Control of lymphatic filariasis

A manual for health personnel
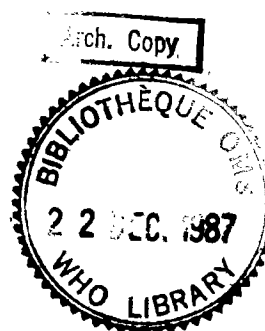
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PREFACE

This manual has been designed as a guide to those who plan, manage and carry out filariasis control programmes. It is in three parts. Part 1 deals with the life cycle and vectors of filarial parasites and with the distribution, clinical manifestations, diagnosis, and treatment of lymphatic filariasis. Part 2 covers the basic concepts and general guidelines for controlling filariasis and describes a systematic approach to developing national strategies for filariasis control programmes that are community-oriented, simple, and economical. Part 3 contains specific methods and techniques for collecting sound epidemiological data for assessment and evaluation of filariasis. The names of the mosquito vectors of human lymphatic filarial parasites in the major endemic zones of the world are listed in Annex 1.

Bibliographical references have not been included but a list of some key works for further reading is given on page 81.

The manual takes full account of the primary health care and community participation approaches to filariasis control, as well as describing the methodology for vertically organized campaigns. It is not specifically directed towards the primary health care worker but rather to those concerned with health training and organization, pointing out the manner in which the community health worker can become involved in filariasis control, and the extent to which control measures may be implemented by means of community participation.

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PART 1. GENERAL INFORMATION

PART 1

General information

1. Definition of lymphatic filariasis

Human lymphatic filariasis results from infection with the nematode parasites *Wuchereria bancrofti, Brugia malayi* and/or *B. timori*. The juvenile and adult worms normally live in the lymph vessels and lymph nodes, and the microfilariae are found in the blood. The adult parasites can live for many years (probably up to 10 years, but a 40-year life-span has been reported). The life-span of the microfilariae is about a year at the most.

2. Life cycle of the parasites (Fig. 1)

The parasite is transmitted by mosquitos which serve as intermediate hosts in which microfilariae develop to the infective stage. In most endemic areas, the highest level in the human circadian or 24-hour cycle of peripheral microfilaraemia (periodicity) coincides with the biting activity of the local vector. Some of the microfilariae ingested by the mosquito shed their sheaths, penetrate the stomach wall, migrate to the muscles of the thorax, and develop there without multiplication.

The slender active microfilaria transforms to the short thick inactive sausage-stage or L_1 larva. The L_1 larva has a cuticle which forms a conspicuous slender tail, characteristic of this stage. In the genus *Brugia* one or two nuclei are present inside the tail.

After the first moult, the larva grows rapidly in length and width and becomes potentially more active, although usually it does not move. It has a thin cuticle which can be seen at the caudal end forming a short tail. This L_2 or pre-infective larva is recognized by its short tail and the presence of one or two papillae at the caudal end.

After a second moult the parasite no longer has a visible cuticle and is known as an infective or L_3 larva. The L_3 larva grows further in length but not in width, moving actively in the haemocoelic cavity of the mosquito, first towards the abdomen and later to the head and proboscis (where

Fig. 1. Life cycle of human lymphatic filarial parasites

Development from L_3 to L_4 larva to adult
worm in skin and lymphatic channels
(3 - 9 months)

Adult worms (male and female) in
lymphatic channels (live for
5 - 10 years)

Infective larvae (L_3) enter person's
skin through wound made by
proboscis of mosquito

Females bring forth microfilariae
into bloodstream, often showing
periodicity in peripheral blood
(live for up to 12 months if
not ingested by mosquito)

Development in mosquito
(8 - 14 days) from microfilaria to
sausage larva (L_1) to preinfective
larva (L_2) to infective
larva (L_3)

Microfilariae
ingested by female
mosquito along with
blood-meal

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most of them are found upon dissection). The caudal end of *Wuchereria bancrofti* is characterized by having three teat-like papillae of equal size. In the genus *Brugia*, the central papilla at the caudal end is most prominent, while the two ventrolateral ones are less conspicuous.

