) pyrrolidin-2-one from marine *Streptomyces* VITSVK5 sp. against *Rhipicephalus* (Boophilus) *microplus*, *Anopheles stephensi*, and *Culex tritaeniorhynchus*. Parasitol Res 112(1):215–226

Service MW (2000) Medical entomology for students, 2nd edn. Cambridge University Press, Cambridge, pp 52–55

Tanvir R, Sajid I, Hasnain S (2014) Larvicidal potential of Asteraceae family endophytic actinomycetes against *Culex quinquefasciatus* mosquito larvae. Nat Prod Res 28(22):2048–2052

Thajuddin N, Muralitharan G, Dhanasekaran D, Muhammad Ilyas MH (2015) Microbial symbionts of plants. Plant biology and biotechnology. In: Bahadur B, Venkat Rajam M, Sahijram L, Krishnamurthy KV (eds) Volume I: plant diversity, organization, function and improvement. Springer, New York, pp 281–306. https://doi.org/10.1007/978-81-322-2286-6-11

Vijayakumar R, Murugesan S, Cholarajan A, Sakthi V (2010) Larvicidal potentiality of marine actinomycetes isolated from Muthupet mangrove, Tamilnadu. India Inter J Microbiol Res 1(3):179–183

Vijayan V, Balaraman K (1991) Metabolites of fungi and actinomycetes active against mosquito larvae. Indian J Med Res 93:115–117

WHO (1997) Dengue haemorrhagic fever: Diagnosis, treatment, prevention and control, 2nd edn. WHO, Geneva, p 48

Yang YC, Lee SG, Lee HK, Kim MK, Lee SH, Lee HS (2002) A piperidine amide extracted from *Piper longum* L. fruit shows activity against *Aedes aegypti* mosquito larvae. J Agric Food Chem 50:3765–3767

Zizka Z, Weiser J, Blumauerova M, Jizba J (1989) Ultra structural effects of macroterrolides of *Sterptomyces griseus* LKS-1 in tissues of *Culex pipiens* larvae-monactin, dinactin, triactin and nonactin preparation; insecticide activity. Cytobios 58:85–89

Nanopesticides: A Boon Towards the Control of Dreadful Vectors of Lymphatic Filariasis

19

Prabhakar Mishra, A. P. B. Balaji, B. K. Tyagi, Amitava Mukherjee, and N. Chandrasekaran

Abstract

Ever since insects, particularly the mosquitoes, had been discovered to mediate transmission of dreadful diseases, such as lymphatic filariasis, dengue and malaria during the late nineteenth century, it had been a continuous battle between man and these vector insects for survival of the fittest! Their mandatory involvement in disease transmission makes these tiny insects very important in the whole disease control efforts, drawing focused attention of entomologists, health workers and sanitarians worldwide. Significance of vector control in disease management was first demonstrated in case of malaria, and it is considered significantly important in the elimination and monitoring of lymphatic filariasis (LF). The human lymphatic filariasis, popularly referred to as elephantiasis, is one of the most debilitating neglected tropical diseases that continues to be a major health concern globally, in tropical and subtropical countries including India bearing almost 40% of the global disease burden. In India the disease is caused by *Wuchereria bancrofti* and *Brugia malayi*, and the third parasite *B. timori* is prevalent in Indonesia and the neighbouring islands. In India it is *W. bancrofti* which is causing over 95% infection. The key vector for this disease worldwide is *Culex quinquefasciatus*. Even though the disease is generally not fatal, nevertheless the kind of debilitation and incapacitation, apart from social stigmatization, lymphatic filariasis has assumed a great significance socially and economically, next only to malaria. The infection is largely controllable through medication (diethylcarbamazine @6 mg/kg body wt), yet the residual parasitic load remained uncontrolled in the vector mosquitoes in nature continues to warrant novel technologies and tools for an

P. Mishra · A. P. B. Balaji · A. Mukherjee · N. Chandrasekaran (✉)
Centre for Nanobiotechnology, Vellore Institute of Technology, Vellore, Tamil Nadu, India
e-mail: nchandrasekaran@vit.ac.in

B. K. Tyagi
Department of Environmental Biotechnology, School of Environmental Sciences, Tiruchirappalli, Tamil Nadu, India

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_19

efficient control of vectors to achieve the target of disease elimination. The nanopesticides have recently attracted the attention of disease managers for their novel pathways to reach the target sites to bring about a kill in vectors. Nanopesticide, majorly classified as synthetic and bio-based, is an efficient system which can be applied in controlling vectors without causing concerns of environmental pollution or affecting adversely nontarget organisms. In the present context of vector control, a few examples of nanopesticides are nanopermethrin and other bio-based oil nanoemulsions. The applications of these nanopesticides can be made either as larvicidal or adulticidal formulation, based on their mode of action. The properties of the nano-formulated pesticides like specificity, target delivery and low residual pollution make it propitious for the integrated pest management strategy. Therefore, the application of the nanopesticide in comparison to the conventional pesticides can become an efficient step for the control of lymphatic filariasis vector in the Indian subcontinent.

19.1 Introduction

The human lymphatic filariasis (LF), commonly referred to as 'elephantiasis', is one of the most debilitating neglected tropical diseases, next only to malaria in global disease burden. It is caused by the parasitic nematodes (*Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*) belonging to the superfamily, Filarioidea. The adult filarial worms of this kind of parasite prevail in the lymphatic system of the cutaneous tissues, hence the phrase lymphatic filariasis. The prolonged accumulation of adult worm in certain cavities may incapacitate human being, while gathering of millions of microfilariae (mf) in the lymphatics may cause blockage resulting in 'elephantiasis' and 'hydrocele', besides several other disorders like chyluria, etc. Among all these three nematodes, only *W. bancrofti* and *Brugia malayi* are prevalent in the Indian mainland, transmitted through the ubiquitous vector, *Cx. quinquefasciatus*. Filariasis caused *W. bancrofti* in India accounts for 99.4% of the disease burden problem (WHO 2005). This infection is found in both rural and urban parts of the country. *Culex quinquefasciatus* breeds mainly in the polluted water bodies of variety, though if forced can also breed in open organically rich waters. This quality requires novel technologies and products to stem out its populations in different polluted waters, and the nanopesticide technology has emerged in a potential manner.

19.2 Vectors for the Filariasis

The lymphatic filarial parasites are transmitted to human being by a host of mosquito species which are termed as 'vectors'. These species of vectors may belong to genera *Culex*, *Mansonia*, *Anopheles* and *Aedes*. All these vector species feed accidentally or preferentially on human blood to bring about the transmission, after allowing parasite

its development (larval stages $L_{1–3}$) in mosquitoes' body. *Culex quinquefasciatus* is the most significant vector for *W. bancrofti* across the world. This mosquito mediates transmission of not only the human filarial nematode but also transmits a variety of other infections such as the dog heartworm (*Dirofilaria*) and West Nile virus. Besides being a strong vector, *Cx. quinquefasciatus* is also a source of intranquility through its painful bites, particularly depriving people of their sleep and robbing them of their working hours. Its bite also results in irritation and allergy and gives much vexation and disquietness. Often the population density of this vector is linked to deforestation and urbanisation process (Albuquerque 1993).

Culex quinquefasciatus (Diptera: Culicidae) is a nocturnal species, biting at night times, a characteristic that is a primary requirement for picking up filarial microfilariae (*mf*) from the infection carriers since the microfilariae appear in the peripheral blood periodically in the night only (Fonseca et al. 2006). In Andaman and Nicobar islands, the vector of lymphatic filariasis, a subperiodic form of the disease, is *Aedes niveus*.

Culex quinquefasciatus lays its eggs and grows in the organically enriched stagnant and slowly running water. Therefore, this vector is associated with the substandard housing conditions, improper sanitation, sullage and other sources (Barbosa et al. 2007; Correia et al. 2012). This mosquito breeds abundantly in the water bodies like cesspools, burrow pits, wells, drains, septic tanks, cesspools, pits, etc. with rich organic component. At the optimum physicochemical conditions, the life cycle of this mosquito comprising egg, larva and pupa gets initiated in aquatic ecosystem, followed by the aerial adult stage. After emergence to adult, the first thing the mosquito undertakes is to go for feeding on a suitable host such as man or cattle (Dibo et al. 2011). *Culex quinquefasciatus* is highly anthropophilic in nature which results in the easy transmission of filarial nematode causing the lymphatic filariasis (Pérez-Arellano et al. 2010).

19.3 Filariasis Scenario in India

According to the estimated survey carried out till 1995, globally there were 1100 million people residing in the endemic areas of lymphatic filariasis and exposed to the risk of infection. About 120 million people were estimated to be infected with either microfilaraemia or chronic filarial disease (Michael et al. 1996). The causative parasite, i.e. *W. bancrofti*, accounted for around 95% of filarial cases in the country. According to the studies carried out by Indian Council of Medical Research (ICMR), in different states and union territories of India, 22 states were found endemic for this disease. Among these endemic states, nine states (Andhra Pradesh, Bihar, Gujrat, Kerala, Maharashtra, Odisha, Tamil Nadu, Utter Pradesh and West Bengal) contributed more than 95% of the total infections. Currently an overall 553 million people are at risk of this infection. The disease/infection is mainly occurring along the eastern, southern and western states/UTs along the sealine.

19.4 Role of WHO in Elimination of the Lymphatic Filariasis

According to the World Health Organization (WHO) resolution popularly named as the Global Programme for Elimination of Lymphatic Filariasis (GPELF), the infection is slated to be eliminated from the face of the world by 2020. India, taking the lead based on its stupendous efforts, had even resolved to eliminate lymphatic filariasis by 2015 (which now postponed to 2018), 5 years in advance to global pronouncement. The human lymphatic filariasis will thus be the second vector-borne, but the first mosquito-borne, infection that would be eliminated from the world following dracunculiasis (Guinea-worm disease) in the late 1990s. Besides effective mass drug administration (MDA), vector control using integrated vector management (IVM) has also been demonstrated to be highly effective in maintaining the low microfilaraemia in endemic regions.

19.5 Vector Control Strategies

The prevention or the reduction in the parasitic interaction totally depends on the strategies applied to control or interrupt the human-vector contact. One of such strategies is the IVM (integrated vector management) (Apple and Smith 1976). The IVM stresses upon cautious contemplation of all available vector control strategies and successive integration of the suitable measures that help to create complications in the vector development, also keeping the intervention level economically and ecologically safe. The several principles of this strategy include the:

(a) Accepted vector level which defines the control of the vector not the total eradication of vector species, as eradication attempt may become expensive and unsafe to the environment.
(b) Preventive cultural practices which deal with the selection of agricultural practices that are beneficial for the healthy human environment maintenance.
(c) Vector control through mechanical (barriers, traps, vacuuming and breeding disruption), chemical (usage of synthetic insecticides such as pyrethroids, organophosphates, organochlorines, juvenile hormone analogues) and biological (fish, dragonfly larvae, biological insecticides such as *Bacillus thuringiensis* var. *israelensis* (*Bti*), entomopathogenic fungi and nematodes) approaches.

Even though certain insecticides are considered highly effective against *Cx. quinquefasciatus*, nevertheless due to their high costs, early development of resistance in vectors and environment and human health concerns, there has been a constant desire to find out more suitable tools and molecules. Nano-insecticides have become an alternative choice at present, although more studies are required to be carried out in this field before putting the technology in practice.

19.6 Role of Nanopesticides in the Vector Control

Nanotechnology exhibits its potential role by forming nanopesticides with an eco-friendly approach which includes natural products integrated with active ingredients of the toxic chemical (Angajala et al. 2014; Rawani et al. 2013). These kinds of approaches are efficient to develop pest control strategies with minimal toxicity levied towards the environment or nontarget organisms. These nanopesticides are formulated using two different approaches involving the presence of active ingredient of a known synthetic pesticide and other which is an active ingredient derived from the vegetation. Few examples of nanopesticides used in controlling mosquito population are nanopermethrin and certain essential oil-based nanoemulsions (e.g. neem oil nanoemulsion, eucalyptus oil nanoemulsion, etc.) (Anjali et al. 2012; Ghosh et al. 2013). Sugumar et al. (2014) describe the potential of nanometric emulsions formulated using essential oils against the vector mosquitoes like *Culex* and *Aedes* species.

Nanometric emulsion like neem oil nanoemulsion, basil oil nanoemulsion, and eucalyptus oil nanoemulsion are few of the examples carrying larvicidal properties against larval population. The biochemical present in these oils tends to characterize their potential as a larvicide. Compounds like azadirachtin in neem oil, eugenol in basil oil and eucalyptol in eucalyptus oil are few examples of the natural biochemical constituents which enhance the larvicidal property of the nanometric emulsions against *Culex quinquefasciatus* and *Aedes aegypti* (Veerakumar et al. 2014). The development of nanoemulsion is also one of the types of nanoformulation for the vector control strategy. Different nanotechnologies exist for formulating varied nanopesticides, like the formation of the nanoparticle by polymerization (Pavel 2004), solid lipid nanoparticles (Gasco et al. 2009), precipitation in an aqueous droplet (Destrée and Nagy 2006) and direct solvent evaporation (Margulis-Goshen and Magdassi 2012). Figure 19.1 describes the potential benefits of the nanopesticides upon the conventional bulk pesticides which prove them to be less toxic to the environment.

19.6.1 Nanopermethrin

Nanopermethrin is the water dispersible powder form of permethrin. This nanoformulation is achieved using the solvent evaporation of the O/W microemulsion consisting of pesticide in an organic phase. The composition of the obtained O/W microemulsion was permethrin as an active ingredient, n-butyl acetate and sec-butyl alcohol as an organic solvent and ammonium glycyrrhizinate and soybean lecithin as a surfactant and water. The obtained microemulsion is further lyophilized at −95 °C and <1 mbar for 24 h which resulted in water dispersible nanopowder along with surfactants. The nano-formulated permethrin powder consisted of permethrin (13%), AG (29.5%), SbPC (29.5%) and sucrose (28%) by weight. Permethrin in its nanoparticulate (NP) form exhibited amorphous nature as reported by Anjali et al. (2010). The mean hydrodynamic size of the obtained nanopermethrin is found to be

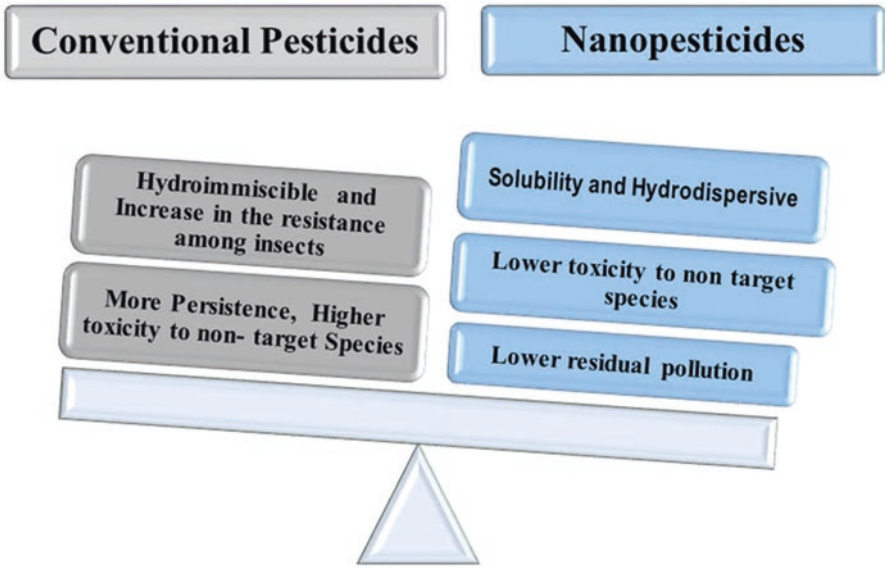


Fig. 19.1 Potential benefits of the nanopesticides upon the conventional bulk pesticides

151 ± 27 nm. Formulated nanopermethrin exhibits a potent larvicidal property against *Cx. quinquefasciatus*. Similarly the NP has exerted the improved efficacy against the adult mosquitoes as compared to its bulk form. The median knock-down values of NP (60 min) for *Cx. tritaeniorhynchus*, *Cx. quinquefasciatus* and *Ae. albopictus* were found to be 0.98×10^4 mg/L, 1.17×10^4 mg/L, and 0.05×10^3 mg/L (Balaji et al. 2015).

19.6.1.1 Mode of Action of Nanopesticide Formulations

The nanoformulation of permethrin acts as an efficient larvicidal agent. The active ingredient of permethrin is a major class of neurotoxic insecticides. It is a synthetic analogue of naturally occurring esters of type II pyrethrins, which is naturally found in the flowers of *Chrysanthemum cinerariifolium*. The permethrin ingredients act as a potent neurotoxic agent, resulting in lethality to the insect. Permethrin tends to have its action on both peripheral and central nervous systems of the insects. The insecticidal action initially stimulates the nerve cells which tend to produce repetitive discharges of stimuli and ultimately leads to insect's paralysis. This type of neurotoxicity shown by the action of permethrin is much more pronounced in nanoemulsions than in conventional pesticide formulations.

The nanoformulation of this pesticide leads to enhancement in the insecticidal action of the pesticide. The reduced particle size and the increase in surface area lead to its easier permeation into the host body improvising the insecticidal property (Schrof et al. 2003). Figure 19.2 describes the proposed mechanism of nanopermethrin which defines its enhanced potential against the vector *Cx. quinquefasciatus*.

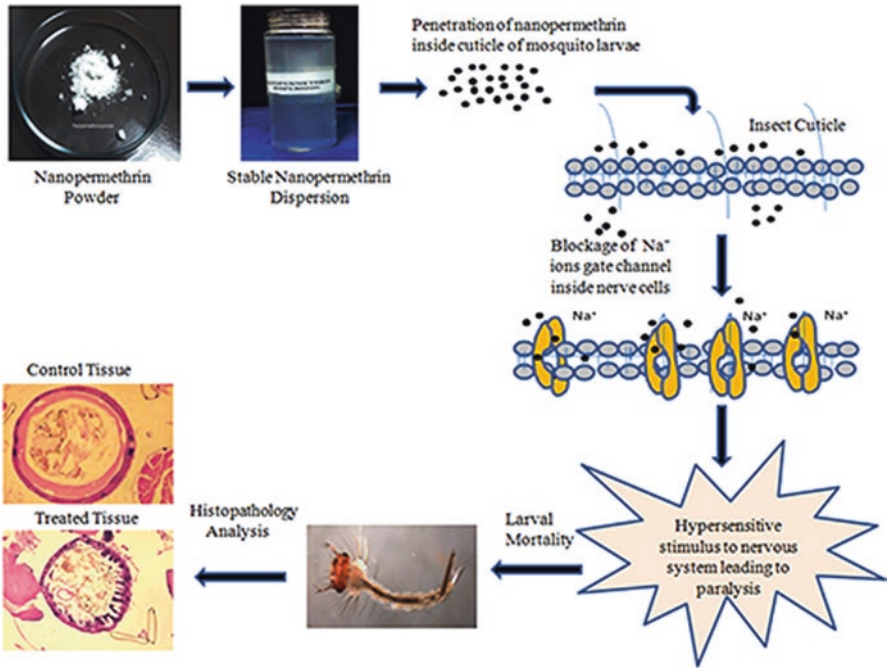


Fig. 19.2 Mechanistic approach of nanopermethrin as a larvicidal agent

19.6.2 Nanoemulsions

An emulsion is a liquid dispersion of two different immiscible liquids. The macroscopic separation of the two different phases is controlled through addition of the surfactant. This emulsion system when tends to attain the nanometric size of 20–200 nm is termed as 'nanoemulsion'. The term 'nanoemulsion' defines a kinetically stable system which is isotopically clear dispersion system of two immiscible liquids. This nanoformulation comprises of an oil phase and aqueous phase which is stabilized through the addition of interfacial film formed by surfactant molecules. The dispersed phase present in the nanoemulsion involves small droplets attaining a size of 20–200 nm with a low interfacial tension between the phases. The nanoemulsions are optically transparent in nature due to the presence of these nanometric droplets which are 25% less to that of wavelength of the visible light.

The surfactant has its vital role in the formation of these nanometric emulsion systems. The interfacial tension generated due to the immiscibility of two different liquid systems is lowered through the use of surfactant. This property of lowering down the interfacial tension between the phases tends to make the surfactant as an essential part of nanoemulsion system. This formulation process for the nanoemulsion is carried out through low- or high-energy emulsification method. The low-energy method includes phase inversion method, while the high-energy method includes ultrasonication and high-pressure homogenization. Nanoemulsion has its

versatile application in several fields, for example, pharmaceutical, cosmetics, agriculture, etc. In the current scenario, different approaches in the development of the nanoemulsion with an efficient insect repellant and larvicidal activity are being carried out (Anjali et al. 2012; Nuchuchua et al. 2009). Sugumar et al. (2014) describe the mosquito control properties of the essential oil nanoemulsions.

19.6.3 Essential Oil Nanoemulsion

Neem oil nanoemulsion is one of the promising tools for the control of vector population. The anti-ecdysone activity exhibited by the neem oil makes it propitious for this application. The formulation of the neem oil nanoemulsion was carried out using the methodology opted by Anjali et al. (2012). The nanoemulsion was prepared using the neem oil, nonionic surfactant Tween 20 and Milli-Q water. The coarse emulsion obtained after the combination of oil and aqueous phase was subjected to ultrasonication for the duration of 20 min to achieve the final nanosized emulsion system. The formulated nanoemulsion exhibited the mean hydrodynamic size of 30.12 ± 1.3 nm with the polydispersity index of 0.262. The nanoemulsion system exhibited its noteworthy larvicidal activity against the dreadful filarial vector *Cx. quinquefasciatus*. The lethal indices (LC_{50}) for the formed nanoemulsion was found to be 11.75 mg/L for 24 h. Nanoformulated emulsion of neem oil with the smallest droplet diameter was efficient than its bulk counterpart, i.e. neem oil. This property of nanosized emulsion system can become an efficient alternative for the vector and vector-borne diseases' control.

