WORLD HEALTH ORGANIZATION
GLOBAL PROGRAMME TO ELIMINATE
LYMPHATIC FILARIASIS

LYMPHATIC FILARIASIS:
PRACTICAL ENTOMOLOGY

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A HANDBOOK
FOR NATIONAL ELIMINATION PROGRAMMES

World Health Organization

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Preface

The goal of the World Health Organization's (WHO's) Global Programme to Eliminate Lymphatic Filariasis (GPELF) is to eliminate the disease as a public health problem by 2020 (*1*). The aims of the Programme are (i) to interrupt transmission with mass drug administration and (ii) to manage morbidity and prevent disability. In mass drug administration, all eligible people in all endemic areas are given a single dose of two medicines together once a year for at least 5 years.

The GPELF has scaled up its activities more rapidly than almost any other global public health programme. By the end of 2011, 53 of 73 endemic countries were implementing mass drug administration, and more than 3.9 billion treatments had been delivered to 952 million people (*2*). Challenges remain, however, in meeting the goal of eliminating the disease. Vector control is a possible complementary strategy in countries or areas where mass drug administration has not started, such as those where loiasis is co-endemic; where the burden is heaviest and mass drug administration must be rapidly scaled up or where the expected impact of drug administration has not been achieved; and where local transmission has been interrupted to prevent recurrence. As the GPELF does not yet provide guidance on the use of vector control in national programmes, a WHO expert consultation meeting in 2012 (*3*) recommended that a practical entomological handbook be prepared to guide programme managers.

Aims of the handbook

The document explains why vector control is important in national programmes and describes the preparation of a tailor-made vector control plan for national programmes. It outlines entomological procedures for regular and specific vector control and how data should be analysed for better overall

understanding of filarial transmission and vectors. The document will also be useful for teaching personnel in lymphatic filariasis programmes about the use and value of entomological procedures in overall epidemiological appraisal in the context of elimination.

The document is designed as a practical handbook for national lymphatic filariasis elimination programme managers and for entomologists and parasitologists. It will also be useful for programme staff working at regional and district levels, including those involved in vector control; development and technical agencies; nongovernmental organizations; and other organizations that support national programmes.

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Glossary

antigenaemia: presence of an antigen circulating in the bloodstream.

at-risk population: total population in the endemic implementation unit(s).

endemic area: implementation unit where the average resident population or any subunit of population, has an antigenaemia or microfilaraemia positivity rate equal to or greater than 1%.

gonotrophic cycle: the cycle of blood-feeding, egg maturation and oviposition.

human blood index: proportion of females of a given species found to have human blood in their stomachs.

infection rate: proportion of mosquitoes infected with any stage of lymphatic filariasis worm.

infective rate: proportion of mosquitoes infected with L3 stage lymphatic filariasis larvae.

implementation unit (IU): the administrative unit in a country which is used as the basis for making decisions about implementing MDA. The IU must be defined before mapping takes place.

integrated vector management: a rational decision-making process to optimize the use of resources for vector control.

L1, L2 and L3: filarial larval stages 1, 2 and 3; L3 is the infective stage.

lymphatic system: the network of nodes and vessels that maintain the delicate fluid balance between the tissues and blood. It is an essential component of the body's immune defence system.

mass drug administration (MDA): a modality of preventive chemotherapy in which anthelminthic medicines are administered to the entire population of an area (e.g. state, region, province, district, sub-district, village) at regular intervals, irrespective of the individual infection status.

microfilariae: microscopic larval stage of lymphatic filariasis parasites that circulates in the blood and is transmitted by mosquitoes.

microfilaraemia: presence of microfilariae in the blood.

morbidity: clinical consequences of infections and diseases that adversely affect the health of individuals. Lymphatic filariasis causes chronic morbidity through damage to the lymphatic system, kidneys, arms, legs or genitals (especially in men).

neglected tropical diseases: a group of primarily infectious diseases that thrive in impoverished settings, especially in the heat and humidity of tropical climates. They have been largely eliminated elsewhere and thus are often forgotten. WHO focuses on the control of 17 neglected tropical diseases: dengue, rabies, trachoma, Buruli ulcer, endemic treponematoses, leprosy, Chagas disease, human African trypanosomiasis, leishmaniasis, cysticercosis, dracunculiasis, echinococcosis, foodborne-trematode infections, lymphatic filariasis, onchocerciasis, schistosomiasis and soil-transmitted helminthiases.

preventive chemotherapy: the use of anthelminthic drugs, either alone or in combination, as a public health tool against helminth infections. MDA is one modality of preventive chemotherapy.

recrudescence: a new outbreak of infection after a period when transmission is controlled.

surveillance: the ongoing, systematic collection and evaluation of data describing the occurrence and spread of disease. The part of the programme aimed at the discovery, investigation and elimination of continuing transmission, the prevention and cure of infections, and the final substantiation of claimed absence of transmission.

transmission assessment survey: a survey designed to measure whether evaluation units has lowered the prevalence of infection to a level where recrudescence is unlikely to occur, even in the absence of mass drug administration interventions.

verification: the procedure for countries to present evidence for external verification of absence of lymphatic filariasis transmission and receive official recognition for the success of their efforts.

xenomonitoring or xenosurveillance: method in which the infection rate in the mosquito population is used to determine whether transmission is still occurring in the human population.

Section 1

Background

Lymphatic filariasis is a neglected tropical disease caused by infection with the mosquito-borne, thread-like, parasitic filarial worms *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*. It is an ancient disease, with significant social and economic consequences for affected individuals, families and communities. The worst symptoms of chronic disease generally appear in adults, in men more often than in women, and include damage to the lymphatic system, arms, legs or genitals, which causes significant pain, wide-scale loss of productivity and social exclusion. Research during the past 20 years has led to new treatment regimens, strategies and diagnostic tools, which have dramatically changed the prospects of lymphatic filariasis control and led to the launch of the Global Programme to Eliminate Lymphatic Filariasis (GPELF) in 2000. The core objective of GPELF is to interrupt transmission of lymphatic filariasis through mass drug administration.

Lymphatic filariasis is transmitted by many species of mosquitoes in four principal genera—*Anopheles*, *Culex*, *Aedes* and *Mansonia* (*Annex 1*), the distribution, ecology, biology and transmission potential of which vary greatly. As transmission efficiency differs considerably by vector species, it is important to understand the entomological aspects of transmission of lymphatic filariasis.

1.1. Goals, strategies and challenges

The GPELF was launched in 2000 in response to World Health Assembly resolution WHA50.29, which requests Member States to initiate activities to eliminate lymphatic filariasis (*Box 1*). The goal of the GPELF is to eliminate the disease. Its aims are to interrupt transmission by delivering a combination of two medicines to entire populations at risk, by mass drug administration, and to manage morbidity and prevent disability (*1*).

Box 1. Target for elimination and definition of lymphatic filariasis

Strategic plan 2010–2020

The target for elimination of lymphatic filariasis was based on the status of control in countries and published in the *Progress report 2000–2009 and strategic plan 2010–2020* (*1*) of the Global Programme to Eliminate Lymphatic Filariasis.

Table 1. Targets in the Global Programme to Eliminate Lymphatic Filariasis for interrupting transmission in endemic countries, by year

YEAR	CATEGORY (OBJECTIVE)[a]			
	Starting (implementation begun)	Scaling up MDA (full geographical coverage achieved)	Stopping interventions and starting surveillance (MDA stopped and post-MDA surveillance established)	Verifying absence of transmission (countries verified as free of lymphatic filariasis)
2012	85	70	25	20
2014	100	75	40	20
2016	100	100	70	40
2018	100	100	75	45
2020	100	100	100	70

MDA, mass drug administration

[a]Values are proportions of national programmes that should achieve the specified indicators of interrupted transmission.

Roadmap for neglected tropical diseases, 2012

In January 2012, WHO published *Accelerating work to overcome the global impact of neglected tropical diseases: a roadmap for implementation* (*4*), which set the targets for prevention, control, elimination and eradication of 17 neglected tropical diseases or conditions, including lymphatic filariasis.

Roadmap for lymphatic filariasis

The GPELF is a vital player in the control of lymphatic filariasis and interruption of transmission by regular mass drug administration. In some countries, this intervention might have to be supported by vector control.

Despite significant successes, achieving the goal of elimination by interrupting transmission remains a challenge in the many places where clinical cases persist. If current levels of intervention are maintained, elimination in all Pacific islands except Papua New Guinea can be achieved by 2015.

By 2017, 70% of all endemic countries will have met the criteria for stopping interventions and will have entered the post-intervention (MDA) surveillance phase.

By 2020, all endemic countries will have been verified as free of transmission or will have entered post-intervention (MDA) surveillance.

Second report on neglected tropical diseases, 2013

The second report on neglected tropical diseases (*5*) provided definitions and indicators for elimination of lymphatic filariasis as defined in the roadmap.

Target: Global elimination of lymphatic filariasis by 2020

Scope: Global elimination of lymphatic filariasis as a public health problem (*6*)

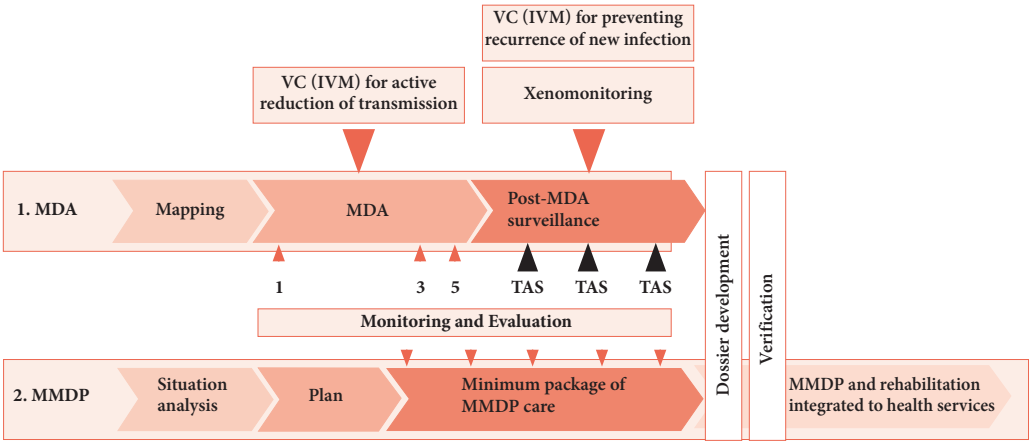
Operational definition: Prevalence of infection with *Wuchereria bancrofti*, *Brugia malayi* or *B. timori* below target thresholds in all endemic areas in all countries

Indicators: Prevalence as defined for the various species and vector complexes in transmission assessment surveys (*Box 2*)

Figure 1 illustrates the overall framework and programme steps of the GPELF, which has two pillars: mass drug administration and morbidity management and disability prevention. The figure also illustrates the roles of two entomological approaches, vector control and xenomonitoring or xenosurveillance. Four sequential steps are recommended by WHO (*1*):

- mapping the geographical distribution of the disease;
- mass drug administration for ≥ 5 years to reduce the number of microfilariae in blood to levels that will prevent mosquito vectors from transmitting infection;
- surveillance after mass drug administration has been discontinued; and
- verification of interruption of transmission.

Figure 1. Overall framework of the GPELF and sequential programme steps recommended by WHO



IVM, integrated vector management; MDA, mass drug administration; MMDP, morbidity management and disability prevention; TAS, transmission assessment survey; VC, vector control

The recommended regimen for mass drug administration is annual treatment with a single dose of two medicines given together for at least 5 years:

- ivermectin (150–200 µg/kg) plus albendazole (400 mg) in areas where onchocerciasis is co-endemic, or
- DEC (diethylcarbamizine) (6 mg/kg) plus albendazole (400 mg) in areas where onchocerciasis is not endemic.

The effectiveness of mass drug administration in reducing the prevalence of microfilariae in the community is directly related to coverage with treatment.

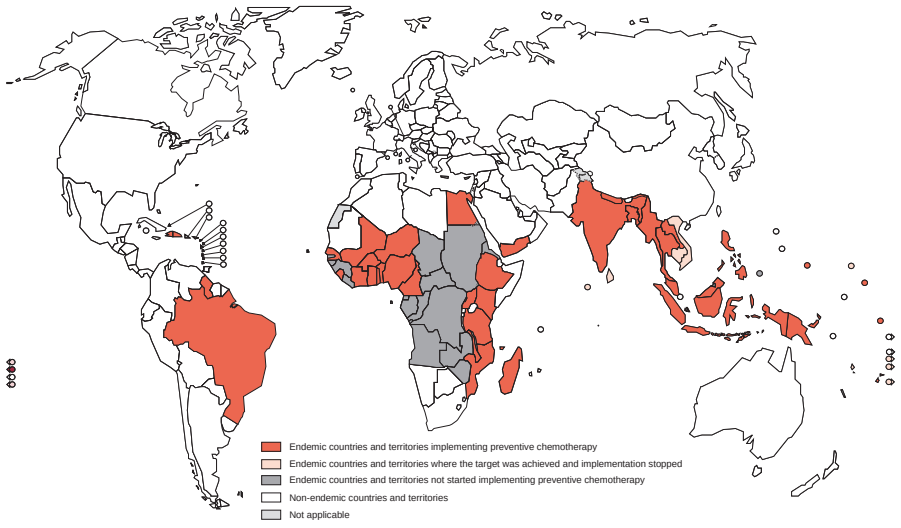
While annual mass drug administration for the prevention of microfilariae transmission from person to mosquito remain the standard intervention for interrupting transmission, use of vector control to reduce the number of potential mosquito vectors is increasingly recognized as a complementary strategy in some situations (*9*, *10*).

1.2. History and impact of the Programme during the first 10 years

In 2010, WHO published the *Progress report 2000–2009 and strategic plan 2010–2020 of the Global Programme to Eliminate Lymphatic Flariasis* (*1*), an assessment of progress made in the first 10 years of GPELF, lessons learnt, major challenges and future opportunities with an updated strategic plan for the next 10 years.

The GPELF is one of the most rapidly expanding global public health programmes in history. During its first decade, it focused on launching the Programme, which involved preparing guidelines based on existing information, initiating programmes in every WHO region in which the disease was endemic and scaling up the programme as rapidly as possible. By the end of 2011, 53 of 73 endemic countries were implementing mass drug administration, of which 12 had moved to the surveillance phase. During 2000–2011, more than 3.9 billion doses of medicine were delivered to a cumulative targeted population of 952 million people (*Figure 2*); (*2*).

Figure 2. Distribution and status of mass drug administration for lymphatic filariasis worldwide, 2011



While significant progress has been made in the past decade, the focus in the second decade will be to accelerate the initiation and scaling-up of interventions, using effective tools and strategies, including vector control, to move to the surveillance phase with transmission assessment surveys (*Annex 2* and *Box 2*). The global target is for 100% of endemic countries to stop mass drug administration and move to the surveillance phase and for 70% of endemic countries to be verified for absence of transmission by 2020 (*1*).

Challenges remain in achieving the goal of eliminating the disease, and vector control may be a complementary strategy for this purpose.

Box 2. Transmission assessment surveys

In 2011, WHO published a standard method called the 'transmission assessment survey' for assessing whether a series of rounds of mass drug administration had successfully reduced the prevalence of infection to levels equal to or below critical cut-off thresholds for the various vector species and complexes. In areas where *W. bancrofti* is endemic and *Anopheles* and *Culex* is the principal vector, the target threshold is <2% antigenaemia prevalence. In Bancroftian areas where *Aedes* is the primary vector, the target threshold is <1% antigenaemia prevalence. In areas where *Brugia* spp. is endemic, the target threshold is <2% antibody prevalence. The results are used to decide whether mass drug administration can be stopped. Transmission assessment surveys should be a standard component of monitoring and evaluation for elimination programmes (*7, 8*). The objectives of a transmission assessment survey are to:

- use a simple, robust survey design for documenting that the prevalence of lymphatic filariasis among 6–7-year-old children is below a predetermined threshold;
- provide evidence for programme managers that mass drug administration can be stopped; and
- assure national governments that national programmes have achieved their elimination goal.

WHO recommends that all Member States in which lymphatic filariasis is endemic implement transmission assessment surveys at the end of the mass drug administration phase in order to move to the surveillance phase.

1.3 Elimination of lymphatic filariasis in the integrated approach

In 2007, WHO published the *Global plan to combat neglected tropical diseases 2008–2015* (*11*), containing its vision of "a world free of neglected tropical diseases and zoonoses" through a strategy of "integrated approaches". The second report, published in 2012 (*5*), highlighted the work of overcoming neglected tropical diseases on the basis of five public health strategies:

- preventive chemotherapy, integrating approaches and packages to deliver multiple interventions;
- innovative, intensified disease management, ensuring that all people have free, timely access to high-quality medicines, diagnostic and preventive tools and services;
- vector control and pesticide management, strengthening and building capacity for integrated vector management (*Box 3*);

- safe drinking-water, basic sanitation and hygiene services and education; and
- veterinary public health services

Although one approach may predominate for the control of a specific disease or group of diseases, evidence suggests that control is more effective when several approaches are combined and used locally.

Box 3. Integrated vector management for lymphatic filariasis: WHO's position

Integrated vector management is defined as a "rational decision-making process for the optimal use of resources for vector control" (*12*). In this concept, several vector control tools are used to control one or more vector-borne diseases.

WHO published a general position statement on integrated vector management in 2008 to support it as a key component of vector-borne disease control (*12*). In 2011, WHO published the *Integrated vector management to control malaria and lymphatic filariasis* (*13*). In view of the overlapping geographical distribution of these diseases in large areas of Africa, Asia and the Americas and the fact that *Anopheles* mosquitoes transmit both malaria and lymphatic filariasis, the document recommended use of vector control in integrated vector management in:

- areas co-endemic for malaria and lymphatic filariasis; and in
- areas in which the vectors of the two diseases are affected by the same vector control interventions.

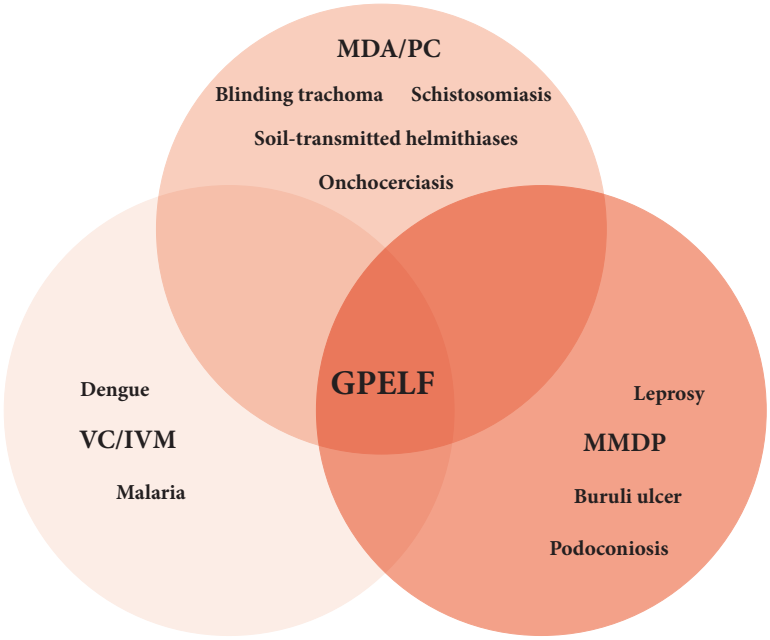
Integrated control of the *Aedes* mosquito vectors of dengue has also been recommended by WHO (*14*). The document cites examples of control of *Ae. aegypti* in some urban areas combined with control of *Culex quinquefasciatus*, an important urban vector of lymphatic filariasis.

The GPELF is now part of a comprehensive programme of neglected tropical disease control, in which mass drug administration, vector control and morbidity management are being integrated and delivered as a multi-intervention package at global, national and local levels (*15*). This intersectoral integrated approach presents an opportunity for even greater synergy between programmes for the elimination of lymphatic filariasis and other health programmes and of further extending the benefits of the GPELF to neglected populations who often suffer from several diseases linked to poverty. *Figure 3* shows three overlapping strategies used in the GPELF to control and eliminate neglected tropical diseases, while *Figure 4* shows mechanisms for integrating preventive chemotherapy and vector control into programmes for malaria, lymphatic filariasis and soil-transmitted helminthiases.

In March 2012, an expert consultation was held in Accra, Ghana, to discuss and identify strategies for interrupting lymphatic filariasis transmission in countries endemic for loiasis, for which mass administration of ivermectin may be unsafe. The meeting provisionally recommended biannual treatment with albendazole (400 mg) in combination with vector control (*3*), as most areas that are endemic for lymphatic filariasis and loiasis are also endemic for malaria, and the same vector species transmit the causative agents of malaria and lymphatic filariasis in these areas.

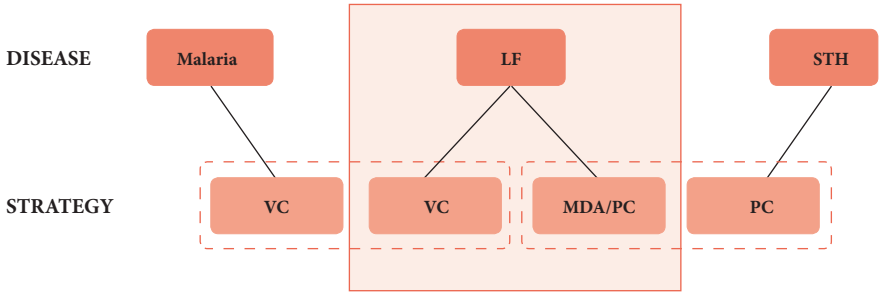
As the GPELF lacks guidance on the use of vector control in national programmes, the consultation and the Strategic and Technical Advisory Group for Neglected Tropical Diseases (STAG-NTD) recommended also preparation of a practical entomology handbook to guide programme managers in preparing vector control plans.