The duration of larval development is affected by the ambient temperature; generally, the warmer it is the more rapid the development. It usually takes 10–14 days for *W. bancrofti* to reach the infective stage, and 7–10 days for *B. malayi* and *B. timori*.

When the now infective mosquito takes a blood-meal, some or all of the infective larvae escape from the proboscis and actively enter the human host through the wound made by the mosquito. The L_3 larva develops in the lymphatic system to the L_4 stage, to the young adult stage, and finally to the mature adult worm, male or female. After fertilization, the female worms produce microfilariae, which find their way from the lymphatic system to the bloodstream. The pre-patent period (from the entrance of L_3 to the appearance of microfilariae in the peripheral blood) is estimated at about 3 months for *Brugia* and 9 months for *W. bancrofti*.

3. Distribution of filariasis

Geography

The distribution of the endemic areas of human lymphatic filariaris is shown in Fig. 2, which also outlines the geographical zones of the vector mosquitos listed in Annex 1.

Bancroftian filariasis (caused by *Wuchereria bancrofti*) is the most widespread human lymphatic filarial infection. The largest number of people both "at risk", and infected, live in India, but the disease is a severe problem in many other Asian countries, notably Bangladesh, Burma, China, Indonesia, Malaysia, Papua New Guinea, the Philippines, Sri Lanka, Thailand and Viet Nam. Localized foci of infection are also common in: parts of East, Central and West Africa and Egypt; Madagascar and neighbouring islands; the northern parts of South America, including two foci in Brazil; parts of Central America, and some Caribbean islands; and many of the Pacific islands.

Brugian filariasis (caused by *Brugia* spp.) has a more restricted distribution which overlaps in places with bancroftian filariasis. *Brugia malayi* infections are found in southern China, India, Indonesia, Malaysia, the Philippines, the Republic of Korea, Thailand and Viet Nam. *B. timori* is localized in the Lesser Sunda Islands of eastern Indonesia.

Ecotypes

Nocturnally periodic *W. bancrofti* is mainly found in the slum areas of cities (urban type) and in rural areas (rural type). A nocturnally subperiodic strain of *W. bancrofti* is found locally in Thailand and Viet Nam. The diurnally subperiodic type is rural, occurring in the eastern Pacific islands.

B. malayi and *B. timori* are found only in rural areas. The subperiodic type of *B. malayi* is mostly found in areas with irrigated rice fields.

Periodicity

In humans, the microfilariae show a characteristic periodicity in the course of the circadian or 24-hour cycle. They live mainly in the pulmonary capillaries, from whence a proportion of them escape into the peripheral blood where they may be detected during the hours of their periodicity.

The nocturnally periodic type shows a marked peak of microfilarial density in the peripheral blood during the night hours; very few, if any, microfilariae can be found during the day in the standard volume of blood taken for routine examination. In the nocturnally subperiodic and