Similarly another essential oil that proves its mosquito control activity against the *Cx. quinquefasciatus* is eucalyptus oil nanoemulsion. According to Sugumar et al. (2014), nanoemulsion prepared using the eucalyptus oil shows an efficient larvicidal property against the larvae of *Cx. quinquefasciatus*. The eucalyptus oil nanoemulsion was formulated using 1:3 ratio of eucalyptus oil: Tween 80 which resulted in the translucent nanoemulsion system. The mean hydrodynamic size of the nanoemulsion was estimated to be 9.4 nm. Later through the estimation of its larvicidal potency, it was found out that this nanosized emulsion also exhibits an efficient larvicidal property. The larvicidal activity exhibited by nanoemulsion was observed at the concentration of 250 mg/L which resulted in 98% mortality within 4 h of treatment.

The larvicidal activity exhibited by the nanoemulsions against the filariasis vector can be attributed to the essential oil components such as azadirachtin, eucalyptol, etc. which consists of chemical constituents like terpene hydrocarbons, such as monoterpenes and sesquiterpenes, and oxygenated compounds such as phenols, alcohols, aldehydes, ketones, esters, lactones, ethers and oxides. This distinct variety of kinetically stabilized formulation comprises the two different phases that are immiscible. According to Solans et al. (2005), these nanoemulsions attain the translucent property with smaller droplet dimeter of 20–200 nm, therefore attaining bluish aspect (Forgiarini et al. 2000). These nanosized emulsions of essential oils tend to attain the insect control property (Wang et al. 2007). Figure 19.3 describes the

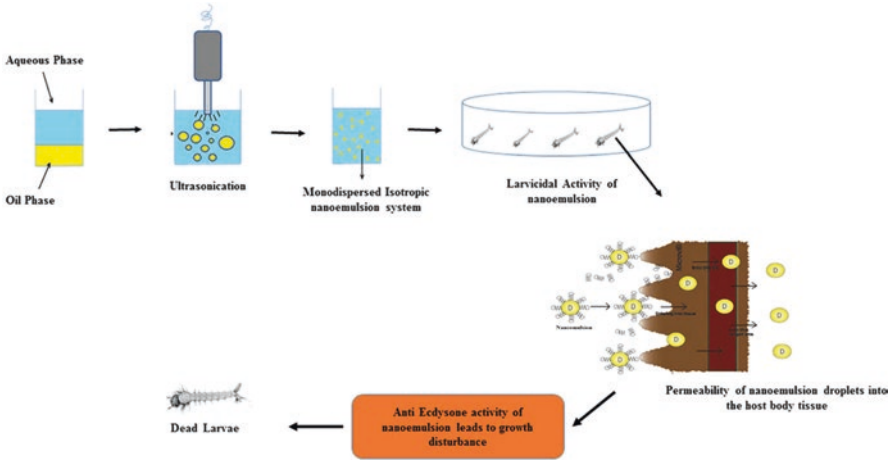


Fig. 19.3 Mechanistic approach of the essential oil nanoemulsion as a larvicidal agent

mechanism behind the effective property of the essential oil nanoemulsion as a larvicidal agent. The chemical components present in the nanoemulsion, for example, azadirachtin present in neem oil, attain the growth inhibitory property. The azadirachtin tends to have action on the ecdysone hormone, which is a key hormone for the growth of insects. The nanometric size of the nanoemulsion enhances its specificity and target delivery resulting in more effectiveness than bulk pesticides. The unregulated growth hormone tends to stop the moulting of the insects, consequently leading to their mortality.

The bio-safe property of these essential oil nanoemulsions makes them a noteworthy insect control agent. Sugumar et al. (2014) describe the bio-safety property of the eucalyptus oil nanoemulsion against the *Rhizobium leguminosarum*. The tested strain exhibited no zone of inhibition depicting the eco-safe property of the nanoemulsion. Similarly the neem oil nanoemulsion exhibited the bio-safe property against the fingerlings of *Labeo rohita* (Mishra et al. 2014). Therefore, from this study it can be concluded the nanopesticide can be an efficient tool with an eco-safe property and can be safely applied for the control of the vector, *Cx. quinquefasciatus*.

19.7 Conclusion

Nano-modification of the conventional pesticides, which are hydro-immiscible in nature, significantly improves the pesticidal life. It also improves their specificity to target organisms. Nanoformulation requires less amount of the conventional pesticide in developing a formulation, and also the use of the bio-surfactants makes the nanopesticide propitious to the environment. The elimination of the volatile organic solvent from the pesticidal formulation enhances its bio-safe property and makes it a ‘greener’ strategy for the vector control. A higher degree of target specificity and

delivery and reduced eco-toxicity make these nanometric pesticides highly eco-friendly. The greater effectiveness of these nanometric pesticides against the filariasis vector, *Cx. quinquefasciatus*, with its lower residual toxicity to the environment, can make it an efficient tool towards the control of *Cx. quinquefasciatus* population. This strategy can serve as an important component in the effective implementation integrated vector management.

References

Albuquerque MFP (1993) Urbanization, slums, and endemics: the production of filariasis in Recife, Brazil. Cad Saude Publica 9:487–497

Angajala G, Ramya R, Subashini R (2014) In-vitro anti-inflammatory and mosquito larvicidal efficacy of nickel nanoparticles phytofabricated from aqueous leaf extracts of Aegle marmelos Correa. Acta Trop 135:19–26

Anjali C, Khan SS, Margulis-Goshen K, Magdassi S, Mukherjee A, Chandrasekaran N (2010) Formulation of water-dispersible nanopermethrin for larvicidal applications. Ecotoxicol Environ Safe 73:1932–1936

Anjali C, Sharma Y, Mukherjee A, Chandrasekaran N (2012) Neem oil (Azadirachta indica) nanoemulsion—a potent larvicidal agent against *Culex quinquefasciatus*. Pest Manag Sci 68:158–163

Apple JL, Smith RF (1976) Integrated pest management. Springer, New York, pp 1–16

Balaji A, Mishra P, Kumar RS, Ashu A, Margulis K, Magdassi S, Mukherjee A, Chandrasekaran N (2015) The environmentally benign form of pesticide in Hydrodispersive Nanometric form with improved efficacy against adult mosquitoes at low exposure concentrations. Bull Environ Contam Toxicol 95:734–739

Barbosa RM, Souto A, Eiras AE, Regis L (2007) Laboratory and field evaluation of an oviposition trap for Culex quinquefasciatus (Diptera: Culicidae). Mem Inst Oswaldo Cruz, Rio de Janeiro 102(5):523–529

Correia JC, Barbosa RMR, Oliveira CMFd, Albuquerque CMRd (2012) Residential characteristics aggravating infestation by Culex quinquefasciatus in a region of Northeastern Brazil. Rev Saude Publica 46:935–941

Destrée C, Nagy J (2006) Mechanism of formation of inorganic and organic nanoparticles from microemulsions. Adv Colloid Interf Sci 123:353–367

Dibo MR, Menezes RMTd, Ghirardelli CP, Mendonça AL, Chiaravalloti Neto F (2011) The presence of Culicidae species in medium-sized cities in the State of São Paulo, Brazil and the risk of West Nile fever and other arbovirus infection. Rev Soc Bras Med Trop 44:496–503

Fonseca DM, Smith JL, Wilkerson RC, Fleischer RC (2006) Pathways of expansion and multiple introductions illustrated by large genetic differentiation among worldwide populations of the southern house mosquito. Am J Trop Med Hyg 74:284–289

Forgiarini A, Esquena J, González C, Solans C (2000) Studies of the relation between phase behavior and emulsification methods with nanoemulsion formation. Progr Colloid Polym Sci 115:36–39

Gasco MR, Priano L, Zara GP (2009) Solid lipid nanoparticles and microemulsions for drug delivery: the CNS. Prog Brain Res 180:181–192

Ghosh V, Mukherjee A, Chandrasekaran N (2013) Formulation and characterization of plant essential oil based Nanoemulsion: evaluation of its Larvicidal activity against Aedes aegypti. Asian J Chem 25:321–323

Margulis-Goshen K, Magdassi S (2012) Organic nanoparticles from microemulsions: formation and applications. Curr Opin Colloid Interface Sci 17:290–296

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

Mishra P, Jerobin J, Thomas J, Mukherjee A, Chandrasekaran N (2014) Study on antimicrobial potential of neem oil nanoemulsion against Pseudomonas aeruginosa infection in Labeo rohita. Biotechnol Appl Biochem 61:611–619

Nuchuchua O, Sakulku U, Uawongyart N, Puttipipatkhachorn S, Soottitantawat A, Ruktanonchai U (2009) In vitro characterization and mosquito (Aedes aegypti) repellent activity of essential-oils-loaded nanoemulsions. AAPS PharmSciTech 10:1234–1242

Pavel FM (2004) Microemulsion polymerization. J Dispersion Sci Technol 25:1–16

Pérez-Arellano J, Carranza-Rodríguez C, Vieira-Lista C, Muro A (2010) Nematodosis I: filariosis. Medicine-Programa de Formación Médica Continuada Acreditado 10:3729–3738

Rawani A, Ghosh A, Chandra G (2013) Mosquito larvicidal and antimicrobial activity of synthesized nano-crystalline silver particles using leaves and green berry extract of Solanum nigrum L.(Solanaceae: Solanales). Acta Trop 128:613–622

Schrof W, Heger R, Koltzenburg S, Bratz M, Zagar C, Horn D (2003) Nanoparticles comprising a protection agent. Patent Scope, WO/2003/039249

Solans C, Izquierdo P, Nolla J, Azemar N, Garcia-Celma M (2005) Nano-emulsions. Curr Opin Colloid Interface Sci 10:102–110

Sugumar S, Clarke S, Nirmala M, Tyagi B, Mukherjee A, Chandrasekaran N (2014) Nanoemulsion of eucalyptus oil and its larvicidal activity against Culex quinquefasciatus. Bull Entomol Res 104:393–402

Veerakumar K, Govindarajan M, Rajeswary M, Muthukumaran U (2014) Low-cost and eco-friendly green synthesis of silver nanoparticles using Feronia elephantum (Rutaceae) against Culex quinquefasciatus, Anopheles stephensi, and Aedes aegypti (Diptera: Culicidae). Parasitol Res 113:1775–1785

Wang L, Li X, Zhang G, Dong J, Eastoe J (2007) Oil-in-water nanoemulsions for pesticide formulations. J Colloid Interface Sci 314:230–235

WHO (2005) Guidelines for laboratory and field testing of mosquito larvicides. (CDS/WHOPES/GCDPP/05.13)

20

Identification and Mapping of Breeding Habitats of the Filariasis Vector, *Culex quinquefasciatus* Say, 1823, Using Remote Sensing and GIS Technologies: A Case Study from the Endemic Tamil Nadu State, India

P. Suganthi, M. Govindaraju, Rajiv Das Kangabam, K. Suganthi, V. Thenmozhi, and B. K. Tyagi

Abstract

Mosquitoes act as a vector to transmit the life threatening diseases to humans. Mosquito control and management practice have played an essential role in the significant reduction of mosquito-borne diseases. The present study is focusing on the mapping of filariasis vector surveillance in urban environment (Tiruchirappalli City Corporation) and forest environment (Sitheri Hills) using remote sensing and GIS techniques. The study in urban environment reveals that the presences of risk occurrence of diseases like malaria, chikungunya and dengue. At Sitheri Hills the highest number of mosquito species was observed due to the presence of more number of mosquito breeding sites. Moreover, spatial analysis and entomological study results show that the people were affected by lymphatic filariasis. Therefore, the study concludes that the monitoring and controlling of filariasis vector are needed to solve public health problems.

P. Suganthi · M. Govindaraju (✉) · R. D. Kangabam · K. Suganthi · B. K. Tyagi
Department of Environmental Biotechnology, School of Environmental Sciences
Bharathidasan University, Tiruchirappalli, Tamil Nadu, India
e-mail: mgrasu@bdu.ac.in

V. Thenmozhi
Centre for Research in Medical Entomology (ICMR), Madurai, Tamil Nadu, India

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_20

20.1 Introduction

Lymphatic filariasis (LF), endemic in over 80 countries, has been long identified as a major public health problem worldwide, particularly in India which accounts for 40% of the global disease burden, next only to malaria. It is currently estimated that up to 120 million people are infected with *Wuchereria bancrofti*, 1 of the 3 causative agents, in about 83 endemic countries (Michael et al. 1996). Of these, it is estimated 40 million people have evidence of chronic manifestations such as hydrocele, lymphoedema/elephantiasis, etc. In addition, the affected individuals suffer repeated episodes of adeno lymphangitis (acute attacks) which result in marked loss in their economic productivity (Ravindran 2003). Improved therapies and diagnostic methods have led to the realization that it should be possible to interrupt transmission and eliminate LF by repeated, annual cycles of mass drug administration (MDA) with single dose combination regimens comprising diethylcarbamazine citrate (DEC) plus albendazole (Alb) (Ottesen 2000). Thus, in 1997 the World Health Assembly passed a resolution calling for strengthening of activities leading to the elimination of LF as a public health problem and gave the movement a name of Global Programme for Elimination of Lymphatic Filariasis (GPELF) (Molyneux and Taylor 2001). Elimination of lymphatic filariasis by providing foolproof MDA is carried out with optimum community compliance (>85%), along with proper vector control to stain benefits of the MDAs over the years. However, the vector control cannot be effectively carried out without a proper surveillance system. The geographic information system, GIS, is able to facilitate proper monitoring of vector breeding and therefore is also the most appropriate vector management strategy.

20.2 Vector of Lymphatic Filariasis

The rapid growth of large cities raises also the problem of disposal of sewage. The immature stages of *Cx. quinquefasciatus* and *Mansonia annulifera/M. uniformis*, as the vectors of lymphatic filariasis, predominantly inhabit sewage drains, cess pits and pools filled with eutrophic waters. Lymphatic filariasis persists as a major cause of clinical morbidity and a significant impediment to socioeconomic development in much of Asian countries (Becker 1996; Mwangangi et al. 2012). At least 120 million people are infected with the nematodes *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*, of which only the first two prevail in India, particularly *W. bancrofti* which is able to transmit approximately 99.4% of all cases in the country. *Wuchereria bancrofti* is transmitted by *Cx. quinquefasciatus* (Becker et al. 2010).The disease manifests often in bizarre swelling of legs and hydrocele and the cause of a great deal of social stigma (Phumee et al. 2011). These parasites after getting deposited on the skin by the infective vector mosquitoes penetrate on their own or through the opening created by mosquito bites to reach the lymphatic system.

20.3 Role of Remote Sensing and GIS for Mapping of Lymphatic Filariasis

Geographic information system (GIS) and remote sensing offer powerful tools for describing, illustrating, explaining and predicting epidemiological phenomena, which can be used to develop or improve surveillance, prevention and control strategies. With the availability of multispectral, multi-temporal and real-time satellite data products, GPS-assisted geo-referenced epidemiological data are being integrated under the umbrella of the GIS software for mapping LF distribution. This technique has been significantly developed for the past 25 years. The remote sensing and GIS for mapping land use/land cover and changes over the period of time interval in association with vector habitats and mapping vector abundance are epidemiologically important for disease control (Ahmad et al. 2011). The survival and longevity of infected mosquitoes and the prevalence of the disease are spatially determined and definitely controlled by the geoclimatic variables. The remote sensing of Landsat TM, IRS LISS I, LISS II, LISS III, IRS CARTOSAT, SPOT, IKONOS, NOAA-AVHRR, etc. are used to map vector breeding habitats and analyse vector abundance as a major parameter to correlate with LF incidence (Palaniyandi 2012).

20.4 Mapping Filariasis Transmission Risk

The remote sensing information derived from the calibrations of NDVI from the red and infrared spectral DN values alone has insignificance with filariasis distribution, because there are complexes of several phenomena influencing the filariasis transmission (Rejmánková et al. 2013). However, soil moisture with vegetation cover information of the remote sensing false colour composite DN values from 145 to 158 are valuable and statistically significant, whereas GIS has the efficient utility value in the application of geostatistical modelling to generate a "filariasis transmission risk map", using the selected environmental variables (Abdel-Hamid et al. 2009). We can demonstrate the filariasis spatial pattern, the quantified clustering and the potential of GIS application in vector-borne disease epidemiology. The appreciation of GIS is for optimum allocation of the patients to the health service centres with <1 km distance coverage for filariasis morbidity management and control (Hassan and Onsi 2004; Ceccato et al. 2005; Reiter and Lapoint 2007). GIS is the rapid method for prediction and mapping the potential breeding habitats of *Culex* species vectors of filariasis in both urban and rural environments. GIS is also used to generate data for predicting the real picture of filariasis situation. Huge sample points are needed at <10 km interval for classifying the areas correctly with the real situation of the filariasis transmission risk in the country. The results obtained showed >93.4% accuracy and 100% sensitivity.

The remote sensing datasets and the geoclimatic environmental data are integrated with LF data, and the epidemiological data for geostatistical analysis to generate the information where no information is available or the areas are at remote and difficult-to-reach locality, predicting and mapping vector-borne disease transmission risk areas, are analysed using GIS expert engine.

20.4.1 Case Study 1: Vector Surveillance Mapping in Urban Environment

20.4.1.1 The Tiruchirappalli City Corporation

The study area, Tiruchirappalli, is the fourth largest city in Tamil Nadu (India), which is situated on the banks of the river Cauvery at 10.5°N, 78.43°E. It spreads over an area of 5114 Sq. km with the population of the city is 1,021,717 of which 507,180 are males and 514,537 are females. Tiruchirappalli City Corporation consists of 60 wards and 4 zones, 15 wards to each zone, namely, Srirangam, Ariyamangalam, Ponmalai and K. Abishekapuram (Fig. 20.1). For maintaining better health resources, the population data, vector-borne disease and its prevalence, land use/land cover (2001), sanitation and other related factors were collected during the study period. From the population density analysis, areas including Srirangam, Chatram bus terminus, KK Nagar, etc. have been found to be on the higher side, while outer regions of the city remain to be less populated.

20.4.1.2 Attribute Data Analysis

The distribution of mosquitoes (*Culex*, *Aedes*, *Anopheles* spp.) in the wards of Tiruchirappalli was done, and the data obtained from the Department of Public Health and Preventive Medicine during 2009–2012. It has been mapped to find out the most vector-prone habitats. The data have been presented in Fig. 20.1.

20.4.1.3 Land Use/Land Cover (LU/LC) Map

With the help of visual interpretation key, change in satellite image was detected to process and identify the changes in LU/LC class based on multi-dated satellite data during the periods of may 2001 (Fig. 20.2) and November 2012 (Fig. 20.4). The images were visually interpreted on system and classified base on the interpretation key prepared during ground truth verification and attributed to the corresponding LU/LC changes. The classified maps are shown in Figs. 20.3 and 20.5.

20.4.1.4 Observation

From the attribute data analysis results, the presence of the vectors has been identified in the wards 1–4, 17, 21, 28, 37–39, 44, 48, 49, 51 and 52. These wards include the areas adjacent to solid waste disposal sites, Ariyamangalam and Panchapur. Also wards in Srirangam and Chatram bus terminus are more prone for vector distribution and preponderance. Therefore, the risk of occurrence of diseases like malaria, chikungunya and dengue in those wards is much higher than the rest. LU/LC results clearly indicate that there is a significant change in settlement area. It may be due to increasing human population which leads to higher vector density. Urban legislative body, all municipal corporations are to develop and implement programme for water supply, sewage, drainage and solid waste management to keep the environment free from vector breeding. So, the diseases surveillance and control

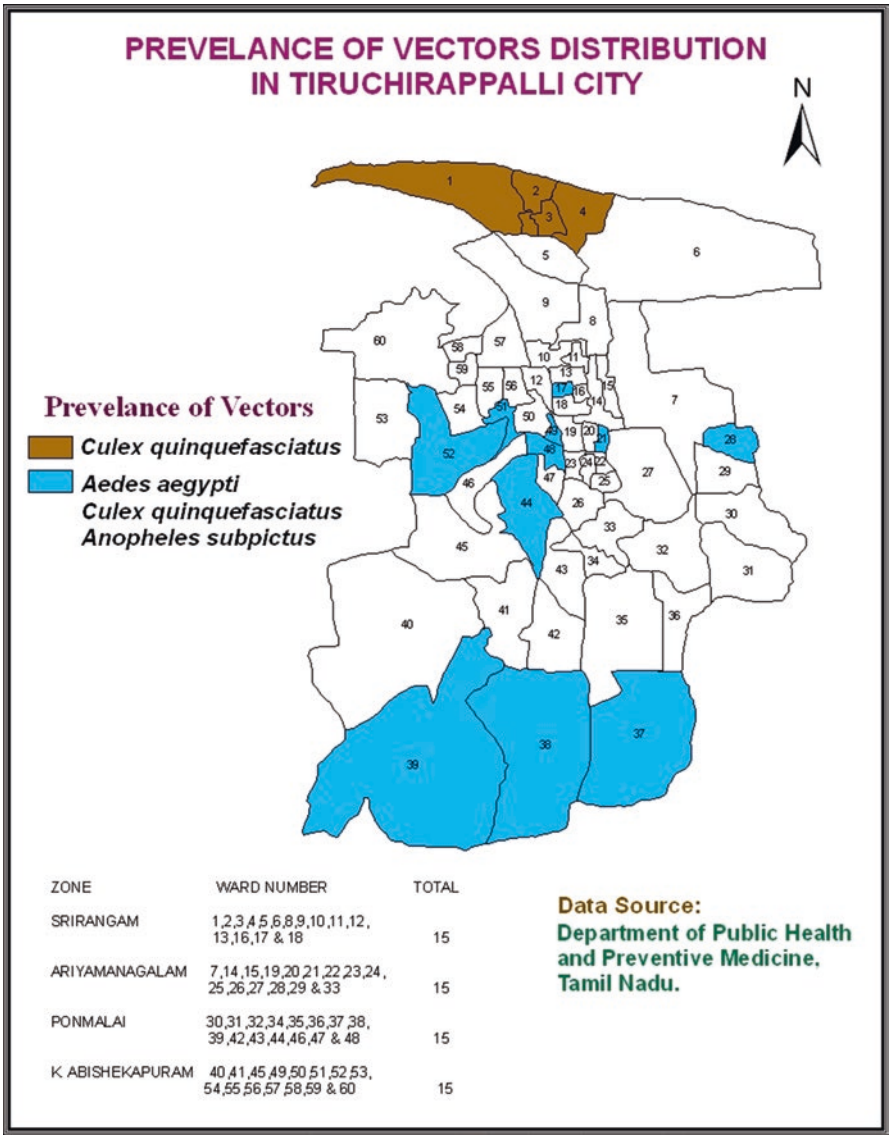


Fig. 20.1 Prevalence of LF vector distribution in Tiruchirappalli city during 2009–2012

activities require advanced technological applications with professional analysis to achieve the target in short term with maximum geographical area covered. In urban settings high-risk areas can be identified using remote sensing and GIS technology where otherwise it would be difficult to control vector-borne diseases rapidly.