Figure 3. Three overlapping strategies used by the GPELF for elimination and control of neglected tropical diseases



GPELF, Global Programme to Eliminate Lymphatic Filariasis; IVM, integrated vector management; MDA, mass drug administration; MMDP, morbidity management and disability prevention; PC, preventive chemotherapy; VC, vector control

Figure 4. Mechanisms for integrating preventive chemotherapy (PC) and vector control (VC) with programmes for control or elimination of malaria, lymphatic filariasis (LF) and soil-transmitted helminthiases (STH)



Source: reference (*16*)
MDA, mass drug administration

Section 2

Role of entomology in the Global Programme

In 2002, participants in an informal consultation in Geneva, Switzerland, discussed the potential role of entomology in designing effective vector control against lymphatic filariasis. They identified operational research and reviewed the evidence for interventions (*16*). The current handbook translates the evidence into practice to support national programmes in formulating tailor-made vector control plans as appropriate.

2.1. Vector control

Lymphatic filariasis is transmitted when microfilariae circulating in the blood of an infected human are taken up in a blood-meal by a mosquito, where the microfilariae develop into infective larval stages. When the mosquito bites a human, the infective larvae migrate into the lymph system and adult worms develop. Therefore, transmission can be interrupted by killing adult worms, by killing microfilariae or by killing mosquito vectors or preventing them from biting humans.

As none of the drugs used in mass administration kill all adult worms, the aims of the current global strategy for interrupting transmission are (i) to reduce, using a combination of two microfilaricides, the density of microfilariae in the blood of infected people to a level at which they can no longer be transmitted by mosquito vectors to a new human host; and (ii) to reduce the prevalence of microfilariae in the community to a level at which transmission can no longer be sustained, even in the presence of mosquito vectors. These objectives are achieved by annual mass drug administration for at least 5 years, which is generally considered to be the reproductive lifespan of an adult worm in an infected human host.

While mass drug administration is the mainstay of lymphatic filariasis elimination, it is complicated biologically and logistically and by insufficient resources in some areas. Vector control could play a complementary role in lymphatic filariasis elimination

programmes at two stages: during mass drug administration, when it would complement the reduction in microfilariae density and prevalence by actively reducing transmission by mosquitoes; and during surveillance, by preventing recurrence or new infections after transmission has been interrupted (*Box 4*).

Box 4. Evidence of effectiveness of vector control in controlling lymphatic filariasis

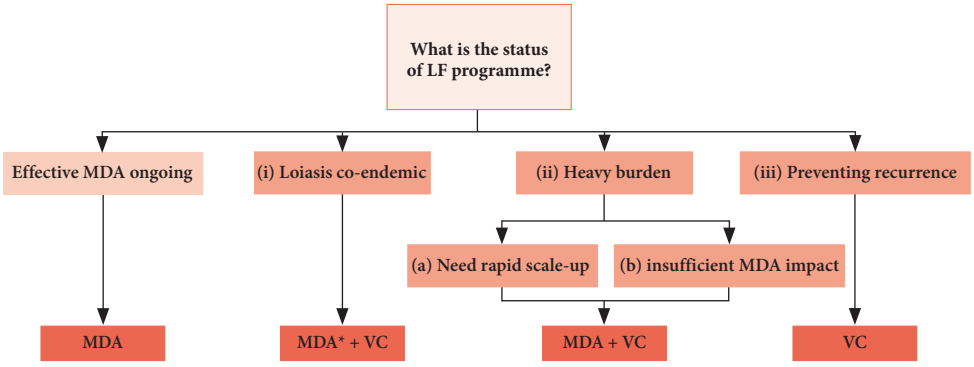
Before the GPELF began and before mass drug administration was used for interruption of the transmission of lymphatic filariasis, vector control appeared to be effective in some situations (*10*). In areas where malaria and lymphatic filariasis are transmitted by the same species of *Anopheles* vector, interventions for malaria, such as distribution of insecticide-treated bed nets and indoor residual spraying, had a significant impact, which may have been even greater against lymphatic filariasis than malaria. For example, after several years of indoor residual spraying with DDT for malaria control, lymphatic filariasis was apparently eradicated in the Solomon Islands (*17*). Further, use of untreated bed nets reduced vector infection rates and microfilariae prevalence in Papua New Guinea (*18, 19*).

Insecticide-treated nets are now widely used against malaria; however, for lymphatic filariasis, they are usually combined with mass drug administration (*20, 21*). Therefore, even if the vector infective biting rate and transmission potential are frequently reduced in such situations, the independent effect of vector control cannot be measured directly. In a recent trial in Nigeria, in an area where mass drug administration could not be used because of the presence of loiasis, full coverage of all sleeping spaces with long-lasting insecticidal nets alone halted the transmission of lymphatic filariasis (*22*). In India, 5 years of environmental management reduced transmission and microfilaria prevalence appreciably (*23*).

To determine the usefulness of vector control in lymphatic filariasis elimination programmes, the status of mass drug administration must be evaluated. Vector control can be beneficial in areas in which (*Figure 5*):

- mass administration of DEC or ivermectin cannot be initiated because lymphatic filariasis is co-endemic with loiasis,
- the burden of lymphatic filariasis is heavy and either mass drug administration must be rapidly scaled up or was not effective despite a number of rounds; and
- local transmission has been interrupted to prevent recurrence.

Figure 5. Steps in deciding whether vector control is required for elimination of lymphatic filariasis in countries where the disease is endemic



LF, lymphatic filariasis; MDA, mass drug administration; VC, vector control
* Mass drug administration with albendazole alone (400 mg) once or twice a year, twice a year being preferable

2.1.1 MDA phase

The first two situations listed above involve mass drug administration (*Figure 5*). In countries in which lymphatic filariasis is co-endemic with loiasis, which are situated mainly in Central and West Africa, the drugs given by mass administration cannot be used because patients co-infected with loiasis can develop serious reactions. The strategy prescribes the use of albendazole alone and vector control in these areas (*3*). Vector control can be done in coordination with malaria vector control programmes (see section 6).

In countries in which mass drug administration rounds are conducted annually, operational issues may compromise the required coverage and compliance rates. In this situation, countries should consider incorporating vector control. Countries in which the expected impact of mass drug administration has not been achieved, despite lengthy implementation, might also consider incorporating a vector control element. Vector control can increase the effectiveness of the elimination strategy in these situations by an additional effect on transmission, thus contributing to achieving or accelerating the process of elimination.

2.1.2 Post-MDA surveillance phase

Vector control can also be beneficial in lymphatic filariasis programmes in areas in which local transmission has been interrupted. The risk for recurrence of lymphatic filariasis after mass drug administration has stopped is unknown, but vector control could be a useful complementary strategy to maintain a transmission-free status and reduce the risk for re-introduction.

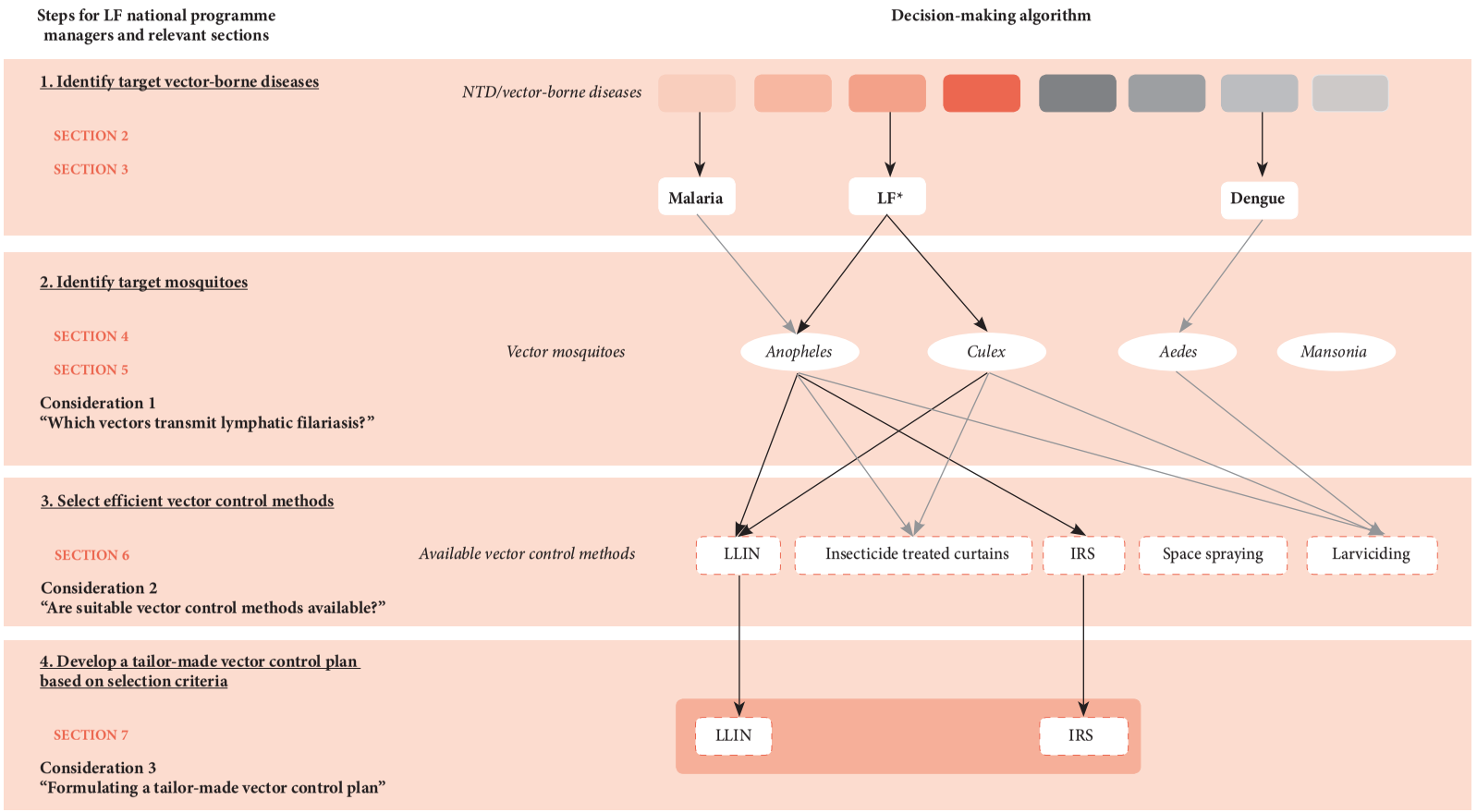
2.1.3 Considerations in preparing a tailor-made vector control plan

Once a decision is taken to incorporate vector control into a lymphatic filariasis programme, the following points should be considered in formulating a tailor-made vector control plan (*Figure 6*).

Consideration 1: Which vectors transmit lymphatic filariasis?

See section 4 for details. The appropriate strategies for vector control depend on a number of variables. The first is the mosquito genus or genera that is the main vector or vectors of lymphatic filariasis locally. The mosquitoes most frequently responsible for transmission are discussed by geographical region in section 3 (see also annexes 1 and 2), but the list is probably incomplete, and other mosquitoes may be involved locally. In areas where lymphatic filariasis is transmitted mainly by *Anopheles* mosquitoes, the practical aspects of vector control may be different from those in areas where the disease is transmitted by *Culex*, *Aedes* or *Mansonia* mosquitoes. Section 5 summarizes the main methods for sampling vectors if the vectors in your area are unknown and genus identification is required.

Figure 6. Example of steps in formulating a tailor-made vector control plan



IRS, indoor residual spraying; LF, lymphatic filariasis; LLIN, long-lasting insecticidal net; MDA, mass drug administration; NTD, neglected tropical disease; VBD, vector-borne disease
* To determine the usefulness of vector control for eliminating lymphatic filariasis in countries where the disease is endemic. (See also *Figure 5*).

Consideration 2: Are suitable vector control methods available?

All vector control methods are not equally effective against *Anopheles*, *Culex*, *Aedes* and *Mansonia* mosquitoes. The appropriate methods for each vector genus are discussed in detail in section 6. The effectiveness of a method depends on the local breeding ecology and behaviour of the vector and on the biting and resting behaviour of local species. Human behavioural and domestic conditions (e.g. activity period, self-protection behaviour, sanitation, housing conditions) are also important.

In general, *Anopheles* and *Culex* bite predominantly at night, and a number of anopheline species prefer to bite humans and to rest indoors. Each species can therefore be targeted with insecticide-treated nets, long-lasting insecticidal nets or indoor residual spraying campaigns. Nevertheless, insecticide resistance is a rising problem in many countries, and resistance can be particularly high in populations of *Anopheles* and *Culex*. Indoor residual spraying should not be done with an insecticide to which local mosquitoes are resistant (see *Annex 6*).

Aedes species bite mainly during the day; consequently, interventions to protect sleepers from adult mosquitoes are not very effective in reducing transmission. Control of larvae may be effective, particularly where breeding is concentrated in locations that are easily identified and treated. For example, *Culex* that breed in urban environments could be controlled by reducing larval sources, perhaps with active community involvement. *Aedes* species commonly breed in human environments by occupying small water-filled containers; control of these breeding sites requires intensive, systematic, routine removal, preferably by the community or with biological control agents.

Changes in the domestic environment can also reduce contact between vectors and humans. The measures include mosquito-proofing houses, improving self-protection and reducing larval sources. These methods are generally demanding and require training and considerable resources to achieve coverage of at-risk populations.

Consideration 3: Formulating a tailor-made vector control plan

Vector control experts are an important asset in vector control units in a health department or programme. Their presence is another consideration in deciding whether the lymphatic filariasis programme should include vector control in its strategy. Many countries in which malaria and lymphatic filariasis are co-endemic already have vector control programmes that target *Anopheles* mosquitoes for malaria control (*24, 25*). Effective coordination between the two programmes can ensure optimal use of resources to benefit both. Entomological surveillance, including monitoring of insecticide resistance and management, is essential for both malaria and lymphatic filariasis vector control.

When there is no vector control programme or expert, a decision must be made about whether investment in vector control will pay off in terms of removing lymphatic filariasis as a public health problem in the long term. This requires careful

analysis of the costs of training and infrastructure for vector control that is not limited to lymphatic filariasis but can be adapted for any other vector-borne disease in the context of an integrated vector management strategy and plan (see *section 7*).

2.2 Xenomonitoring or xenosurveillance

Entomological techniques are also useful for lymphatic filariasis programmes in a more indirect way. Direct assessment of worms in vector mosquitoes with polymerase chain reaction (PCR) techniques is increasingly used to detect recurrence of new infections during post-MDA surveillance (*26, 27*). This tool is called xenomonitoring or xenosurveillance.

As the threshold for lymphatic filariasis elimination is so low (1–2% antigenaemia prevalence in the human population (*Box 2*)), large numbers of mosquitoes must be collected and processed for testing with this method. The samples are usually examined in pools, the pool size being determined by the estimated prevalence of infection. The collection sites must be representative and widespread because of the heterogeneity in infection rates in humans. A standard protocol for sampling and testing is still not available.

2.3 Methodological issues in mosquito collection

As large numbers of mosquitoes must be collected in order to determine the low vector infection rates after mass drug administration, manual collection is not feasible, and various methods are being tested. Gravid traps are efficient for collecting large number of *Culex* mosquitoes (*28*), pyrethrum spray collection and CDC light traps are used routinely for collecting *Anopheles* mosquitoes in sub-Saharan Africa, and BG-sentinel mosquito traps baited with BG-Lure were used successfully to collect *Aedes* vector mosquitoes in American Samoa (*27*).

2.4 Other issues

The number of mosquitoes to be collected depends on the objective. In order to estimate an infection rate of 0.30% in *Culex* mosquitoes, about 5000 mosquitoes must be sampled and processed (*29*). Monitoring the small changes in infection rate expected during the surveillance phase requires processing about 10 000 mosquitoes. Vector infection thresholds differ by species, and the number of mosquitoes to be collected and processed is higher the lower the threshold.

Currently, a pool of 25 mosquitoes is often used for PCR processing in determining infection. Two-stage sampling is usually used, and the numbers of clusters and households per cluster to be sampled depends on the situation. Mass crushing of fresh adult mosquitoes is a rapid technique for identifying low levels of infection, provided the species of infective larvae is confirmed by microscopy or PCR (*30*).

Additional areas in which more research is needed are listed in *Box 5*.

Box 5. Potential areas for operational research in the entomology of lymphatic filariasis

Several priorities for research in lymphatic filariasis entomology would strengthen the evidence base for deciding on vector control strategies:

Vector-parasite compatibility

- The distribution and role of *Culex* mosquitoes in lymphatic filariasis transmission in Africa should be defined to guide vector control and integrated vector management. The literature suggests that West African *Culex quinquefasciatus* is refractory to infection, unlike those in other parts of Africa.
- More information is required on the distribution and role of *Mansonia* mosquitoes in lymphatic filariasis transmission.
- As many vector species are involved in the transmission of *W. bancrofti* filariasis in Africa, laboratory and field studies are needed to understand the vector–parasite relations for effective vector control.

Vector control

- In view of the increasing threat of insecticide resistance, non-insecticidal vector control methods should be a priority.
- The role of environmental management in vector control in specific settings should be established.
- Modelling studies are needed on the impact of vector control on lymphatic filariasis transmission.

Monitoring and implementation

- Various mosquito sampling protocols should be tested to identify robust sampling strategies for different vector areas.
- The PCR technique should be standardized for wider use by the network of regional reference laboratories in endemic countries.

Techniques

- Simple field methods are needed for measuring the vectorial capacity of different vector species.
- New tools should be developed and tested to simplify mosquito age-grading.
- Appropriate new methods should be developed for simple mass screening of large numbers of mosquitoes and for detection of infection in mosquitoes.

Section 3

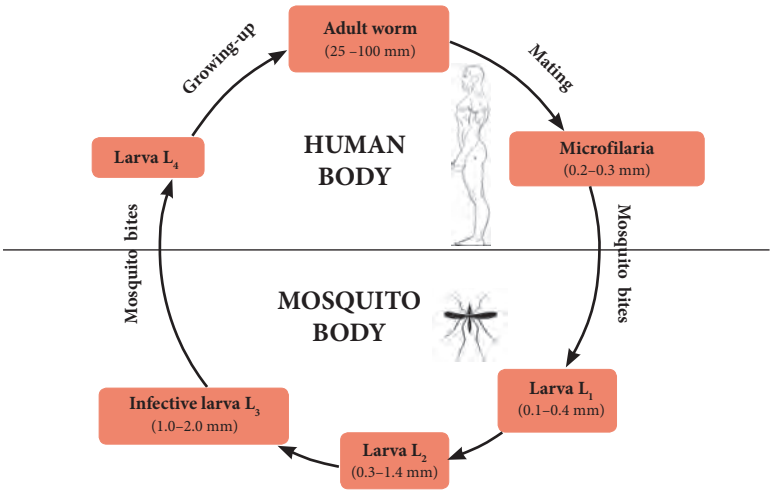
Dynamics of transmission of lymphatic filariasis

To define the role of entomology in GPELF, the dynamics of transmission of lymphatic filariasis parasites between their human host and their mosquito vector, the geographical distribution of filarial parasites and vector competence must be understood.

3.1 Filarial parasites

Lymphatic filariasis is caused by three species of parasitic worm, *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*, which have generally similar life cycles (*Figure 7*).

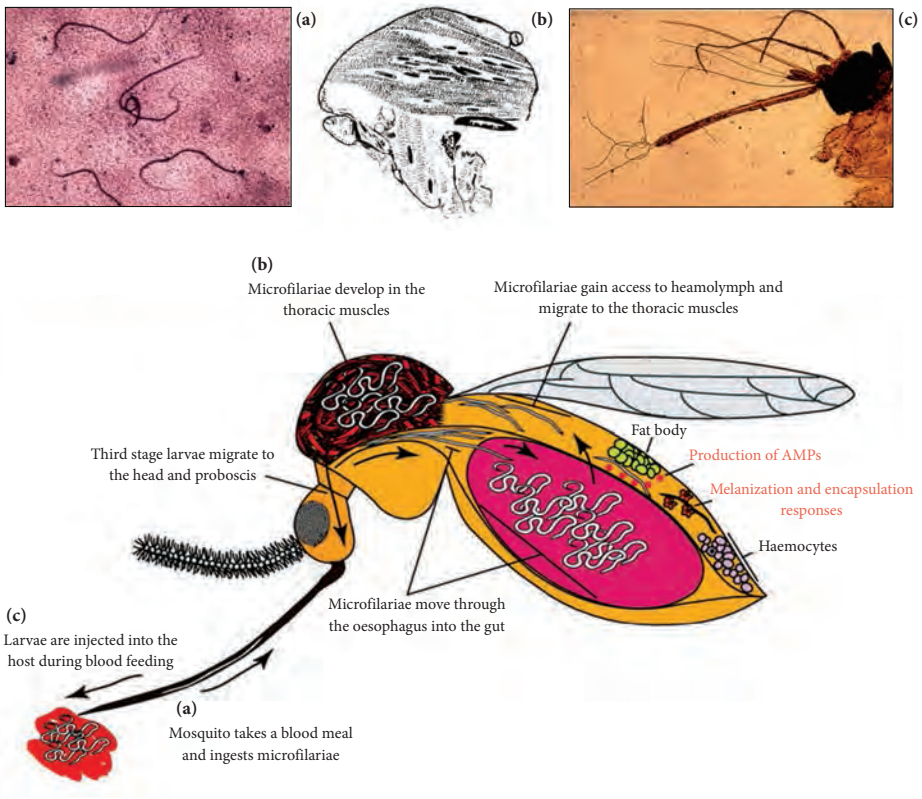
Figure 7 Filariasis life cycle: *Wuchereria bancrofti*



Source: reference *(31)*

In the human body, adult worms (male and female) live in nodules in the lymphatic system and, after mating, produce numerous microfilariae, which circulate in the bloodstream. The lifespan of adult worms is 4–6 years. Microfilariae migrate between the lymph system and blood channels to reach the peripheral blood vessels, often at times of the day that coincide with the peak biting activity of local vectors. When female mosquitoes ingest a blood meal, they consume microfilariae with the blood. In the mosquitoes' stomachs, they lose their sheath, and some of the parasites migrate through the stomach wall to reach the thoracic flight muscles, where they develop into first-stage larvae (L1). The larvae grow and moult into second-stage larvae (L2) and moult again to produce highly active infective third-stage larvae (L3), a process that takes 10–12 days from the L1 stage to the L3 stage. The infective larvae migrate to the mosquito's proboscis, where they can infect another human host when the mosquito takes a blood meal. The L3 are deposited on the skin and find their way through a bite wound. The L3 develop to fourth-stage larvae (L4) as they migrate through the human body to the lymphatic vessels and lymph nodes, where they develop into adult worms. See *Figure 8*.