Fig. 2. Distribution of lymphatic filariasis and vector zones (for explanation of the numbered vector zones see Annex 1)
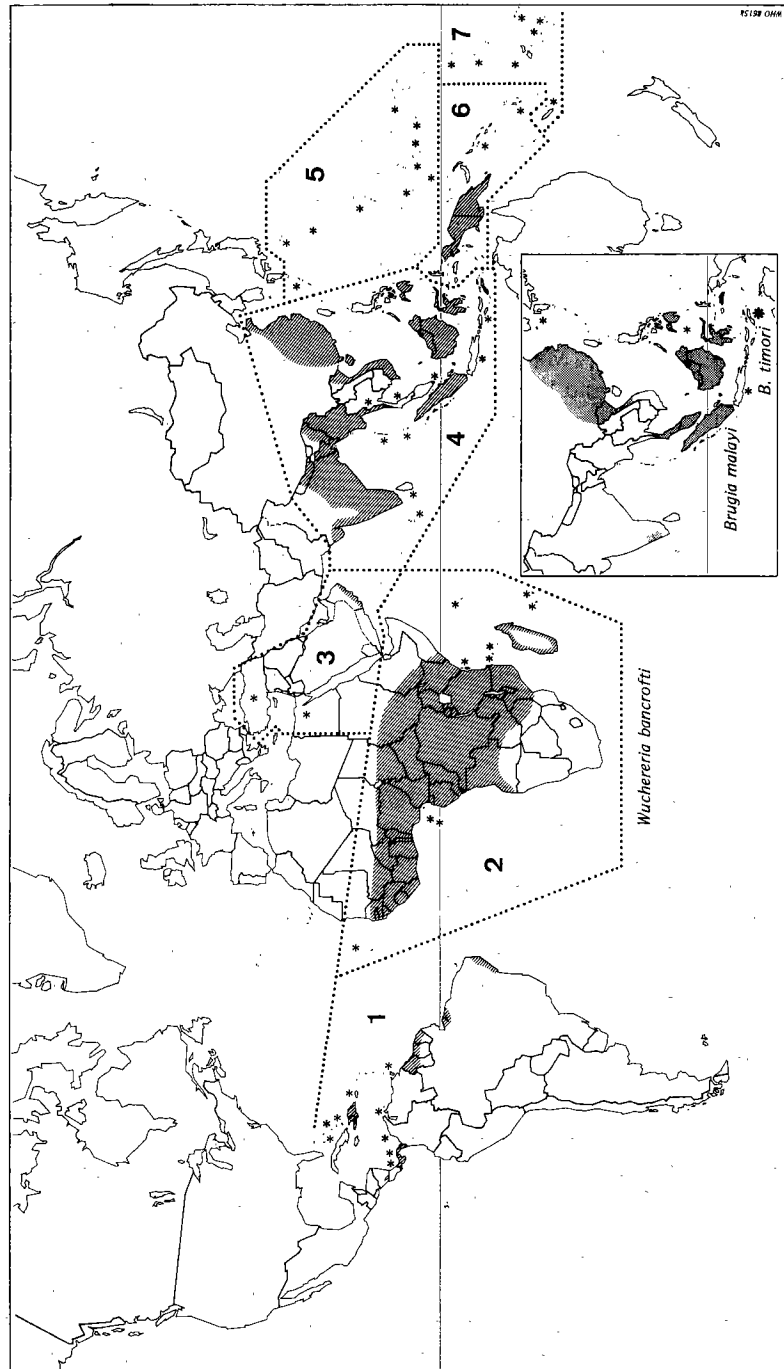
diurnally subperiodic types, microfilariae can be found in the peripheral blood at all hours but their density increases slightly during the night or day respectively.

The practical reasons for determining microfilarial periodicity are to know the best time to take blood samples to detect microfilaraemia and, in the case of nocturnally subperiodic *B. malayi*, to be aware of the possibility of animal reservoir hosts.

Vectors

Urban *W. bancrofti* is transmitted mainly by *Culex quinquefasciatus* (formerly known as *C. pipiens fatigans*) in tropical regions and by *C. pipiens pallens* and *C. p. molestus* in subtropical regions. The periodicity of the microfilariae is nocturnal.

Rural *W. bancrofti* is nocturnally periodic over most of its range. It is mainly transmitted by several species of *Anopheles*, occasionally by *Aedes* spp., and rarely by *Mansonia uniformis*. The diurnally subperiodic type is transmitted predominantly by several species of *Aedes*, and the nocturnally subperiodic type by mosquitos of the *Aedes niveus* group.

The nocturnally periodic type of *B. malayi* is transmitted in certain regions by *Mansonia* spp., and in other regions by *Anopheles barbirostris* or *A. campestris. Aedes togoi* is a vector in coastal parts of the Republic of Korea, and parts of southern China. The nocturnally subperiodic type is transmitted by *Mansonia* spp.

B. timori is transmitted by *Anopheles barbirostris*.

Reservoir hosts

The occurrence of zoonotic *B. malayi* in domestic cats and in *Macaca* and *Presbytis* monkeys living close to human habitations, and the presence of other, morphologically similar *Brugia* species (e.g., *B. pahangi*), in the same or related animals, complicates the epidemiology of *B. malayi*, and leads to difficulty in identifying the infective larvae found in wild mosquitos.