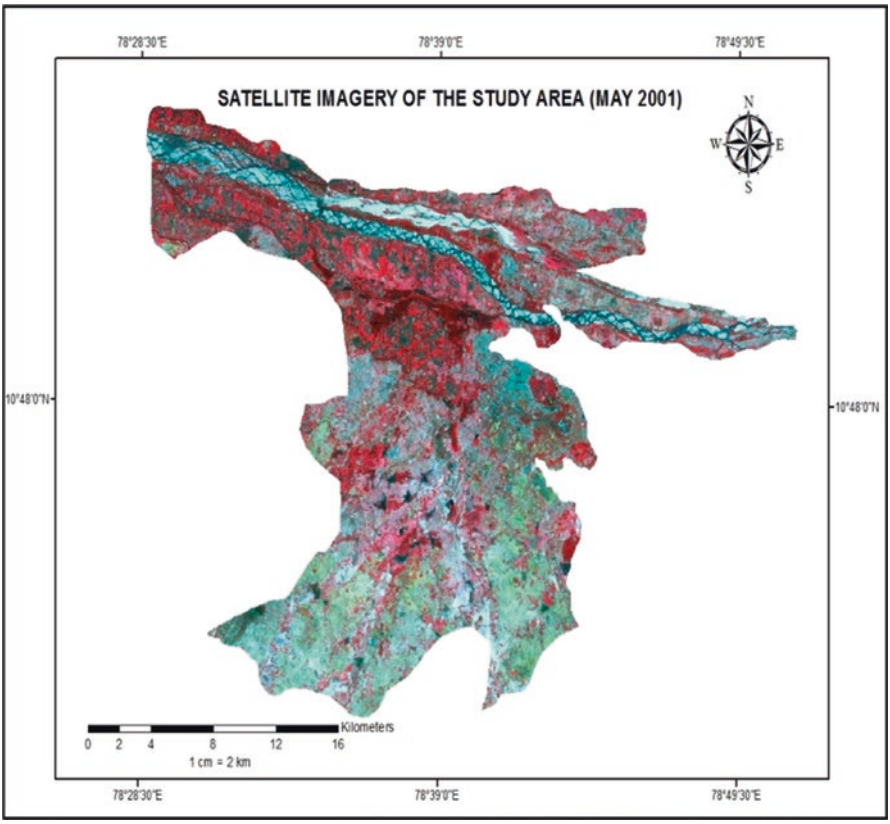


Fig. 20.2 Satellite imagery during May 2001

20.4.2 Case Study 2: Vector Surveillance Mapping in Forest Environment

20.4.2.1 Sitheri Hills

Sitheri Hills form one of the segments of Eastern Ghats in Tamil Nadu, within the geographical limit of 78°15′00″–78°45′00″E longitude and 11°44′00″–12°08′00”N latitude. The total area is about 736.18sq. km situated at Pappireddipatti Taluk, 28 km distance from Harur town in Dharmapuri district. It is situated at an altitude of 1097.3 m (3,600 ft). The area comprises various vegetation types such as evergreen, semievergreen, riparian, dry deciduous scrub and southern thorn scrub forests. The total area of Sitheri Hills consists of 59 hamlets, and all the hamlets are under the control of one panchayat president. It has one primary health centre which is located in Sitheri village and four health subcenters, and they are situated in Suriyakadai, Nochikuttai, Ammapalayam and Kalasapadu. According to the health department records, the total population is 9045: male 4656 and female 4389 with 1908 houses.

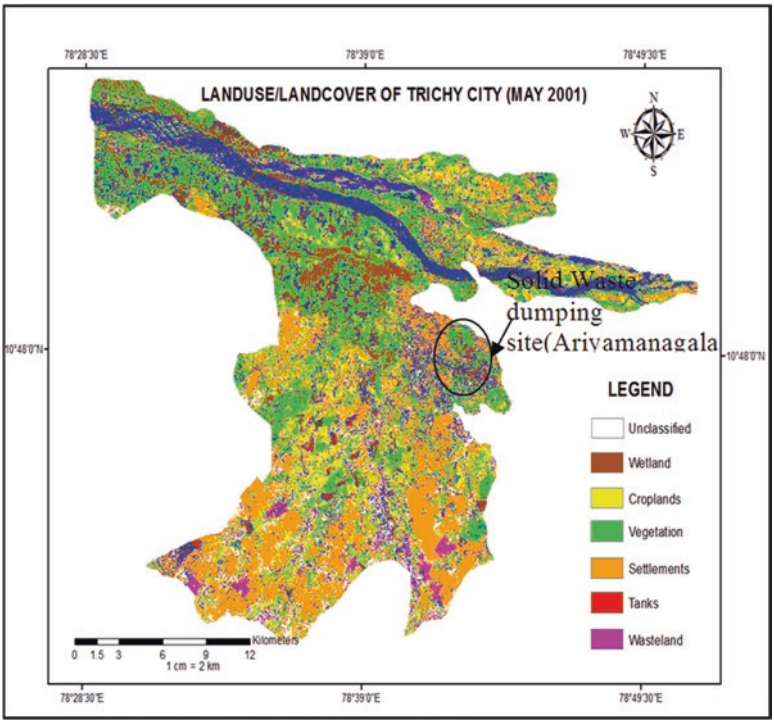


Fig. 20.3 Land use/land cover map

20.4.2.2 Spatial Distribution Analysis

Mosquito vector species' abundance and surveillance in the study area were mapped by inverse distance weighted (IDW) and Kriging interpolation technique. The analysis was done for the data of indoor resting and dusk collection methods. For this analysis, nonspatial entomological survey data were used, and a number of mosquitoes sampled were identified. The result obtained from this study was used for the spatial distribution analysis by converting nonspatial data of mosquito abundance into spatial form. The maps are presented in Figs. 20.6, 20.7 and 20.8.

20.4.2.3 Observation

The spatial and temporal distributions of mosquitoes are varied based on the climatic factors and man-made activities. The highest number of mosquito species is observed in settlement, agricultural plantation and forest area due to the presence of more number of mosquito breeding sites. The potential mosquito-abundance map and spatial data base are essential for vector control and also useful for monitoring of lymphatic filariasis in remote areas.

Moreover, spatial analysis, entomological and people perception survey results show the people were affected by lymphatic filariasis, but no official records were found. Ammapalayam, Perperi, Pudur, Selur and Thekkalpatti are sensitive villages

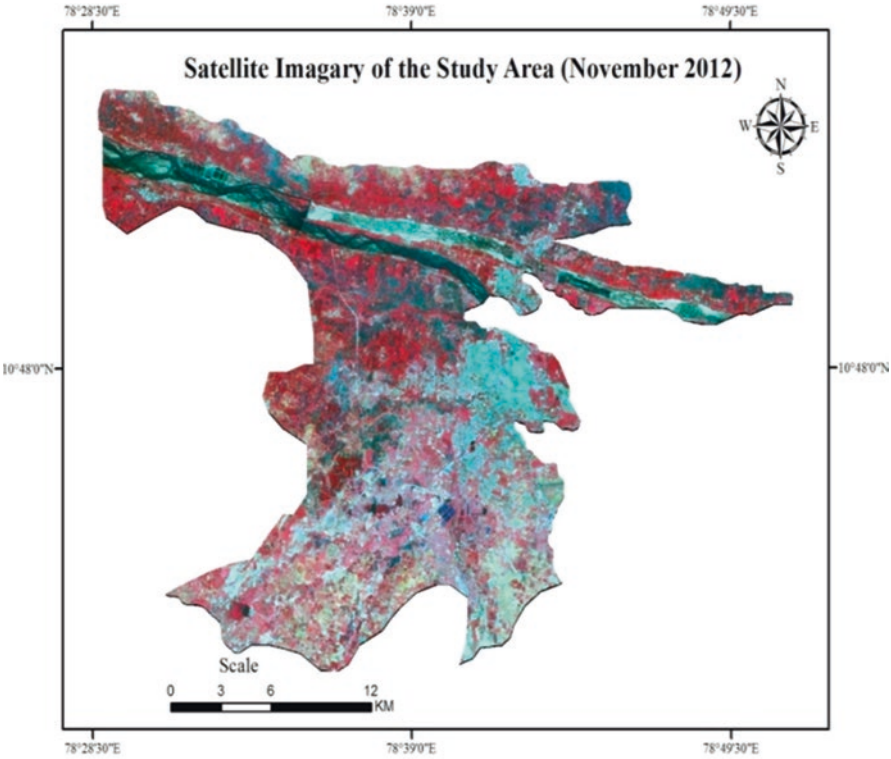


Fig. 20.4 Satellite imagery during November 2012

for LF vector abundance. The study area had less number of health centres with inadequate treatment facilities keeping in view the LF risk in the area. The remote sensing plays a key role in ascertaining the vector distribution and identification as well as dissemination of a vector-borne disease like lymphatic filariasis in a cost-effective manner. So this study recommends that prevention mechanisms for such vector-borne diseases need be established with better health facilities, developmental programmes to improve socioeconomic status, and awareness about vector-borne diseases should be implemented to make the people become aware of the national initiative.

20.5 Conclusion

Our investigations reveal that remote sensing and GIS play a major role in establishing vector density, mapping and monitoring of lymphatic filariasis transmitting vectors in a cost-effective way. Also it provides an accurate and scientifically valuable data/solution for environment and health planners. Development of health information system using GIS is an excellent data integrated document for the purpose of disease emergence period. Further, it is an excellent interactive analytical tool that

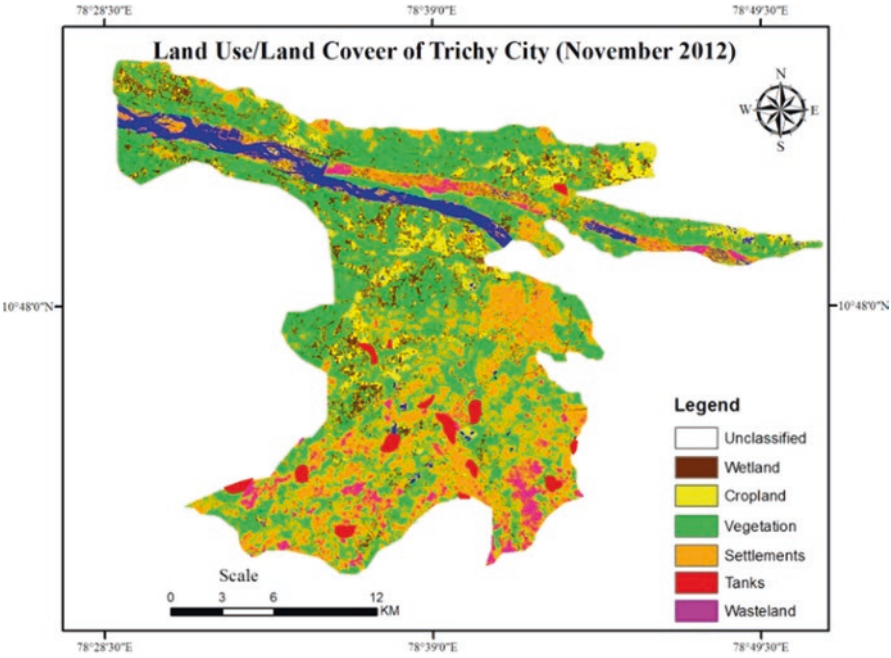


Fig. 20.5 Land use/land cover map

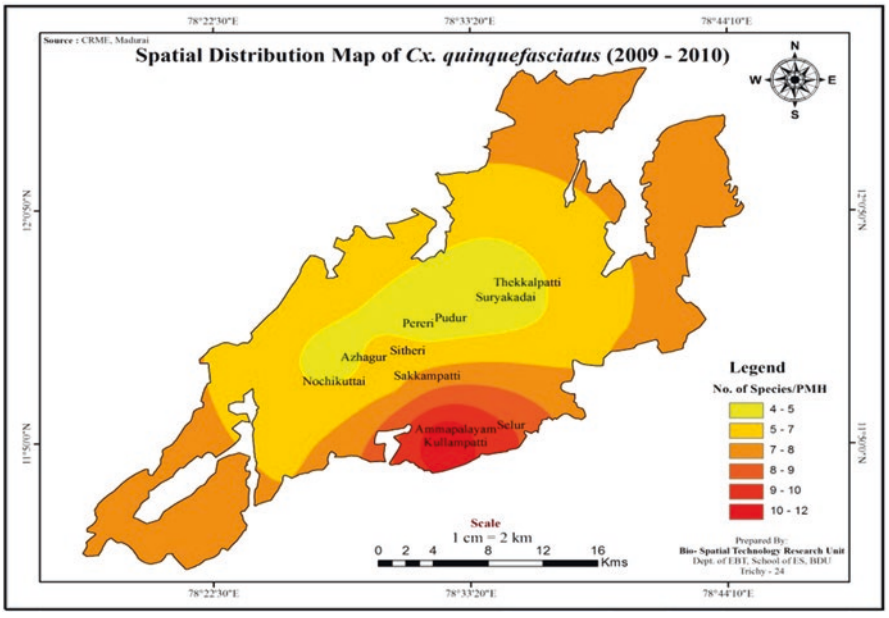


Fig. 20.6 Spatial distribution of LF vectors (2009–2010)

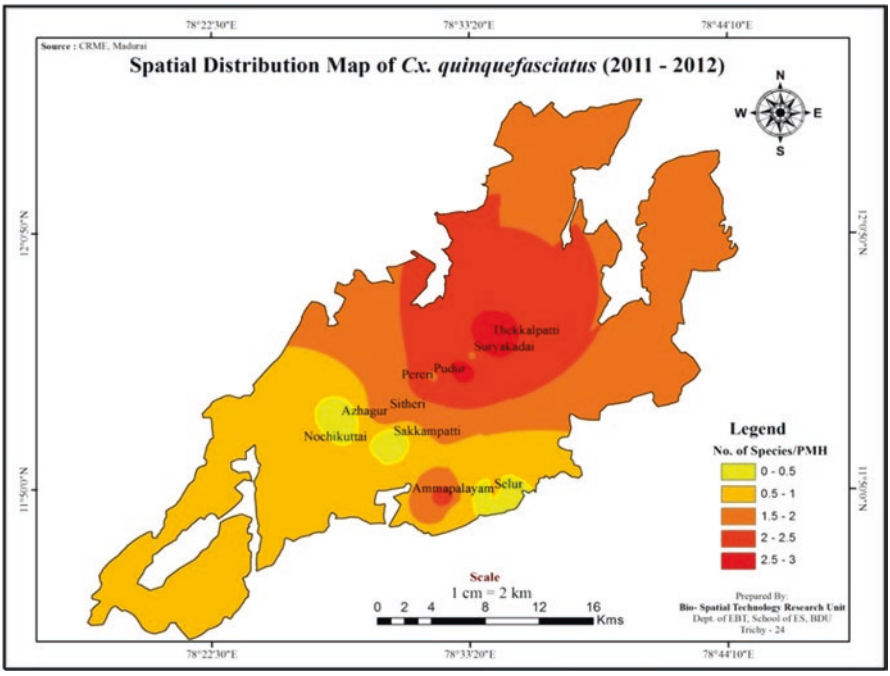


Fig. 20.7 Spatial distribution of LF vectors (2011–2012)

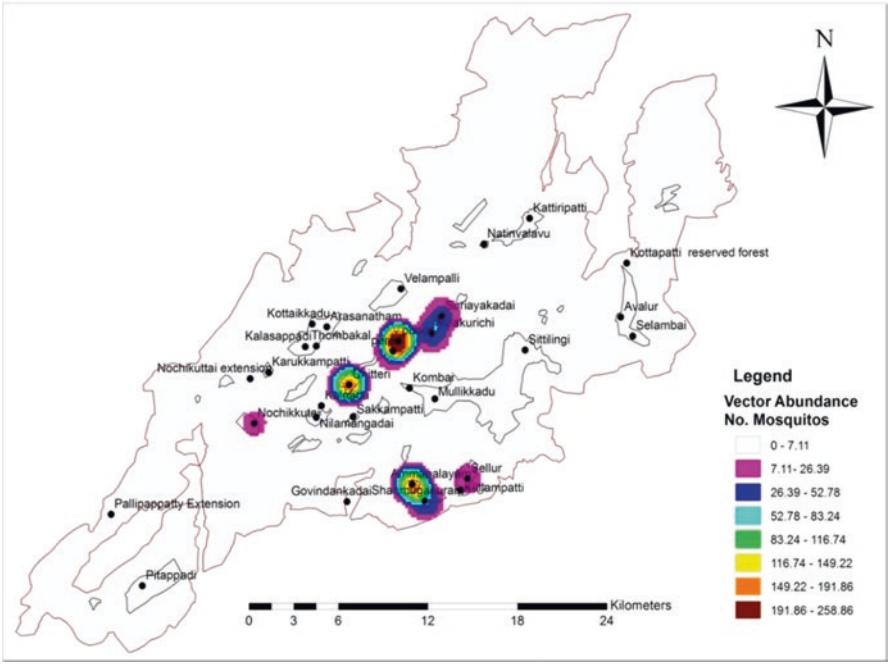


Fig. 20.8 Prevalence of LF vectors in Sitheri Hills

enables the decision-makers to solve public health problems on time-bound basis. The distribution map for vectors and associated filariasis cases in the study areas can be made available to health planners for vector control, with more information on their potential to transmit lymphatic filariasis as well as the choices for the control strategies in the near future.

Acknowledgement The authors gratefully acknowledge the help rendered by the Tribal Health Division, Sitheri Hills, Tamil Nadu. They also express their gratitude to the Indian Council of Medical Research, New Delhi, for providing financial support to carry out this important piece of research.

References

Abdel-Hamid YM, Soliman MI, Allam KM (2009) Spatial distribution and abundance of culicine mosquitoes in relation to the risk of filariasis transmission in El Sharqiya Governorate, Egypt. Egypt Acad J Biol Sci 1:39–48

Ahmad R, Ali WNWM, Nor ZM, Ismail Z, Hadi AA, Ibrahim MN, Lim LH (2011) Mapping of mosquito breeding sites in malaria endemic areas in Pos Lenjang, Kuala Lipis. Malaria J 10(1):361. https://doi.org/10.1186/1475-2875-10-361

Becker N (1996) Microbial control of mosquitoes in urban areas. In: Wildey KB (ed) Proceedings of the second international conference on urban pests, pp 91–96

Becker N, Petric D, Zgomba M, Boase C, Madon M, Dahl C, Kaiser A (2010) Mosquitoes and their control. Springer, London, p 577

Ceccato P, Connor S, Jeanne I, Thomson MC (2005) Application of geographic information systems and remote sensing technologies for assessing and monitoring malaria risk. Parasitologia 47(1):81–96

Hassan AN, Onsi HM (2004) Remote sensing as a tool for mapping mosquito breading habitats and associated health risk to assist control efforts and development plans: a case study in Wadi El Natroun, Egypt. J Egypt Soc Parasitol 34(2):367–382

Michael E, Bundy DA, Grenfell BT (1996) Re-assessing the global prevalence and distribution of lymphatic filariasis. Parasitology 112(Pt 4):409–428

Molyneux DH, Taylor MJ (2001) Current status and future prospects of the Global Lymphatic Filariasis Programme. Curr Opin Infect Dis 14:155–159

Mwangangi JM, Midega J, Githure J, Mbogo CM, Beier JC (2012) Mosquito species abundance and diversity in Malindi, Kenya and their potential implication in pathogen transmission. Parasitol Res 110:61–71. https://doi.org/10.1007/s00436-011-2449-6

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

Palaniyandi M (2012) The role of remote sensing and GIS for spatial prediction of vector-borne diseases transmission : a systematic review. J Vector Borne Dis 49(4):197–204

Phumee A, Preativatanyou K, Kraivichain K, Thavara U, Tawatsin A (2011) Morphology and protein profiles of salivary glands of filarial vector mosquito Mansonia uniformis; possible relation to blood feeding process. Asian Biomed 5(3):353–360. https://doi.org/10.5372/1905-7415.0502.046

Ravindran B (2003) Aping Jane Goodall. Insights into human lymphatic filariasis. Trends Parasitol 19:105–109

Reiter ME, Lapoint DA (2007) Landscape factors influencing the spatial distribution and abundance of mosquito vector Culex quinquefasciatus (Diptera: Culicidae) in a mixed residential-agricultural community in Hawaii. J Med Entomol 44(5):861–868

Rejmánková E, Grieco J, Achee N, Roberts DR (2013) Chapter 13: ecology of larval habitats. https://doi.org/10.5772/55229. http://www.intechopen.com/books/anopheles-mosquitoes-new-insights-into-malaria-vectors/ecology-of-larval-habitats

Scenario of Lymphatic Filariasis (LF) in Bangladesh: A Scientific Approach

21

Mohammad Nazrul Islam and Moazzem Hossain

Abstract

Lymphatic filariasis is highly endemic in the northern part of Bangladesh. Long-term disability, social embarrassment, and preterm deaths are ultimate outcome of the neglected tropical disease. The Bangladesh government has taken different positive and constructive initiatives to reduce the DALYs, chronic devastating consequence, and mortality rate of the disease. It has been observed that budgetary constraints, lack of skilled persons or health volunteers, proper interventions, knowledge regarding vector control, public health, the inadequate infrastructural development, high level social stigma, and unexpected poor socioeconomic condition in the highly endemic districts are interrupting to eliminate LF in Bangladesh. Periodic mass drug administration and NTD strategies are facing multidimensional obstacles. So, strategic changes and improving the IEC system to the highly endemic districts are needed to achieve the desired goal of LF elimination in Bangladesh.