Figure 8. *Wuchereria bancrofti* microfilaria L1 in human blood; (b) L2 larvae (sausage stage) in the thoracic muscles; and (c) L3 worms emerging from the proboscis of the mosquito



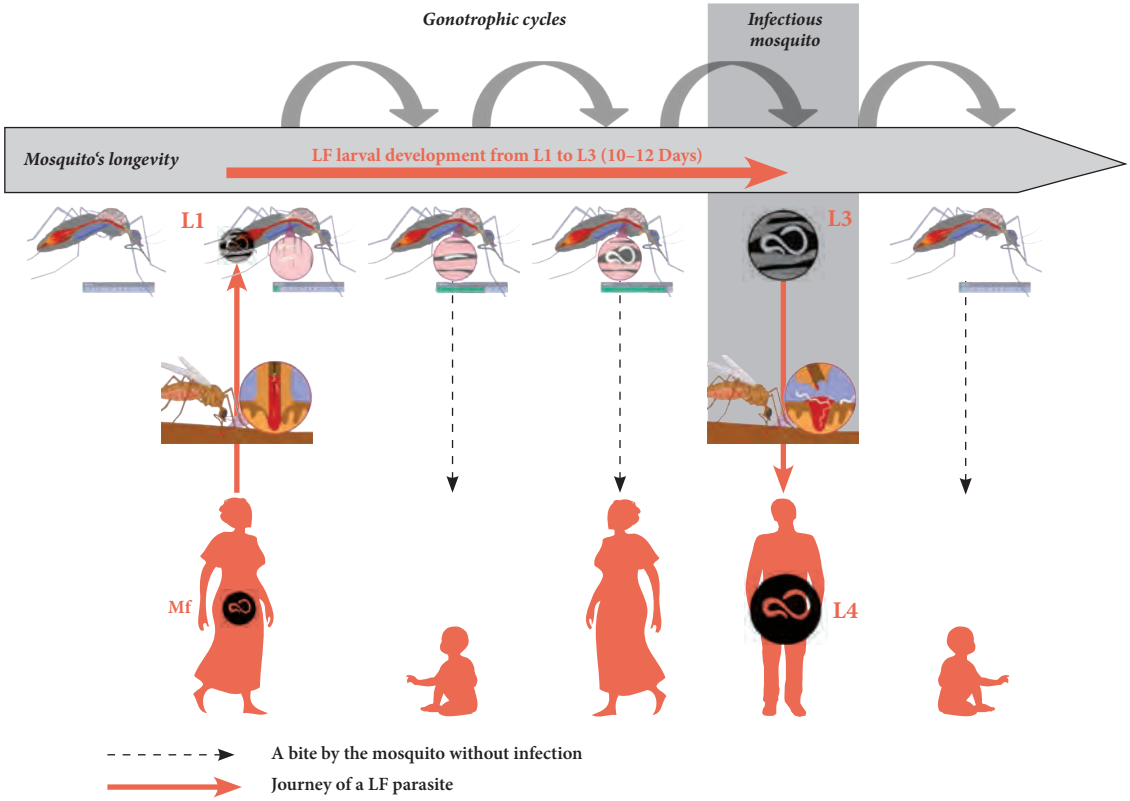
Images adapted from references (*31*) and (*32*).
AMP, antimicrobial peptide
Blood meal volume depends on the size of the mosquito and is around 2–4 microlitres.

3.2 Transmission in the vector

The transmission dynamics of lymphatic filariasis are complex, involving two genera of parasite (*Wuchereria* and *Brugia*) and a number of genera of mosquito carriers. The four main genera are *Anopheles*, *Culex*, *Aedes* and *Mansonia*.[1] The biological features of the vector–parasite relationship should be understood in order to define the entomological variables critical to lymphatic filariasis transmission and the threshold for interrupting transmission. Unlike the transmission of malaria and arboviruses, that of lymphatic filariasis is inefficient, and a large number of bites from infectious mosquitoes is required to initiate a new infection with microfilaraemia (*33*).

Many factors contribute to the inefficient transmission of lymphatic filariasis (*Figure 9*). Firstly, microfilariae do not multiply in the mosquito body; hence, the number of L3 is limited by the number of microfilariae ingested. Second, only those mosquitoes that survive more than 10 days will contribute to transmission of the parasites (*Figure 9* and *Box 6*). Those mosquitoes that die before the L3 develop cannot play a role in the transmission cycle. Third, the L3 are deposited on the skin and have to find their way into the bite wound (rather than being injected with the mosquito saliva like malaria sporozoites). In view of all these factors, the transmission of lymphatic filariasis parasites is considered to be less efficient than that of other vector-borne parasites, such as malaria and dengue.

Figure 9. Development and life stages of a lymphatic filariasis parasite in a mosquito



Source: reference *34*; images from reference *35*. In this figure, the gonotrophic cycle is defined as the time between two blood-feedings (see section 4.1.6) (*36*).
Mf, microfilariae

The rate of uptake of microfilariae by a mosquito vector from a human host depends on the prevalence and intensity of infection in the community and the biting rate of the mosquito. In general, the greater the number of infectious hosts available in a community with a moderate-to-high density of circulating microfilariae in their peripheral blood and the higher the biting rate, the higher the chance of a mosquito picking up microfilariae from a human host and causing transmission. Extremely high levels of microfilariae in the blood may, however, result in a substantial number of mosquito deaths as the larvae develop.

When a mosquito ingests large volumes of microfilariae, considerable damage can be caused to the mosquito's internal structures as the parasites pass through the stomach to reach the thoracic muscles. Furthermore, as L3 larvae emerge from the flight muscles they can cause irreversible harm, hindering the mosquito from flying and causing the death of the vector. The implications of these effects in the field require further research. The intensity of lymphatic filariasis transmission therefore depends on the biting rate of the vector, the proportion of mosquitoes with L3 larvae and the number of individuals carrying microfilariae.

In order to interrupt filariasis transmission, the intensity of microfilariae or the vector density must be driven below a threshold to ensure that no new infection occurs. The thresholds are likely to vary in different parts of the world because of the heterogeneity of the vector–parasite relationship. Local environmental conditions also affect transmission: rainfall, temperature, humidity and soil type can all affect the production of breeding sites and the survival of adult mosquitoes.

3.3 Transmission potential

Transmission potential can be calculated as the product of the mean number of infective larvae (L3) per infective mosquito and the estimated biting rate of the vector for a given period. It represents a summary of important vector and parasite characteristics in a combined measure of the risk of transmission over a particular period, which may be monthly or annual. As monthly transmission potential fluctuates seasonally with biting density, a full year's measurements should be used for the annual estimate.

The annual transmission potential is a useful indicator of risk for lymphatic filariasis transmission but has not been evaluated in detail with respect to the incidence of the disease, the transmission efficiency of each species of vector or the thresholds for elimination of lymphatic filariasis. More operational research is needed to determine competence and transmission risk. The critical level of control to prevent new cases of lymphatic filariasis infection will vary according to the vector species involved.

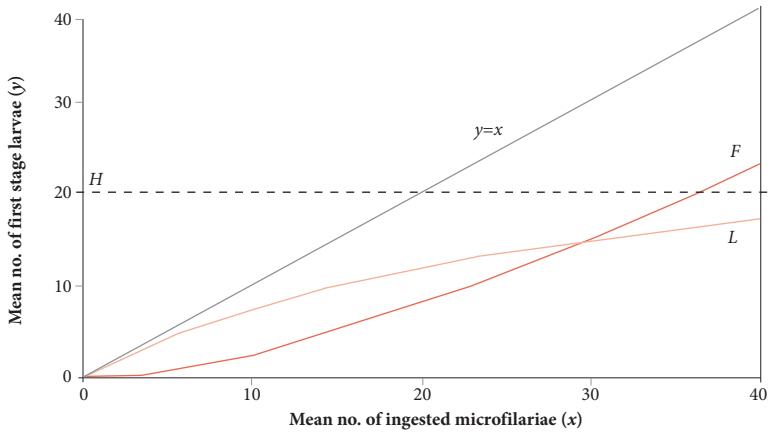
Further details on estimating annual transmission potential and other transmission parameters are given in *Annex 4*.

Box 6. Factors that affect the efficiency of lymphatic filariasis transmission in different vector genera

Facilitation and limitation

The rate of successful development from ingested microfilariae to infective L3 larvae in the mosquito vector depends on the number of microfilariae ingested and the genus of mosquito vector. Two types of vector–parasite relation, called facilitation and limitation, have been described (*Figure 10*).

Figure 10. Diagrammatic representation of limitation (L) and facilitation (F)



Source: reference (*37*)

In *Anopheles* mosquitoes, the proportion of microfilariae that reaches the L3 stage increases as the number of ingested microfilariae increases (facilitation). Low densities of microfilariae are associated with a much lower rate of development to L3. In contrast, in *Aedes* vectors of filariasis, low densities of ingested microfilariae have a high likelihood of survival but by a process known as limitation, whereby the proportion of ingested microfilariae that survive to become L3 larvae decreases as more microfilariae are ingested.

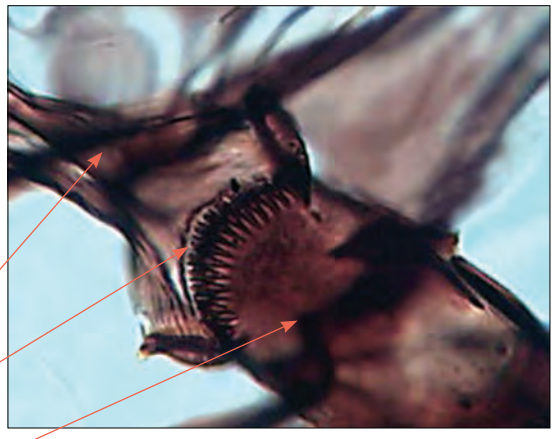
Reducing the microfilarial density (for example by mass drug administration) below a critical equilibrium between adult worms and microfilariae will eliminate the parasite population. As shown in *Figure 10*, this equilibrium is harder to reach for Aedes than for *Anopheles* mosquitoes.

Pharyngeal armature

The digestive tract of many filariasis vectors contains teeth-like structures (armatures) that protrude from the gut wall into the lumen (*Figure 11*). In anopheline mosquitoes, the pharyngeal armature is well developed, so that microfilariae are damaged when they are ingested.

In some species, these armatures serve as first lines of defence against filarial infection, by inflicting lethal injuries on microfilariae. The difference between culicines and anophelines with respect to the success of development of low densities of microfilariae to L3 may be associated with differences in the structure of the pharyngeal armature.

Figure 11. Pharyngeal and cibarial armature of *Anopheles gambiae* s.l.



Source: D. Boakye

Section 4

Vector biology and profile

Several species of four genera of mosquitoes—*Anopheles, Culex, Aedes* and *Mansonia*—play major roles in the transmission of lymphatic filariasis in different endemic regions (*annexes 1* and *2*). The distribution and the ecological and biological characteristics of these genera differ widely. Within a genus, differences in biting and feeding behaviour, resting and breeding preferences, seasonal abundance and affinity to human habitations determine their transmission potential. Understanding these characteristics helps in selecting appropriate vector control tools and strategies. The ecological and biological characteristics of each of the four genera are presented below.

4.1 Vector biology

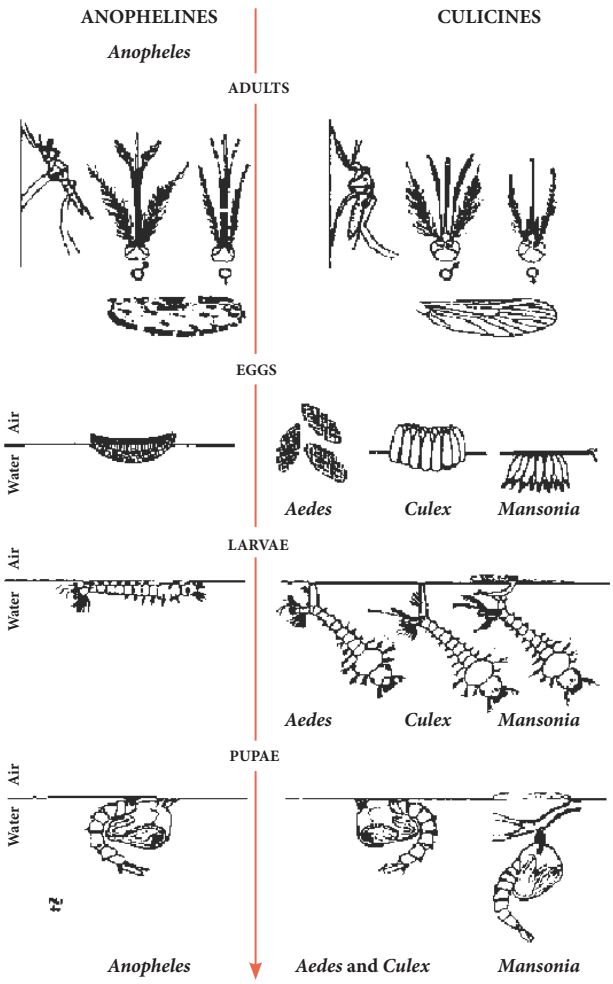
This section describes the biological characteristics of the mosquito vectors that are relevant to selecting appropriate vector control strategies:

- mosquito life cycle
- breeding sites and larval habitats
- flight range
- microfilarial periodicity and mosquito biting behaviour
- resting behaviour
- gonotrophic cycle
- parity rate

4.1.1 Mosquito life cycle

The mosquito life cycle has four stages: egg, larva, pupa and adult (*Figure 12*). While adults fly, the other three stages survive only in aquatic bodies and require 8–14 days to develop completely. Adult mosquitoes live for up to 1 month, but some do not survive beyond 1–2 weeks.

Figure 12 Mosquito life cycle



Source: reference *(38)*

Eggs

Almost all mosquito species lay eggs on water or moist surfaces. When deposited, the eggs are white, but they turn black or brown within 12–24 h. The eggs of most species measure about 0.5 mm. The incubation period, i.e. the time between deposition of eggs and their hatching into first-instar larvae, varies widely with species. It is usually around 2–3 days but is influenced by temperature. *Anopheles* have floats on either side of the egg and lay their eggs singly, while *Culex* mosquitoes lay them together in the form of a raft. Many *Aedes* species lay their eggs on moist surfaces or above the water line. As the eggs hatch only when the surface is submerged in water, the incubation period ranges from a few days to 1 year. In the absence of water, eggs remain dry and viable for many weeks without desiccating.

Larvae

Once the eggs hatch, the larvae grow in four stages known as the first, second, third and fourth instars. The larvae shed their skin at the end of each stage to allow further growth in a process known as 'moulting'. The instars have no legs but have well-developed heads with mouth brushes, and their bodies are covered with hairs. They feed actively on algae, yeasts, bacteria, organic matter and small aquatic organisms and swim by making sweeping movements with their bodies, giving them the nickname 'wrigglers'. Each larva has a siphon through which it takes in oxygen. *Anopheles* mosquito larvae have a rudimentary siphon and breathe horizontal to the water surface. *Mansonia* larvae do not have to surface to obtain air, as they breathe by inserting their siphons into plants growing in the water and remain there most of the time. The duration of the larval stage depends mainly on the temperature and ranges from 5 to 8 days. Upon full development, fourth-instar larvae moult into pupae.

Larvae occur in various habitats, ranging from very fresh to highly polluted water, and the water body can vary from small containers (e.g. egg shells, small puddles or discarded containers) to large streams, lakes and swamps.

Pupae

Unlike those of many other insects, mosquito pupae are very active. The pupa has a comma-shaped body with two distinct parts, the cephalothorax and the abdomen, with a pair of respiratory trumpets on the upper part. The abdomen consists of movable segments and carries a pair of paddle-like appendages at the tip. Pupae have no functional mouth part and do not feed but come to the water surface frequently to get oxygen. The pupal stage usually lasts 1–3 days, during which time all the larval tissues transform into adult tissues. When the pupae are mature, their skin splits on the surface of the water, and fully developed adult mosquitoes emerge.

Adults

The adult mosquito body consists of three parts: the head, thorax and abdomen. The head has a pair of eyes and a pair of antennae and has evolved to recognize sensory information. The antennae sense host odours and the odours emanating from breeding habitats. The head also has a pair of sensory palpi and mouth parts, which are well developed in female mosquitoes to pierce the host's skin and suck blood. The abdomen is evolved for digestion of food and development of eggs.

Newly emerged adult mosquitoes rest for a while in nearby shelters. After the body parts have firmed up, mating takes place. Mating occurs only once in lifetime. The adult mosquito is entirely terrestrial and capable of flying long distances. Both male and female mosquitoes feed on plant nectar, from which they derive energy. Males generally live for a shorter time than females—often not more than 1 week. Female mosquitoes bite and feed on the blood of warm-blooded birds and mammals (some species also feed on cold-blooded animals), as blood is essential for the development of eggs. A blood-fed

female mosquito gradually digests the blood and simultaneously develops eggs over 2–3 days. A female mosquito with fully developed eggs is termed 'gravid', and it searches for an appropriate habitat to lay the eggs. After laying the eggs, the mosquito takes a fresh blood-meal, a second batch of eggs develops, and the life cycle continues. Most mosquitoes survive to lay at least two batches of eggs, and only a small proportion live beyond a fourth egg laying. The survival and longevity of adult mosquitoes depend on temperature, humidity and the ability to find a host for blood-feeding after each egg laying.

4.1.2 Breeding sites and larval habitat

It is important to know where breeding sites are, as this is where eggs are laid by gravid mosquitoes and hence the source of new vectors for lymphatic filariasis transmission. The sites depend on the genus and species of mosquito and vary widely; therefore, larval control should be specific and suitable to the local setting. Not all breeding sites are clearly visible to humans, especially as some species prefer temporary water bodies (*Aedes* spp.) while others prefer more permanent ones (*Anopheles*, *Culex*, *Mansonia* spp.).

4.1.3 Flight range

The flight range indicates how far a mosquito species can travel to seek a host and, when gravid, deposit eggs. It therefore is a measure of the area over which vector control must be applied for effective control. *Anopheles* mosquitoes can usually travel further than *Aedes* spp.

4.1.4 Microfilarial periodicity and mosquito biting behaviour

The concentration of microfilariae in the peripheral blood of a host shows a daily pattern, called 'microfilarial periodicity', which differs for the three worm species responsible for lymphatic filariasis (*Table 2*). In 'nocturnal periodic filariasis', the microfilarial density is high during the night and almost inexistent during the day. 'Diurnal periodic filariasis' has the opposite periodicity, with microfilarial density high during the day. In 'diurnal sub-periodic filariasis', microfilarial density is higher at night, but microfilariae are still present during the day. The aperiodic form shows no specific periodicity, with the same microfilarial density at all times.

Microfilarial periodicity corresponds to the biting habits of the principal vectors, which ensures transmission. Furthermore, some mosquitoes seek blood-meals inside houses (endophagy), while others prefer to feed on hosts outside (exophagy). In order to protect humans from being bitten, a vector control method must be applied at the appropriate time of the day and in the correct place.

Anopheles mosquitoes, for example, characteristically bite more during the night and inside houses, therefore control strategies that are suitable for the day and outdoors are not appropriate control strategies for interrupting lymphatic filariasis transmission where these mosquitoes are the major vector (*Figure 13*). Correctly targeted vector control prevents host-seeking mosquitoes from finding a suitable human to feed from and to develop the eggs and find a place to lay them.