The same applies to other *Brugia* species infecting animals, such as *B. patei*, which is confined to Pate Island off Kenya where it is found in cats, dogs and genet cats; *B. ceylonensis*, found in cats and dogs in India and Sri Lanka; *B. buckleyi* in the wild hare in Sri Lanka; and *B. tupaiae* in the tree shrew in Malaysia, Thailand and Viet Nam.

Wuchereria kalimantani, found in leaf monkeys (*Presbytis cristata*) in South Kalimantan, Indonesia, poses similar problems in the epidemiology of bancroftian filariasis.

The relationship between periodicity and the animal reservoir in Brugia spp.

Until recently, it used to be thought that the nocturnally subperiodic form of *B. malayi*, which is zoonotic, could be distinguished from the periodic form by the fact that most of the microfilariae in the latter shed their sheaths in blood films. However, sheath shedding is not a feature of the non-zoonotic periodic form found in India, and it has recently been demonstrated that, in Indonesia, a number of zoonotic forms of the parasite exist that are not necessarily subperiodic. The zoonotic types, whatever their periodicity pattern, are difficult to control.

The microfilariae of *B. malayi* are morphologically very similar to those of other *Brugia* species of animal origin in standard stained blood films, but are different from those of *B. timori*. The infective larvae of *B. malayi*, of other *Brugia* species of animal origin, and of *B. timori* are morphologically very similar, which may create confusion in the evaluation of entomological data in places where these parasites occur together. So far, *B. timori* has not been found in animals, and *Brugia* spp. from animals have not been reported in areas where *B. timori* is endemic.

W. bancrofti

Although *W. bancrofti* has recently been transmitted experimentally to leaf monkeys (*Presbytis* spp.), no natural infection with this parasite has been reported in wild monkeys.

In wild mosquitos, the infective larvae of *W. bancrofti* may be confused with those of *W. kalimantani*, a recently discovered natural parasite of the leaf monkey.

Potential importance of non-human primate models

Non-human primates can be naturally or experimentally infected with *B. malayi* and other *Brugia* species, and *W. kalimantani* occurs naturally in the leaf monkey *P. cristata*. In the past few years, several non-human primate species have been successfully infected with *W. bancrofti*. These non-human primate models offer certain advantages for drug screening and for immunological and pathological studies.

4. Vectors

The vectors of filariasis include a wide variety of mosquitos of the genera *Culex, Anopheles, Aedes* and *Mansonia* (see Annex 1). The purpose of this section is to provide concise information on the most important and widespread vectors of filariasis, dealing mainly with the important bionomic aspects that are relevant to the control of filariasis.

Culex spp.

C. quinquefasciatus (formerly known as *C. pipiens fatigans*)

Distribution: widespread in tropical countries.

Vector of: nocturnally periodic *W. bancrofti*, especially the urban type.

Ecology: breeds in polluted waters, sullage water, drains, pit latrines, etc.

C. pipiens pallens

Distribution: China, Japan.

Vector of: nocturnally periodic *W. bancrofti*, especially the urban type.

Ecology: similar to *C. quinquefasciatus*.

C. p. molestus

Distribution: east Mediterranean.

Vector of: nocturnally periodic *W. bancrofti*, especially the urban type.

Ecology: similar to *C. quinquefasciatus*.

Aedes spp.

A. poicilius

Distribution: Philippines.

Vector of: nocturnally periodic *W. bancrofti*.

Ecology: Breeds in leaf axils, especially abaca and banana plants; rests indoors and outdoors.

A. polynesiensis

Distribution: Polynesia and the eastern Pacific.

Vector of: diurnally subperiodic *W. bancrofti*.

Ecology: breeds in small natural and artificial containers; rests outdoors; bites outdoors by day.

A. samoanus

Distribution: American Samoa and Samoa.

Vector of: diurnally subperiodic *W. bancrofti*.

Ecology: breeds in leaf axils; rests outdoors; bites outdoors at night.