M. N. Islam (✉)
Hamdard University Bangladesh, Gazaria, Bangladesh

Filaria Hospital & CDC, Nilphamari, Bangladesh

M. Hossain
Filaria & General Hospital, Savar, Bangladesh

IACIB, Dhaka, Bangladesh

Disease Control, Government People's Republic of Bangladesh, Dhaka, Bangladesh

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_21

21.1 Introduction

Bangladesh is a prompt developing country in the Southeast Asian Region. After acheived independence and sovereignty in 1971, it has been initiated several steps to ameliorate the socioeconomic conditions and upgrade the living standards of the peoples. Geographical conducive environment, poor socioeconomic condition, low literacy rate, climatic variations, river, rivulets, and plenty of rainfalls are prominent predisposing factors to increase the prevalence of the neglected tropical diseases (NTDs) in Bangladesh. Lymphatic filariasis (LF) is a disfiguring and stigmatizing neglected tropical disease (NTD) and inflicts severe social and economic burden on the affected communities. A total of 34 districts of Bangladesh with an estimated 70 million population are living in the endemic areas of LF. Out of 34 districts, 5 northern districts are highly endemic and 15 are low endemic. A total of 35 million people are living in the 15 low endemic districts. *Culex quinquefasciatus* is the only vector for bancroftian filariasis in Bangladesh. From 2001 to till now, around 8–11 rounds of mass drug administration (MDA) have been conducted in the highly endemic districts but until new case of LF has observed. By changing the strategic framework and developing effective information, education and communication system to the highly endemic region will be a prime approach to eliminate LF filariasis in Bangladesh.

21.2 Epidemiological Pattern of LF in Bangladesh

Entomological investigations showed that the "tropical house mosquito" *Culex quinquefasciatus* is the only vector for bancroftian filariasis in Bangladesh. A study conducted in Ache and Madarganj village of Dinajpur district found that out of 35 mosquito species, 11, 10, 8, and 3 were *Culex*, *Anopheles*, *Aedes*, and *Mansonia*, respectively. Filarial infection was found in *Culex quinquefasciatus* and *Culex vishnui* complex mosquitoes. *Mansonia* species are sporadic potential vectors in the various parts of Bangladesh especially in Chittagong division (Sasa 1976). Night blood survey in the northern part of Bangladesh revealed that the prevalence of microfilaremia is up to 16.4% (Aslam Khan and Wolfe 1972). *Wuchereria* bancrofti and *Brugia malayi* parasites are both nocturnally periodic. A multi-district study indicated that LF is prevalent in many parts of the country including some localities of Dhaka (Ahmed et al. 1986). About one-tenth of the sampled population showed the chronic manifestations of the disease. The study found that higher prevalence of LF in male (>2times) than females. Hydrocele was a very predominant clinical manifestation for male patient. The infection and infectivity rates of the former species were 10.5% and 1.1% (n = 3545) (Aslam Khan and Wolfe 1972). In Mirpur area of Dhaka, 43 (1.2%) were found infected with any stage larvae and 7 (0.2%) with infective stage larvae (Ahmed et al. 1986). These studies demonstrated that active transmission of LF in the communities. One study conducted in the 19 unions of Nilphamari, Kishorgonj, and Sayedpur showed that 1.34% people were infected

with LF. Age-specific prevalence showed that for the ages between 26 and 45, the highest rate of infection was 53.07%, and for the age group 3–15 years, the lowest rate of infection was 0.92% (Saha and Mohanta 2011).

21.3 Endemicity of LF in Bangladesh

Endemic districts of LF in Bangladesh are operationally divided into three categories:

(a) *Non-endemic districts:* There are 64 districts in Bangladesh. Out of the 64 districts, 30 districts were declared non-endemic, 13 based on historical evidence (no or low number of clinical cases) and 17 on the basis of antigen prevalence less than 1.0%.
(b) *Very low endemic districts:* Fifteen districts showed >1.0% antigen prevalence.
(c) *Endemic districts*: Nineteen districts were declared endemic, based on historical and/or empirical evidence, i.E., the presence of considerable number of people affected with clinical disease and/or high prevalence of microfilaremia.

The endemic districts are situated in the four divisions of Bangladesh such as:

1. *Barisal division* (*five districts*): Barguna, Barisal, Jhalokati, Patuakhali, and Pirojpur.
2. *Khulna division* (*three districts*): Chuadanga, Kushtia, and Meherpur.
3. *Rajshahi division* (*four districts*): Chapai Nawabganj, Pabna, Rajshahi, and Sirajganj.
4. *Rangpur division* (*seven districts*): Dinajpur, Panchagarh, Thakurgaon, Nilphamari, Rangpur, Lalmonirhat, and Kurigram.

21.4 Socioeconomic Conditions and the Prevalence of LF in Bangladesh

Socioeconomic conditions are strongly associated with prevalence of LF in Bangladesh. A hospital-based randomized study showed that 69 (n = 1359) percent of the subjects belonged to lower socioeconomic class, 21 (n = 413) percent belonged to middle class, 7 (n = 139) percent were enrolled under upper middle class, and 3 (n = 59) percent were upper class by following the Kuppuswamy modified socioeconomic class scale 2011. Another study conducted in 19 unions of Nilphamari, Kishorgonj, and Sayedpur revealed that the highest prevalence of LF was found in Alam Biditor (1.35%) under Kishoreganj Thana and the lowest prevalence in the Sayedpur (0.21%). Among the affected population, 69.48% were poor, 28.88% were middle class, and 1.63% were rich (Saha and Mohanta 2011).

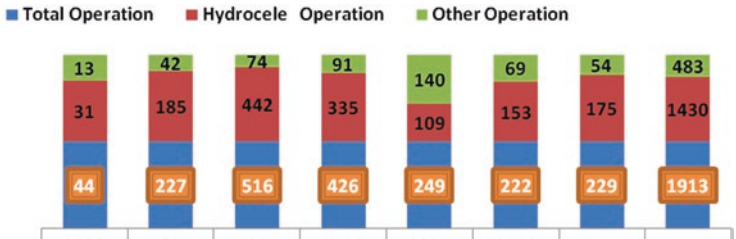
21.5 Contribution of Institute of Allergy and Clinical Immunology of Bangladesh (IACIB) for Elimination of LF in Bangladesh

The Institute of Allergy and Clinical Immunology of Bangladesh (IACIB) was formed in 1995. It is a nongovernment, nonpolitical, and nonprofit-making organization. IACIB is the only organization in Bangladesh which has been working for elimination of filariasis in collaboration with the Ministry of Health and Family Welfare (MoHFW), Government of Bangladesh (GoB). Filaria Hospital & C.D.C. (30 beds), Saidpur, Nilphamari, Bangladesh, was one of the successful projects of IACIB which was established in 2002 with the grant from the Government of Japan and functioning since January 1, 2003. It was the only filaria hospital in Bangladesh. In 2012, IACIB established another hospital, Filaria and General Hospital, Savar (50 beds), for proper management of LF in Bangladesh. More than 3000 hydrocele operations were conducted by IACIB in filaria hospitals, Saidpur and Savar, from inception till now for reducing the disease-related disability, and also played a pivotal role for rehabilitation. Total numbers of hydrocele operation were done in FH & C.D.C., Saidpur from January 2003 to July 2009 in Table 21.1.

21.6 Evaluation of Elimination Program in Bangladesh

The MoHFW, Bangladesh, launched the MDA-based LF elimination program in 2001. It coincided with the launching of the global program to eliminate LF in 2000. The government followed the technical guidelines provided by WHO and framed a policy to eliminate LF by the year 2015. The advent of single-dose treatment, community-wide mass drug administration became the most feasible and affordable option, and the major antifilarial drug (diethylcarbamazine) was also administered

Table 21.1 Total numbers of hyrdrocele operation done in FH & CDC, Sayedpur between January 2003 and July 2009



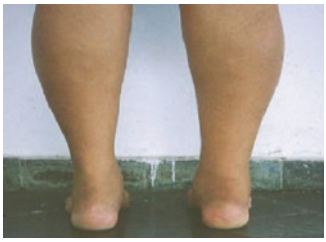
	2003 (January - December)	2004 (January - December)	2005 (January - December)	2006 (January - December)	2007 (January - December)	2008 (January- December)	2009 (January- July)	Total
Other Operation	13	42	74	91	140	69	54	483
Hydrocele Operation	31	185	442	335	109	153	175	1430
Total Operation	44	227	516	426	249	222	229	1913

through fortification of salt, a challenge strategy intern of logistics (Adinarayanan et al. 2007). The drugs used for mass drug administration (MDA) were DEC 6 mg/kg body weight plus albendazole 400 mg in the areas with LF. It was one of the first countries to start MDA in 2001 and continued 8–11 rounds. By the end of 2010, all districts received at least 3 MDAs and 12 received >6 MDAs. The program is unique in that there was no "MDA holiday" in any district, once it started the activity. In Panchagarh, MDA was started in 2001, received ten MDAs by 2010. The transmission assessment survey (TAS) and Mf survey were conducted to evaluate the impact of the MDA program. TAS was conducted in the five LF endemic districts among the 1500–1700 schoolchildren of grades 1 and 2 by immunochromatographic test on the basis of population size. Cutoff value of ICT test was set at 18–20. Result of ICT test among the survey groups was found below the cutoff point in all five districts which was indicating the interruption of transmission (L. Hafiz 2012).

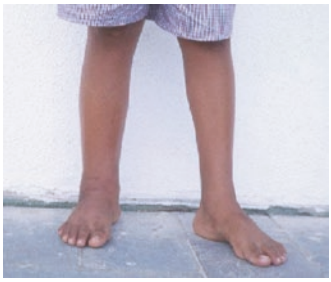
21.7 Stage Specifications and Diagnostic Procedure Adopted to Confirm the Case of LF in Bangladesh

Clinical case of LF has been diagnosed by clinical examinations and stage-specific clinical manifestations. Stage specification is assumed by the following criteria:

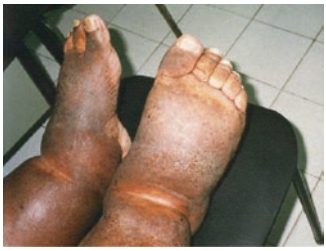
1. **Stage 1: Lymphedema of the Leg**

Swelling reverses at night Skin folds—absent Appearance of the skin—smooth, normal	

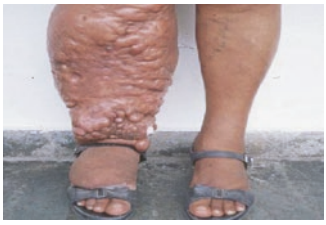
2. **Stage 2: Lymphedema of the Leg**

Swelling not reversible at night Skin folds—absent Appearance of the skin—smooth and normal	

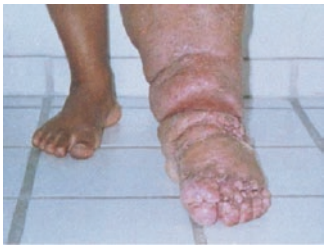
3. **Stage 3: Lymphedema of the Leg**

Swelling not reversible at night Skin folds shallow Appearance of the skin—smooth, normal	

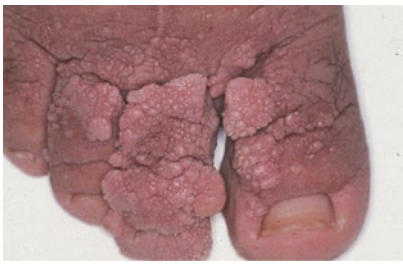
4. **Stage 4: Lymphedema of the Leg**

Swelling not reversible at night Skin folds—shallow Appearance of the skin—irregular, knobs, nodules	

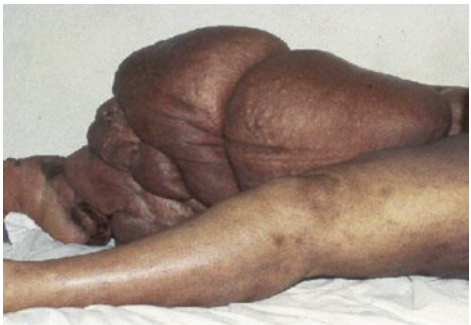
5. **Stage 5: Lymphedema of the Leg**

Swelling not reversible at night Skin folds—Deep Appearance of the skin—Smooth or irregular	

6. **Stage 6: Lymphedema of the Leg**

Swelling not reversible at night Skin folds—Absent, shallow, deep Appearance of the skin—Wartlike lesions on the foot or top of the toes	

7. **Stage 7: Lymphedema of the Leg**

Swelling not reversible at night Skin folds—Deep Appearance of the skin—Irregular Needs help with daily activities, e.G., walking, bathing, using bathroom Dependent on family or health-care system	

Examination of nocturnally collected blood has been a widely used method for diagnosis of infection/microfilaremia in endemic areas of Bangladesh. Mf used to see in thick and thin blood film with G-stain on the basis of specific periodicity. Antigen detection by using ICT card test has been also used for diagnosis, mapping and monitoring, and evaluation of LF elimination programs. Polymerase chain reaction (PCR) and filarial antigenic assay are also used to detect circulating filarial antigen in LF patient. The DEC provocation test was performed to obtain satisfying number of parasite in daytime samples. X-ray, USG, CT, MRI, lymphoscintigraphy, allergy test, and ELISA for Mf are advised to the patient who can afford the test expenses by expert clinician (if needed). It may be mentioned that the routine diagnostic tests such as ICT and MF are all negative, when morbidity develops – swelling of different parts.

21.8 Treatment Strategies Are Followed to Treat LF in Bangladesh

It is proved that if the patient visits the health center/hospital at stage 3, the disease can be cured by treatment, and if the patients come to the HC/hospital at stages 3–7, the target of the treatment is enrolled in stage limitation. Treatment guideline followed for LF in Bangladesh is as follows:

Age	Tablet DEC (100 mg)	Tablet albendazole (400 mg)
>2–8 years	1	1
>8–12 years	2	1
>12 years	3	1

In addition, symptomatic treatment is given to alleviate the suffering of the patient. Morbidity control by modern specific treatment is done by the following:

1. Extensive washing with soap and water.
2. Elevation of affected organ.
3. Twisting tourniquet therapy.
4. Sequential circulatory therapy and bandage.

21.9 Prospective Strategies to Combat LF in Bangladesh

1. Ensure periodic extensive MDA in the highly endemic areas.
2. Strengthening the infrastructure to combat the disease.
3. Application of tourniquet therapy in large scale to the high endemic districts.
4. Improve information, education, and communication system (mass awareness-raising activities for social mobilization and reducing social stigma, involving print and electronic media, community participations, and cultural activities).
5. Proper vector control by chemical and biological methods (guppy, gambusia, and tilapia fish).
6. Distribution and application of insecticide-treated mosquito bed nets can reduce the transmission of lymphatic filariasis.
7. Maintain personal hygiene (cleaning and dressing with antiseptic and antifungal drugs).
8. Financial compensation, involving income-generating activities, and related rehabilitation (tertiary prevention) are mandatory to reduce the consequences.
9. Ensure active community participation and conduct community interventional study.
10. Comprehensive approaches are needed to adopt and involve private NGOs and funding agencies and government officials and ensure the essential logistics support for reducing the disability-adjusted life years (DALYs) of lymphatic filariasis in Bangladesh.

Acknowledgment We would like to express our gratitude to employees of IACIB, government officials, and local representatives for their enormous support.

References

Adinarayanan S, Critchley J, Das PK, Gelband H (2007) Diethylcarbamazine (DEC) medicated salt for community based control of filariasis. Cochrane Database Syst Rev 24(1):CD003758

Ahmed T et al (1986) Filariasis in Mirpur area of Dhaka city. Bangladesh Med Res Council Bull 12:83–94

Aslam Khan M, Wolfe MS (1972) Bancroftian filariasis in two villages in two villages in Dinajpur district, East Pakistan. Entomological investigations. Am J Trop Med Hyg 21:30–37

Hafiz L (2012) Elimination of LF -Bangladesh experience. Int Soc Infect Dis 16:18–19

Saha AK, Mohanta MK (2011) Bancroftian elephantiasis in Nilphamari, Bangladesh. Mymensingh Med J 20(1):40–44

Sasa M (1976) Human Filariasis. University of Tokyo Press, Tokyo, pp 500–501

22

Biological Control of *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae), the Ubiquitous Vector for Lymphatic Filariasis: A Review

B. Reddya Naik

Abstract

Culex quinquefasciatus Say is the most annoying and important vector of human lymphatic filariasis. It is one of the most widespread mosquitoes in the world that prefers to breed generally in polluted water such as sewage drains, septic tanks, cesspits, cesspools, etc. Infection to man occurs when an infected *Cx. quinquefasciatus* bites, and in the process transmits filarial parasites. Notwithstanding the fact that lymphatic filariasis is controllable in humans through mass drug administration (MDA) of diethylcarbamazine citrate (DEC), alone or in combination with albendazole (Alb), vector control is considered to play a pivotal role in breaking down the host-parasite link to arrest the spread of lymphatic filariasis. The major stumbling block in this process is however that *Cx. quinquefasciatus* has in recent times developed multiple and cross-resistance to various insecticides including organochlorines, organophosphates, carbamates, and even pyrethroids. Thus, biological control method has emerged the best alternative to these synthetic insecticides and offers great promise in abating the vector population below recognized threshold levels.

22.1 Introduction

The ecological relationship between humans and mosquitoes is there since ages, and because of some species of mosquitoes acting as pest or vector, man considers mosquitoes as their deadliest foe (Spielman and D'Antonio 2002). The war waged by man against these enviable creatures more than a century ago is still continued, and the mosquito has so far proved, much to the chagrin and frustration of human being, to be

B. Reddya Naik (✉)
Department of Zoology, University College of Science, Osmania University, Hyderabad, Telangana, India

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_22

an invincible enemy (Tyagi 2004). Inadequate management of land and water sources and failure to solve the problems of liquid waste management give rise to larger and more productive habitats for mosquitoes which continue to grow and spread several dreadful diseases. Public health is more than the mere absence of disease or pests since it includes the right to have an environment free of mental and physical discomforts that destroy its positive values. Therefore, ideally the primary objective must be to curb the mosquito menace and to preserve or create an environment for existence of prey-predator relationship so as to balance the natural ecosystem.

Culex quinquefasciatus Say, 1823, commonly known as the southern house mosquito, is a major vector of lymphatic filariasis and one of the most widespread annoying mosquitoes in the world. Lymphatic filariasis is a major mosquito-borne disease in Asia caused by three nematode parasites, viz., *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori.* Apart from *Culex* there are other mosquito species of genus *Anopheles*, *Aedes*, and *Mansonia* which also act as vectors. Mostly the infections occur when microfilarial parasites are transmitted to humans through the bite of female *Culex quinquefasciatus* mosquito. It breeds generally in sewage water but can have a large choice to breed in different types of polluted water collections. It is a domesticated species which is often found living in close proximity to humans and does not usually disperse greater than 1 km from a releasing or hatching point. It remains close to breeding habitat and host resources (Reisen et al. 1990). Inadequate sanitation, improper drainage system, crowding, and lack of affordability of personal protection measures contribute to propagation of higher population density/biting of *Culex quinquefasciatus* and thereby facilitate transmission of the diseases and annoyance.

There are many zoonoses, such as avian malaria, St. Louis encephalitis, Western equine encephalitis, and West Nile fever involving humans and both wild and domestic animals, where *Cx. quinquefasciatus* is involved as a vector. Yet, the most significant and debilitating vector-borne infection, bancroftian filariasis caused by *Wuchereria bancrofti*, is attributable to *Cx. quinquefasciatus.* Infection to humans occurs when an infected mosquito bites and, in the process, passes on the parasites (L_4). The parasite may then undergo a long peregrination for approximately 6 months' period in various systems, finally reaching its destination of lymphatic system and developing into male and female adults. The adults mate here in the lymph ducts and nodes and produce thousands of microfilariae *(mf)* which make their way to the peripheral blood during night hours. Adults may live for many more months, and death is calcified generally at places of joints and becomes the reason for excruciating pain on movement or disabled limb due to immobility. At a time when *mf* is circulating in the peripheral blood, the mosquito, *Cx. quinquefasciatus*, a nocturnal creature, may hence bite and pick up the microfilariae during feeding on human blood. In mosquito body it metamorphoses into a saucer-shaped first-instar larva (L_1), followed by more elongated second-instar larva (L_2), and finally to the third-instar larva (L_3) in approximately 8–10 days. The parasite is at this stage ready to get transmitted to an uninfected man with the infected bite of the mosquito.