Table 2. Periodicity and distribution of organisms that cause human lymphatic filariasis

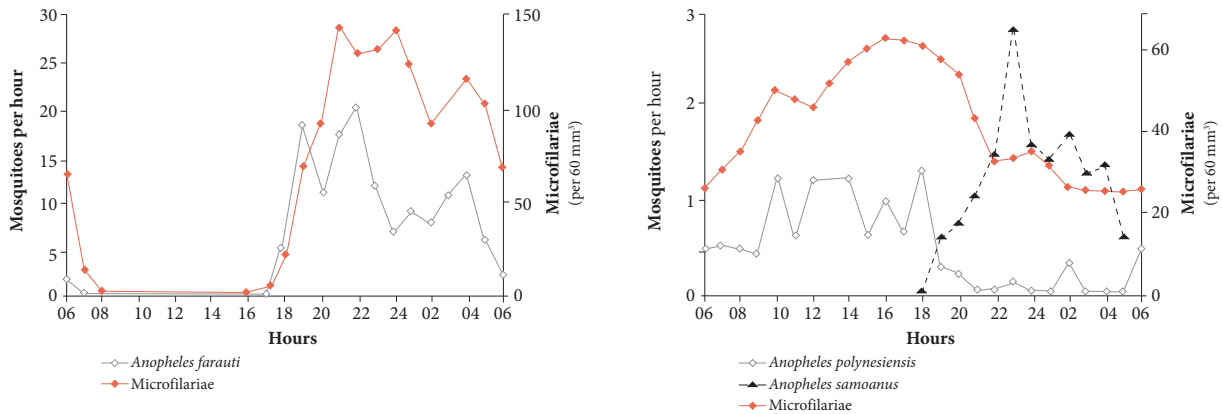
Organism	Periodicity	Distribution	Main vector
Wuchereria bancrofti	Nocturnal periodic	Worldwide, including Africa, Indonesia, Melanesia, Micronesia, Middle East, South America, South Asia	*Anopheles, Culex*
	Nocturnal sub-periodic	South-East Asia	*Aedes*
	Diurnal sub-periodic	Polynesia	*Aedes*
Brugia malayi	Nocturnal periodic	India, Indonesia, South-East Asia	*Anopheles, Mansonia*
	Nocturnal sub-periodic	Indonesia, South-East Asia	*Mansonia*
	Diurnal sub-periodic	Thailand	*Mansonia*
Brugia timori	Nocturnal periodic	Alor, Flores, Indonesia, Roti, Timor	*Anopheles*

Modified from reference (*39*)

4.1.5 Resting behaviour

After taking a blood-meal, female mosquitoes rest until the eggs are fully developed, either indoors (endophily) or outdoors (exophily). This behaviour determines the choice of vector control method; for example, indoor residual spraying or distribution of long-lasting insecticidal nets (see section 6) is suitable for controlling endophilic vectors.

Figure 13. Microfilarial periodicity and mosquito biting patterns



Source: reference *31*

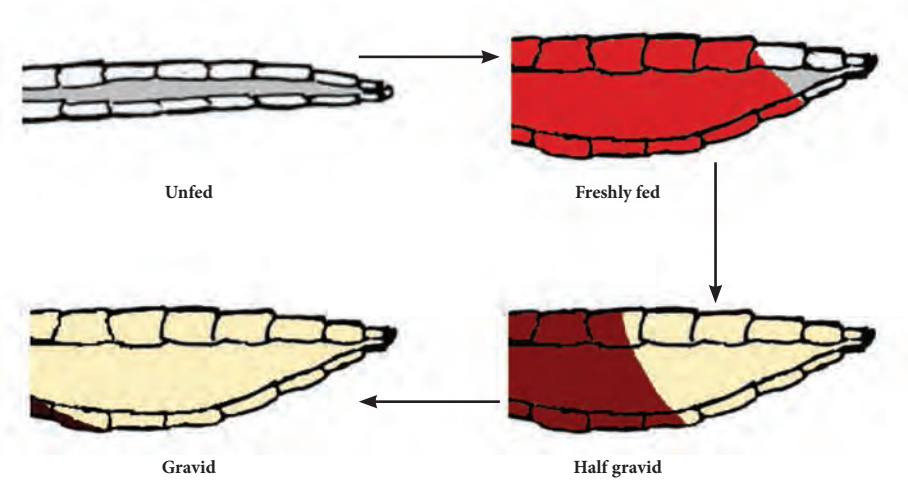
4.1.6 Gonotrophic cycle and lymphatic filariasis transmission

Mosquitoes ingest blood, gradually digest it to become fully gravid (*Figure 14*) and then lay eggs in their preferred breeding habitat. Blood-feeding, egg maturation and oviposition comprise the gonotrophic cycle, which is repeated several times in a mosquito's lifespan. In some cases, the first batch of eggs requires more than one blood-meal to mature, after which blood-meal and oviposition alternate regularly. The length of the gonotrophic cycle varies by genus and also depends on temperature. The duration of the cycle for most mosquito species is generally 3–4 days.

Lymphatic filariasis parasites take 10–12 days to develop from microfilariae to the L3 stage in mosquitoes. Therefore, mosquitoes must survive at least two gonotrophic cycles for 10–12 days in order to pick up microfilariae and transmit L3. The more gonotrophic cycles a mosquito survives, the longer its survival and the higher the probability of transmitting infection. Mosquitoes survive and live longer in favourable climatic conditions, and areas with such conditions are therefore more conducive for transmission of lymphatic filariasis.

Some mosquitoes feed only on humans and are known as 'anthropophilic', while others are less selective and can feed on humans and animals and are described as 'zoophilic'. Feeding on a variety of hosts can protect against the transmission of lymphatic filariasis, as the parasite will not be transmitted from an animal back to a human.

Figure 14. Appearance of the abdomen of a female mosquito by stage of the gonotrophic cycle



Source: reference (*40*)

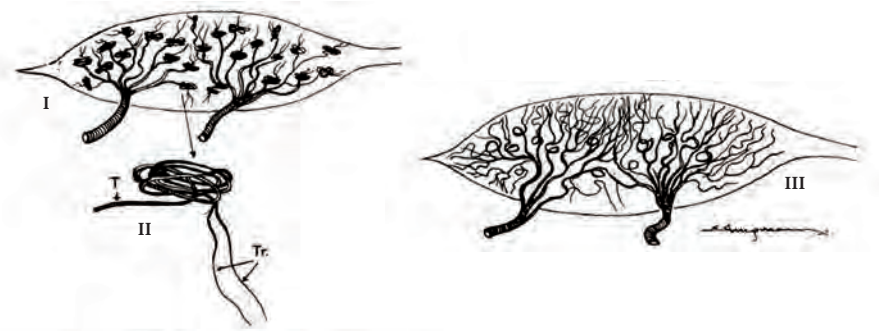
4.1.7 Parity rate

The number of gonotrophic cycles that a mosquito undergoes can be determined by examining the ovarioles. Mosquitoes that have laid eggs at least once and completed one gonotrophic cycle are parous, while those that have not laid eggs are nulliparous. Mosquitoes that have completed 1, 2 and 3 gonotrophic cycles are known as 1-parous, 2-parous and 3-parous, respectively. The higher the proportion of parous mosquitoes, the higher their age and survival and the higher the transmission potential. The parity rate is lower when the mosquito population contains more freshly emerged or nulliparous mosquitoes.

When vector control is successful, the population has a younger age structure, and a higher percentage of mosquitoes are nulliparous, because control prevents mosquitoes from taking a blood-meal, becoming gravid and depositing eggs. When vector control is not fully effective, mosquitoes can survive longer and the population has a higher proportion of older mosquitoes, which have been parous many times.

Parity is determined by dissecting out the ovaries in distilled water on a slide and examining them at 40x magnification. Only females that are unfed or freshly fed are suitable. *Figure 15* shows the appearance of the ovaries of a nulliparous and a parous female.

Figure 15. Parity as determined from the tracheoles (Tr) on a mosquito ovary: I, ovary of nulliparous female; II, end of a fine trachea (T) forming a skein; III, ovary of a parous female



Source: reference *(41)*

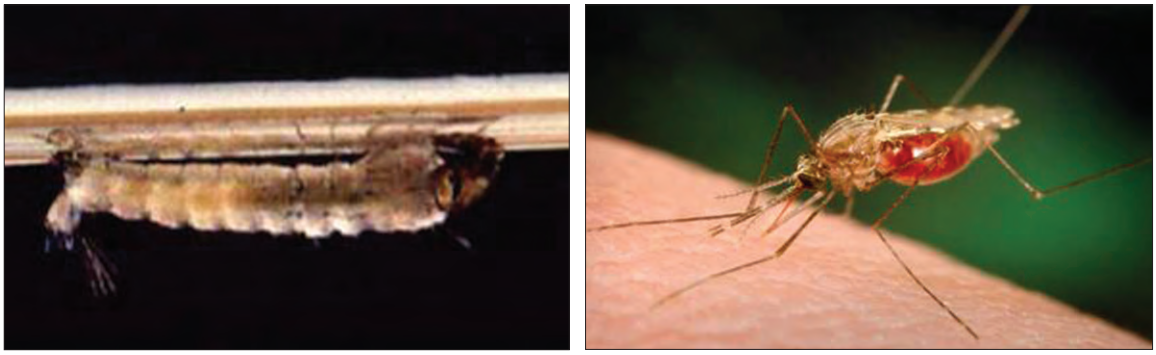
Source: Liverpool Scholl of Tropical Medicine

4.2 Vector profile

(See also 'Further reading' below.)

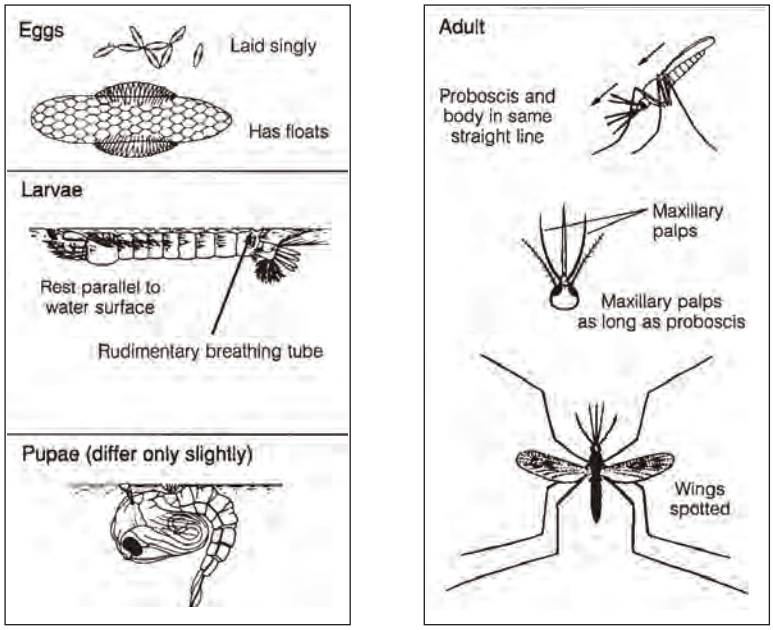
4.2.1 *Anopheles* genus

Figure 16. Larvae (left) and adult (right) of *Anopheles* mosquito



Source: C. Curtis (left) and Centers for Disease Control and Prevention (right)

Figure 17. Identification features of *Anopheles* mosquitoes



Source: reference (*42*)

Breeding sites and larval habitats

An. gambiae occurs in a wide range of habitats, including puddles, drainage channels, ponds and rice fields with small amounts of surface vegetation (*43, 44*), and also in polluted breeding habitats in urban environments (*45*).

An. arabiensis breeds primarily in still water bodies, such as ponds, swamps and wells.

During the dry season, *An. punctulatus* often breeds in seepage areas, ponds, swamps, open wells, springs and streams. During the rainy season, they breed in all types of water bodies including puddles. The water may be clear, turbid, stagnant, brackish or pure rainwater.

An. merus are commonly known as East African salt-water breeders and *An. melas* as West African salt-water breeders.

Biting and resting behaviour

Anopheles mosquitoes bite at night. Some species enter houses to feed (endophagic), while others feed outdoors (exophagic).

After feeding, some species rest indoors (endophilic), while others seek sheltered outdoor resting sites (exophilic) in e.g. vegetation, rodent burrows, cracks and crevices in trees, the ground, caves or under bridges.

Some *Anopheles* species feed on both humans and animals, while others are more likely to feed on humans, e.g. *An gambiae s.s.* in East Africa.

Flight range

1000–3000 m

Figure 18. Rice field

Source: B. Koudou

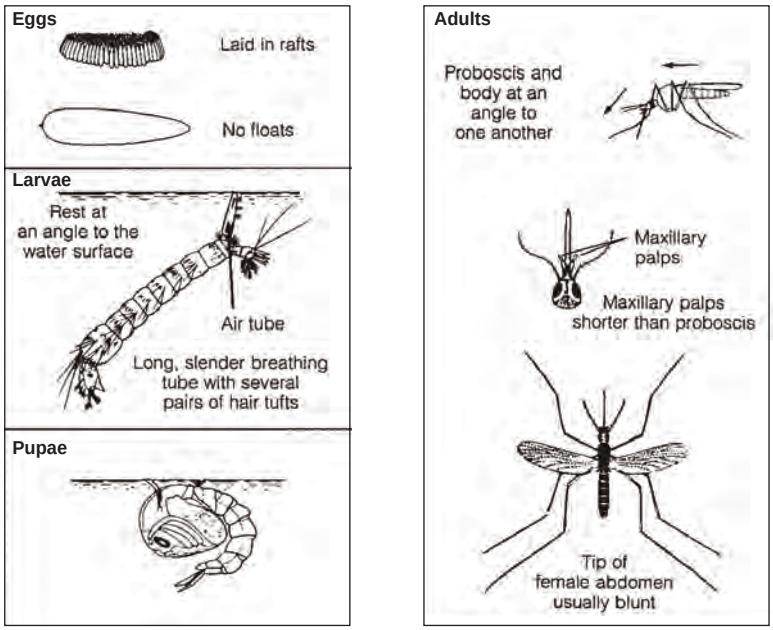
Figure 19. Rain-filled rut

4.2.2 *Culex* genus

Figure 20. Larvae (left) and adult (right) of *Culex* mosquitoes

Source: Liverpool School of Tropical Medicine (left) and Centers for Disease Control and Prevention (right)

Figure 21. Identification features of *Culex* mosquitoes



Source: reference (*42*)

Breeding sites and larval habitats

Culex breed in still water bodies ranging from artificial containers and drainage catchment basins to large permanent water bodies.

Cx quinquefasciatus usually breeds in polluted waters containing organic material such as refuse, excreta and rotting plants. Typical breeding sites are soakaway pits, septic tanks, pit latrines, blocked drains, canals and abandoned wells. Their breeding sites are rapidly expanding in urban areas in which drainage and sanitation are inadequate.

While the genus tolerates organically polluted water in shaded places, species of this genus are also found in clean fresh and brackish water.

Biting and resting behaviour

Cx quinquefasciatus is largely domestic. Adult females bite people and animals throughout the night, indoors and outdoors. The species is mainly inactive during the day, often resting in dark corners of rooms, shelters and culverts. They also rest outdoors on vegetation and in holes in trees in forested areas.

Flight range

< 200 m

Figure 22. Open drainage

Source: Centers for Disease Control and Prevention

Figure 23. Waste-polluted ponds

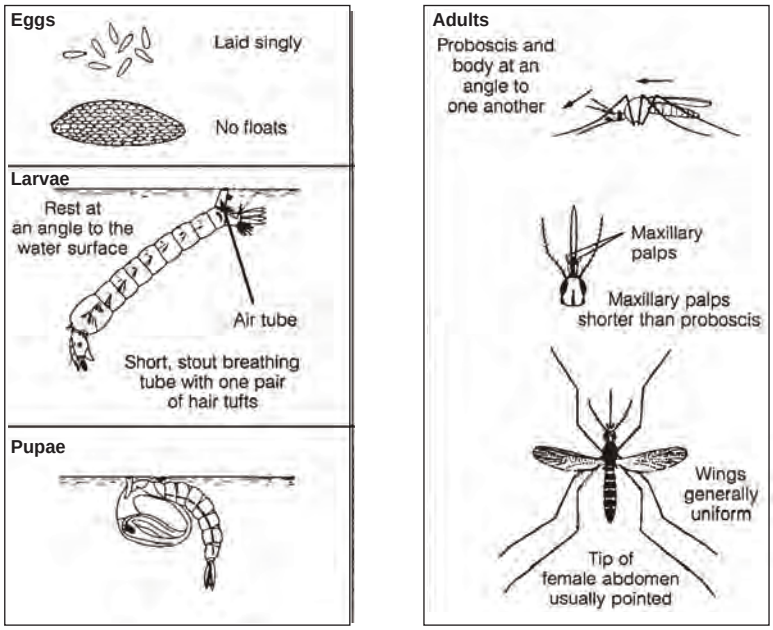
Source: WHO/TDR

4.2.3 *Aedes* genus

Figure 24. Larvae (left) and adult (right) of *Aedes* mosquitoes

Source: G. McCormack, Cook Islands Natural Heritage Project (left) and Centers for Disease Control and Prevention (right)

Figure 25. Identification features of *Aedes* mosquitoes



Source: reference (*42*)

Breeding sites and larval habitats

Some species breed in coastal salt marshes and swamps that are flooded at intervals by high tides or heavy rains. Other species have adapted to agricultural irrigation systems. Some prefer relatively small water bodies in artificial or natural sites.

Ae. polynesiensis and related species are the major filariasis vectors, from Fiji throughout the Polynesian islands. They breed in natural sites such as crab holes, coconut shells and tree holes but are also commonly found in artificial containers (*46*).

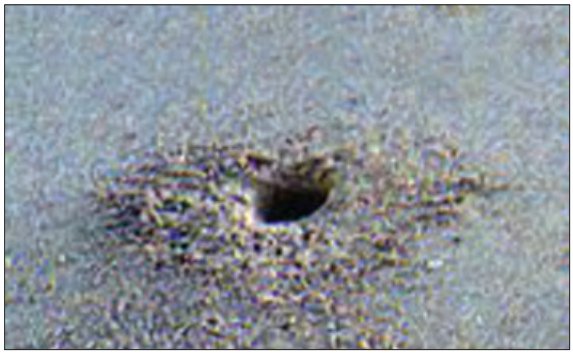
Biting and resting behaviour

Aedes mosquitoes usually bite during the day, often with morning and evening peaks. *Ae. polynesiensis* is mainly exophagic and exophilic but has been recorded feeding and resting in and around houses in towns.

Flight range

Average, < 400 m; *Ae. polynesiensis*, typically < 100 m

Figure 26. Crab hole



Source: K. Ichimori WHO

Figure 27. Unused containers and tyres

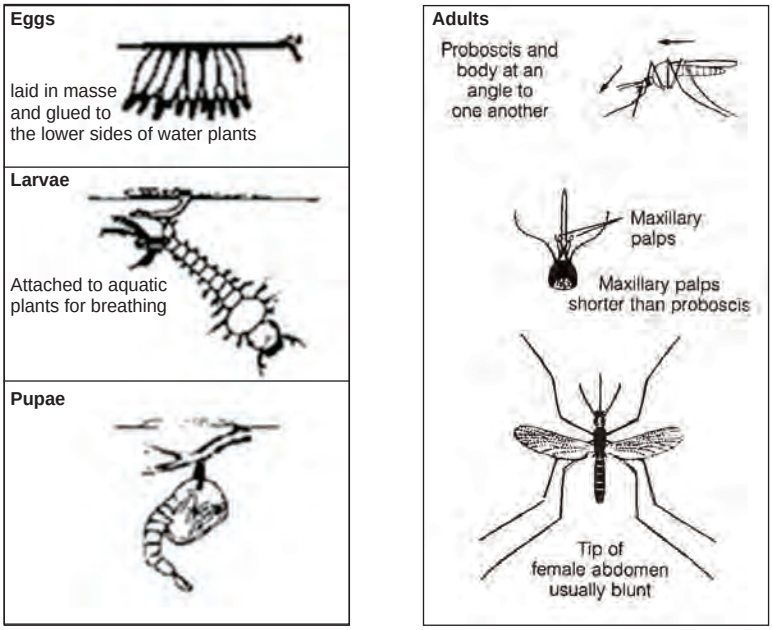
Source: Government of India

4.2.4 *Mansonia* genus

Figure 28. Larvae (left) and adult (right) of *Mansonia* mosquitoes

Source: Centers for Disease Control and Prevention (left) and S.L. Doggett (right)

Figure 29. Identification features of *Mansonia* mosquitoes



Source: reference (*42*)

Breeding sites and larval habitats

Mansonia mosquitoes are most commonly found in marshy areas and usually lay their eggs in masses, glued to the lower sides of plants hanging or floating near a water body.

The larvae and pupae depend on their attachment to aquatic plants for breathing and therefore occur only in water bodies that contain permanent vegetation, e.g. swamps, ponds, grassy ditches and irrigation canals. *Mansonia* larvae occur with floating vegetation, often attached to the underwater parts of floating aquatic weeds and grasses.

Biting and resting behaviour

Mansonia species usually bite at night, mostly outdoors, although some species enter houses. After a blood-meal, the mosquitoes generally exhibit exophilic behaviour.

Flight range

< 2000 m

Figure 30. Clean water pond with vegetation

Source: Centers for Disease Control and Prevention

Figure 31 Swamp with vegetation

Source: K. Ichimori WHO

Section 5

Vector sampling methods

Mosquito sampling is used to study their ecology, determine species abundance and estimate the vector density and transmission levels. Evaluation of changes in the density of adult mosquitoes and immature stages is part of vector control programmes. Adequate numbers of adult mosquitoes must be sampled, collected and processed to determine changes in infection and transmission rates. The mosquito sampling and collection methods widely used in entomological investigations of lymphatic filariasis control and elimination are described below.

Before collecting mosquitoes from peoples' homes or living areas, the purpose of the activity must be explained to them and informed consent provided. House owners should be free to decide whether they agree to having their house or land be used for collection and are free to decline trapping at any time. People who serve as human baits should also provide prior written informed consent (*47*).

5.1 Adults

Because mosquito species have specific behavioural patterns (e.g. resting and biting), the correct method must be selected to ensure that the results of sampling are representative and allow identification of mosquitoes that transmit lymphatic filariasis. Different methods are used to identify vectors for selecting appropriate control measures, for assessing the operational efficacy of the control measures and for determining whether transmission is still active and whether the parasite can be detected in the vector (xenomonitoring). *Table 3* summarizes the main methods that are suitable for sampling adult mosquitoes.