A. togoi

> *Distribution*: coastal Japan, China and Republic of Korea.
> *Vector of*: periodic *B. malayi*; also a vector of periodic *W. bancrofti* in China and formerly in Japan.
> *Ecology*: breeds in brackish rock-pools or artificial water containers; rests indoors; bites indoors and outdoors after sunset.

Anopheles spp.

Many of the anopheline vectors of lymphatic filariasis in the western part of the Pacific also transmit malaria, as do *A. barbirostris* in Indonesia and *A. gambiae* in Africa.

> *Distribution and vector ability.* Important filarial vectors include: *A. barbirostris* for periodic *B. malayi* and *B. timori* in Indonesia; *A. campestris* for periodic *B. malayi* in north-west Malaysia; *A. sinensis* for periodic *B. malayi* in mainland China and the Republic of Korea; *A. farauti* and *A. koliensis* for nocturnally periodic *W. bancrofti* in Papua New Guinea, west Irian (Indonesia), and the Solomon Islands; and other *Anopheles* species with focal distribution in many areas.

> *Ecology.* Anopheline mosquitos breed in a wide variety of water bodies. They mostly rest indoors; and they bite at night, either indoors or outdoors. Many are vulnerable to spraying of houses with residual insecticide in conjunction with malaria control programmes. Larvicides are usually ineffective for their control. Mosquito nets, especially those impregnated with insecticide, and improved housing, with mosquito-screened sleeping quarters, can help to prevent human–vector contact.

Mansonia spp.

> *Distribution and vector ability.* Vectors include *M. annulifera* for periodic *B. malayi* in India and formerly in Sri Lanka; *M. bonneae* and *M. dives* for subperiodic *B. malayi* in Indonesia, Malaysia and the Philippines; and *M. uniformis* for periodic *B. malayi* in India, Indonesia, and north-west Malaysia.

> *Ecology.* The larval stages of *Mansonia* need to attach themselves to aquatic host plants. The adults mainly rest outdoors and bite outdoors by day.

At present, there is insufficient background information concerning operational control of *Mansonia* mosquitos. Reclamation of swamp land may eliminate the breeding sites of some *Mansonia* species.

5. Other filarial parasites of man

There are other filarial worms parasitic in man which may need to be distinguished from the lymphatic filarial parasites. These are:

Onchocerca volvulus in tropical Africa, Central and South America, and Yemen;

Loa loa in West and Central Africa;

Mansonella ozzardi in parts of Latin America and the Caribbean; *M. perstans* in tropical Africa and Latin America; *M. semiclarum* in Zaire; and *M. streptocerca* in West and Central Africa.

Onchocerca volvulus

This parasite is of very considerable public health importance because of its ability to cause onchocerciasis ("river blindness") and itching and disfiguring skin lesions. Hanging groins and hydroceles may develop as a result of onchocerciasis, and, in the Zaire River Basin, scrotal elephantiasis has been observed in onchocerciasis patients. The adult worms of *O. volvulus* live in subcutaneous or deep-lying nodules, and the microfilariae (mf) occur normally in the upper layers of the skin. They have also been demonstrated in the cornea and anterior chamber of the eye, in the urine, and in various body fluids. They are rarely found in the blood except at relatively low densities in heavily infected patients (up to 30 mf/ml recorded) or for a few days after beginning treatment with diethylcarbamazine citrate (up to 300 mf/ml recorded). The infection is transmitted by blackflies (*Simulium* spp.).

Loa loa

Loa loa causes fugitive "Calabar" swellings, often in the hands or arms. Migrating adult worms may occasionally be seen crossing the conjunctiva or in the eyelids. The sheathed microfilariae are found in the blood, and show a marked diurnal periodicity. Transmission is by tabanid flies of the genus *Chrysops*, commonly known as "red flies".

Mansonella ozzardi

Although sometimes held to be responsible for irritating skin eruptions, this is generally a harmless parasite. It is most common in Brazil and the Caribbean. Its microfilariae occur mostly in the blood and occasionally in the skin. They are non-periodic and are transmitted by *Simulium* spp. blackflies in South America and by *Culicoides* spp. midges in the West Indies.