Culex quinquefasciatus breeds in virtually all kinds of stagnant and polluted waters such as the drains, septic tanks, pit latrines, cesspits, wells, coir pits, marshy

swamps, and empty containers that get filled with rainwater, as well as tree holes, which are known to support the aquatic stages of life cycle (Das and Shenoy 2008). Having known the complex life cycle of mosquito, no single control measure can cope up to reduce the mosquito density below tolerable thresholds. Chemical control of mosquitoes should only be used as a fire fighting measure to curb the mosquito-borne disease epidemics and not as a regular phenomenon, because most chemical insecticides are prone to develop the insect resistance and thereby cause environmental pollution. According to the local conditions, initiation of the action level is independently determined by each mosquito control program (Anonymous 2008). Generally mosquito control program can be categorized as ovicidal/larvicidal/pupicidal (against aquatic stages) and/or adulticidal (against terrestrial or aerial stage). However, using adulticides for the control of mosquitoes is not a prudent strategy as these adult stages occur alongside human habitation and they can easily escape from control measures (Service 1992). On the other hand, most of the insecticides available commercially are synthetic chemical products apart from their high-cost and persistent applications which have unintended implications including the production of resistant strains of mosquitoes, ecological imbalance, and elimination of nontarget organisms in the environment (Anyaele and Amusan 2003). Moreover, *Cx. quinquefasciatus* has developed resistance to organochlorine, pyrethroids, and carbamates. The resistance is very strong with DDT, permethrin, and deltamethrin (Agnes Yadouléton et al. 2015). Thus, biological control method is the best alternative to synthetic insecticides especially as larvicides. In general terms, "biological control" is the use of a specifically chosen living organisms to suppress the vector population below the tolerable thresholds either by pathogens, parasites, predators, or plant extracts that will attack the target mosquito during the "aquatic development stage."

22.2 Biological Control

In view of the fact that chemical insecticides were largely proving ineffective against *Cx. quinquefasciatus*, on one hand, their persistent use affected adversely the environment, on the other. At the same time, biological means of vector control when considered from the ecological viewpoint seemed to offer great promise. DeBach (1964) defined biological control as a phase of natural control can be defined as the action of parasites, pathogens, or predators in maintaining another organism's population density at a lower average than would occur in their absence. The twentieth century is considered as a modest beginning to implement biological control of medical pests and vectors (Lamborn 1890). However, the momentum of biological control had gained importance only after the Rachel Carlson's "Silent Spring" in 1962. Historically, the growth of discipline was much slow and painful, and only after the advent of resistance among an increasing number of insects to modern insecticides in the late 1950s, the importance of biological control as an alternative strategy was seriously understood. Legner et al. (1974) reviewed literature on biological control listing for the first time and the known natural enemies of medically

important arthropods which generated great interest in these predators of mosquitoes. When site modification or elimination is not possible, Biological control of mosquito larvae is a preferable method in many permanent mosquito breeding sites (WHO 2016). Therefore, Biological control of mosquitoes necessitates a positive consideration of mosquito breeding habitats which included sewage drains, septic tanks, cesspits, cesspools, and so forth, so as to get rid from the mosquito menace. In consideration of environmental pollution, biological control does not cause any pollution to the environment and it is just a natural way to manipulate nature to increase a desired effect of human living conditions (Hoffmann and Frodsham 1993).

Biological control of vector mosquitoes can be achieved successfully by using predators, pathogens, plant extracts, and natural mechanisms wherein the role of human involvement is desirous. Therefore, having a comprehensive knowledge of the ecology of predator-prey and pathogen-host relationships will be much useful in designing the mosquito intervention program (Service 1983). Every imaginable water container would serve a potential mosquito breeding habitat; thus they exploit a wide breadth of different aquatic habitats for their propagation. Accordingly, a biological control agent may confine to a much thinner range of environmental activity than that of the target species. In such circumstances a number of different biological control agents and/or appropriate methods will be required for successful control of even a single species of mosquito across its range of exploitable breeding sources. The most popular agents used to suppress the *Culex quinquefasciatus* populations include bacteria (*Bacillus thuringiensis* var. *israelensis*), fungi (*Lagenidium* and *Culiciomyces* spp.), mermithid nematode (*Romanomermis culicivorax*), larvivorous fish (*Poecilia* spp.), predatory aquatic insects like odonata (*Crocothemis servilia*, *Bradinopyga geminata*), plant extracts (phytochemicals), and silver nanoparticles (AgNPs).

22.2.1 Bacteria

Many bacterial groups are reported to have antimosquito activity. Of all these bacteria, *Bacillus thuringiensis* var. *israelensis* and *B. sphaericus* have become globally available. *Bacillus thuringiensis* var. *israelensis* is the most thoroughly explored bacillus among all mosquitocidal bacteria. It is in this bacterium that isolation, nomenclature, mosquito target range, as well as the genetics and biochemistry of the toxins have been most extensively dealt with. The discovery of the spore-forming bacterial pathogen, *Bacillus thuringiensis* var. *israelensis*, which was isolated from the waning larvae of mosquito collected from the groundwater puddles and the toxin produced subsequently found to be an effective and prospective microbial insecticide against *Culex quinquefasciatus* mosquitoes, has a stimulating story of subtle observation to tell behind this historic quest for Ariadne's thread to exit from the labyrinth of mosquito larval control, as narrated in person by Margalit to a group of scientists including Dr. Norbert Becker from Germany and Dr. B.K. Tyagi from

India, during a field expedition in the outskirt of Prague, Czech Republic, in 1995 (Goldberg and Margalit 1977; Jayapriya and Gricilda Shoba 2014; Tyagi, *pers. comm.*). During a routine entomological expedition, Margalit spotted floating upside-down dead mosquito larvae in a small stagnated water pond in a riverbed in Israel in 1976. The subtlety of observation worked wonders for him since he not only successfully isolated the bacterium from the samples but later also extracted the toxin specific to mosquito, *Cx. quinquefasciatus*. The Bti affects the larvae of the mosquito in a very quaint manner; it releases crystals which are toxic when mosquito larvae ingest them. These crystals' toxins are activated by the surrounding mélange of an alkaline environment and the enzymes in the midgut, where it ruptures cells in the larvae's gut and eventually brings about killing of the target larvae within 4–24 h. The toxicity action targets the midgut epithelium where it binds to microvilli in the midgut, causing cellular hypertrophy and lysis causing inhibition of metabolism leading to the death of the affected larval population (Bravo et al. 2013). On the contrary, the bacterium *Bacillus sphaericus* Neide, discovered with larvicidal activity against mosquito species (Kellen et al. 1965; Mulla et al. 1984), is active primarily against *Culex* mosquitoes compared to those of *Anopheles* and *Aedes* (DesRochers and Garcia 1984, Baumann et al. 1991, Federici 1995). Moreover, its toxin is having a binary moiety which is proteolytically activated in the mosquito midgut to release peptides. In case of both the above referred bacteria, the protein endotoxins are produced during sporulation and assembled into paraspo-ral bodies. On release in the midgut, they bring about fatal cellular alterations (Davidson 1984; Charles 1987).

Since adult biology and ecology of most vector mosquitoes is extensively explored with clear understanding about their resting sites, zoophilic and/or anthropophilic behavior and biting rhythms, mostly emphasis, have been laid on developing adulticides with higher residual effect and low toxicity to mammals and other nontarget organism. The development of a larvicide for use in public health programs demands selectivity due certain behavioral traits of the vector, e.g., breeding in hideous and/or concealed areas where adulticides could not have arrived in any form. To improve efficacy and better transportation and reduced costs all larvicides, innocuous to nontarget organisms (e.g., fish, frog, beneficial aquatic insects like dragonflies, mayflies, etc.) must be essentially dissolved in water (Bulla Jr 1975; Couch 2000). It should also ensure biological stability of the active ingredient and must have an adequate shelf life. There are two forms of administration:

(i) Liquid formulations (emulsion, aqueous suspension).
(ii) Solid formulations (wettable powder, powder, granules, water dispersible granules, and briquettes).

Several formulations of spore-forming larvicidal bacteria are presently available from different commercial firms and their efficacies under different environmental conditions and problems associated with its use have been intensively studied by Garcia (1987) and Lacey and Undeen (1986).

22.2.2 Fungi

There are many fungi that have proven excellent biotools for controlling serious vectors, such as *Lagenidium giganteum* and *Culiciomyces clavisporus* which have been extensively studied for the control of *Culex quinquefasciatus* mosquito larval stages to affect mosquito populations. There are, however, many other fungi that upon penetration form a fungal mycelium inside the larval hemocoel, resulting in the death of the mosquito larva (Becker and Rettich 1993). *Lagenidium giganteum*, first described by Couch (1935), is a facultative parasite of mosquito larvae (Rueda et al. 1990; McCray et al. 1973). *Lagenidium* is capable of recycling in standing bodies of water through asexual (zoospores) and sexual (oospores) alternate cycles in mosquito larvae. Its resistance to desiccation and long-term stability of sexually produced oospore offer a promising phase for commercial production (Thomas and Fisher 1999). Maximum virulence of *L. giganteum* against *Cx. quinquefasciatus* was noted at concentrations of >150 zoospores ml/1 of water, at water temperatures between 20 °C and 30 °C. No infection occurred at 17 °C and less than 20% larval mortality occurred at 19 °C for any age of zoospores (Federici 1981; Domnas et al. 1982; Jaronski and Axtell 1983; Service 1983; Sur et al. 2001). *Culicinomyces clavisporus* is another active fungus against a wide range of mosquito species (Knight 1980; Sweeney et al. 1973; Sweeney 1981; Couch et al. 1974). The life cycle of *C. clavisporus* is asexual, and usually it begins with the ingestion of conidia that adhere to and penetrate through the chitinous wall in the foregut or hindgut of the larva (Sweeney 1981; Federici 1981). Septate, branched hyphae of Mycelium at a concentration of 105 conidia ml-1 may kill within 2–7 days (Suh and Axtell 1999; Sweeney 1981, 1983; Debenham and Russell 1977; Goettel et al. 1984). The culture filtrates of *C. clavisporus* were pathogenic to *Cx. quinquefasciatus* (LC_{50} at 2. 5 μl/cm^2) after exposure for 24 h, with conidia remaining on the cadavers for more than 3 weeks and viability about 70–90% (Singh and Prakash 2011; Cooper and Sweeney 1986).

22.2.3 Larvivorous Fish

Of all the biotools, fishes are arguably the best known for controlling mosquitoes, and the most commonly used fish species for mosquito control are the guppy (*Poecilia reticulate*) in sewage water where *Cx. quinquefasciatus* breeds (Koldenkova et al. 1989; Garcia et al. 1991; Shim and Self 1973) and gambusia (*Gambusia affinis holbrooki*) deployed in controlling filariasis vector, *Cx. quinquefasciatus*, in water ponds and tanks in endemic South India (Bay et al. 1976; Menon and Rajagopalan 1978). The average feeding rate of *P. reticulata* on *Cx. quinquefasciatus* larvae was recorded as 102.93 ± 6.43 per day in laboratory and 29.6 ± 6.49 per day in sewage water (Rupp 1996; Reddya Naik and Umamaheshwara Reddy 2005). Mosquito larval feeding efficiency of larvivorous fish (female) *G. a. holbrooki* and *P. reticulata* in freshwater was recorded as 127 larvae per day.

22.2.4 Plant Extracts

Plants are rich source of bioactive organic chemicals to kill the insects and offer an advantage over synthetic insecticides as they are easily biodegradable. Insecticides derived from botanical sources are mainly secondary metabolites and include various alkaloids, terpenoids, phenolics, etc. (Hartzell and Wilcoxon 1941; Jacobson 1958; Mittal and Subbarao 2003; Rajkumar and Jebanesan 2005; Promsiri et al. 2006; Elimam et al. 2009; Chantraine et al. 1998; Cavalcanti et al. 2004; Amer and Mehlhorn 2006, Rahuman et al. 2009). Whole body of little herbs or various parts like fruits, leaves, stems, barks, roots, etc. of larger plants or trees are sources of phytochemicals some of which were found effective for mosquito control (Shaalan et al. 2005). Many plant families, e.g., Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Meliaceae, Oocystaceae, and Rutaceae, have members that have various types of larval, adulticidal, or repellent activities against *Cx. quinquefasciatus* mosquitoes. The pyrethrum (*Chrysanthemum cinerariifolium*) flowers are the root information for future pyrethrins and many synthetic (Hartzell and Wilcoxon 1941; Campbell et al. 1933). Nicotine is documented as one of the earliest plant extracts used against mosquito larvae of *Culex quinquefasciatus*. Later, anabasine, methylanabasine, and iupinine extracted from *Anabasis aphylla* and *Phellodendron amurense* were also added to this property (Haller 1940; Wilcoxon et al. 1940). The fern, *Aspidium filix-mas*, yielded a toxic constituent, filicin, a phloroglucinol propyl ketone, which proved toxic to *Cx. quinquefasciatus*). Tyagi et al. (1994, 1998) have found important alkaloids in *Tagetes minuta* and *Cymbopogon* species against vectors of malaria, dengue, and filariasis. Some phytochemicals interfere with growth and reproduction or act on the olfactory receptors, eliciting responses of attractancy or repellency (Sukumar et al. 1991).

22.2.5 Silver Nanoparticles (AgNPs)

Nanotechnology deals with design, synthesis, and manipulation of particle structure ranging from approximately 1 to 100 nm, such as silver nanoparticles (AgNPs), and has opened a novel fundamental and applied frontier, including the synthesis of nanoscale materials and exploration or utilization of their exotic physicochemical and optoelectronic properties (Colvin et al. 1994; Wang and Herron 1991; Schmid 1992; Hoffman et al. 1992; Mansur et al. 1995; Klaus-Joerger et al. 2001). Many plants like *Annona squamosa*, *Carica papaya*, *Chloroxylon swietenia*, *Mentha arvensis*, *Pongamia pinnata*, *Morinda tinctoria*, and *Vitex negundo* have been experimented with biosynthesize AgNPs (Shivshankar et al. 2003; Njagi et al. 2011). Bacteria, fungi, and yeast are known to reduce silver ions into AgNPs by both extra- and intracellularly (Absar et al. 2005; Thirunavukkarasu et al. 2011; Naik et al. 2014). Mishra et al. (2017) have used the permethrin in colloidal state for controlling *Cx. tritaeniorhynchus* nanotechnologically. Nanosized particles can

pass through biological membranes and penetrate even very small capillaries throughout the body (Daniel and Astruc 2004; Bogunia-Kubik and Sugisaka 2002; Zharov et al. 2005; Ji et al. 2007). Synthetic larvicides like malathion is effective at 16 ppm concentration, but plant extracted AgNPs are effective at less than 2 ppm (Naik et al. 2014; Chitra et al. 2015). *Caulerpa scalpelliformis* extract at 31.38 ppm, LC_{50} values, were found effective against *Cx. quinquefasciatus* (Murugan et al. 2015; Soni and Prakash 2014; Adesuji et al. 2016). Kumar et al. (2014) synthesized AgNPs against third-instar larvae of *Culex quinquefasciatus* which was recorded as LC_{50} at 1.44 ppm.

22.3 Conclusion

Lymphatic filariasis is a great social stigma and morbidity in Asian countries. Mosquitoes are not only dreadful disease vectors but also causes a terrible annoyance due to which man has to forego even a pleasure of patio. Mostly, the tropical countries are facing immense disease preponderance and annoyance of mosquitoes. Man could land on Mars but fails to outwit a tiny devil. Mosquito control is a crucial activity to interrupt of the host-parasite link and to restrict the spread of lymphatic filariasis. Though there are varieties of time-tested mosquito control measures, outwitting the mosquitoes has become a challenging task due to their complex life cycle, i.e., terrestrial and aquatic. Nevertheless, the terrestrial form of mosquito control measures could be taken not only as a firefighting measure to control the epidemics but to maintain the mosquito population below the tolerable thresholds; aquatic mode of life can be attacked to curb the mosquitoes especially through biological control measures. Among the above listed biological control measures, silver nanoparticle is emerging as one of the very promising alternatives to the existing mosquito control measures. According to Naik et al. (2014), the plant extract of *Pongamia pinnata* has shown the Lc90 at 100 ppm in solvent (ethanol) extraction, and the plant-mediated AgNPs of *Pongamia pinnata* has shown significantly lower concentration of larvicidal property at 1.0 ppm (Lc90). Presence of 9-Octadecenoic acid (Z) and n-hexadecanoic acid as reducing and capping ligands of the synthesized silver nanoparticles are thought be **responsible for** the larvicidal activity, as they might have entred through oral route and destroyed the intestinal tissue.

References

Absar A, Satyajyoti S, Khan MI, Rajiv K, Sastry M (2005) Extra-intracellular biosynthesis of gold nanoparticles by an alkalotolerant fungus, *Trichothecium* sp. J Biomed Nanotechnnol 1(1):47–53

Adesuji ET, Oluwaniyi OO, Adegoke Haleemat I, Roshila M, Labulo AH, Bodede Olusola S, Oseghale CO (2016) Investigation of the larvicidal potential of silver nanoparticles against *Culex quinquefasciatus*: a case of a ubiquitous weed as a useful bioresource. J Nanomater:2016

Amer A, Mehlhorn H (2006) Larvicidal effects of various essential oils against Aedes, Anopheles, and Culex larvae. Parasitol Res 99:466–472

Anonymous (2008) Overview of mosquito control practices in California. Vector-Borne Disease Section, California Department of Public Health, pp 15
Anyaele OO, Amusan AAS (2003) Toxicity of hexanoic extracts of *Dennettia tripetala* (G. Baxer) on larvae of *Aedes aegypti* (L.). Afr J Biomed Res 6:49–53
Baumann P, Clark MA, Baumann L, Broadwell AH (1991) *Bacillus sphaericus* as a mosquito pathogen: properties of the organism and its toxins. Microbiol Rev 55:425–436
Bay EC, Berg CO, Chapman HC, Legner EF (1976) Biological control of medical and veterinary pests. In: Huffaker CB (ed) Theory and practice of biological control. Academic, New York/ London, pp 457–479 pp 788
Becker N, Rettich F (1993) Biological control of mosquitoes and flies in the urban environment. In: Wildey KB, Robinson Wm H (eds), Proceedings of the first international conference on urban pests, pp 455–463
Bogunia-Kubik K, Sugisaka M (2002) From molecular biology to nanotechnology and nanomedicine. Biosystems 65:123–138
Bravo A, Gómez I, Porta H, García-Gómez BI, Rodriguez-Almazan C, Pardo L, Soberón M (2013) Evolution of *Bacillus thuringiensis* Cry toxins insecticidal activity. Microb Biotechnol 6(1):17–26
Bulla LA Jr (1975) Bacteria as insect pathogens. Ann Rev Microbiol 29:163–190
Campbell FL, Sullivan WW, Smith LN (1933) The relative toxicity of nicotine, Anabasine, methyl anabasine and Lupinine for Culicine mosquito larvae. J Econ Entomol 26:500–509
Cavalcanti ESB, Morais SM, Lima MA, Santana EWP (2004) Larvicidal activity of essential oils from Brazilian plants against *Aedes aegypti*. Mem Inst Oswaldo Cruz 99:541–544
Chantraine JM, Laurent D, Ballivian C, Saavedra G, Ibanez R, Vilaseca LA (1998) Insecticidal activity of essential oils on *Aedes aegypti* larvae. Phytother Res 12:350–354
Charles JF (1987) Ultrastructural midgut events in culicidae larvae fed with *Bacillus sphaericus* 2297 spore/crystal complex. Ann Microbiol 138:471–484
Chitra G, Balasubramani G, Ramkumar R, Sowmiya R, Perumal P (2015) *Mukia maderaspatana* (Cucurbitaceae) extract-mediated synthesis of silver nanoparticles to control *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). Parasitol Res 114(4):1407–1415
Colvin VL, Schlamp MC, Alivisatos AP (1994) Light-emitting diodes made from cadmium selenide nanocrystals and a semiconducting polymer. Nature 370(6488):354–357
Cooper R, Sweeney AW (1986) Laboratory studies on the recycling potential of the mosquito pathogenic fungus Culicinomyces. J Invertebr Pathol 48:152–158
Couch JN (1935) A new saprophytic species of Coelomomyces, with notes on other forms. Mycologia 27:376–387
Couch TL (2000) Industrial fermentation and formulation of entomopathogenic bacteria. In: Entomopathogenic Bacteria: from laboratory to field application. Springer, Dordrecht, pp 297–316
Couch JN, Romney SV, Rao B (1974) A new fungus which attacks mosquitoes and related Diptera. Mycologia 66:374–379
Daniel MC, Astruc D (2004) Gold nanoparticles: assembly, supramolecular chemistry, quantum-size related properties, and applications towards biology, catalysis and nanotechnology. Chem Rev 104:293
Das PK, Shenoy RK (2008) Helminthic disease: Filariasis. In: Griffiths J, Maguire JH, Heggenhougen K (eds) Public health and infectious diseases. Elsevier Inc, Amsterdam, p 2010
Davidson EW (1984) Microbiology, pathology and genetics of *Bacillus sphaericus*: biological aspects which are important to field use. Mosq News 44:147–152
DeBach P (1964) The scope of biological control. In: DeBach P (ed) Biological control of insect pests and weeds. Chapman and Hall Ltd, London, pp 3–20 pp. 844
Debenham ML, Russell RC (1977) The insect pathogenic fungus Culicinomyces in adults of the mosquitoes *Anopheles amictis hilli*. Journal of Australian Entomological Society 16:46
DesRochers B, Garcia R (1984) Evidence for persistence and recycling of *Bacillus sphaericus*. Mosq News 44:160–165

Domnas AJ, Fagan SM, Jaronski ST (1982) Factors influencing zoospore production in liquid cultures of the mosquito pathogen *Lagenidium giganteum* (Oomycetes: Lagenidiales). Mycologia 75:820–825

Elimam AM, Elmalik KH, Ali FS (2009) Efficacy of leaves extract of Calotropis procera Ait. (Asclepiadaceae) in controlling Anopheles arabiensis and *Culex quinquefasciatus* mosquitoes. Saudi J Biol Sci 16:95–100

Federici BA (1981) Mosquito control by the fungi culicinomyces, coelomomyces and coelomomyces. In: Burges HD (ed) Microbial control of pests and plant diseases 1970–1980, vol 29. Academic, London, pp 555–572

Federici BA (1995) The future of microbial insecticides as vector control agents. J Am Mosq Control Assoc 11:260–268