Table 3. Main methods used for sampling adult mosquitoes, by genus

Sampling method	Type of mosquito collected	*Anopheles*	*Culex*	*Aedes*	*Mansonia*
Hand catch with oral or mechanical aspirator (indoors or outdoors)	Resting adults	√	√	√	√
Pyrethrum spray sheet	Resting adults	√	√		√
Human landing collection	Host-seeking adults	√	√	√	√
Attractant traps: light traps, baited traps (CO2 traps, BG traps)	Adults (host-seeking, resting dependent on placement)	√		√	
Gravid traps	Gravid females		√	√	
Entry–exit trap	Host-seeking adults	v	√		√
Sweep net	Outdoor resting adults			√	
Emergence trap	Emerging adults	v	√		√
Sweep net	Resting adults	√	√	√	√

BG, Biogents AG

5.1.1 Hand catches with an oral or mechanical aspirator

Mosquitoes are collected from their resting places with mouth- or battery-operated mechanical aspirators (*48; Figure 32*) by torch-light. This type of collection provides information about usual resting places, resting density and seasonal changes in density. It also provides live specimens for susceptibility tests and bioassays and for observations of mortality among mosquitoes from insecticide-treated houses or houses with insecticide-treated bed nets. Resting mosquitoes collected outdoors in pit shelters, on other suitable structures or on plants or trees can also be used to estimate the human blood index or assess parity and survival.

Figure 32. Mechanical aspirator

Source: Centers for Disease Control and Prevention

The materials needed are a mouth or mechanical aspirator (*Figure 32*), a torch, test tubes, cotton wool, rubber bands and a cardboard container. Mosquitoes resting indoors are usually collected in the morning. The house is searched or, if it is too large, searched for 15 min, and each room is checked for mosquitoes with a torch. For each house, a separate test tube or cup should be used for collection, each labelled clearly with the locality, date and time of collection, time spent, house number or householder's name and type of structure.

5.1.2 Pyrethrum spray sheet collection

In this method (*Figure 33*), all the resting mosquitoes in a given space are knocked down. Members of selected households are informed, and the room or house is visited early the next morning. Light-coloured sheets are laid on the floor of the selected area to completely cover it, with the windows and doors closed. The rooms are sprayed with a non-residual insecticide (e.g. pyrethroid), and, 5–10 min later, the knocked-down mosquitoes on the sheet are picked up with forceps or tweezers and placed on moist paper in labelled Petri dishes. The mosquitoes are then counted, identified, and dissected or tested by PCR.

Figure 33. Pyrethrum spray sheet collection

Source: Liverpool School of Tropical Medicine

5.1.3 Human landing collection

Collection by human landing is useful for assessing the degree of human–vector contact, host attractiveness, mosquito survival and infection and infectivity rates. Individuals sit on a chair, exposing their legs, and wait for mosquitoes to land. Then, using an aspirator, they collect the mosquitoes coming to bite, usually separating them by hour of the catching period (*Figure 34*). For *Anopheles* mosquitoes, a first group of collectors usually works from 18:00 to 24:00 and a second group of collectors from 24:00 to 6:00, in 6-h shifts. The collectors are rotated between shifts to allow for variation in collecting efficiency. If this method is used in areas endemic for malaria, the collectors should be given appropriate antimalarial drugs and followed for several weeks after collection has stopped to determine whether they have been infected.

Figure 34. Pyrethrum spray sheet collection

Source: Liverpool School of Tropical Medicine

5.1.4 Attractant traps

Attractant traps consist of baited traps containing light, CO_2 or chemical attractants. Mosquitoes attracted to the light or bait are captured in a bag or container. Light and CO_2 traps capture all genera of mosquitoes, while chemical baits (such as BG sentinel traps) are frequently used outdoors for *Aedes*. The source of CO_2 for these traps is either a gas tank or dry ice. Some traps contain rechargeable batteries to power a fan.

Figure 35 Light trap

Source: Centers for Disease Control and Prevention

CDC light traps (*Figure 35*) can be set either indoors or outdoors, although they work more efficiently indoors. If used indoors, they should be positioned with the light 1 m above the ground next to someone sleeping under a bed net, at the foot of the bed. All the occupants of the room should be provided with nets. The mosquitoes are collected in a bag, which may be protected in areas of heavy rain with a plastic bag over it; the open end of the bag must allow unobstructed air-flow through the trap and collecting bag. Mosquitoes in the trap are held alive until they can be frozen or dried.

The number and species of mosquito collected in a given type of light trap is affected by the trap placement, including the height and proximity to breeding sites. For example, the efficiency of traps for Culex can be increased by placing them near an oviposition site attractive to female Culex mosquitoes.

5.1.5 Gravid traps

Figure 36. Gravid trap

Source : Centers for Disease Control and Prevention

Gravid traps are highly efficient for sampling *Culex* species (*Figure 36*). The trap attracts females with an oviposition attractant medium contained in a pan below the trap. The trap creates an upward current of air from within the pan, so that the mosquitoes are blown into the collection bag while they examine the oviposition medium.

A hay infusion is made by adding 0.5 kg of hay to 114 l of tap water and allowing the infusion to incubate for 5 days. While placing the trap, care must be taken to protect it from sun and rain. The trap is placed at the collection site at least 1 h before sunset, and the pan is filled with 4 l of infusion solution. Captured mosquitoes are removed early the next morning

to ensure maximum survival. The medium is replaced each night. The traps must be placed near mosquito resting areas.

5.1.6 Entry–exit trap

Window exit traps (*Figure 37*) are rectangular boxes made of a wooden or wire frame covered in Teflon®-coated woven fibreglass netting, with a slit-shaped rectangular tilted wire opening at one side for entry of mosquitoes and a sealable cotton sleeve aspirator inlet on the other side. The trap is first attached to a window or, if there is no window, to a hole in a plywood or canvas sheet fixed to an external door. The edges of the plywood can be wrapped with a foam seal to cover the gap between the board and the wall of the house and also to protect the wall from being scratched by the board.

Figure 37. Entry–exit trap

Source: B. Koudou

The traps are installed only in experimental huts, houses with no intact screens and houses whose owners have provided informed consent. Mosquitoes are retrieved from the trap with a hand-held aspirator through a sealable sleeve between 07:00 and 08:00.

Entry–exit traps are useful for sampling mosquitoes with exophilic behaviour and to trap endophilic mosquitoes that leave houses for oviposition.

5.1.7 Emergence traps

Emergence traps (*Figure 38*) have been widely used for sampling aquatic insects in both deep and shallow water. These traps can be either completely submerged in water, such as funnel traps, or float on or be positioned over the water, such as floating conical box traps, cages erected over the water and sticky traps (*48*). Only emergence traps that are positioned over the water surface are used to sample mosquitoes. They can be used to detect mosquito breeding in inaccessible habitats, such as crab holes, pit latrines and deep wells, to study daily and seasonal patterns of emergence and to obtain estimates of adult productivity.

Figure 38. Emergence trap

Source: S.W. Lindsay

Use of emergence traps to calculate the proportions of a population emerging daily or to estimate total productivity is, however, problematic, as habitat size can change rapidly

due to desiccation or flooding, and the numbers caught in the traps must be related to the total surface area of the breeding place that produces emerging adults. This area is not necessarily equivalent to the area of free water, because some pupae may survive in waterlogged leaf litter and give rise to adults.

5.1.8 Sweep nets

Sweep nets are designed to collect mosquitoes that rest outdoors, particularly in thick vegetation. The net is swept from side to side in a full 180° arc. One stroke is swept per step as the collector walks through the sampling area. In short vegetation, the net is swung as deeply as possible; in taller vegetation, the net is swept only deeply enough to keep the upper edge of the net opening even with the top of the plants. In general, the net should not be swept more than 25 cm below the top of the plants. The numbers of sampling sites and sweeps depends on vegetation type and height and the density of resting mosquitoes.

5.2 Larvae

Mosquito larvae are sampled to identify the main types of breeding site in an area, assess the impact of larval control programmes and obtain specimens for testing the resistance of larvae and adults to insecticides. The methods available for sampling larvae are dipping, netting and pipetting. All three methods can be used to trap mosquitoes, except for *Aedes* mosquitoes, which cannot be trapped by netting.

The sampling method depends on the nature and type of breeding site. In a larval survey, all kinds of water body must be sampled, especially pit latrines and underground bodies such as septic tanks. Larvae collectors must approach breeding sites carefully and not cast a shadow on the water, as any disturbance will cause the larvae and pupae to swim downwards and become inaccessible.

5.2.1 Dipping

This method is usually used to sample relatively large water bodies, such as swamps, ditches, streams and rice fields. The dipper should be lowered gently at an angle of about 45° to minimize disruption. It should either skim the top of the water or be gently lowered to allow the water and nearby larvae to flow into it (*Figure 39*). Larvae should be collected from the dipper with a pipette and transferred to a labelled bottle or vial. The number of dips at each breeding place should be noted for calculation of larval density; note also the time spent on collection.

Figure 39. Larval collection by dipping

Source: reference (*40*)

5.2.2 Netting

This method involves use of a fine mesh net mounted on a handle, with a plastic bottle or tube tied to one end. It is usually used to collect larvae and pupae in water bodies such as ponds and small lakes. The net should be held at about 45° to the water surface and be dragged across the surface. Larvae and pupae are collected in the plastic bottle.

5.2.3 Pipetting

This method is used for collecting larvae at small breeding sites, such as puddles, hoof-prints, containers, plant axils and tree holes (*Figure 40*).

Figure 40. Larval collection by pipetting

Source: reference (*40*)

Section 6

Vector control methods

Vector control can play an important role in lymphatic filariasis elimination (*section 2*), and programme managers should be knowledgeable about the available methods. Vector control for the elimination of lymphatic filariasis should focus on complementing, or replacing in some situations, mass drug administration, and several examples demonstrate the effectiveness of vector control in this situation (see *Box 4*). The choice of vector control method depends on the vector species and local ecology (see *section 7*); the chemical products for vector control must be those recommended in WHOPES (see also *40* and *42*).

6.1 Adults

A variety of methods is available to control adult mosquitoes, each of which could reduce the transmission of lymphatic filariasis by interrupting stages of the mosquito life cycle. The methods suitable for introduction by governments include long-lasting insecticidal nets and indoor residual spraying; communities can use also personal or household protection (e.g. household insecticides, repellents or house screening). The introduction of vector control in the GPELF retains a focus on populations living in areas endemic for lymphatic filariasis.

Community sensitization is a priority before interventions are begun. The community must be informed about the campaign, and their knowledge and understanding of lymphatic filariasis transmission must be improved to ensure acceptance of vector control methods. Cultural factors, such as ownership, must be considered (*49*).

The discussion below covers both classical (widely accepted) and innovative methods for vector control, for which widespread effectiveness must still be demonstrated, are discussed below. The method chosen should be specific to the genus and behavioural patterns of the mosquito. *Table 4* summarizes the main methods that can be used for effective control of specific genera.

Table 4. Main methods of control of adult mosquitoes, by genus

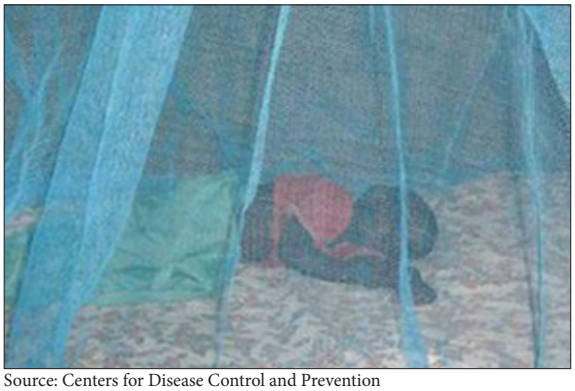
Method	*Anopheles*	*Culex*	*Aedes*	*Mansonia*
Long-lasting insecticidal nets	√	√	√	√
Insecticide-treated curtains and wall linings	√	√	√	
Indoor residual spraying	√			
Space spraying			√	
House screening	√	√	√	√
Household insecticides	√	√	√	√
Repellents, coils, vaporizing mats	√	√	√	√

6.1.1 Long-lasting insecticidal nets

While untreated nets provide a barrier to human–vector contact, nets treated with insecticide maintain a barrier even if they have some holes. The killing and contact repelling effect of the insecticide improves the efficacy of nets for both individual prevention and area-wide reduction in transmission (*50*). These nets are now the main weapon used to control malaria vectors and are highly effective (*51*). Long-lasting insecticidal nets and other impregnated materials for malaria control can be delivered through community-based activities, such as mass drug administration and other campaigns. Mass campaigns to deliver a certain number of nets per household or per person are effective in achieving high coverage and replacement.

Long-lasting insecticidal nets (*Figure 41*) provide a long-term solution, as they last for about 3 years. As only one class of insecticide (pyrethroids) is used on mosquito nets, however, the development of pyrethroid resistance threatens their long-term efficacy (*52*). The sensitivity of mosquitoes to insecticides varies by species.

Figure 41. Child sleeping under an insecticide-treated net



Source: Centers for Disease Control and Prevention

6.1.2 Insecticide-treated curtains and wall linings

In areas where the predominant vectors for lymphatic filariasis are *Culex* and *Anopheles* mosquitoes, insecticide-treated curtains or wall linings may be suitable. Curtains should be adapted to the type of house. Wall linings must be re-dipped or replaced at regular intervals to ensure complete coverage in areas endemic for lymphatic filariasis.

6.1.3 Indoor residual spraying

Indoor residual spraying (*Figure 42*) is useful mainly in areas of *Anopheles* transmission, where it has been shown to be effective in malaria control (*53*), and in areas in which:

- the rates of lymphatic filariasis infection remain high, despite several rounds of mass drug administration with full coverage;
- the vectors are highly resistant to pyrethroids;
- long-lasting insecticidal nets are not adequately used; and
- indoor residual spraying is already conducted within a malaria control programme.

Figure 42. Indoor residual spraying

Source: Liverpool School of Tropical Medicine

Four classes of insecticide are available for indoor residual spraying: organochlorines, carbamates, organophosphates and pyrethroids (*54*). Blood and urine samples should be collected for analysis before and after spraying in a district endemic for lymphatic filariasis. All spraymen and some inhabitants should be checked regularly by an experienced medical doctor and undergo biochemical and haematological analyses including kidney function tests, urine analysis, erythrocyte volume fraction and blood analysis.

Figure 43. Space spraying

Source: Liverpool School of Tropical Medicine

6.1.4 Space spraying

Space spraying (*Figure 43*) is used mainly during outbreaks, as the effects are not long-lasting and the procedure must be repeated frequently. It is usually applied in or around houses, some outdoor resting places and dense vegetation or salt marshes.

6.1.5 House screening

The use of screens is particularly useful in the control of *Anopheles* and *Aedes* mosquitoes. In a trial in the Gambia, screening reduced malaria anaemia by 50%, suggesting that it would also reduce lymphatic filariasis transmission by *An. gambiae* (*55*). The screens are a physical barrier and should be placed on all openings, including windows, doors and eaves. Householders should be encouraged to improve the condition of their house (closing holes, filling crevices in walls), as this will also decrease the opportunity for mosquitoes to enter or rest.

6.1.6 Household insecticides

Aerosol insecticide sprays or flit guns are commercially available for use by individuals and households. The spray should be applied within a room with all exits closed. The person who is spraying should leave the room and wait for a few minutes while the mosquitoes are being knocked down. The method has limitations, as opening a window or door again may re-introduce vectors. It also has no residual killing effect. The impact of household insecticides on transmission of lymphatic filariasis is unknown.

6.1.7 Repellents, coils and vaporizing mats

Coils are a popular, inexpensive method of vector control. They burn slowly and steadily release insecticide into the air over 6–8 h. Vaporizing mats work on the same principle but are powered by electricity, and their advantage over coils is that they release no visible smoke. The porous paper pad is impregnated with an insecticide (usually a pyrethroid), which both repels and kills mosquitoes.

Natural methods include certain fast-growing, densely foliated plants that repel mosquitoes. Production of the plants is cheap and self-sustaining, but botanical toxins may have health effects, and the efficacy of these methods is unknown.

Repellents are also available in the form of creams, lotions and sprays, which can be bought and applied as required by individuals. The strength of a product and the environmental and behavioural patterns of the vectors will determine how long the product remains effective. Their impact on transmission of lymphatic filariasis is unknown.

Box 7 outlines the progress made in vector control over the past decade, and *Box 8* describes the problems in control of *Culex* vector species.

Box 7. Progress in vector control: *World Malaria Report 2012*

During the past decade, coverage with vector control interventions increased substantially in sub-Saharan Africa. Owing to fewer deliveries of nets and increasing mosquito resistance to insecticides, however, the successes in malaria vector control may be jeopardized (*56*).

Insecticide-treated nets

By 2011, 32 countries in the WHO African Region and 78 other countries had adopted the WHO recommendation to provide insecticide-treated nets to all people at risk for malaria. A total of 89 countries, including 39 in Africa, distribute insecticide-treated nets free of charge.

The percentage of households that owned at least one insecticide-treated net in sub-Saharan Africa is estimated to have risen from 3% in 2000 to 53% in 2011 and 2012. The proportion of the population sleeping under an insecticide-treated net, representing the population directly protected, also increased, from 2% in 2000 to 33% in 2011 and 2012.

Indoor residual spraying

Indoor residual spraying remains a powerful tool for reducing and interrupting malaria transmission. In 2011, 80 countries, with 38 in the African Region, recommended indoor residual spraying for malaria control. In 2011, 153 million people were protected worldwide, representing 5% of the global population at risk. In the African Region, the proportion of the at-risk population protected rose from less than 5% in 2005 to 11% in 2010 and 2011, with 77 million people benefiting from the intervention.

Insecticide resistance

Resistance to at least one insecticide used for malaria control has been identified in mosquitoes in 64 countries. In May 2012, WHO and Roll Back Malaria released the *Global plan for insecticide resistance management in malaria vectors*, involving a five-pillar strategy for managing the threat of insecticide resistance.

Box 8. Discussion on *Culex* vectors

Participants in a WHO meeting in Liverpool, United Kingdom, on 4–5 March 2013 discussed the problems and prospects of control of *Culex* vector species, which are responsible for lymphatic filariasis transmission in many countries, especially in urban areas. A strategy and plan were prepared for vector control in areas in which *Culex* is a major vector.

The conclusions were:

- On the basis of the goal of the lymphatic filariasis elimination programme and the challenges encountered, areas in which vector control might be useful have been identified.
- A clear policy for *Anopheles* control has been drawn up, in collaboration with malaria control programmes.
- As more than 50% of countries endemic for lymphatic filariasis are at risk for infection by *Culex* mosquitoes, a number of research questions have been identified to address the role of *Culex* mosquitoes in lymphatic filariasis transmission, particularly in the context of mass drug administration.

The recommendations are:

- Encourage academic institutions to review and analyse existing evidence and experience in vector control, particularly with regard to *Culex* vectors, in order to identify the optimal vector control methods and to prepare policy guidelines for vector control in lymphatic filariasis elimination programmes.
- Encourage operational research on the priorities identified during the meeting.
- Urge WHO to continue to provide clear guidance on planning and implementation of vector control in national lymphatic filariasis programmes.
- Urge WHO to promote capacity-building on lymphatic filariasis vector control in the context of integrated vector management and lymphatic filariasis entomological techniques.

6.2 Larvae

Reducing the sources of mosquitoes by environmental modification or management is a first step in vector control, especially in urban areas. When breeding sites cannot be controlled in this way, however, larval control should be considered, if the breeding sites are relatively few, fixed and findable.. Larval control is less likely to be effective in areas with extensive breeding sites, such as large-scale irrigated rice fields or the flood plains of major rivers.

Larval control reduces the number of vectors and therefore affects the parasite life cycle. Many methods are available, but coverage of all larval habitats is usually difficult, and this method should not be used as the sole control strategy. Like control strategies for adult vectors, some methods are suitable for use by governments and agencies on an area-wide scale, while others are suitable for use by communities. Larval populations are naturally dependent on density, i.e. when the numbers in a particular site are reduced, others develop to take their place. The main issue in larval control is covering enough breeding sites and achieving sufficient reduction in larval numbers to affect the adult biting population.

As larval control can benefit from community involvement, the social mobilization strategy must be informative. A community health worker should be available to motivate community members, highlight the benefits of the methods and encourage them to sustain the control methods. Various larval control methods are listed in *Table 5*.

Table 5. Methods for larvae control suitable for each genus of mosquito

Method		*Anopheles*	*Culex*	*Aedes*	*Mansonia*
Environmental management	Filling mosquito breeding sites	√		√	
	Draining mosquito breeding sites	√		√	√
	Water level fluctuation	√			
	Elimination and modification of breeding sites	√		√	
	Removal of water plants and vegetation				√
	Use of expanded polystyrene beads in closed sites, such as pit or soakage latrines	√			
Larvicides	Insecticides	√		√	√
	Mosquito larvicidal oils	√		√	
	Insect growth regulators	√			
	Microbial larvicides (*Bacillus thuringiensis* subsp. *israelensis, B. sphaericus*)	√		√	

6.2.1 Environmental management

Environmental management is an overall term for a wide variety of methods, such as removal of vegetation, eliminating unnecessary water bodies, fluctuating water levels and draining swamp areas. This may be a cost-effective strategy for larval control when it focuses on the elimination of a limited number of categories of larval habitat that are known to contribute to the adult population. Use of insecticides and biological control agents could supplement the strategy.

Use of expanded polystyrene beads (*Figure 44*) is suitable in areas where Culex species that breed in pit latrines and soakage pits are the primary vector for lymphatic filariasis. Integrated use of mass drug administration and expanded polystyrene beads in Zanzibar and India was shown to reduce transmission (*57*).

Figure 44. Application of polystyrene beads for vector control

Source: C. Curtis

As in any larval control method intended to reduce the adult mosquito population in a community, a large proportion of breeding sites within the mosquito flight range should be found and treated before polystyrene beads are introduced.

6.2.2 Larvicides

Larviciding is killing larvae with chemical or biological agents. This should be considered a temporary method rather than environmental modification to remove breeding sites. The advantages and disadvantages are:

Advantages

- Mosquito larvae are killed before they reach the adult stage.
- Larviciding operations can be conducted quickly.
- Many effective larvicides are available on the market.
- Larvicides can be applied by hand or on a large scale with agricultural sprayers and hand-spray pumps.