Mansonella perstans

M. perstans seems to be an almost harmless parasite. Its microfilariae are found in the blood. They show no periodicity and are transmitted by *Culicoides* spp., midges commonly but erroneously known as "sandflies". *M. semiclarum* is a very similar species confined to Zaire.

Mansonella streptocerca

This species is occasionally responsible for skin irritation but is generally non-pathogenic. The microfilariae have so far only been found in the skin. It is transmitted by *Culicoides* spp.

6. The clinical manifestations of lymphatic filariasis

Lymphatic filariasis is characterized by a wide spectrum of clinical manifestations with signs and symptoms often differing from one endemic area to another. The clinical course of filariasis can be divided into asymptomatic, acute, and chronic stages, generally progressing in that order. However, in an endemic community the different stages of the disease are frequently interwoven with each other and, in certain groups of people originating from non-endemic filarial areas, the disease may be characterized by an acute initial stage followed directly by the chronic stage within a relatively short period of time.

Exposure versus infection

In most areas where filariasis is endemic, there is always a certain proportion of the population who, despite having been exposed to infective larvae, do not show any clinical manifestation of filariasis or any microfilaraemia with the presently available diagnostic procedures. It is impossible, in the absence of a reliable immunodiagnostic test, to determine whether people in this group have subthreshold microfilaraemia or subclinical infection, or whether they are free from infection.

The asymptomatic stage

This is characterized by the presence of microfilariae in the peripheral blood, although there are no clinical manifestations of filariasis. Some individuals remain asymptomatic for years, while others progress more rapidly to the acute and chronic stages.

The acute stage

The acute clinical manifestations of filariasis are characterized by recurrent attacks of fever associated with inflammation of the lymph nodes (lymphadenitis) and lymph vessels (lymphangitis).

In bancroftian filariasis, recurrent attacks of fever associated with lymphadenitis are less frequently seen than in brugian filariasis. In addition to the lymph nodes in the inguinal, axillary and epitrochlear regions, the lymphatic system of the male genitalia is frequently affected, leading to funiculitis, epididymitis or orchitis, or to a combination of these.

In brugian filariasis, the affected lymph nodes are mostly situated in the inguinal and axillary regions, with inflammation along the course of the distal lymphatic vessels. Quite often, an attack of lymphadenitis is precipitated by hard physical work.

The course of infection

Typically, each attack of fever and lymphadenitis lasts for several days and usually subsides spontaneously following bed-rest. However, it may be followed by a more or less characteristic retrograde lymphangitis, oedematous infiltration of the surrounding subcutaneous tissues, or even formation of abscesses, which may in turn ulcerate and lead to scarring. In contrast to bacterial infections, the ulcer in filariasis is relatively clean, and produces a serosanguinous fluid. Lymphoedema is quite often present in these fulminant episodes. Usually, the oedema subsides after each episodic attack, but with repeated attacks the oedema persists, leading to chronic lymphoedema.

The social aspects of acute filariasis

The acute clinical course of filariasis may last for several days or up to 4-6 weeks with a fulminating episode, and may result in prolonged inability to work. As filariasis is more prevalent in rural areas and in the slums of cities, and as it affects predominantly the young and active working section of the population, it can result in significantly decreased productivity of the poorer sectors of the community—those who can least afford it.

The chronic stage

The chronic signs of filariasis do not usually develop before the age of 15 years and only a small proportion of the infected community is affected. However, immigrants from areas where filariasis is not endemic tend to develop elephantiasis more often and much sooner (sometimes within 1-2 years) than do the indigenous populations of endemic areas.

During the chronic stage, microfilariae are usually absent from the blood.

In bancroftian filariasis, the occurrence of the major chronic signs—hydrocele, chyluria, lymphoedema and elephantiasis—may differ from one area to another. The most common are hydrocele and swelling of the testis, followed by elephantiasis of the entire lower limb, the scrotum, the entire arm, the vulva, and the breast, in descending order of frequency.

In brugian filariasis, the leg below the knee is characteristically affected, and sometimes the arm below the elbow. Genital involvement has not been reported, except in areas where brugian filariasis occurs together with *W. bancrofti*.