Garcia R (1987) Strategies for the management of mosquito population with *Bacillus thuringiensis* var. *israelensis* (H-14). In: Proceedings of the 4th Symposium Arbovirus Research in Australia (1986), St. George TD, Kay BH, Blok J (eds) Q.I.M.R., Brisbane, pp 145–150

Garcia AI, Koldenkova L, Santamarina MA, Gonzalez BR (1991) The introduction of the larvivorous fish *Poecilia reticulata* (Peters) (Cyprinodontiformes: Poeciliidae), a bioregulator of culicids in oxidation ponds and contaminated drainage ditches on the isla de la Juventud. Rev Cubana Med Trop 43(1):45–49

Goettel MS, Sigler L, Carmichael JW (1984) Studies on the mosquito pathogenic hyphomycete Culicinomyces clavisporus. Mycologia 76:614–625

Goldberg LJ, Margalit J (1977) A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii, Uranotaenia unguiculata, Culex univitattus, Aedes aegypti* and *Culex univitattus*. Mosquto News 37:355–358

Haller HL (1940) Insecticidal properties of the fruit of Phellodendron spp. J Econ Entomol 33:941

Hartzell A, Wilcoxon F (1941) A survey of plant products for insecticidal properties. Contrib Boyce Thompson Inst 12:127–I41

Hoffman AJ, Mills G, Yee H, Hoffmann MR (1992) Q-sized cadmium sulfide: synthesis, characterization, and efficiency of photoinitiation of polymerization of several vinylic monomers. J Phys Chem 96(13):5546–5552

Hoffmann MP, Frodsham AC (1993) Natural enemies of vegetable insect pests. Cooperative Extension, Cornell University, Ithaca, 63 pp

Jacobson M (1958) Insecticides from plants. A review of the literature 1941–1953. U. S. Department of Agriculture H and b.154

Jaronski ST, Axtell RC (1983) Persistence of the mosquito fungal pathogen *Lagenidium giganteum* (Oomycetes; Lagenidiales) after introduction into natural habitats. Mosq News 43:332–337

Jayapriya G, Gricilda Shoba F (2014) Evaluation of *Gambusia affinis* and *Bacillus thuringiensis var. israelensis* as *Culex quinquefasciatus* control agents. J Entomol Zool Stud 2(3):121–125

Ji JH, Jung JH, Kim SS, Yoon JU, Park JD, Choi BS, Chung YH, Kwon IH, Jeong J, Han BS, Shin JH, Sung JH, Song KS, Yu IJ (2007) Twenty-eightday inhalation toxicity study of silver nanoparticles in Sprague-Dawley rats. Inhal Toxicol 19:857–871

Kellen WR, Clark TB, Lindegren JE, Ho BC, Rogoff MH, Singer S (1965) *Bacillus sphaericus* Neide as a pathogen of mosquitoes. J Invertebr Pathol 7:442–448

Klaus-Joerger T, Joerger R, Olsson E, Granqvist CG (2001) Bacteria as workers in the living factory: metal accumulating bacteria and their potential for materials science. Trends Biotechnol 19(1):15–20

Knight AL (1980) Host range and temperature requirements of *Culicinomyces clavosporus*. J Invertebr Pathol 36:423–425

Koldenkova L, Famthingoc D, Garcia AI, Garcia GI (1989) Feeding the young of the larvivorous fish *Poecilia reticulata* (Cyprinodontiformes: Poecillidiae) in a natural breeding site of Culex quiquefasciatus (Say, 1823). Rev Cubana Med Trop 41(1):40–48

Kumar KR, Nattuthurai N, Gopinath P, Mariappan T (2014) Synthesis of eco-friendly silver nanoparticles from *Morinda tinctoria* leaf extract and its larvicidal activity against *Culex quinquefasciatus*. Parasitol Res 114(2):411–417

Lamborn RH (1890) Dragon flies vs. mosquitoes. D. Appleton Co, New York, 202 pp

Legner EF, Sjogren RD, Hall IM (1974) The biological control of medically important arthropods. Crit Rev Environ Control 4(1):85–113

Mansur HS, Grieser F, Marychurch MS, Biggs S, Urquhart RS, Furlong DN (1995) Photoelectrochemical properties of 'Q-state'CdS particles in arachidic acid Langmuir–Blodgett films. J Chem Soc Faraday Trans 91(4):665–672

McCray EM, Womeldorf DJ, Husbands RC, Eliason DA (1973) Laboratory observations and field tests with *Lagenidium* against California mosquitoes. Proc Calif Mosq Control Assoc 41:123–128

Menon PKB, Rajagopalan PK (1978) Control of mosquito breeding in wells by using *Gambusia affinis* and *Aplocheilus blochii* in Pondicherry town. Indian J Med Res 68:927–933

Mishra P et al (2017) Stability of nano-sized permethrin in its colloidal state and its effect on the physiological and biochemical profile of *Culex tritaeniorhynchus* larvae. Bull Entomol Res 107:676. https://doi.org/10.1017/S0007485317000165

Mittal PK, Subbarao SK (2003) Prospects of using herbal products in the control of mosquito vectors. ICMR Bull 33(1):1–10

Mulla M, Darwazeh HA, Davidson EW, Dulmage HT (1984) Efficacy and persistance of the microbial agent *Bacillus sphaericus* against mosquito larvae in organically enriched habitats. Mosq News 44:166–173

Murugan K, Benelli G, Ayyappan S, Dinesh D, Panneerselvam C, Nicoletti M, Hwang JS, Kumar PM, Subramaniam J, Suresh U (2015) Toxicity of seaweed-synthesized silver nanoparticles against the filariasis vector *Culex quinquefasciatus* and its impact on predation efficiency of the cyclopoid crustacean *Mesocyclops longisetus*. Parasitol Res 114(6):2243–2253

Naik BR, Guntreddi S, Yuvo Singh G, Satyavathi Sambashiva Daravath R, Ramachandra Reddy P (2014) Bio- synthesis of silver nanoparticles from leaf extract of *Pongamia pinnata* as an effective Larvicide on dengue vector *Aedes albopictus* (Skuse) (Diptera: Culicidae). Adv Entomol 2:93–101

Njagi EC, Huang H, Stafford L, Genuino H, Galindo HM, Collins JB, Hoag GE, Suib SL (2011) Biosynthesis of iron and silver nanoparticles at room temperature using aqueous *Sorghum* bran extracts. Langmuir 27:264–271

Promsiri A, Naksathit AK, Thavara U (2006) Evaluations of larvicial activity of medicinal plant extracts to Aedes aegypti (Diptera: Culicidae) and other effects on a non-target fish. Insect Sci 13:179–188

Rahuman AA, Bagavan A, Kamaraj C, Vadivelu M, Zahir AA, Elango G, Pandiyan G (2009) Evaluation of indigenous plant extracts against larvae of *Culex quinquefasciatus* say (Diptera: Culicidae). Parasitol Res 104(3):637–643

Rajkumar S, Jebanesan A (2005) Larvicidal and adult emergence inhibition effect of Centella asiatica Brahmi (Umbelliferae) against mosquito *Culex quinquefasciatus* say (Diptera: Culicidae). Afr J Biomed Res 8:31–33

Reddya Naik B, Umamaheshwara Reddy P (2005) Survival of Larvivorous fish (*Poecilia reticulata*) in sewage water. J Theoretical Expt BiolElias Academic Publishers 2(1):1–4

Reisen WK, Meyer RP, Tempelis CH, Spoehel JJ (1990) Mosquito abundance and bionomics in residential communities in Orange and Los Angeles counties. Calif J Med Entomol 27:356–367

Rueda LM, Patel KJ, Axtell RC, Stinner RE (1990) Temperature-dependent development and survival rates of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). J Med Entomol 27(5):892–898

Rupp HR (1996) Adverse assessments of *Gambusia affinis*: an alternative view for mosquito control practitioners. J Am Mosq Control Assoc 12(2 pt 1):155–166

Schmid G (1992) Large clusters and colloids. Metals in the embryonic state. Chem Rev 92(8):1709–1727

Service MW (1983) Biological control of mosquitoes–has it a future? Mosq News 43:113–120

Service MW (1992) Importance of ecology in *Aedes aegypti* control. Southeast Asian J Trop Med Public Health 23:681–688

Shaalan EAS, Canyon D, Younes MWF, Abdel-Wahab H, Mansour AH (2005) A review of botanical phytochemicals with mosquitocidal potential. Environ Int 31:1149–1166

Shim JC, Self LS (1973) Toxicity of agricultural chemicals to larvivorous fish in Korean rice fields. Trop Med 15:123–130
Shivshankar S, Rai A, Ahmad A, Sastry M (2003) Rapid synthesis of au, ag and bimetallic au core-ag shell nanoparticles using neem (*Azadirachta indica*) leaf broth. J Colloid Inter Sci 275:496–502
Singh G, Prakash S (2011) Studies on fungal cultural filtrates against adult *Culex quinquefasciatus* (Diptera: Culicidae) a vector of Filariasis. J Parasitol Res 2011:1–5
Soni N, Prakash S (2014) Green nanoparticles for mosquito control. Sci World J 2014:1–6
Spielman A, D'Antonio M (2002) Mosquito: the story of man's deadliest foe. Hachette Books, New York, p 272
Suh CP, Axtell RC (1999) Coelomomyces giganteum zoospores: effects of concentration, movement, light, and temperature on infection of mosquito larvae. Biol Control 15:33–38
Sukumar K, Perich MJ, Boobar LR (1991) Botanical derivatives in mosquito control: a review. J Am Mosq Control Assoc 7(2):210–237
Sur B, Bihari V, Sharma A, Joshi AK (2001) Studies on physiology, zoospore morphology and entomopathogenic potential of the aquatic oomycete: *Coelomomyces giganteum*. Mycopathologia 154:51–54
Sweeney AW (1981) Preliminary field tests of the fungus Culicinomyces against mosquito larvae in Australia. Mosq News 41:470–476
Sweeney AW (1983) The time-mortality response of mosquito larvae infected with the fungus Culicinomyces. J Invertebr Pathol 42:162–166
Sweeney AW, Lee DJ, Panter C, Burgess LW (1973) A fungal pathogen for mosquito larvae with potential as a microbial insecticide. Search 4:344–345
Thirunavukkarasu S, Abdul AR, Govindasamy R, Sampath M, Asokan B, Chidambaram J, Abdul AZ, Gandhi E, Chinnaperumal K (2011) Synthesis of silver nanoparticles using *Nelumbo nucifera* leaf extract and its larvicidal activity against malaria and filariasis vectors. Parasitol Res 108:693–702
Thomas, Fisher (1999) Handbook of biological control: principles and applications of biological control (Fisher TW, Thomas S, eds). Bellows Pub. Academic Press, San Diego, p 941
Tyagi BK (2004) The invincible deadly mosquitoes. Scientific Publisher, Jodhpur, p 272
Tyagi BK, Ramnath T, Shahi AK (1994) Evaluation of repellency effect of *Tagetus minuta* (Family: Compositae) against the vector mosquitoes *Anopheles stephensi* (Liston), *Culex quinquefasciatus* (Say) and *Aedes aegypti* (L). Int Pest Control 39(6):184–185
Tyagi BK, Shahi AK, Kaul BL (1998) Evaluation of repellent activities of *Cymbopogon* essential oils against mosquito vectors of Malaria, Filariasis and dengue fever in India. Phytomedicine 5(4):324–329
Lacey LA, Undeen AH (1986) Microbial control of black flies and mosquitoes. Annu Rev Entomol 31:265–296
Wang Y, Herron N (1991) Nanometer-sized semiconductor clusters: materials synthesis, quantum size effects, and photophysical properties. J Phys Chem 95(2):525–532
WHO (2016) http://www.who.int/mediacentre/factsheets/fs102/en/
Wilcoxon F, Hartzell A, Wilcoxon F (1940) Insecticidal properties of extract of male fern (*Aspldium filixmas* (L.) SW). Contrib Boyce Thompson Inst 11:1–4
Yadouléton A, Badirou K, Agbanrin R, Jöst H, Attolou R, Srinivasan R, Padonou G, Akogbéto M (2015) Insecticide resistance status in *Cx. quinquefasciatus* in Benin. Parasit Vectors 8:17
Zharov VP, Kim J-W, Curiel DT, Everts M (2005) Self-assembling nanoclusters in living systems: application for integrated photothermal nanodiagnostics and nanotherapy. Nanomedicine 1:326–345

23

Chemical Control of *Culex quinquefasciatus* (Say, 1823), the Principal Vector of Bancroftian Filariasis, with Emphasis on Resistance Development Against Insecticides in India

T. Mariappan and B. K. Tyagi

Abstract

Lymphatic filariasis is under the era of elimination from many countries including India. India has contributed towards the control of disease through various methods including chemotherapy and vector control with main emphasis on larvicides against vectors. Several groups of larvicides including carbamates, organochlorines, organophosphorus and pyrethroids have been used against vector *Culex quinquefasciatus* for several decades, and the development of resistance to various compounds was documented in several published articles. However, the present review is aiming to highlight resistance development in *Cx. quinquefasciatus* against insecticides in India. Also the mosaic pattern of application of various insecticides, essential in order to delay the resistance against a particular compound, is emphasized upon. The in-depth review leads to conclude that not only the chemical insecticides but also other compounds, namely, biocides like plant extracts need to be used in a continuous process to reduce the vector population.

T. Mariappan
Centre for Research in Medical Entomology (ICMR), Madurai, Tamil Nadu, India

B. K. Tyagi (✉)
Department of Environmental Biotechnology, Bharathidasan University, Tiruchirapalli, Tamil Nadu, India

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_23

23.1 Introduction

Bancroftian filariasis, transmitted by the ubiquitous mosquito *Culex quinquefasciatus*, is the commonest of all kinds and accounts for 96% of the infection. Rapid urbanization and industrialization with improper drainage facilities are mainly considered responsible for the preponderance of *Cx. quinquefasciatus* in various ecosystems of India (Singh 1967). Being a serious nuisance mosquito and a vector for several deadly vector-borne/zoonotic diseases the world over, *Cx. quinquefasciatus* has been the subject of intensive control programmes, particularly by insecticides. In India, the mosquito had been under insecticidal impact since the 1950s during the National Filaria Control Programme (NFCP). Although a large number of chemical products and formulations have been explored to mitigate its population, the larvicides like fenthion and adulticides like deltamethrin have stayed for a long time and are still deployed in various different formulations. Obviously, therefore, *Cx. quinquefasciatus* has been under insecticidal pressure for many decades forcing mosquito to develop the resistance in varied spectrum. The present communication aims to draw attention to the challenges coupled with chemical use, with special emphasis to assess the resistance status of *Cx. quinquefasciatus* to conventional insecticides belonging to carbamates, pyrethroids, organochlorines and phosphates as well as other biocides such as insect growth regulators, plant-based extracts, etc.

23.2 Filariasis Vector Control

Since filarial transmission takes place throughout the year, adulticidal measures were required to be applied more frequently, which is not only impracticable and uneconomical but also increased the risk of precipitating resistance in the vector mosquito to insecticides. Therefore, residual insecticidal spray against adult mosquitoes for filariasis control was discontinued in 1959, and larval control operation using ML oil was made the main plank of operations against the vector. Decrease in vector density, infection and infectivity rates in mosquitoes were shown to be well marked in areas where antilarval measures were efficiently carried out up to 1959 (ICMR Assessment Committee Report on NFCP 1961). Excessive industrialization, rapid urbanization, and unplanned growth of cities in India, however, have resulted in production of large number of mosquitogenic habitats enhancing the breeding of a variety of disease vectors, such as that of *Cx. quinquefasciatus*, vector of bancroftian filariasis. Vector-borne diseases control solely depends upon control measures with emphasis on insecticides as one of the major weapons for the past several decades. However, recent methods for controlling most vector species including *Cx. quinquefasciatus* have largely based on the use of insecticides (either adulticides or larvicides). Although insecticides are highly effective in reducing vector population, other factors such as low quality of insecticides and other operational

deficiencies hamper in achieving the desired results (FAO/WHO 2001), warranting to explore new alternatives which are eco-friendly. Also, the importance of vector control towards lymphatic filariasis (LF) elimination is stressed upon to achieve the desired results in India (Mariappan 2007).

23.3 History of Insecticide Usage During LF Control in India

Controls of most of the vector-borne diseases (malaria, filariasis, dengue, chikungunya, Japanese encephalitis, yellow fever, onchocerciasis and leishmaniasis) are covered under the use of insecticides to reduce the burden. Even though the current strategy to eliminate lymphatic filariasis (LF) is by use of mass drug administration (MDA), it is understood lately that a complete annihilation of the parasite would not be possible without supplementing it by transmission control interventions. These interventions include largely larvicides against vector of bancroftian filariasis. However, it was the DDT which was first widely used against malaria vectors, but also other vector mosquitoes. *Culex quinquefasciatus* however soon developed resistance against this wonder insecticide, DDT, besides many others. Insecticides like organophosphates, carbamates and pyrethroids were subsequently pressed in use, but they also met with the same fate sooner or later, i.e., *Cx. quinquefasciatus* developed resistance against all of these insecticides. For use of these insecticides for many years, mosquitoes have developed resistance against major insecticides utilized in public health programmes due to intense selection pressure. Tolerance pattern of *Cx. quinquefasciatus* against various insecticides like carbamates, organochlorines, organophosphorus compounds, pyrethroids, IGRs, biocides, plant extracts, etc. has been extensively documented.

23.4 Choice of Chemical Insecticides

Chemical insecticides are considered essential in abating vector populations in case of most of the vector-borne diseases, especially where disease bursts out as an epidemic! Although alternative means like environmental, biological, and genetical methods bring about good results in the long run, all of them are however unable to meet the emergencies of disease outbreaks. Integrated vector management (IVM) of vector population, best suited in the case of *Cx. quinquefasciatus*, strongly supports location, time, space and species-specific choice of insecticidal use, along with other adequate methodologies. Currently, the utility of various types of insecticides used in vector control programmes in the country has indicated that *Cx. quinquefasciatus* is highly resistant to DDT, malathion and initial resistance to synthetic pyrethroids (deltamethrin, cyfluthrin, permethrin and lambda-cyhalothrin) (Kumar et al. 2011).

23.5 Emergence of Resistance

Vector resistance development against insecticides is currently the singular most important reason for failure in mitigating vector-borne diseases. Therefore, as suggested by Van Bortel et al. (2008), it is imperative to widen our knowledge of vector resistance and the changing trends of resistance against vector species as well as some practical key terms under practice/usage including insecticide, resistance, cross resistance, multiple resistance, biochemical-metabolic resistance, resistance cost, insecticide resistance management, insecticide combination, insecticide mixture, insecticide mosaic, synergist, etc., relevant to studies on resistance pattern (Suman et al 2010a, 2010b). These terms with explanations on a potential threat to global public health have been stated elaborately (Karunamoorthi and Sabesan 2013).

23.6 Insecticide Resistance Mechanisms

Vector populations, in general, proliferate in shorter life span within few generations. One female mosquito reproduces thousands of progeny within a period of few weeks (Bellinger 2011). Exposure of vectors under chemical control either alone or along with other bioecological applications/methods (i.e. integrated vector management – IVM) always leads to crisis on insecticide resistance. In this context various vector control tools are applied to reduce disease burden to achieve the sustainability (Georghiou 1994; WHO 2004). It has been reported that for delaying resistance against target species biological control agents *Metarhizium anisopliae* and *Beauveria bassiana* could also be practiced (Hougard et al. 2003). The resistance in vectors warrants development of newer insecticides for mosquito control besides the use of other countermeasures. Studies related to determining the susceptibility of adult and larvae of *Cx. quinquefasciatus* mosquitoes are being done elsewhere as continuous process and considering introducing rotation of insecticides as a management strategy to increase the duration of the usage of the current insecticides. Furthermore, a rationalized use of insecticides coupled with regularly examining resistance mechanism is suggested to mitigate rapid emergence of insecticide resistance.

23.7 Assessment of Resistance

Before the introduction of pyrethroids in the 1990s in public health programmes, DDT and HCH were the major weapons to control vector-borne diseases in India. However, detection of resistance against DDT and HCH, including multiple insecticides resistance to vectors, was reported in the 1950s (Singh et al. 2002). Resistance in vectors is assayable by a number of reliable methods including microplate assay (detection of resistance and its mechanism even at low frequency). These methods lead to the beneficial detection of commonly used chemical insecticides (Brogdon 1989; Cordón-Rosales et al. 1990). One of the earliest methods in vogue was popularized by the World Health Organization is the resistance monitoring kit as shown in Fig. 23.1.

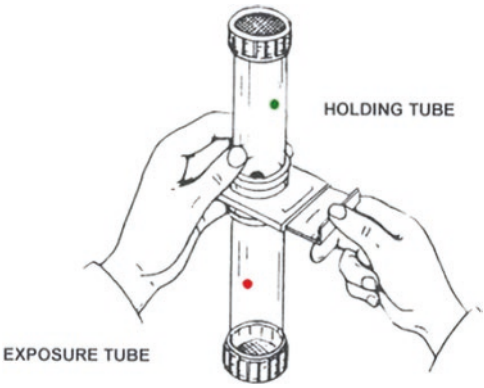


Fig. 23.1 Insecticide resistance monitoring kit. (*Source*: WHO)

23.8 Various Insecticides Used in the National Control Programme or Major Control Activities

23.8.1 Demonstration Projects

Carbamates, organochlorines, organophosphates, pyrethroids, insect growth regulators (IGRs), biocides plant extracts and surfactants including oils, were used to kill larvae and adults under vector control programmes in different schemes organized in India. In addition, the LLIN (long-lasting insecticide-impregnated net) is being practiced in many parts of the north-eastern states in India for the past three decades (Table 23.1).