Disadvantages

- Larviciding provides temporary control and must be repeated, which can be costly.
- Some larvicides harm other organisms, including natural enemies of mosquito larvae.
- Larvicides may be toxic to humans; therefore, education and training are necessary.

Larviciding can be conducted with insecticides, larvicidal oils, insect growth regulators or microbial larvicides.

Insecticides

Identified mosquito breeding sites should be treated with insecticides such as temephos (Abate), a larvicide with little toxicity for mammals and fish. The larvicide should be applied on water by ground or aerial spraying when in liquid form, while granules and pellets should be applied by hand or by portable blowers and briquettes impregnated with insecticide by hand (*Figure 45*). Other larvicides that are used include organophosphates (*58*), which are effective against culicine mosquitoes that breed in polluted water.

Figure 45. Application of larvicide

Source: S.W. Lindsay

Mosquito larvicidal oils

A thin layer of oil is applied to cover the surface of the water completely. Different types of oil are recommended, depending on the setting or environment. The method is expensive and short-lived (often lasting only a few hours) but is highly effective in killing larvae.

Mosquito larvicidal oils kill larvae in two ways: by suffocating them when they rise to the surface to breathe and by poisoning them with toxic vapour. This method is not effective against *Mansonia*, as the larval and pupal stages do not come to the surface.

Insect growth regulators

Insect growth regulators mimic or inhibit the effect of insect hormones, such as juvenile hormone, or inhibit insect skeleton formation, thus preventing normal development of larval and adult stages. Examples are methoprene and pyriproxifen. They act more slowly but are considered to be less toxic to other insects in the environment than conventional insecticides. They are applied similarly.

Microbial larvicides

Bacillus *thuringiensis* subsp. *israelensis* and *B. sphaericus* are the two main microbial larvicides suitable for controlling mosquito larvae. They are applied in the same way as chemical insecticides. *Bacillus thuringiensis var. israelensis* is tasteless and therefore suitable for use in drinking-water.

Boxes 9 and *10* describe case studies of vector control for lymphatic filariasis.

Box 9. *Aedes polynesiensis* vector control: a case study

Ae. polynesiensis (also known as Polynesian tiger mosquito) is a member of the *Ae. scutellaris* group, which is found only on island chains in the Polynesian region of the South Pacific. It is a vector of both dengue virus and *Wuchereria bancrofti* (diurnally sub-periodic). Particular features of this vector species include daytime biting, highly efficient transmission (*see Box 6*), a short flight range and diverse oviposition and breeding habitats in natural and artificial sites, including tree holes, coconut shells, leaf axils, banana stumps, cacao pods and canoes. A major larval habitat used to be flooded burrows of land crabs, but breeding site preferences appear to be changing to extensive use of discarded man-made containers (*46*).

Mass drug administration alone may not be sufficient to eliminate lymphatic filariasis sustainably in areas where it is transmitted by *Ae. polynesiensis* vectors (*59*). Numerous vector control strategies have been tested, especially in French Polynesia, and it was found that insecticide has only limited effectiveness, because *Ae. polynesiensis* adults tend to bite and rest outdoors, and their wide variety of breeding grounds, including crab holes, which provide protection against spraying of insecticide, makes it difficult to target control interventions. Some options that have been proposed are:

- larvicidal baits designed so that land crabs carry them back into their burrows, thus eliminating the need for burrow location and treatment by humans (*60*);
- biological control agents, including copepods (*Mesocyclops aspericornis*), larvivorous mosquito fish (*Gambusia affinis* and *Poecilia reticulata*) and predatory mosquitoes (*Toxorhynchites*); and
- mechanical methods, such as treating standing water with polystyrene beads to limit hatching and larval habitat and house screening to prevent biting.

While some of these methods were promising in small-scale trials, large-scale application and evidence of their effect on the prevalence and incidence of lymphatic filariasis are lacking.

Box 10. *Mansonia* (Mansonioides) larval control: best practices

Mansonia species breed in swampy areas, canals, ponds and pools, where their larvae breathe air through a siphon attached to the roots or stems of certain water plants, such as *Pistia stratiotes* and *Salvinia auriculata*. The larvae of most other mosquito species use their breathing siphons to take in air from the water surface.

Most conventional methods for controlling the immature, aquatic stages of mosquitoes have proved unsatisfactory against *Mansonia (61*). Removal of host plants by community participation and the establishment of proper drainage and sanitation systems by local authorities are the practices in use.

A classic example of weed removal for the control of *Mansonia* larvae was documented in Shertallai, Kerala, India, by Rojagopalan and colleagues (*62*). The community cleared the weeds and replaced them with the fast-growing terrestrial legume *Glyricedia*, which can be used to fertilize coconuts. The Government also introduced incentives for weed clearing and fish culture. The obvious economic benefit motivated the community to undertake fish farming in the ponds, thereby rendering them larvae-free.

Mansonia larvae can also be controlled by environmental manipulation. For example, in Kresek, Indonesia, Oemijati et al. (*63*) reported that the number of *M. indiana* and, subsequently, the number of cases of Brugian filariasis decreased after an irrigation development programme that resulted in conversion of swamp land into rice fields and brought about semi-permanent ecological changes. In another example, Samarahan Division, Sarawak, Malaysia, was highly endemic for Brugian filariasis in the 1980s, before rapid development turned the low-lying swampy areas into agricultural land and satellite towns (*64*). The breeding sites for *M. uniformis* and *M. indian* , the primary vectors of filariasis in the area, were largely eliminated by the late 1990s, after filling and clearing of swamp land and removal of the water plants *Pistia stratiotes* and *Salvinia* by the Government.

Section 7

Formulating a tailor-made vector control plan

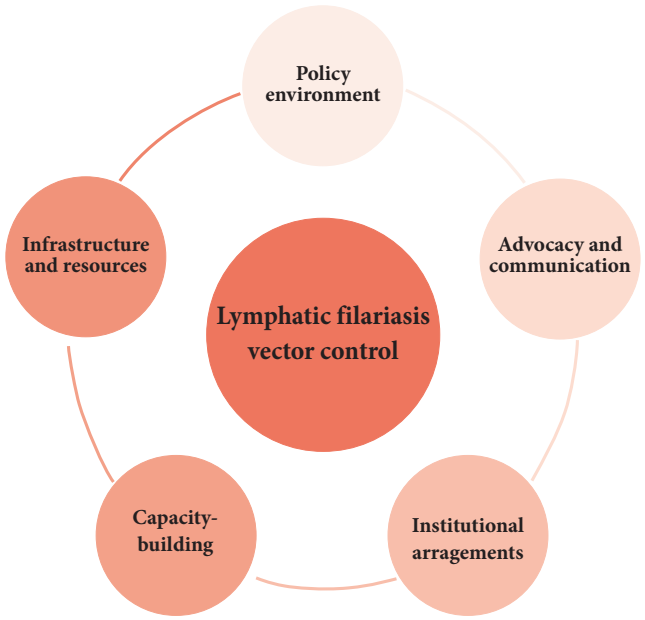
This section explains the steps involved in preparing a lymphatic filariasis vector control plan for informed decision-making and locally appropriate strategies. The steps follow the approach of integrated vector management, which is a rational decision-making process for optimal use of resources for vector control. The aim of the approach is to make vector control more cost-effective, ecologically sound and sustainable (*12*). In many countries, capacity for vector control already exists in programmes for other diseases (e.g. malaria) that are transmitted by the same or similar vectors. Hence, capitalizing on or collaborating with these vector control programmes can result in synergistic or complementary effects and sharing of resources.

7.1 Organization and management

Effective vector control depends on how techniques and methods are selected and on the policy and institutional environment (*65*). Consequently, ensuring the organization and management of vector control determine the success of operations to eliminate lymphatic filariasis.

A systematic approach should be used to analyse existing systems of vector control and identify constraints and opportunities for improvement or collaboration. The elements to be considered are: the policy environment, advocacy and communication, institutional arrangements, capacity-building and infrastructure and resources (*Figure 46*).

Figure 46. Basic components of the organization and management of lymphatic filariasis vector control



7.1.1 Policy environment

The first step is to analyse the policy environment to establish which elements are favourable and unfavourable for preparing a lymphatic filariasis vector control strategy (*66*). *Table 6* presents a framework for use by countries to identify activities relevant to lymphatic filariasis control and, for each activity, existing policies, guidelines and programmes. The opportunities for lymphatic filariasis vector control should be highlighted and gaps identified.

Table 6. Proposed framework for analysing the policy environment for lymphatic filariasis vector control

Relevant activities (examples)	Existing policies, guidelines or programmes	Opportunities for vector control	Gaps to be addressed
Malaria vector control			
Dengue vector control			
Integrated vector management			
Insecticide use			
Environmental health			
Waste collection			
Water sanitation improvement			
Integrated control of neglected tropical diseases			

7.1.2 Advocacy and communication

If vector control is considered beneficial, it should be accompanied by advocacy to the people involved in policy-making, decision-making and implementation of national lymphatic filariasis elimination programmes. Coordination with other relevant vector control programmes should be sought, supported by clear messages about the value of sharing resources to improve efficiency and optimize benefits for public health.

In regions where *Anopheles* species are the vectors of both lymphatic filariasis and malaria, advocacy should be used to ensure that malaria vector control interventions (e.g. long-lasting insecticidal nets, indoor residual spraying) apply to the distribution and prevalence of both diseases. Advocacy should be used to ensure that the impact of interventions on prevalence is evaluated. If lymphatic filariasis is transmitted by *Culex*, locally appropriate vector control methods (e.g. larval source management) and community participation should be emphasized. Where *Aedes* is the primary vector of lymphatic filariasis, reducing potential breeding sites (e.g. natural and artificial small water containers) can reduce mosquito-biting densities. Community involvement is essential to implement such control measures, as lack of motivation can quickly reverse any progress made. Hence, appropriate communication and education strategies are required at community level to increase compliance and participation in vector control and personal protection.

In preparing an advocacy and communication plan, the target audience must be identified, advocacy methods selected and the expected outcomes spelt out. *Table 7* outlines a plan for advocacy that could be used by countries.

Table 7. Outline to assist countries in planning advocacy and communication on lymphatic filariasis vector control, with examples of target audiences, methods and expected outcome

Level	Target audience	Method	Expected outcome
Policy	Health sector decision-makers, donors	Policy briefs, messages, success stories	Awareness, budget allocation, coordination
Programme	Managers of disease-specific programmes	Messages, success stories	Collaboration on vector control and monitoring and evaluation
Research	Medical entomologists	Forum discussion	Research on vector control
Community	Community health workers and volunteers	Training sessions	Awareness, commitment
	Community at large	Information, education and communication	Awareness, behaviour change

7.1.3 Institutional arrangements

When vector control is added to a lymphatic filariasis elimination programme, the institutional arrangements should be reviewed and roles and responsibilities in relation to vector control determined. Functional coordination is essential among programmes and sectors involved in integrated vector management. Coordination on lymphatic filariasis vector control might also be required across national borders to synchronize elimination efforts in neighbouring countries. *Table 8* lists institutional arrangements that would have to be adapted to accommodate vector control and could be used by countries in making their own assessment.

Table 8. Four examples of institutional arrangements that would require adaptation to accommodate lymphatic filariasis vector control

Institutional arrangement	Current situation	Desired situation	Steps to be taken	Benefits
Coordination between lymphatic filariasis and malaria vector control programmes	No coordination	Joint mapping, planning, monitoring and evaluation	High-level meeting; initiate vector control core group	Opportunities for lymphatic filariasis vector control are used
Tasks of community health workers and volunteers	Mass drug administration	Involvement in surveys to increase use of long-lasting insecticidal nets	Training, supervision, monitoring and evaluation	Improved bed net use; data collection
Linkage between research and operations	Research detached from operations	Research addresses priorities for lymphatic filariasis	Coordination on research priorities	Improved evidence base
Intersectoral coordination on integrated vector management	No functional coordination	Effective coordination on lymphatic filariasis vector control	Inter-ministerial meeting; form integrated vector	Sectors contribute to lymphatic filariasis vector control management committee

7.1.4 Capacity-building

General requirements for capacity-building at all levels (senior, programme, research and community) are assessed. *Table 9* aims to assist national programmes in conducting their own assessment.

Many of the competencies required for vector control for lymphatic filariasis are the same as those for other mosquito-borne diseases and may already be present in vector control programmes for malaria and dengue. Hence, lymphatic filariasis vector control requires coordination with other programmes through work sharing.

However, some competencies needed for vector control are unique to lymphatic filariasis, such as assessment of filarial infection rates and measurement of transmission efficiency and transmission potential. Therefore, specific training with specialized technical support may need to be conducted.

Table 9. Example of the requirements for capacity building on lymphatic filariasis vector control

Level	Target group	Required competency	Capacity building	
			Human resources development	**Infrastructure development**
Senior	Technical staff	Advocacy; communication; curriculum development on LF vector control	Training; study visits	Not appricable
Programme	Lymphatic filariasis Programme managers	Planning, targeting of LF vector control interventions	Training	Lymphatic filariasis mapping; data sharing
	Technical staff	Basic LF entomological techniques; monitoring and evaluation	Training	Equipment
Research	Entomologists	LF entomological techniques; M&E	Postgraduate/ diploma courses	Laboratories and equipment
Community	Community-based workers, volunteers	Monitoring of bednet use; health promotion	Training; supervision	Networking; use of training facilities

7.1.5 Infrastructure and resources

The infrastructure and the financial, human and technical resources that are already available in other programmes or at community level should be ascertained.

In areas where *Anopheles* mosquitoes transmit both lymphatic filariasis and malaria, strong programme infrastructure and ample resources from the malaria control programme may be available for lymphatic filariasis, including technical expertise on vector control. In exchange, a community-based infrastructure of drug distributors in the lymphatic filariasis programme may be an asset for the malaria programme, as they could help to enhance net use and maintenance.

In areas where *Culex* or *Aedes* species transmit lymphatic filariasis, strong community participation in the removal of breeding sites and personal protection measures will be important in reducing transmission of lymphatic filariasis. Opportunities for coordination with community programmes should be explored, including programmes for sanitation, hygiene and dengue control.

7.2 A tailor-made vector control plan

A national plan for lymphatic filariasis vector control should be prepared by the national programme, and partners should be involved in its implementation. If appropriate, the timing of vector control and mass drug administration might be harmonized in order to make optimal use of resources and infrastructure and to maximize the benefits for public health.

7.2.1 Disease and vector assessment

Prerequisites of a national programme to eliminate lymphatic filariasis are mapping the presence of the disease and assessing the progress of mass drug administration in implementation units, such as districts. The presence of other vector-borne diseases should also be taken into account. For example, the geographical distribution of lymphatic filariasis overlaps to a large extent with that of malaria, particularly when the two diseases are transmitted by *Anopheles* mosquitoes (i.e. in sub-Saharan Africa and the Western Pacific Region). As interventions will have an impact on both diseases, such co-endemicity should be an additional criterion in planning malaria vector control.

Where lymphatic filariasis is transmitted by *Aedes* vectors, a study should be conducted to determine whether it is co-endemic with dengue or another arboviral disease transmitted by related species of *Aedes*. Some species of *Aedes* that are vectors of lymphatic filariasis in the Pacific region share breeding sites with *Ae. aegypti*, the main vector of dengue. Therefore, larval source management for the two diseases could be combined.

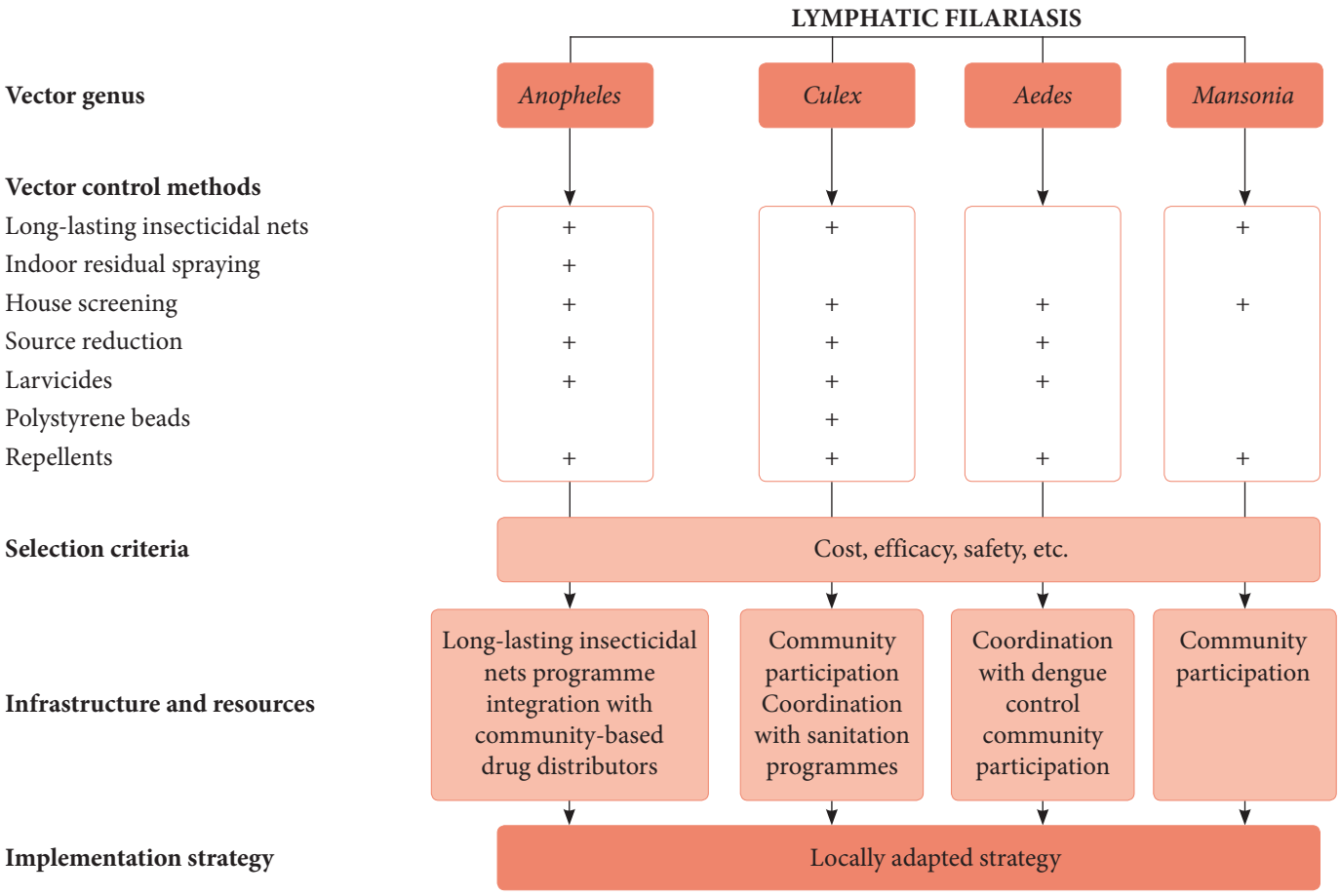
7.2.2 Selection of vector control methods

Once the disease situation and vector species are known, information about the biology, ecology and behaviour of the genus should guide the choice of intervention methods (*65*), as the vector control methods selected depend on species, habitat preference, behaviour and susceptibility to insecticides. The criteria that should be used to select the most suitable vector control methods are: environmental factors, cost, efficacy, support by policy and logistics (e.g. current campaigns or programmes), human and environmental safety, risk for development of resistance, acceptability by the community and human behaviour. The latter can be ascertained from answers to a questionnaire on the main activities of householders, sleeping hours, low and high transmission periods and bedtime during high mosquito density (*Figure 47*).

A combination of more than one vector control method will probably enhance the impact on vector populations and transmission reduction, particularly if the methods address different stages of the mosquito's life cycle or have a different mode of action. Hence, under certain conditions, larval source management or insect repellents can have an important incremental impact on transmission if they complement use of long-lasting insecticidal nets, particularly if some of the adult mosquito vectors bite outdoors.

The vector control plan should be adjusted to changes in insecticide resistance. *Cx quinquefasciatus* has developed high levels of resistance to the pyrethroid insecticides used in long-lasting insecticidal nets in many countries, although the physical barrier of bed nets will continue to provide some degree of protection. The effectiveness of these nets and of indoor residual spraying against *Anopheles* mosquitoes is also being threatened by insecticide resistance in many

Figure 47. Example of a tailor-made vector control plan, showing selection of control methods on the basis of genus and infrastructure, resources and implementation strategy



countries. Resistance should be detected at an early stage and alternative insecticides used to avoid spread of resistance genes within vector populations (*67*; see also *Annex 5*).

7.3 Monitoring and evaluation

Monitoring and evaluation studies showing effect of vector control on lymphatic filariasis have been described previously (*68*). The strategies require setting realistic targets and baseline measures for indicators, and the programme should be monitored with 'process', 'input' and 'outcome' indicators. Suitable indicators are not listed here but should be devised by each programme, depending on the vector targeted and interventions used.

Impact indicators are a measure of the success of a programme and are divided into impacts on the disease and on the vector. Methods for measuring these indicators are summarized below, and the formulae for calculating each indicator are given in *Annex 4*.

7.3.1 Impact on parasites in humans

The transmission of lymphatic filariasis in an area and the impact of vector control can be quantified by examining blood samples to determine the prevalence of microfilariae or antigen, and incidence of infection in the human population. The methods for determining prevalence, the recommended timing of surveys and the meaning of the two measures for determining endemicity and interruption of transmission are discussed elsewhere (*7*).

7.3.2 Impact on vectors

The impact on vectors can be measured as vector biting density, vector longevity (parity rate) and the human blood index or mosquito feeding.