The social aspects of chronic filariasis

The chronic clinical manifestations of filariasis are characterized by an absence of physical pain and suffering when there is no associated adenolymphangitis. However, patients with elephantiasis or hydrocele have many social and marital problems. They have less chance of getting married or, when the disease manifests itself during their married life, it may lead to divorce. Moreover, persons with extremely large legs or hydrocele are not able to work or even to take care of themselves. They are a burden on the family and the community.

The chronic stage of elephantiasis is not a static condition. In an endemic area, persons with elephantiasis are exposed to infective larvae in a similar way to those who have no elephantiasis, although their bodies appear to be counteracting these infections, most probably through immunological mechanisms. It is therefore not unusual for patients with elephantiasis to continue to have recurrent adenolymphangitis, leading to incapacity and work loss. These attacks are known as acute-on-chronic infections.

Tropical pulmonary eosinophilia of chronic filariasis

Tropical pulmonary eosinophilia may be caused by human or non-human filarial parasites. It is characterized by immunological hyper-responsiveness of the human host to the parasite, especially the microfilariae. There is a marked increase in the production of IgE and IgG antiparasite antibodies and a pronounced hypereosinophilia. In some areas, it is associated with paroxysmal nocturnal cough, breathlessness and wheezing, occasionally accompanied by a radiological picture of diffuse patchy infiltration of the lungs. In other areas, it is associated with lymphadenopathy, and sometimes hepatosplenomegaly. Microfilariae are almost never present in the blood, but remnants of microfilariae surrounded by aggregates of eosinophils are sometimes found in the spleen, liver, lymph

nodes or lungs. The syndrome responds quite well to treatment with diethylcarbamazine citrate (DEC).

Filarial granulomata

Painless lumps in the breasts, testicles or subcutaneous tissues, and a "coin" shadow in the lung, can reflect granulomatous reactions around adult or developing adult worms of human or animal filarial parasites. They should be regarded as filarial infections in an unusual location or in an abnormal host.

Other syndromes of uncertain relation to filariasis

In certain areas, arthritis has been suspected as being a sign of filariasis. Typically it is described as a mono-arthritis, most commonly affecting the knee, with soft tissue swelling and transudation, frequently painless and appearing quite suddenly. It is reported that this syndrome responds favourably to DEC. Its causal association with filariasis is not yet conclusively established.

Similarly, a variety of other syndromes that coexist with filariasis or are found in filarial-endemic areas, have been suspected as being manifestations of filarial disease. These include endomyocardial fibrosis, tenosynovitis, thrombophlebitis, dermatoses, lateral popliteal nerve palsy, and others. In the absence of further evidence, such syndromes cannot as yet be attributed to a filarial etiology.

Differentiation from other syndromes

Lymphoedema versus elephantiasis

Lymphoedema and elephantiasis are often difficult to differentiate clinically except when, in the former condition, the swelling proves to be transient. Elephantiasis is characterized by hyperplasia and fibrosis of the subcutaneous tissue and thickening of the skin. However, in barefoot farming communities, skin thickening is quite common.

Other etiologies of elephantiasis, chyluria and hydrocele

Other conditions that cause compression of the lymphatic vessels may reduce lymph flow and produce subsequent lymphoedema or even elephantiasis of the affected part of the body, distal to the compression. These include tumours and fibrotic tissue formation as a result of burns, irradiation, or surgical lymphadenectomy. Onchocerciasis may also cause elephantiasis. In Africa, elephantiasis with a physicochemical etiology

(absorption of silicate particles through the skin) has been reported in certain areas where filariasis is not endemic.

Compression of the thoracic duct above the lymphatic vessels of the kidneys may produce chyluria or chylocele. Acute trauma of the testicle may produce haematocele; and chronic trauma may produce hydrocele, as among the drivers of *beca* tricycles in Indonesia. The clinical history and serological tests for the detection of antifilarial antibodies may aid in the differentiation of these conditions from filariasis (see Section 7). The finding of microfilariae in urine (in chyluria) or hydrocele fluid is conclusive support for the diagnosis of filariasis.