23.9 Strategies Adopted Towards Resistance Management

The strategies involved on insecticide resistance management through various means are:

(a) Current status of monitoring susceptibility status to understand the development of insecticide resistance is essential to avoid any threat.
(b) Suitable usage of techniques (biochemical or molecular) to verify various tendencies of resistance.
(c) Application of selection pressure to exclude genes responsible for resistance.
(d) Delay in evolution of resistance to avoid dependence on chemical insecticides and finding out alternative tools for controlling vectors.
(e) Public health experts, researchers, medical entomologists, manufacturers of insecticides and other personnel involved in vector control activities need to be coordinated to highlight drawbacks on excess insecticide use and resistance development.

Table 23.1 Types of chemical compounds used in various control programmes

Sl. No	Chemical group	Compounds used in programmes/schemes
1	Carbamates	Carbaryl, propoxur, bendiocarb
2	Organochlorines	DDT, dieldrin, gamma HCH, lindane, hexachlor
3	Organophosphorus compounds	Fenthion, malathion, phenthoate, temephos, dichlorvos (DDVP), fenitrothion, Sumithion: Activities of organophosphorus insecticides include both contact and stomach poisons. Those compounds used both agriculture and public health. Some of them are used as a larvicide to control mosquitoes in potable water, midges, black flies and other insects in public health and to control fleas on dogs and cats
4	Pyrethroids	(permethrin, etofenprox, fenvalerate, deltamethrin, cyfluthrin, pyrethrum, fendona, lambda-cyhalothrin): Pyrethroids are a nonsystemic one used both in agricultural and public health mosquito abatement programmes in numerous residential areas, both indoor and outdoor, and on pests controlling various developmental stages of insects
5	Insect growth regulators	Methoprene, diflubenzuron
6	Biocides	*Bacillus sphaericus*, *Bacillus thuringiensis*, Spinosad, *Metarhizium anisopliae*, and *Beauveria bassiana*
7	Plant extracts	*Ocimum bascilicum Vinca rosea,* azadirachtin; nerium; oleander; various types of oils include Calamus, cinnamon, citronella, clove, eucalyptus, lemon, mentha and orange; *Acorus calamus*; *Bambusa arundinacea*; *Lantana camera*, *Adathoda* spp.
8	Surfactants	Monoxi, Malariol

23.10 Results

The collection of data from various works with the high priority of larvicidal form against *Cx. quinquefasciatus* in India is presented in Tables 23.2, 23.3 and 23.4. The LC_{50} values presented are only indicative for each group of compounds.

A total of eight different types of categories belonging to carbamates, organochlorines, organophosphates, pyrethroids, IGRs, biocides, plant extracts and surfactants were identifiable in India. Both larvae and pupae as well as adults of *Cx. quinquefasciatus* were used for analysis. Almost for each category no less than few observations to maximum 69 were obtained for analysis.

The DDT invention has supported the control of malaria vectors, with collateral output towards control of *Cx. quinquefasciatus.* In view of search of alternatives to insecticides several groups of insecticides emerged towards disease control. Currently, NVBDCP (2015) follows undermentioned choice of control tools in various parts of the country. The details are given in Table 23.5 (A–D).

Table 23.2 LC50 values for carbamates, IGRs, organochlorines and organophosphates

Chemical insecticides				
		LC_{50} (mg/l)		
Insecticide	Type	High	Low	Average
Carbamate	Bendiocarb	107.000	68.000	93.667
	Carbaryl	0.670	0.410	0.503
	Propoxur	0.000	0.000	0.000
IGR	Diflubenzuron	0.001	0.000	0.000
	Lufenuron	0.001	0.000	0.000
	Methoprene	1.000	0.000	0.000
	Triflumuron	0.001	0.000	0.000
Organochlorine	DDT	0.617	0.040	0.162
	Gamma HCH	0.684	0.128	0.272
Organophosphorus	Chlorpyrifos	0.007	0.000	0.004
	DDVP	0.050	0.030	0.037
	Fenitrothion	0.049	0.000	0.025
	Fenthion	0.413	0.002	0.040
	Folithion	0.008	0.004	0.006
	Malathion	0.630	0.020	0.175
	Phenthoate	0.013	0.005	0.008
	Temephos	0.020	0.000	0.003
	Zolone	0.467	0.098	0.212

23.11 Discussion

The selection and cost-effective use of insecticides depend upon a number of criteria, both technical and operational. The susceptibility of the vector populations, to be targeted (controlled) by insecticides available for public health use, is a major determinant of the selection of the insecticide, of the vector response and of the efficacy and impact of the insecticide used. Measurement of resistance status of the target vector populations therefore becomes an essential component in the planning, monitoring and evaluation of any disease control programme using insecticides. Based on the nature and extent of insecticide use in a particular place information on proper collection, interpretation and use of data from insecticide resistance monitoring is of necessary practical value in guiding insecticide application in a particular disease control programme. As per WHO criteria, 98–100% mortality indicates susceptibility of the population tested against a particular insecticide (Vatandoost and Vaziri 2004). According to the WHO criteria (WHO 2003) for characterizing insecticide susceptibility, evidence for resistance in *Cx. quinquefasciatus* reported an increase in tolerance against permethrin. Resistance of *Cx. quinquefasciatus* against malathion has been documented in a study from Rajahmundry town, India

Table 23.3 LC50 values for biocides and plant extracts and derivatives

Biocides and plant extracts/derivatives				
		LC_{50} (mg/l)		
Insecticide	Type	High	Low	Average
Biocide	*Bacillus sphaericus*	18.612	0.087	0.001
	Bacillus thuringiensis	0.467	0.349	0.408
	Spinosad	0.000	0.000	0.000
Plant extract	*Acorus calamus*	33.700	0.000	16.850
	Adathoda sp.,	57.300	0.000	28.650
	Azadirachtin	0.024	0.017	0.021
	Bambusa arundinacea	68.500	0.000	34.250
	Bugsac	74.100	0.000	37.050
	Calamus oil	40.400	0.000	20.200
	Cinnamon oil	67.160	0.000	33.580
	Citronella oil	91.230	0.000	45.615
	Clove oil	58.920	0.000	29.460
	Eucalyptus oil	64.640	0.000	32.320
	Lantana camera	9.400	0.000	4.700
	Lemon oil	43.790	0.000	21.895
	Mentha oil	42.250	0.000	21.125
	Nerium Oleander AS	2758.870	0.000	1379.435
	Nerium Oleander HS	102.540	0.000	51.270
	Ocimum bascilicum	62.800	0.000	31.400
	Orange oil	63.250	0.000	31.625
	Vinca rosea	66.300	0.000	33.150
	Vitex negundo	7.700	0.000	3.850

Table 23.4 LC50 values for pyrethroids derived from plants

Chemical insecticides derived from plants				
		LC_{50} (mg/l)		
Insecticide	Type	High	Low	Average
Pyrethroid	Alphamethrin	0.000	0.000	0.000
	Cyfluthrin	0.001	0.000	0.001
	Deltamethrin	0.175	0.000	0.028
	Fenfluthrin	0.001	0.000	0.001
	Fenvalerate	0.011	0.000	0.006
	Lambda Cyhalothrin	0.000	0.000	0.000
	Permethrin	0.115	0.002	0.023

(Mukhopadhyay et al. 2006). A study at Nagpur district has shown that diagnostic concentrations of deltamethrin 0.05%, DDT 4% and alpha-cypermethrin 0.10% led to a high level of resistance against *Cx. quinquefasciatus* (Mukhopadhyay et al. 1993). Similar observation was made by Sarkar et al. (2009) on resistance of *Cx. quinquefasciatus* against DDT in the city of Patna (Bihar state). The use of DDT has been discontinued in most parts of India due to the development of resistance in

Table 23.5 (**A**–**D**) Showing choices of control tools adopted by NVBDCP

(A) Mosquito adulticides and formulations		
S. No.	**Adulticide**	**Rate of application in square metre –preparation of suspension in water and dosage per square metre of active ingredient (a.i.) in mg in parentheses**
(i)	DDT 50% wp	1 kg/10 lit/500 (100)
(ii)	Malathion 25% wp[a]	2 kg/10 lit/500 (200)
(iii)	Deltamethrin 2.5% wp	400 gm/10 lit/500 (20)
(iv)	Cyfluthrin 10%wp	125 gm/10 lit/500 (25)
(v)	Lambda-cyhalothrin 10% wp	125 gm/10 lit/500 (25)
(vi)	Alpha-cypermethrin 5%wp	250gm/10 lit./500 (25)
(vii)	Bifenthrin 10% WP	125gm/10 lit/500 (25)
(B) Mosquito Larvicides and formulations		
S. No.	**Larvicide**	**Rate of application in square metre –preparation of suspension in water and dosage per square metre of active ingredient (a.i.) in mg in parentheses**
(i)	MLO	20 ml/1 square metre (20)
(ii)	Temephos (abate)	20 ml in 10 lit. Water/1 square metre(20)
(iii)	*Bacillus thuringiensis* var. *israelensis*	5 kgs in 200 lit. Water/hectare (500)
(iv)	*Bacillus thuringiensis* var. *israelensis 12* aqueous suspension (12AS)	1 lit. In 200 lit. Water/hectare (100) or (200)
(v)	Diflubenzuron 25% WP	100 gms in 100 lit. Water/hectare (200)
(vi)	Pyriproxyfen	2 kg./hectare (200)
(C) Insecticides (Pyrethroid) and formulations: Adulticide		
S. No.	Insecticide	**Rate of application – Preparation of suspension in water and dosage per square metre of active ingredient in mg in parentheses**
1.	Pyrethrum extract (2%)	1:19, i.e.1 part of 2% pyrethrum extract in 19 parts of kerosene (50 ml in 1 litre K.Oil)
2.	Cyphenothrin 5% EC	0.5 mg a.I per sq.mt.(20 ml in 1 litre kerosene oil)
(D) Insecticide used for outdoor fogging		
S. No.	**Insecticide**	**Rate of application – Preparation of suspension in water and dosage per square metre of active ingredient (a.i.) in mg in parentheses**
1.	Malathion (Technical)	1:19, i.e.1 part of malathion tech in 19 parts of diesel (50 ml in 1 litre diesel)
2.	Cyphenothrin 5% EC	3.5 g a.i. Per hectare (7 ml in 1 litre diesel)

Source: http://nvbdcp.gov.in/malaria3.html

[a]For three rounds

vector populations. However, it is still being used for vector control of Kala-azar and also some parts of north eastern India against malaria vectors. Nevertheless, determination of DDT in the environment may have resulted in the continued selection for resistance (Sarkar et al. 2009a, b). Earlier, it has been reported about biocides wherein observations exist on cross resistance to three different bacterial strains (Bs 2397, Bs 2362, *Bs* IAB59) against Bs 1593 M in resistant population of

Cx. quinquefasciatus (Poopathi et al. 1999). The insect growth regulators (IGRs) pyriproxyfen and triflumuron have been reported to cause sterility in adult female mosquitoes. A pyrethroid-resistant individual which is not killed by one particular dose would be prevented from passing on its resistant genes, and selection for resistance by the treated nets would thus be prevented (Langley et al. 1988; Howard and Wall 1995). The reported study indicated the possible development of resistance against temephos in the larvae of *Cx. quinquefasciatus* in most of the urban areas. This may be because of continuous use of temephos for over two decades as part of the Urban Malaria Scheme (UMS). Under UMS, these areas were sprayed with temephos on a weekly basis. The development of resistance may also be because of spraying of under dose of temephos in stagnant water over a period of time, coupled with the constant use of related organophosphate compounds in the agricultural sector in and around Delhi. Such development of resistance warranted a detailed evaluation of the efficacy of temephos against the larvae of *Cx. quinquefasciatus*. Such a study may help in reformulating control strategies, including rescheduling application of insecticide or replacing the larvicide with other suitable compounds under the programme. Also report suggests that alternative insecticides could also be used to control epidemic situations. IGRs are efficient but show very low persistence on *Cx. quinquefasciatus* (Jambulingam et al. 2008; Sadanandane et al. 2012). In control operations, this insecticide was recommended to overcome resistance, and *Cx. quinquefasciatus* did not show resistance for pirimiphos-methyl either as larvicide or adulticide (Das et al. 1987).

In northern India, a few trials had been carried out using pirimiphos-methyl 50% EC against *Cx. quinquefasciatus*, and based on the observation at 24 h post-treatment, Wattal et al. (1975) recommended 12.5 g (ai)/ha as an effective dosage. Multiple insecticide (deltamethrin, cyfluthrin, permethrin, lambda-cyhalothrin, malathion and DDT) resistance was reported in filaria-endemic areas of northern India and proved for high resistance to DDT and malathion. In an earlier report regarding the susceptibility of five species of mosquitoes to deltamethrin at Mysore, an LC_{50} value of 0.00078 was observed for *Cx. quinquefasciatus* which is more susceptible to deltamethrin (Vijayan et al. 2007). However, experiments need to be made in order to determine whether *Cx. quinquefasciatus* could develop resistance to this insecticide. Effective control measures could be adopted based on the decrease in insecticidal susceptibility of *Cx. quinquefasciatus* against temephos up to 10.8-folds, fenthion up to 6.94-folds, neem compound up to 5.29-folds, cypermethrin up to 3.19-folds, α-cypermethrin up to 5.48-folds and λ-cyhalothrin up to 3.33-folds as observations obtained from Bathinda, Bikaner, Jodhpur and Jamnagar in India (Suman et al. 2010a). Laboratory studies on efficacy (LC_{50}) of diflubenzuron 25WP formulation against *Cx. quinquefasciatus* (0.0011 mg/l) in comparison with 22SL formulation against *Cx. quinquefasciatus* (0.0008 mg/l) and also diflubenzuron have also shown good efficacy against various mosquito species in field studies (Ansari et al. 2005). Efficacy of IGR has been demonstrated in the field at Pondicherry (Amalraj et al. 1988). High efficacy of triflumuron (IE_{50}: 0.0002–0.0003 mg/l) against organophosphorus-resistant *Cx. quinquefasciatus* belonging to different geographical areas of India has been eliciting attention for effective use of

this IGR. The earlier laboratory evaluation of triflumuron has shown high LC_{90} (0.007 mg/l) (Mulla 1995). A study at Delhi demonstrated 50% adult emergence inhibition at 0.0003 mg/l against IGR compound Starycide 480SC (triflumuron) for *Cx. quinquefasciatus* (Batra et al. 2005). Invariably, most of the IGR compounds are more effective against *Cx. quinquefasciatus* populations in various breeding habitats such as drains and pools found in Rajasthan (Batra et al. 2005; Suman et al. 2010a). In South India, resistance to malathion was detected against *Cx. quinquefasciatus* populations, and the resistance ratios of larval and adult stages were not particularly high; however, the lethal concentrations were 0.94 to 3.07 in larval stage and from 0.93 to 34.68 in adult stage. Also, *Cx. quinquefasciatus* is known to develop high levels of resistance to synthetic biolarvicides within a short duration (Rao et al. 1995, Yebakima et al. 1995a, b, Gopalan et al. 1996, Poopathi et al. 1999). Insecticide susceptibility tests on adults and larvae conducted in Panaji, Goa, revealed that *Cx. quinquefasciatus* adults were resistant to DDT, dieldrin, malathion and fenitrothion, and larvae found to be highly resistant to DDT, however, showed low resistance to malathion and fenitrothion (Thavaselvam et al. 1993).

Proper management of insecticide resistance in field populations could be achieved through effective vector control only (Raghavendra and Subbarao 2002). Various recommended/suggested insecticides/biocides are practiced by different states and Union territories depending upon the requirement and gravity of the situation from time to time. However, organophosphorus compounds have been extensively used by the programme authorities for many decades in reducing malaria incidence but it collaterally also exposed *Cx. quinquefasciatus* population simultaneously in villages as well as in cities/urban areas. In addition, technical and operational deficiencies are well known to facilitate or promote development of resistance in *Cx. quinquefasciatus* populations.

23.12 Conclusion

Though insecticides have been used against mosquito vector species for a long time, in order to slow down the selection pressure of insecticides, applying insecticide on rotational basis is the most essential component. Data have shown that *Cx. quinquefasciatus* is still sensitive to temephos. Therefore, vector control units should consider introducing rotation of insecticides as a management strategy to increase the duration of the usage of the currently used insecticides. Furthermore, a rationalized use of insecticides coupled with regular monitoring of insecticide resistance is recommended to mitigate the rapid emergence of insecticide resistance. Proper management of insecticide resistance of mosquitoes in the wild populations can only achieve effective vector control. For the implementation of this approach/policy, though adopting alternative method of vector control like use of biological control and bioenvironmental methods may help to avoid the problem of insecticide resistance, yet application of any one type of insecticides is the most essential part towards control of bancroftian filariasis. Under the current situation of elimination of LF in India, vector control through application of insecticide is the most essential

component against *Cx. quinquefasciatus* in order to reduce the microfilaria (mf) rate in endemic areas. Thereby selection of rotation of insecticides plays a significant role to avoid/delay resistance development against *Cx. quinquefasciatus*.

In order to overcome the development of resistance both quantity of insecticide use and insecticidal selection pressure need to be monitored periodically. Mosaic pattern of application of chemical insecticides is so much necessitated apart from other environmental and biological control methods against *Cx. quinquefasciatus*.

Acknowledgement We wish to express our thanks to Shri. V. Rajamannar, Senior Technical staff, and Miss. S. Karthigai Selvi, Technical staff of CRME, for their technical assistance.

References

Amalraj D, Vasuki V, Kalyanasundaram M, Tyagi BK, Das PK (1988) Laboratory and field evaluation of three insect regulators against mosquito vectors. Indian J Med Res 87:24–31.3

Ansari MA, Razdan RK, Sreehari U (2005) Laboratory and field evaluation of Hilmilin against mosquitoes. J Am Mosq Control Assoc 21:432–436

Batra CP, Mittal PK, Adak T, Ansari MA (2005) Efficacy of IGR compound Starycide 480SC (Triflumuron) against mosquito larvae in clear and polluted water. J Vect Borne Dis 42:109–116

Bellinger RG (2011) Pesticide resistance Management. Available from: http://www.clemson.edu/extension/rowcrops/pest/files/2011pmhpest_resist_mgmt.pdf

Brogdon WG (1989) Biochemical resistance detection: an alternative to bioassay. Parasitol Today 5:56–60

Cordón-Rosales C, Beach RF, Brogdon WG (1990) Field evaluation of methods for estimating carbamate resistance in *Anopheles albimanus* mosquitoes from a microplate assay for insensitive acetylcholine esterase. Bull WHO 68:323–329

Das M, Srivastava BN, Rao CK, Thapar BR, Sharma GK (1987) Field trial of the effectiveness of indoor-spraying with pirimiphos-methyl emulsion for malaria control in a tribal area of Phulbani district, Orissa state, India. Med Vet Entomol 1:289–295

Georghiou GP (1994) Principles of insecticide resistance management. Phytoprotection 75(4):51–59

Gopalan N, Prakash S, Bhattacharya BK, OP A, Rao KM (1996) Development of malathion resistance in *Culex quinquefasciatus* Say. (Diptera: Culicidae). Indian J Med Res 103:84–90

Hougard JM, Corbel V, N'Guessan R, Darriet F, Chandre F, Akogbeto M, Baldet T, Guillet P, Carnevale P, Traoré-Lamizana M (2003) Efficacy of mosquito nets treated with insecticide mixtures or mosaics against insecticide resistant Anopheles gambiae and *Culex quinquefasciatus* (Diptera: Culicidae) in cote d'Ivoire. Bull Entomol Res 93(6):491–498

Howard J, Wall R (1995) The effects of triflumuron, a chitin synthesis inhibitor, on the housefly *Musca dornesfico*. Bull Ent Res 85:71–77

ICMR (1961). Assessment committee report of the assessment committee on National Filaria Control Programme (NFCP)

Jambulingam P, Sadanandane C, Boopathi Doss PS, Subramanian S, Zaim M (2008) Field evaluation of an insect growth regulator, pyriproxyfen, 0.5% GR against *Culex quinquefasciatus*, the vector of Bancroftian filariasis in Pondicherry, India. Acta Trop 107:20–24

Karunamoorthi K, Sabesan S (2013) Insecticide resistance in insect vectors of disease with special reference to mosquitoes: a potential threat to global public health. Health Scope 2(1):1–18

Kumar K, Sharma AK, Kumar S, Patel S, Sarkar M, Chauhan LS (2011) Multiple insecticide resistance/susceptibility status of *Culex quinquefasciatus*, principal vector of bancroftian filariasis from filaria endemic areas of northern India. Asian Pac J Trop Med 4(6):426–429

Langley PA, Felton T, Oouchi H (1988) Juvenile hormone mimics as effective sterilants for the tsetse fly *Glossina morsitans morsirans*. Med Vet Entomol 2:29–35

Mariappan T (2007) Vector control in lymphatic filariasis elimination programme. Curr Sci 93(8):1061–1062

Mukhopadhyay AK, Sinha SN, RL Y, Narasimham MV (1993) Susceptibility status of *Culex quinquefasciatus* in Patna to insecticides. Indian J Pub Heal 37:57–60

Mukhopadhyay AK, Patnaik SK, Babu PS (2006) Susceptibility status of some culicine mosquitoes to insecticides in Rajahmundry town of Andhra Pradesh, India. J Vector Borne Dis 43(1):39–41

Mulla MS (1995) The future of insect growth regulators in vector control. J Am Mosq Control Assoc 11:269–273

NVBDCP (2015) http://nvbdcp.gov.in/malaria3.html

Poopathi S, Mani TR, RagunathaRao D, Baskaran G, Kabilan L (1999) Cross resistance to *Bacillus sphaericus* strains in *Culex quinquefasciatus* resistant to *B.sphaericus* 1593. M. South East Asian J Trop Med Public Health 30:477–481

Raghavendra K, Subbarao SK (2002) Chemical insecticides in malaria vector control in India. ICMR Bull 32:93–99

Rao DR, Mani TR, Rajendran R, Joseph AS, Gajanana A (1995) Development of a high level resistance to Bacillus sphaericus in a field population of *Culex quinquefasciatus* from Kochi, India. J Am Mosq Control Assoc 11:1–5.2