Vector biting density

The biting density of adult mosquitoes can be monitored by human landing collections, light traps or pyrethrum spray catches for night-biting mosquitoes, such as *Culex* and *Anopheles* (see *section 5.1*). All the collection methods relate to mosquito density in relation to humans directly or indirectly, and their goal is to estimate the average number of bites received by a person during a given time (day, month or year). When combined with the proportion of mosquitoes infected (see below and *Annex 3*), this measure allows estimation of the *infective biting rate*, the number of infective bites received over a given time (*Annex 4*).

Human landing collection allows direct estimates of the average number of bites per person per defined period (e.g. hour or night). Light traps and pyrethrum spray catches are alternative methods for estimating mosquito density (see *section 5*). Light traps sample mosquitoes coming to feed, whereas pyrethrum spray catches trap mostly blood-fed or gravid mosquitoes resting inside houses. Adjustment factors must be applied in order to estimate the mosquito biting density (bites per person per day) from light trap and pyrethrum spray catches. For light traps, a commonly used means of estimating the human biting rate is to multiply the number of mosquitoes caught (per trap, per day) by 1.5, on the basis of the estimate by Lines et al. (*69*) that three light traps catch about the same number of mosquitoes as two human collectors. For pyrethrum spray catches, the average resting density is calculated by dividing the total number of mosquitoes collected by the number of collection days and the number of houses (or rooms) sampled. To transform pyrethrum spray catch density estimates to human biting rate:

1. Separate the freshly fed mosquitoes and count them.
2. Divide the total number of freshly fed mosquitoes by the total number of occupants who spent the night in the rooms used for collection.
3. If the human blood index from indoor resting catches is known, multiply the number of blood-fed mosquitoes by the proportion likely to have fed on humans.

This method is based on the assumption that no blood-fed mosquitoes left the house during the night.

Mosquito larval presence and density can also be monitored, but these are indirect measures of the effect of vector control, especially for methods such as larviciding. The number of breeding sites and their area and volume must be measured before and after application of this type of vector control to estimate indicators such as the proportion of breeding sites with larvae or the density of larvae within them. Consistent sampling is essential for these comparisons. These measures alone are not, however, sufficient to estimate any impact on the infective biting rate or transmission potential, which should be measured in adult mosquitoes.

Vector longevity

Control methods that result in the death of adult mosquitoes, such as indoor residual spraying and use of long-lasting insecticidal nets, affect the average longevity of the vector population. As older mosquitoes are more likely to be parous and if vector control shortens the average lifespan of a mosquito, fewer mosquitoes that are parous will be caught. A decrease in the parity rate in a population of mosquitoes indicates that vector control is reducing the size of the population, although care must be taken to sample consistently to account for seasonal increases and decreases in population size. A growing population will have a low parity rate, while a declining population (with fewer new emergences) will have a high rate, for reasons unrelated to vector control. The simple parity rate in a mosquito population can be determined by the method described in *section 4.1.7* and *Figure 15* and should decrease as mosquito mortality increases.

Human blood index

The human blood index (proportion of meals taken on humans) can be obtained by analysing blood-meals, usually by ELISA, from identified species caught in the field. The human blood index is the proportion of females of a given species found to have human blood in their stomachs. Similar indices can be calculated for any blood-meal type present in the collection analysed. A decrease in the human blood index suggests that mosquitoes are being diverted away from humans to feed on other animals.

7.3.3 Impact on lymphatic filariasis transmission

The impact on transmission can be determined from the vector infection or infective rate, monthly or annual infection or infective biting rate, and monthly or annual transmission potential.

Vector infection or infective rate

Adult mosquitoes collected by any of the methods described in section 5 and above can be used to estimate the infection rate (the proportion of mosquitoes infected with any stage of lymphatic filariasis worm) or the infective rate (the

proportion of mosquitoes infected with L3 stage). Biases in the methods, such as catching either biting or resting mosquitoes, with consequently different average ages and feeding habits, will affect the results; therefore, the type of catch must always be reported. If the mosquito infection rate is low, sufficient numbers of mosquitoes must be sampled in order to detect the level of infection with sufficient power. This usually means hundreds or even thousands of mosquitoes per sample site and time. A statistician should be consulted.

The methods for determining mosquito infection and infective rates are dissection and examination of freshly caught or fixed and stained mosquitoes, or detection by polymerase chain reaction (PCR) analysis of dried mosquitoes. The methods are described in *Annex 3*. The PCR method may be more sensitive but detects all stages of worm, including microfilariae. Detection must be done on mosquitoes individually, while PCR analysis can be done on pools of mosquitoes.

Monthly or annual infection or infective biting rate

The risk for infection in humans depends on the infective rate in mosquitoes and also on the density of mosquitoes biting humans. The infective biting rate is estimated from the mosquito density (bites per person per day, month or year) multiplied by the proportion infected with L3 stage larvae (See *Annex 4* for formulae). If collection was done on a certain number of days or nights per month, the average number of bites per night must be standardized to the average number of days in a month (30.5) or a year (365) to arrive at the infective biting rate (*26*).

Monthly or annual transmission potential

In addition to the proportion of infected or infective mosquitoes, the average number of L3 present in infective mosquitoes is important from the perspective of transmission. This can be determined only by manual dissection of mosquitoes, although a PCR method to detect and quantify L3 is being developed.

The monthly transmission potential is estimated from the average number of L3 in a dissected mosquito sample, multiplied by the biting rate (See *section 3.3* and *Annex 4* for formulae). The annual transmission potential can be estimated from the sum of 12 monthly transmission potentials or from catches conducted all year.

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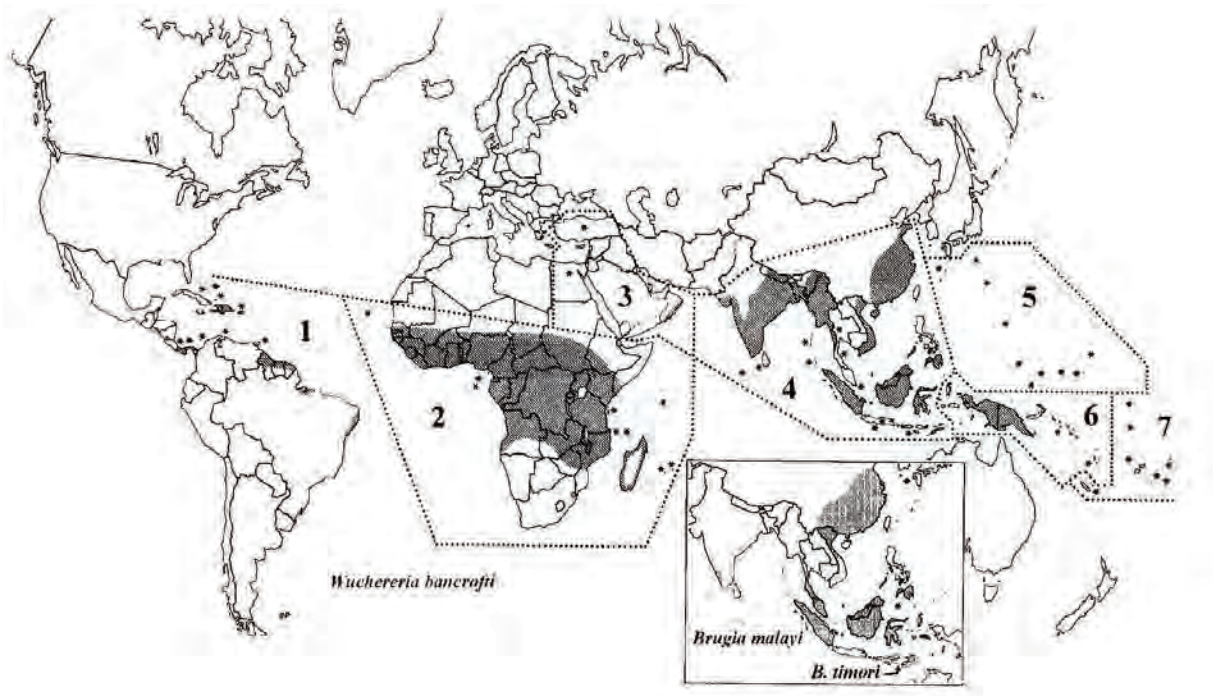
Annexes

Annex 1. Mosquito species



Areas of the world in which Bancroftian filariasis is endemic and, in the inset, where Brugian filariasis is endemic. Large areas of endemicity are shaded; small foci are indicated with an asterisk. Brugian filariasis is caused by *Brugia malayi* in all endemic countries and also by *B. timori* in Nusa Tenggara (Alor, Flores and Timor islands). Zones 1–7 indicate the locations of the species of vector and type of filaria described in the table below. The zones differ with respect to the mosquito species that transmits the parasites and the strain or species of parasite that causes the disease, resulting in unique epidemiological characteristics.

TABLE 1. *The mosquito vectors of the filariae causing human lymphatic filariasis in seven zones shown on the world map (Fig. 1), based on articles by the World Health Organization (1984, 1989, 1992, 2002); the epidemiological characteristics of the filariasis in the zones differ according to the locally endemic filaria–vector combination)*

		Vector species[†]	
Filaria type	Endemic region and (map zone)[*]	Major and widespread	Local or subsidiary
Brugia malayi (periodic)	South Asia (4)	*Anopheles* (*Anopheles*) *barbirostris* *Anopheles* (*Anopheles*) *campestris* *Anopheles* (*Anopheles*) *donaldi* *Mansonia* (*Mansonioides*) *annulata* *Mansonia* (*Mansonioides*) *annulifera* *Mansonia* (*Mansonioides*) *uniformis*	*Anopheles* (*Anopheles*) *anthropophagus*[‡] *Anopheles* (*Anopheles*) *kweiyangensis*[‡] *Anopheles* (*Anopheles*) *nigerrimus* *Anopheles* (*Anopheles*) *sinensis*[‡] *Mansonia* (*Mansonioides*) *bonneae* *Mansonia* (*Mansonioides*) *dives* *Mansonia* (*Mansonioides*) *indiana* *Ochlerotatus* (*Finlaya*) *kiangsiensis*[‡] *Ochlerotatus* (*Finlaya*) *togoi*[‡]
Brugia malayi (sub-periodic)	South Asia (4)	*Mansonia* (*Mansonioides*) *annulata* *Mansonia* (*Mansonioides*) *bonneae* *Mansonia* (*Mansonioides*) *dives*	*Coquillettidia* (*Coquillettidia*) *crassipes* *Mansonia* (*Mansonioides*) *uniformis*
Brugia timori (periodic)	Flores, Timor (4)	*Anopheles* (*Anopheles*) *barbirostris*	
Wuchereria bancrofti (periodic)	Americas (1)	*Culex* (*Culex*) *quinquefasciatus*	*Anopheles* (*Nyssorhyncus*) *albimanus*[§] *Anopheles* (*Nyssorhynchus*) *aquasalis* *Anopheles* (*Nyssorhynchus*) *darlingi*[¶] *Mansonia* (*Mansonia*) *titillans* *Ochlerotatus* (*Ochlerotatus*) *scapularis*[§] *Ochlerotatus* (*Ochlerotatus*) *taeniorhynchus*[§]
	Afrotropical (2)	*Anopheles* (*Cellia*) *funestus* *Anopheles* (*Cellia*) *gambiae* *Culex* (*Culex*) *quinquefasciatus*	*Anopheles* (*Cellia*) *arabiensis* *Anopheles* (*Cellia*) *hancocki* *Anopheles* (*Cellia*) *melas* *Anopheles* (*Cellia*) *merus* *Anopheles* (*Cellia*) *nili* *Anopheles* (*Cellia*) *pauliani* *Anopheles* (*Cellia*) *wellcomei*[§] *Culex* (*Culex*) *antennatus*[§] *Mansonia* (*Mansonioides*) *uniformis*[§]
	Middle East (3)	*Culex* (*Culex*) *pipiens molestus* *Culex* (*Culex*) *quinquefasciatus*	*Culex* (*Culex*) *antennatus*
	South Asia (4)	*Culex* (*Culex*) *quinquefasciatus*	*Anopheles* (*Anopheles*) *anthropophagus*[‡] *Anopheles* (*Anopheles*) *barbirostris* *Anopheles* (*Anopheles*) *donaldi* *Anopheles* (*Anopheles*) *kweiyangensis*[‡] *Anopheles* (*Anopheles*) *letifer* *Anopheles* (*Anopheles*) *leucosphyrus* *Anopheles* (*Anopheles*) *nigerrimus* *Anopheles* (*Anopheles*) *sinensis* *Anopheles* (*Anopheles*) *vagus* *Anopheles* (*Anopheles*) *whartoni* *Anopheles* (*Cellia*) *aconitus* *Anopheles* (*Cellia*) *balabacensis* *Anopheles* (*Cellia*) *dirus* *Anopheles* (*Cellia*) *flavirostris* *Anopheles* (*Cellia*) *jeyporiensis* *Anopheles* (*Cellia*) *maculatus* *Anopheles* (*Cellia*) *minimus*[¶] *Anopheles* (*Cellia*) *philippinensis* *Anopheles* (*Cellia*) *subpictus* *Anopheles* (*Cellia*) *tessellatus* *Culex* (*Culex*) *bitaeniorhynchus*[§] *Culex* (*Culex*) *sitiens*[§] *Ochlerotatus* (*Finlaya*) *poicilius*
	Far East (5) Papuan (6)	*Culex* (*Culex*) *quinquefasciatus* *Anopheles* (*Cellia*) *farauti* *Anopheles* (*Cellia*) *koliensis* *Anopheles* (*Cellia*) *punctulatus*	*Anopheles* (*Anopheles*) *bancrofti*[‡] *Culex* (*Culex*) *annulirostris*[§] *Culex* (*Culex*) *bitaeniorhynchus*[§] *Culex* (*Culex*) *pipiens pallens*[‡] *Mansonia* (*Mansonioides*) *uniformis* *Ochlerotatus* (*Finlaya*) *kochi*

TABLE 1. *Continued*

Filaria type	Endemic region and (map zone)*	Vector species†	
		Major and widespread	Local or subsidiary
Wuchereria bancrofti (sub-periodic)	Nicobar, Thailand (4)	*Ochlerotatus* (*Finlaya*) *niveus* *Ochlerotatus* (*Finlaya*) *harinasutai*	*Aedes* (*Stegomyia*) *annandalei* *Aedes* (*Stegomyia*) *desmotes* *Aedes* (*Stegomyia*) *imitator*
	Polynesia (7)	*Aedes* (*Stegomyia*) *polynesiensis*	*Aedes* (*Stegomyia*) *cooki* *Aedes* (*Stegomyia*) *horrescens* *Aedes* (*Stegomyia*) *kesseli* *Aedes* (*Stegomyia*) *marshallensis* *Aedes* (*Stegomyia*) *rotumae* *Aedes* (*Stegomyia*) *tabu* *Aedes* (*Stegomyia*) *tongae* *Aedes* (*Stegomyia*) *pseudoscutellaris* *Ochlerotatus* (*Finlaya*) *fijiensis* *Ochlerotatus* (*Finlaya*) *oceanicus* *Ochlerotatus* (*Finlaya*) *samoanus* *Ochlerotatus* (*Finlaya*) *tutuilae* *Ochlerotatus* (*Finlaya*) *upolensis* *Ochlerotatus* (*Ochlerotatus*) *vigilax*

*The map zones are indicated in Figure 1.
†For mosquito nomenclature and taxonomy see http://wrbu.si.edu.
‡No longer involved in transmission, because lymphatic filariasis has been eliminated from the species' range.
§Doubtfully or rarely implicated in transmission.
¶No longer involved in transmission, because species has been eliminated from all areas where lymphatic filariasis occurs.

Source: Zagaria N, Savioli L. Elimination of lymphatic filariasis: a public health challenge. *Annals of Tropical Medicine and Parasitology*, 2002, 96(Suppl. 2):S3–S13.

Annex 2. Filarial parasites and vectors in countries endemic for lymphatic filariasis

Country or area	Programme progress[a]			Parasite[b]					Vector[c]			
				Wb		*Bm*		*Bt*				
	Mapping	MDA	Post-MDA surveillance	P	Sp	P	Sp	P	*An*	*Cx*	*Ae*	*Ma*
WHO AFRICAN REGION												
Angola	√											
Benin	√	√		√					√			
Burkina Faso	√	√		√					√			
Cameroon	√	√		√					√			
Central African Republic	√											
Chad	√			√								
Comoros	√	√		√						√		
Congo	√			√								
Côte d'Ivoire	√	√		√					√			
Democratic Republic of the Congo	√			√					√			
Equatorial Guinea	√			√					√			√
Eritrea	√			√					√			
Ethiopia	√	√		√					√			
Gabon	√			√								
Gambia	√			√					√			
Ghana	√	√		√					√			√
Guinea	√			√					√			
Guinea-Bissau	√	√		√					√			
Kenya	√	√		√					√	√		
Liberia	√			√					√			
Madagascar	√	√		√					√			
Malawi	√	√		√					√	√		
Mali	√	√		√					√			
Mozambique	√	√		√								
Niger	√	√		√					√			
Nigeria	√	√		√					√			
Sao Tome and Principe	√			√								
Senegal	√	√		√					√			
Sierra Leone	√	√		√					√			
Togo	√	√	√	√					√			
Uganda	√	√		√					√			
United Republic of Tanzania	√	√		√					√	√		
Zambia	√			√					√			
Zimbabwe	√			√								

Annex 2. Filarial parasites and vectors in countries endemic for lymphatic filariasis (continued)

Country or area	Programme progress[a]			Parasite[b]					Vector[c]			
				Wb		*Bm*		*Bt*				
	Mapping	MDA	Post-MDA surveillance	P	Sp	P	Sp	P	*An*	*Cx*	*Ae*	*Ma*
WHO REGION OF THE AMERICAS												
Brazil	√	√		√						√		
Dominican Republic	√	√		√						√		
Guyana	√	√		√						√		
Haiti	√	√		√						√		
WHO EASTERN MEDITERRANEAN REGION												
Egypt	√	√		√						√		
South Sudan	√			√						√		
Sudan	√			√						√		
Yemen	√	√								√		
WHO SOUTH-EAST ASIA REGION												
Bangladesh	√	√		√						√		
India	√	√		√		√				√		√
Indonesia	√	√		√		√	√	√	√	√		√
Maldives	√	√	√	√						√		
Myanmar	√	√		√						√		
Nepal	√	√		√						√		
Sri Lanka	√	√	√	√						√		
Thailand	√	√			√	√	√				√	√
Timor-Leste	√	√		√		√		√	√	√		
WHO WESTERN PACIFIC REGION												
American Samoa	√	√	√		√						√	
Brunei Darussalam	√					√						√
Cambodia	√	√	√	√					√			
Cook Islands	√	√	√		√						√	
Fiji	√	√			√						√	
French Polynesia	√	√			√						√	
Kiribati	√	√		√						√		
Lao People's Democratic Republic	√	√		√								
Malaysia	√	√		√		√	√		√	√		√
Marshall Islands	√	√	√	√						√		
Micronesia	√	√		√						√		
New Caledonia	√				√						√	
Niue	√	√	√		√						√	

Annex 2. Filarial parasites and vectors in countries endemic for lymphatic filariasis (continued)

Country or area	Programme progress[a]			Parasite[b]					Vector[c]			
				Wb		*Bm*		*Bt*				
	Mapping	MDA	Post-MDA surveillance	P	Sp	P	Sp	P	*An*	*Cx*	*Ae*	*Ma*
Palau	√			√						√		
Papua New Guinea	√	√		√					√			
Philippines	√	√		√					√		√	
Samoa	√	√			√						√	
Tonga	√	√	√		√						√	
Tuvalu	√	√			√						√	
Vanuatu	√	√	√	√					√			
Viet Nam	√	√	√	√		√			√	√		√
Wallis and Futuna	√	√	√		√						√	

Ae, *Aedes*; An, *Anopheles*; *Bm, Brugia malayi; Bt, Brugia timori; Cx, Culex*; MDA, mass drug administration; Ma, *Mansonia*; *Wb, Wuchereria bancrofti*; NA, not available P, periodic (nocturnal); Sb, sub-periodic

[a] Source: Global Programme to Eliminate Lymphatic Filariasis: progress report, 2011. *Weekly Epidemiological Record*, 2012, 87:346–356. Mapping, not started or ongoing in any part of the country; MDA, mapping completed in the entire country and MDA ongoing in any part of the country; Post-MDA surveillance, MDA stopped in the entire country

[b] Only the most prevalent parasites cited in the published literature and national or regional reports are included.

[c] Only major vectors cited in the published literature and national or regional reports are included.

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Annex 3. Methods for detecting filarial parasites in mosquitoes

A3.1. Dissection

A3.1.1 Freshly collected mosquitoes

Mosquitoes that are collected dead (usually by pyrethrum spray or light trap) should be dissected within 6 h, or the next day if they are held overnight at 4 °C. Mosquitoes can be held longer if they are collected alive, for example by biting catch, if they are given sugar solution on cotton wool in a humid environment. Mosquitoes should be sorted by date and site of collection and the species identified before dissection. For anopheline vectors, part of the mosquito can be held for molecular confirmation of the species if necessary. One person can dissect 50–100 mosquitoes a day.

Items needed:

- Cups or tubes for mosquito collection and sorting
- Petri dishes
- Cool box, refrigerator or freezer for chilling mosquitoes
- Fine forceps or needles
- Saline (0.9% NaCI in water)
- Droppers and pipettes
- Microscope slides and cover slips
- Dissecting microscope with mirror or light
- Compound microscope

Method: (modified from references (*1*) and (*2*)

1. Anaesthetize mosquitoes one tube or cup at a time, by chilling for a few minutes.
2. Place mosquitoes on a Petri dish; remove the wings and legs using two pairs of needles or forceps with low-power magnification under a stereoscopic dissecting microscope.
3. Place individual mosquitoes on a microscope slide and, with dissecting needles, divide into head, thorax and abdomen, placing each portion of the body in a separate drop of saline solution on the same slide.
4. Tease the three body segments apart and examine for larval stage worms or microfilariae. The mouth parts should be separated with fine needles to allow L3 larvae to escape, when they can be spotted moving in the drop of saline. Although some worms can be seen under a binocular microscope at about 20x magnification, in order to find all the worms it is preferable to cover the saline drops with cover slips and examine at 40x under a compound microscope.
5. Note the location and number of worms in each body section. The L1 and L2 stages are often seen in the flight muscles of the thorax, whereas L3 are commonly found in the head and neck area or coming out of the proboscis, as well as in the thoracic haemocoele (mosquito body cavity).
6. In recently blood-fed mosquitoes, the mid-gut can be removed from the body and the blood cells lysed in distilled water. The microfilariae can then be counted in the blood-meal (under a cover slip under a 40x compound microscope).

A3.1.2 Fixed and stained mosquitoes

If all the mosquitoes caught cannot be dissected immediately or if the catching site is unsuitable for undertaking immediate dissection, they can be fixed and stained for later dissection (*1–3*). Staining makes it easier to see the L1 and L2 stages.

Items needed:

- Ethanol (ethyl alcohol), ≥ 80%
- Distilled water
- Haemalum (Mayer's) stain (VWR, West Chester, Pennsylvania, USA)
- Glycerol
- Glass screw-top vials.
- Measuring cylinder to make up alcohol dilutions of 70%, 55% and 25%
- Glass slides and cover slips
- Dissecting microscope with mirror or light source
- Compound microscope

Method: from reference (*4*), as modified in reference (*1*)

1. After collection and identification, place mosquitoes in 80–98% ethanol in tightly sealed vials. (Alternatively, 70% ethanol with 5% glycerol (v/v) can be used.) They can be placed in individual vials or grouped by species and collection site or time. Up to 200 mosquitoes can be placed together in one 2.5 x 7.6 cm tube. They can be transported and stored indefinitely in alcohol until staining.
2. Wash specimens in descending dilutions of ethanol (70%, 55% and 25%) for 30 min in each dilution, followed by a final 30-min wash in distilled water.
3. Stain for 7 days in Mayer's haemalum stain at room temperature (15–27 °C) .
4. Remove stain, wash specimens for 3 days in distilled water, and store in pure glycerol until dissection.
5. Dissect mosquitoes individually with fine needles in pure glycerol. Dissect the head and thorax separately from the abdomen on a glass slide under a stereoscopic zoom dissecting microscope at 8x–35x magnification.
6. Note the number, location and developmental stage of filarial worms in the head, thorax and abdomen.
7. Re-screen filarial-positive slides at 20x–40x magnification to determine the species of filarial worm. Expert help and examination under a compound microscope at up to 100x may be needed at this stage.
8. Slide preparations can be permanently mounted in glycerol if desired.

   Staining can also be done after fresh dissection of worms (*2*).

Classification of results:

Whether they are dissected fresh or after staining, mosquitoes carrying microfilariae or L1, L2 or L3 larvae are defined as 'infected', while those carrying only L3 are defined as 'infective'.

A3.2. Polymerase chain reaction

PCR is used to identify worms in mosquitoes by the presence of their DNA, which is extracted from ground mosquitoes and amplified. This technique cannot identify the stage of the worm (microfilariae, L1, L2 or L3). It should be done in a laboratory with experience in PCR techniques.

Mosquitoes should be sorted by date, collection site and species, dried thoroughly and stored with silica gel until used. Samples can be dried by placing the tubes in a block or oven at 95 °C or in the sun for 1 h with the cap open. The tubes are then closed and stored in a sealed container with enough silica gel to cover the bottom of the container to keep the tubes dry; the silica gel should not be put into the tubes but into the container holding the tubes. The container can be stored at room temperature or at 4 °C.

A3.2.1 Overview

Firstly, mosquitoes are divided into pools of 5–50 specimens by species, site and time of collection (depending on the number caught and the expected infection rate) for DNA extraction and purification before analyses to amplify DNA. The DNA samples are then run by electrophoresis on agarose gel and stained. Positive and negative controls are run concurrently with the samples to ensure that the PCR amplification is not contaminated, which can result in false positives, as well as to ensure that all the reagents are working properly. The mosquito infection rates are estimated on the basis of the number of pools that give positive results with the statistical software PoolScreen.

The method described below for *W. bancrofti* is that described in reference *1*; additional information on the PoolScreen algorithm and the primers is given in reference *2*. Methods are also available for analysing Brugia-infected mosquitoes, but an experienced laboratory should be consulted.

A3.2.2 Extraction of DNA from mosquitoes

The supplies needed per pool of mosquitoes are:

- 1 zinc-plated .177 calibre (4.5 mm) steel airgun shot
- 2-ml tube for grinding
- 1 Qiagen DNeasy column/wash tube (provided in kit)
- 2 additional wash tubes (provided in kit)
- 180 µl phosphate-buffered saline
- 40 µl proteinase K (some provided in kit; may need extra)
- 200 µl lysis buffer (provided in kit)
- 200 µl 95–98% ethanol
- 1000 µl buffer AW1 (provided in kit; may need extra)
- 500 µl buffer AW2 (provided in kit)
- 240 µl buffer AE (provided in kit)
- Parafilm
- Pipettor and sterile tips (20, 200 and 1000 µl)
- Eppendorf centrifuge
- Racks
- DNA thermal cycler

1. Extract DNA using a modification of the Qiagen DNeasy kit protocol (Qiagen, Hilden, Germany).
2. Sterilize by autoclaving 2-ml microcentrifuge graduated tubes with flat snap-top caps (e.g. Fisher Scientific) and 0.177 calibre 4.5-mm zinc-plated ball-bearings (gun pellets, e.g from Walmart, USA).
3. Place dried mosquitoes for grinding in sterile tubes, with one pool per tube.
4. Add 180 µl phosphate-buffered saline and one zinc-plated ball-bearing to each tube. Wrap Parafilm around the cap of each tube to prevent leakage or contamination.
5. Vortex the tubes with mosquitoes for 15 min to macerate them, e.g. on a Fisher Vortex Genie 2 mixer (Fisher Scientific, Waltham, Massachusetts, USA) with a Mo Bio Horizontal vortex adapter (Mo Bio, Carlsbad, California, USA).
6. Spin the tubes briefly before adding 200 µl of lysis buffer (buffer AL) and 20 µl of proteinase K to each sample. Vortex the samples briefly, and incubate at 70 °C for 10 min.
7. Add an additional 20 µl of proteinase K to each tube, and incubate the samples at 56 °C for 60 min. Then spin the incubated material at 13 000 g for 5 min, remove the supernatant and add it to 200 µl of 98% ethanol.
8. Apply this mixture of supernatant and ethanol to the Qiagen DNeasyspin column, and then wash the column twice with buffer AW1 and once with buffer AW2.
9. Elute DNA from the column into a labelled 1.5–2.0-ml tube (e.g. Eppendorf tube) by adding 125 µl of AE elution buffer (performed twice).

The purified DNA is then ready for use in the PCR assay. Known positive and negative control DNA samples are recommended.

A3.2.3 PCR amplification with *W. bancrofti* primers:

1. PCR assays can be performed with NV-1 and NV-2 primers (*5*). The target sequence for these primers is the SspI repeat, a gene present at ~500 copies per haploid genome. Amplification with these primers yields a 188-bp fragment. Other primers or other (real-time) PCR methods may also be used (*2*).
2. Each 50-µL PCR reaction contains 1× Qiagen Taq buffer, 3 mmol/l MgCl2, 0.20 mmol/l each of dATP, dCTP, dGTP and dTTP, 10 pmol of NV-1 and NV-2 primer, 1.25 U HotStarTaq DNA polymerase and 1 µl genomic DNA.
3. Run PCR reactions on a cycler (e.g. BioRad I Cycler, BioRad, Hercules, California, USA), with reaction conditions consisting of a single step of 95 °C for 15 min, followed by 54 °C for 5 min. After these initial steps, the reactions are subjected to 35 cycles of 72 °C for 30 s, 94 °C for 20 s and 54 °C for 30 s. The final step is a 5-min extension at 72 °C.
4. Size-fraction the PCR products on 2% agarose gels stained with GelRed (Biotium, Hayward, California, USA). Run agarose gels at 70 V for 1 h and visualize under ultraviolet light.
5. Verify samples that are positive for the *W. bancrofti* SspI repeat by repeating the PCR reaction.
6. A pool is confirmed to be positive if at least one of the two repeat samples is also positive.
7. The vector infection rate is determined from the proportion of positive pools, using PoolScreen software available from http://www.soph.uab.edu/bst/poolscreen (reference (*6*), as discussed in reference (*2*)).

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Annex 4. Entomological indicators

A4.1 Impact on parasites in human

1. **Microfilaria prevalence:** percentage of blood slides from humans found positive for microfilariae:

$$\frac{\text{Number of individuals whose slides are positive for microfilariae}}{\text{Total number of individuals examined for microfilariae}} \times 100$$

2. **Antigen prevalence:** percentage of antigen-positive people out of the number examined by ICT card test (or other antigen detection test):

$$\frac{\text{Number of individuals found positive with ICT cards}}{\text{Total number of individuals examined for filarial antigen by ICT cards}} \times 100$$

A4.2 Impact on vectors

3. **Vector biting density:** number of mosquitoes biting a human per unit time (usually hour or day)

4. **Vector resting density:** number of female resting mosquitoes collected per unit of time, per room or per house

5. **Parity rate:** percentage of mosquitoes that are parous (have laid eggs at least once), calculated from:

$$\frac{\text{Number of parous mosquitoes}}{\text{Number of parous and nulliparous mosquitoes}} \times 100$$

6. **Monthly vector biting rate:** estimated number of mosquitoes biting a human per month.

If collections are not made every night of the month, this can be estimated from the vector-biting density per person per day (indicator 3) multiplied by the number of days in a month (average, 30.5).

7. **Annual vector biting rate:** estimated number of mosquitoes biting a human per year

If collections are not made every night of the month, this can be estimated from the vector-biting density per person per day (indicator 3) multiplied by the number of days in a year (365) or by the monthly vector biting rate (indicator 6) multiplied by 12, taking into account seasonal variation.

8. **Human blood index:** proportion of mosquito blood-meals taken on humans, calculated from:

$$\frac{\text{Number of human feeds}}{\text{Number of human feeds + number of feeds on other animals}} \times 100$$

For example, assuming that blood-meal analysis by ELISA of a given *Anopheles* species reveals that 83 fed on humans, 1 fed on chickens and 36 females fed on dogs:

$$\frac{83}{(83+11+36)} = 0.63$$

A4.3 Impact on LF transmission

9. Vector infection rate: percentage of mosquitoes infected with filarial worms (any stage), calculated from:

$$\frac{\text{Number of mosquitoes with any stage (microfilariae or L1 or L2 or L3) of the parasite}}{\text{Number of mosquitoes dissected}} \times 100$$

10. Vector infective rate: percentage of mosquitoes infected with L3 infective stage filarial worms, calculated from:

$$\frac{\text{Number of mosquitoes with L3 stage of the parasite}}{\text{Number of mosquitoes dissected}} \times 100$$

11. Monthly infective biting rate: estimated number of infective mosquitoes biting a human per month, calculated from the vector infective rate (indicator 10) and the monthly vector-biting rate (indicator 6) from:

Vector infective rate x monthly vector-biting rate

12. Annual infective biting rate: estimated number of infective mosquitoes biting a human per year, calculated from the vector infective rate (indicator 10) and the annual vector-biting rate (indicator 7) from:

Vector infective rate x annual vector-biting rate

13. Monthly transmission potential: indicator of risk for infection per month that includes the number of infective larvae rather than the proportion of infective mosquitoes. It is calculated from the monthly vector-biting rate (indicator 6) from:

$$\frac{\text{Total number of infective larvae (L3)}}{\text{Number of mosquitoes dissected}} \times \text{monthly vector biting rate}$$

14. Annual transmission potential: indicator of risk for infection per year that includes the number of infective larvae rather than the proportion of infective mosquitoes. It is calculated from the annual vector-biting rate (indicator 7) from:

$$\frac{\text{Total number of infective larvae (L3)}}{\text{Number of mosquitoes dissected}} \times \text{monthly vector biting rate}$$

It can also be estimated as the sum of monthly transmission potentials (indicator 13) obtained during each of the 12 months of the year.

A4.4 Indicators for *Aedes* mosquitoes

The commonest survey methods for monitoring the impact of vector control on Aedes mosquitoes involve larval sampling procedures (active immature specimens, including pupae) rather than collecting eggs or adults. The basic sampling unit is a house or premises, which is systematically searched for water-holding containers. The containers are examined for the presence of mosquito larvae, pupae and larval and pupal skins.

15. House (premises) index: proportion of houses infested with *Aedes* larvae or pupae, calculated from:

$$\frac{\text{Number of infested houses} \times 100}{\text{Number of houses inspected}}$$

16. Container index: percentage of mosquitoes infected with L3 infective stage filarial worms, calculated from:

$$\frac{\text{Number of positive containers} \times 100}{\text{Number of containers inspected}}$$

17. Breteau index: number of containers infested with *Aedes* larvae or pupae per 100 houses inspected, calculated from:

$$\frac{\text{Number of positive containers} \times 100}{\text{Number of houses inspected}}$$

Annex 5. Methods for monitoring and managing resistance to insecticides

A5.1 Monitoring insecticide resistance

Levels of resistance in mosquitoes must be monitored in order to decide when to change a product or class of insecticide before insect control fails and the risk for disease transmission rises. The strategy might be changed not only with regard to the insecticide but also to the type, such as from an adulticide to a larvicide, or method. Monitoring must also include assessment of cross-resistance, as changing from a product that has failed to one to which there is cross-resistance is a waste of both time and money.

Monitoring of insecticide resistance in vector control programmes has three objectives:

- before the start of the programme, to provide baseline data for programme planning and choice of insecticide;
- to detect resistance at an early stage, so that resistance can be managed (If resistance is detected only after control has failed, the problem is defined but there is no strategy for management.); and
- to determine levels of resistance over time in order to compare them with the baseline data and therefore evaluate the effects of control operations on resistance.

While monitoring and accurate assessment of the susceptibility of the vector population is fundamental, failure may be due to a variety of reasons other than resistance, including poor application technique, underdosing and application at the wrong time of day (space sprays). These deficiencies must be eliminated first.

Resistance may be localized. Therefore, before the identification of resistance leads to panic, its distribution should be assessed. Monitoring and detection of resistance have little value unless a management strategy has been defined and an action plan prepared to react to any build-up of resistance.

The efficacy of indoor residual spraying and insecticide-treated nets depends partly on the proportion of vectors that rest on a sprayed surface for a sufficient time and on the susceptibility of the vectors to the insecticide used. It is therefore important to monitor the extent of insecticide resistance in a given vector population.

Physiological resistance to insecticides has been defined as the "ability of a population of insects to tolerate doses of an insecticide that would prove lethal to the majority of individuals in a normal population of the same species". Susceptibility tests are conducted to determine the proportion of the vector population that is physiologically resistant to a particular insecticide. Methods for monitoring resistance are described below.

A5.1.1 WHO tube tests for adult mosquitoes

The principle of this test is to expose mosquitoes for a given time in a plastic tube lined with filter paper treated with a standard concentration of insecticide. The dose on the paper (diagnostic concentration) is twice the lethal dose required to kill 100% of mosquitoes of a susceptible strain, in order to avoid spurious reports of resistance in the field when none exists. The kit can be used in a laboratory or the field.

The standard WHO method involves checking the mortality of several female lymphatic filariasis vector mosquitoes of a known species that have been exposed in tubes to filter papers impregnated with a lethal concentration (known as the 'discriminating dose') of an insecticide dissolved in mineral oil. The kit and papers can be purchased with full instructions from suppliers and with details from WHO (*1*). A range of treated papers is available. The WHO-recommended diagnostic concentrations for each group of vectors are chosen, so that exposure for a standard time (usually 1 h) followed by a 24-h holding period can be relied upon to cause 98% mortality of individuals of susceptible strains. New diagnostic concentrations must be determined for new insecticides. Full details of the test technique and method are given in reference *2*.

The results with standard papers are interpreted as follows: 98% mortality = susceptible; 80–97% mortality = resistance suspected but verification or confirmation required; and < 80% mortality = resistant individuals present. When mortality is < 95% in tests conducted under optimum conditions with a sample of > 100 mosquitoes, resistance can be strongly suspected.

A5.1.2 Centers for Disease Control and Prevention bottle bioassay

The bottle bioassay of the United States Centers for Disease Control and Prevention (CDC) is based on measures of the time it takes for an insecticide to penetrate an arthropod, traverse its intervening tissues, reach the target site and act on that site. Anything that prevents or delays the compound from achieving its objective—killing arthropods—contributes to resistance. Information derived from the CDC bottle bioassay can provide initial evidence that an insecticide is losing its effectiveness. This method should be considered for routine use even before an insecticide is considered and procured for vector control.

The CDC bottle bioassay can be performed on arthropod populations collected in the field or on those reared in an insectary from larval field collections. It is not recommended for use on mosquitoes that have emerged from eggs laid in an insectary.

A major advantage of this bioassay is that different concentrations of an insecticide can be evaluated. Furthermore, the technique is simpler, faster and cheaper than other alternatives. The CDC bottle bioassay can be used as part of a broader insecticide resistance monitoring programme, which may include the WHO paper-based bioassay and biochemical and molecular methods.

Detailed web-based instructions are available from the National Center for Infectious Diseases, CDC (*3*).

A5.2 Insecticide efficacy monitoring

A5.2.1 WHO cone bioassay

The residual efficacy of an insecticide on a treated surface (walls or nets) is determined in bioassays, by checking mortality among target mosquito vectors exposed to a sprayed surface at intervals of weeks or months after spraying. The technique can also be used to evaluate the quality of a residual spraying operation and to determine the residual efficacy of an insecticide on bed nets to determine when the nets should be re-treated and to assess the quality of treatment.

Procedure:

Cone bioassays are performed at the beginning of an intervention and at intervals during the intervention to test the efficacy of vector control. Susceptible and resistant malaria vectors and nuisance Culex strains are tested separately. Batches of five or six unfed, 2–5-day-old resistant and susceptible strains are placed inside the plastic cones and exposed simultaneously and in a similar manner to different parts of unwashed and washed nets (roof and sides) or walls for 3 min, before being transferred from the cones to holding containers. To minimize disturbance of mosquitoes during the short exposures, batches of only five females are introduced into each of four to five cones applied to the same net sample or wall site.

For nets, a total of 10 replicates with five female mosquitoes are performed for each sample tested, for a total of 50 female mosquitoes per sample of net. After exposure, survivors are maintained in 150-ml plastic cups in a climatic chamber for 24 h at 27 °C ± 2 °C and 80% ± 10% relative humidity and containing 10% honey solution. The number of mosquitoes knocked down is recorded 60 min after exposure, and the final mortality is recorded 24 h after exposure. An untreated net is used as a negative control.

A5.3 Insecticide resistance management

Although new vector control products can effectively control insect strains that are resistant to currently used insecticides, few new insecticides are being developed. Therefore, the useful life of current insecticides must be protected and extended.

Lymphatic filariasis programme managers should note the recently published Global Plan for Insecticide Resistance Management produced by the Global Malaria Programme in 2012 (*4*). They should become aware of the resistance levels in local lymphatic filariasis vectors and become involved in the strategies being used by the malaria control programme in their country.

The Global Plan draws attention to the threat of insecticide resistance, outlines a collective strategy to prevent and combat it and provides technical recommendations to countries for preparing policy. The recommendations are put into the context of a near-term action plan to avert the potentially severe long-term consequences of resistance, for example to pyrethroids, that would compromise the use of long-lasting insecticidal nets for controlling lymphatic filariasis.

The Plan states (p. 15) that "Several strategies exist for insecticide resistance management for vector control, based on the use of indoor residual spraying and long-lasting insecticidal nets. They include: rotations of insecticides, use of interventions in combination, and mosaic spraying. Potential future strategies include use of mixtures. In some settings, resistance management strategies may be implemented in the broad context of integrated vector management. These strategies can have several effects on populations of resistant vectors: they can delay the emergence of resistance by removing selection pressure (e.g. rotations) or kill resistant vectors by exposing them to multiple insecticides (e.g. mixtures, when they become available)."

The managers of lymphatic filariasis control programmes should know about, understand and support the measures being taken in their country to prevent the emergence of resistance to insecticides.

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The Global Programme to Eliminate Lymphatic Filariasis has scaled up its activities more rapidly than almost any other global public health programme. By the end of 2011, 53 of 73 endemic countries were implementing mass drug administration, and more than 3.9 billion treatments had been delivered to 952 million people. Challenges remain, however, in meeting the goal of eliminating the disease.

Vector control is a possible complementary strategy in countries or areas (i) where mass drug administration has not started, such as those where loiasis is co-endemic; (ii) where the burden of lymphatic filariasis is heaviest and mass drug administration must be rapidly scaled up or (iii) where the expected impact of mass drug administration has not been achieved; and where local transmission has been interrupted to prevent recurrence.

This document explains why vector control is important in national programmes and describes the preparation of a tailor-made control plan for national programmes. It outlines entomological procedures for regular and specific vector control and how data should be analysed for better overall understanding of filarial transmission and vectors. The document will also be useful for teaching personnel in lymphatic filariasis programmes about the use and value of entomological procedures in overall epidemiological appraisal in the context of elimination.

Designed for national lymphatic elimination programme managers and for entomologists and parasitologists, this practical handbook will also be useful for programme staff working at regional and district levels, including those involved in vector control; development and technical agencies; nongovernmental organizations; and other organizations that support national programmes.

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