Sadanandane C, Boopathi Doss PS, Jambulingam P (2012) Efficacy of three formulations of diflubenzuron, an insect growth regulator, against *Culexquinquefasciatus* Say, the vector of Bancroftian filariasis in India. Indian J Med Res 136:783–791

Sarkar M, Bhattacharyya KI, Borkotoki A, Baruah I, Srivastava R (2009) Development of physiological resistance and its stage specificity in *Culex quinquefasciatus* after selection with deltamethrin in Assam, India. Memórias do Instituto Oswaldo Cruz, Rio de Janeiro 104673-677

Sarkar M, Bhattacharyya IK, Borkotoki A, Goswami D, Rabha B, Baruah I et al (2009a) Insecticide resistance and detoxifying enzyme activity in the principal bancroftian filariasis vector, *Culex quinquefasciatus* in north eastern India. Med Vet Entomol 23:122–131

Sarkar M, Borkotoki A, Baruah I, Bhattacharyya IK, Srivastava RB (2009b) Molecular analysis of knock down resistance (kdr) mutation and distribution of kdr genotypes in a wild population of *Culex quinquefasciatus* from India. Tropical Med Int Health 14(9):1097–1104

Singh D (1967) The *Culex pipiens fatigans* problem in South-East Asia with special reference to urbanization. Bull World Health Org 37(2):239–243

Singh OP, Raghavendra K, Nanda N, Mittal PK, Subbarao SK (2002) Pyrethroid resistance in *Anopheles culicifacies in* Surat district, Gujarat, West India. Curr Sci 82:547–550

Suman DS, Parashar BD, Prakash S (2010a) Efficacy of various insect growth regulators on organophosphates resistant immatures of *Culex quinquefasciatus* (Diptera: Culicidae) from different geographical areas of India. J Entomol 7:33–43

Suman DS, Tikar SN, Parashar BD, Prakash S (2010b) Development of insecticide resistance in *Culex quinquefasciatus* mosquito (Diptera: Culicidae) from different locations in India. J Pest Sci 35(10):9–36

Thavaselvam D, Kumar A, Sumodan PK (1993) Insecticide susceptibility status of *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti* in Panaji, Goa. Ind J Malariol 30:182

Van Bortel W, Trung HD, Thuan le K, Sochantha T, Socheat D, Sumrandee C et al (2008) The insecticide resistance status of malaria vectors in the Mekong region. Malar J 7:102

Vatandoost H, Vaziri VM (2004) Larvicidal activity of a neem tree extract (Neemarin) against mosquito larvae in the Islamic Republic of Iran. Eastern Med Health J 10:573–581

Vijayan VA, Sathish Kumar BY, Ganesh KN, Urmila J, Fakoorziba MR, Makkapati AK (2007) Efficacy of piperonyl butoxide as a synergist with deltamethrin on five species of mosquitoes. J Com Dis 39(3):159–163

Wattal BL, Bhatnagar VN, Joshi GC (1975) Efficiency of different larvicides against Anopheles and Culicine mosquitoes in weekly application schedules. J Com Dis 7:358–368

WHO (2001) Amount of poor-quality pesticides sold in developing countries alarmingly high [Media release]. Food and Agricultural Organization of the United Nations/WHO, Rome/Geneva http://www.fao.org/waicent/ois/press_ne/presseng/2001/pren0105.htm

World Health Organization (2003) Specifications and evaluations for public health pesticides. Geneva

World Health Organization (2004) Global strategic framework for integrated vector management. WHO/CDS/CPE/PVC/2004.10

Yebakima A, Raymond M, Marquine M, Pasteur N (1995a) Resistance to organophosphate insecticides in *Culex pipiens quinquefasciatus* from Martinique. J Med Entomol 32:77–82

Yebakima A, Raymond M, Marquine M, Pasteur N (1995b) Resistance to organophosphate insecticides in *Culex quinquefasciatus* from Martinique. J Med Entomol 32:77–82

Perspectives of Lymphatic Filariasis with Special Reference to "Sleepada": An Ayurvedic Loom

24

Kancherla Prameela Devi, P. Srinivas, and Rohini

Abstract

The disease sleepada is analogous to be lymphatic filariasis described in detail in ancient Ayurvedic classics in India. Based on the evidence of literature, it is known that in India, since the time immemorial, filarial population was there. Today India is the largest endemic country of filariasis in the world contributing about 40% of total global burden of filariasis. In Ayurvedic treatises like *Charaka Samhita*, *Sushruta Samhita*, *Astanga Sangraha*, *Astanga Hridaya*, and *Madhava Nidana*, the disease was dealt thoroughly. In Ayurvedic literature, the word sleepada means stony foot, increase in the size of the foot, and a foot resembling the thickness of a lower timber of chariot. The role of vector was not directly mentioned in Ayurvedic classics, but the area in which breeding of the mosquitoes is very common was mentioned (Anonymous 2010). Medicinal plants are being used in India, especially in Ayurveda, from ancient times for the treatment of filariasis. Therapeutic regimens like external application of poultices, heat therapy, purgation, emesis, and bloodletting from specific points in the affected limb are also mentioned.

24.1 Introduction: Etymology of the "Sleepada"

Lymphatic filariasis (LF), the most significant neglected tropical vector-borne disease (VBD) globally, has posed a serious public health problem in India for decades (Anonymous 2012). Lymphatic filariasis is a common disease in India and existed since ancient times. Sushruta has provided the detailed description of Shlipada covering the etiopathogenesis, clinical features, types, and prognosis (Sushruta 2009a,

K. Prameela Devi (✉) · P. Srinivas · Rohini
Research Officer(Ay), Scientist-3, Achanta Lakshmipathi Ayurvedic Research Centre for Ayurveda, CCRAS, Ministry of AYUSH, Government of India, Chennai, India

© Springer Nature Singapore Pte Ltd. 2018

B. K. Tyagi (ed.), *Lymphatic Filariasis*, https://doi.org/10.1007/978-981-13-1391-2_24

b, c). Charaka explained this as a subtype in the chapter of Shotha (Agnivesa Charaka Samhita 2009a, b). Madhava seventh A.D. has given an independent disease entity to Shlipada and explained epidemiology, etiopathogenesis, classification, and prognosis. The age-old Indian science of human medicine, Ayurveda, explains a disease by name Sleepada," which is correlated with lymphatic filariasis (elephantiasis). In Sanskrit, "Shlee" means elephant and "Pada" refers to foot, meaning a disease condition where the foot appears like that of the "foot" of an elephant, termed as "Sheelapada." In **Sabda Kalpadruma** and the lexicon **Sabdasthomamahanidhi** (*Shanaih Shanairghanam Shopamtatprachashate*), it is described that the term Sleepada (= Sheelapada) must be understood as an increase in the size of the foot. Therefore, considering that there is a correlation of disease elephantiasis and Sleepada, it is considered opportune to describe the disease with treatment as available in Ayurveda.

24.2 Concept of VBD Epidemics in Ayurveda

The concept of communicable diseases is well defined and established in Ayurveda. Charaka (1500 BC–400 AD), the great physician of Ayurveda, has mentioned epidemic conditions under the section "Janapadodhwamsa" and dedicated a separate chapter on epidemic disorders named as Janapadodhwamsa Vimanam. The vitiation of factors such as Jala (water), Vayu (air), Desha (Place), and kala (Season) is considered to be responsible for the production of communicable diseases which can be well correlated with modern science. Susruta (1500 BC–500 AD) has very clearly defined such diseases under oupasargikarora, i.e., communicable diseases (Agnivesa Charaka Samhita 2009a, b; Sushruta 2009a, b, c).

24.3 Epidemiology and Etiopathogenesis

Etiological factors for sleepada can be classified as paryavara nidana (environmental factors), Ahara nidana (dietic factors), dosha pradhana (involvement of tridoshas (humors of body like vata, pitta, kapha).

The role of vector was not directly mentioned by Ayurvedic Acharyas, which is responsible for the dreadful disease Sleepada, but they mentioned the area in which breeding of the mosquitoes is very much common. In Ayurveda the incidence of this condition had been specified more in anupadesha (the geographical area surrounded by river banks, canals, having seacoasts, rich of water resources), sitala desha (the geographical areas where the atmospheric temperature is low through out the year), Purna Udakabhuyishtadesa (the geographical areas where there is scope of stagnation of water/improper drainage systems). These conditions provide a suitable environment for the growth of vectors. The diet and attitudes which aggravates the Kapha dosha plays important role in pathogenesis of Sleepada (Madhavakara 2006, Madhavakara 2006, Vagbhata 2000).

24.4 Pathogenesis of Sleepada

Lymphology is found in the ancient books of the Indian system of medicine under the discipline of anatomy and embryology (*Sareerasthana*). *Sushruta Samhita* and *Ashtanga Hridaya* are the two classical ancient books that provide details on lymphology. *Rasa vaha srotas* is comparable to the medical lymphatic system.

Tridoshas (Vata, Pitta, Kapha) are vitiated by the above mentioned etiological factors and takes the path towards groins and gradually spreading to the rest of the parts of the legs. When the aforesaid is associated with fever and severe pain it is known as "Sleepada". Some times pathogenesis may manifest in ears, hands, eyes, penis, lips and nose also.

24.5 Correlation of Ayurvedic and Modern Concepts

The modern concept of plasma, tissue fluid, and lymph may be compared with Rasa dhatu. Rasavaha srotas (microchannels of the body) described in Ayurvedic literature may be compared with "lymphatic channels." "Rasasanchara" procedure, described in Sushruta, bears some importance. During the course of transport, all the "Rasa" get collected into Rasakulya (lymphatic duct) and vama rasa kulya (thoracic duct). Due to vitiation of Doshas, the Rasa comes downward to Vankshna, Uru, Janu, and Jangha and settles there for a certain period. After a long period, it travels toward the Pada (lower extremities), manifests as swelling, and becomes hard like stone. Sushruta and Charaka have classified the Shotha as Ekadeshashotha (localized edema) and Avayavasamutha Shotha (edema restricted to particular anatomical site).

24.6 Lakshnas (Symptoms) of Sleepada in Ayurveda

24.6.1 General Symptoms

The swelling begins at the groins and gradually spreads to the legs associated with fever and severe pain, and these symptoms may also prevail in the hands, ears, eyes, pains, and lips in a few patients.

24.6.2 Specific Symptoms

As a result of the etiological factors, tridoshas are situated and travel towards the groin, thighs, knees and wireless causing Sophia. Based on this, Sleepada is of three types, database, pittance and kaphaja (Table 24.1).

The vataja and kaphaja varieties may be compared with chronic filarial symptoms of modern literature.

Table 24.1 Classification of "Sleepada" in Ayurveda

Vataja	Pittaja	Kaphaja
1. Swollen legs look blackish, skin surface is rough, fissured severe and irregular pain, and fever	Affected part looks yellowish, soft on touch, and patient suffers from burning sensation along with fever	The affected part is oily, white, and pale in color, heavy and hard mild pain present

24.7 Prognosis of Shlipada

The swelling which grows upward like an ant-hill with many sprouts, chronic for more than 1 year and that is very big in size, is to be rejected. The Sleepada patient of Kaphaprakriti, having the habit of Kaphavardhaka ahara and vihara (dietics and life style that increases Kapha dosha), filarial parts with exudation, severe itching and manifested of all the symptoms at a time is also to be rejected.

24.8 Correlation of Ayurveda and Modern Concepts

According to modern literature too, prognosis is bad in most of the deformed chronic filarial legs including ant-hill deformity, legs with thrombosis, third-grade filarial legs, and elephantiasis.

24.9 Ayurvedic Preventive Measures and Principles of Ayurvedic Treatment

Fumigation with Nimbapatra (*Azadirachta indica*) and Tulasipatra (leaves of *Ocimum sanctum*) is to be given. In *Passiflora foetida* leaves which are locally known as "Davaradangi," the juice is used externally as Lepa to prevent mosquito bites (Folklore claim).

The following principles are pursued in practicing Ayurvedic treatment:

The lines of treatment of "Sleepada" as per Bhavaprakasa are langhana (fasting), Kaphaharalepa application (application of pastes), Sweda karma (fomentation), Virechana (purgation), Raktamokshana (bloodletting), Kaphaghna Chikitsa (treatment which reduces the kaphadosha), and Ushnopachara (heat therapy). Charaka has also advised Siraveda (pricking of vein), Kaphaghna Chikitsa (treatment which reduces the kapha dosha), and Sarshapa Lepa (application of paste of mustard seeds) over the affected area. Sushruta specially emphasized Raktamokshana (bloodletting) therapy for three types of "Sleepada" separately in specified areas. The vein situated two to four fingers above the ankle joint of the affected leg should be pricked for bloodletting in Vataja Sleepada. The vein situated below the ankle joint is pricked for bloodletting in Pittaja Sleepada, and the vein near the great toe is to be pricked for bloodletting in Kaphaja Sleepada (Sushruta 2009a, b, c; Bhavaprakasha 2002).

Therapies for each variety of Sleepada are indicated as follows.

24.9.1 Vataja Shlipada

(i) Intake of Erandataila (castor oil) + Gomutra (cow's urine).
(ii) Shuntikshirapaka (boiling of *Zingiber officinale* in milk and making it ¼ part) + Anna (rice) (prepared from old rice).
(iii) Trivritsneha, described in Vatavyadhi cikitsa.
(iv) Snehavasti (oil enema).
(v) Daha karma (cauterization therapy).
(vi) Pricking of vein (Siraveda): the vein situated two to four fingers above the ankle joint of the affected leg should be pricked.

24.9.2 Pittaja Shlipada

In this type along with oleation, mild fomentation is indicated. The treatments which are described for Pittja arbuda and Pittaja visarpa are also to be employed. Pricking of the vein (Siraveda): the vein situated below the ankle joint is pricked.

24.9.3 Kaphaja Shlipada

(i) Intake of astringent substances (Kashaya Dravya) with honey.
(ii) Intake of Haritaki (*Terminalia chebula*) with Gomutra (cow's urine).
(iii) Yogaratnakar indicated Lodhraishta, Pippalyadichurnam, Krishnadimodakam, Vidangaditaila, and Soureswaraghrita.
(iv) Siraveda (pricking of vein) is performed in between the toe and second toe.
(v) Lepa (application of pastes): Bhadradaru (*Cedrus deodara*) + Chitraka (*Plumbago zeylanica*) + Vidanga (*Embelia ribes* + Maricha (*Piper nigrum*) + Arka (*Calotropis procera*) + Nagar (*Zingiber officinale*) + lavana (salt).

Bhava Prakasha has advocated the following:

(i) External application of the paste prepared with sarshapa (mustard seed), shigru (drumstick) (*Moringa oleifera*), devadaru (*Cedrus deodara*), and Shunthi (*Zingiber officinale*) is useful.
(ii) Application of the paste of Punarnava (*Boerhavia diffusa*), Shunthi (*Zingiber officinale*), and sarshapa (*Brassica campestris*) mixed with kanji on affected part of Shlipada is beneficial.
(iii) Intake of decoction of Shakhotaka (*Streblus asper*) + Gomutra is beneficial.
(iv) Intake of Haritaki (*Terminalia chebula*) fried with Erandataila (castor oil) and mixed with Gomutra (cow's urine) is beneficial for all types of Shlipada.
(v) According to Bhaishajyaratnavali and others, Nityanandaras and Shakhotaka Ghana vati are also beneficial in Shlipada.

24.10 Some Important Therapeutic Regimens

24.10.1 External Application (Lepa)

(i) Datturadilepa.
(ii) Swetarkamoolalepa.
(iii) Manjistadilepa.
(iv) Guduchyadilepa.
(v) Paste prepared by mustard seeds + shigru (drum stikc), devadaru+ shunti (dry ginger).
(vi) RupikaMoolaValkalaLepa.
(vii) ChittrakaLepa.
(viii) DevadaruLepa.
(ix) Vidangaditailam.

24.10.2 Internally Used Treatment

1. Powders (Churnam):
   (i) RajaniChoorna.
   (ii) Intake of Erandataila (castor oil) + Gomutra (cow's urine).
   (iii) Pippalyadi Churnam.
   (iv) Vriddha Daruka Churnam.
   (v) Shunti Kshirapaka + old rice.
   (vi) Intake of decoction of Shakhotaka (Streblus asper) and Gomutra (cow's urine).
2. Ghritam (Ghee):
   (i) Saureswara Ghritam.
   (ii) Panchanana Ghritam.
   (iii) Vriddhadaruka Ghritam.
3. Tailam (Oil):
   (i) Vidangaditaila.
   (ii) Panchananan Taila.
4. Swarasa (Fresh juice):
   (a) Guduchiswarasa.
   (b) Puthikauranja Patra Rasam.
5. Few compound formulations:
   (i) Sleepadarilauha.
   (ii) Nityananda Ras.
   (iii) Guda Haridra.
   (iv) Krishnadamodakam.
   (v) Dhanyamulam.
   (vi) Lodharishta.
   (vii) Shakhotaka Ghana Vati.
   (viii) Slipdari Loha.

24.11 Treatment for Irreversible and Chronic Sleepada (Elephantiasis)

- Gets cured by application of pack of Sahadevi (*Vernonia cinerea*) mixed with tala phala rasa.
- Pana (oral intake): wet bolus of seven leaves of Tambula added with salt and consumed along with warm water cures Sleepada.
- Shakhota (*Streblus asper*) bark decoction added with Gomutra (cow's urine) and consumed cures Sleepada.
- Rajani (turmeric powder) mixed with guda and consumed with Gomutra (cow's urine).
- Powder of varshbhu (Punarnava), Triphala, and Pippali mixed with honey and consumed cures sleepada.
- The patient of sleepada who consumes Hareetaki (*Terminalia chebula*) fried in erandataila (castor oil) along with Gomutra (cow's urine) everyday get cures of sleepada.

24.12 Other Treatment Modalities Prescribed by Ayurvedic Treatise

Vaidyacintamani, a treatise on Ayurveda, prescribes daivachikitsa in which one has to perform Chandrayana (moon) vrata up to 1 month by taking only milk. Furthermore, offering Dana by preparing a statue made in the shape of Tripada, holding Dhanus, knife, and falling down posture is to be donated (***ref: vaidyacintamani (53/2***).

24.12.1 Pathya (Do's) Dietetics Beneficial in Sleepada

The old shali and Shastika rice, yava (barley), kulattha (horse gram), Lasuna (garlic), Patola (snake guard), Vartaka (Brinjal), shigru (drumstick), Karavellaka (bitter guard), root of Punarnava (*Boerhavia diffusa*), upodika (*Basella rubra*), Erandataila (castor oil), Surabhijala (cow's urine), Kattu and Tikta rasa pradhanaahara (diets enriched of pungent and bitter taste), and Dipaniyadravya (substances which enhance the digestive capacity) are the main diet for Sleepada patients (Cintamani 2014).

24.12.2 Apathya (Don'ts): Nondietetics Harmful in Sleepada

Pishtanna (food variety prepared from rice floor), dugdhavikara (dairy products), guda (jaggery), Anupamamsha (meat of the animals living in humid atmosphere), diets having Madhura and Amla rasa (sweet- and sour-tasted diets), Guru ahara (heavy diet or the diets which are difficult to digest), Pischchhilaahara (oily and

fatty diet), and Abhishyandiahara (diet which obstructs the srotas, viz., lymph channels) are to be avoided. Also the water of Sindhu River and the rivers which are flowing from Vindya and Mahendra hills should be avoided.

24.13 Conclusion

Though path physiological terms were not known back, classical Ayurveda explains Sleepada with similar clinical feature and pathophysiology of elephantiasis. When the diseases worsened, Ayurveda may support with its multiform drug approach.

Acknowledgment The authors express their sincere gratitude to their organization, the CCRAS, New Delhi, for all the encouragement and help extended in preparing this manuscript.

References

Agnivesa (2009a) Charaka Samhita. Chikitsasthana, 12th Chapter (Vaidya Jadavji Trikamjiacharya, ed). Chowkhambha-prakashan, Varanasi, p 490

Agnivesa (2009b) Charaka Samhita. Vimana Sthana, 2nd Chapter (Vaidya Jadavji Trikamjiacharya, ed). Chowkhambha-prakashan, Varanasi

Anonymous (2010) Reported medical practices on prevention, management of vector- borne and infectious diseases through Ayurveda and Siddha. CCRAS, Department of Ayush, Goverment of India, New Delhi

Anonymous (2012) Perspectives of Indian medicinal plants in the management of lymphatic filariasis. ICMR, Goverment of India, New Delhi

Bhavaprakasha B (2002) (Sanskrita-English translation by Sri Kanthamurthy K.R.), Krishnadas Academy, Varanasi, pp 546–547

Vaidya Cintamani (2014) 53rd Chapter, 30,31 and 32 slokas, 1st edn, Chowkhambha Orientalia, Varanasi, p 496

Madhavakara (2006) Madhavanidanam, 39th Chapter (Sri Brahmashankarashastri Bhishagratna, ed). Sanskruta Samsthan, Varanasi, pp 340–341

Sushruta (2009a) Susruta Samhita. Nidanasthana 12th Chapter (Vaidya Jadavji Trikamjiacharya, ed). Chowkhambha Sanskruta Samsthan, Varanasi, pp 317–318

Sushruta (2009b) Susruta Samhita. Chikitsasthana 19th Chapter (Vaidya Jadavji Trikamjiacharya, ed). Chowkhambha Sanskruta Samsthan, Varanasi, pp 477–785

Sushruta (2009c) Susruta Samhita, Nidanasthana 5th Chapter (Vaidya Jadavji Trikamjiacharya, ed). Chowkhambha Sanskruta Samsthan, Varanasi

Vagbhata (2000) Ashtanga Hridroga with the commentaries of sarvangasundaram of Arunadatta and Ayurveda Rasayana of Hemadri. Reprint. Varanasi Krishnadas Academy