7. Diagnosis

Detection of microfilariae

Microfilariae can be detected in blood, urine (in chyluria), hydrocele fluid, or tissues.

Microfilariae in blood

The time of blood collection should be as close as possible to the peak of the microfilarial periodicity for the species and strain concerned. Measured fingerprick blood samples of 20–120 µl are used, depending upon the purpose of the diagnostic test. For mass survey and treatment a blood sample of 60 µl is recommended. To identify microfilaria-positive cases in a selective treatment programme, a larger volume of blood (2×60 µl blood films or 1 ml for membrane filtration) should be taken for diagnosis.

The blood slide (see page 58). The conventional thick blood film is still the best method for field work. It is particularly important for correct species identification in areas where mixed infections occur. It can be used for malaria detection at the same time. The slide should be perfectly clean, and the blood sample evenly spread, dried, carefully dehaemoglobinized, fixed, stained, correctly labelled and stored for examination.

The counting chamber (see page 66). The counting chamber can be used in areas where the parasite has been previously identified, but preferably not in an area where mixed infections are found.

The diethylcarbamazine citrate (DEC) provocative test. In this test, an adult is given 100 mg of DEC (50 mg for children, i.e., 1.5–2 mg/kg of body weight) by mouth in the daytime, and the peripheral blood is examined for microfilariae 30–45 minutes later. This test "provokes" micro-

filariae in the lung capillaries to invade the peripheral blood. It should only be used in areas where the microfilariae have a nocturnal periodicity and where night-blood collection is impossible. It has a lower sensitivity than the night-blood sample. Furthermore, DEC should not be used in areas where *Wuchereria bancrofti* occurs together with *Onchocerca volvulus* or *Loa loa*, since it may provoke a severe reaction (the "Mazzotti reaction").

Concentration techniques.

(a) Membrane filtration techniques (see pages 62 and 68) using Millipore or Nuclepore membrane filters of 3–5-μm porosity are the most sensitive tools currently available for the detection of microfilariae. Venous blood samples of 1–10 ml or even more have been used by various workers. These methods are expensive and are normally reserved for special purposes, such as for the diagnosis of individual cases, evaluation of treatment, as a research tool or in pilot control studies.

(b) Knott's concentration technique (see page 69) is an alternative concentration method when membrane filters are not available. It has a lower sensitivity than membrane filtration, since microfilariae are more likely to be missed in the viscous sediment.

Microfilariae in hydrocele fluid or urine (see page 69)

Microfilariae are sometimes absent from the blood but present in hydrocele fluid or urine. The specimens should be processed and examined using one of the concentration techniques described above.

Clinical diagnosis

Acute adenolymphangitis in communities

A clinical history of recurrent fever associated with adenolymphangitis is strongly indicative of filariasis and constitutes a syndrome that is usually well known to the inhabitants of endemic areas. The presence of scars at typical locations—e.g., over the inguinal and epitrochlear lymph nodes—supports the diagnosis of filariasis. The presence of clinical cases with acute and chronic manifestations of filariasis in the community provides more conclusive evidence.

Diagnosis of individual cases with clinical manifestations of filariasis

A history of having lived in an endemic area of filariasis should focus attention on a possible filarial etiology of adenolymphangitis. The detection of filarial parasites or antifilarial antibodies provides supporting

evidence for the diagnosis, as does the presence of a microfilaria-positive individual living in the same house as the patient.

Diagnosis of individual cases with bronchial asthma or lymphadenopathy

An eosinophil count of more than 3000 per µl of blood gives rise to a suspicion of occult filariasis. Supportive evidence is a chest X-ray showing suggestive shadowing or the presence of a high titre of antifilarial antibodies of the IgE class. A favourable response to DEC provides additional evidence.

Detection of adult or developing adult worms

Adult or developing adult worms are sometimes found in biopsy specimens of lymph nodes. Knowledge of the microanatomy of adult filarial worms in cross-section is essential for differentiation of human and animal filarial parasites. The presence of microfilariae around the adult worm may help in establishing the species.

Detection of filarial larvae in mosquito vectors

After parasite control with DEC, mass dissection of mosquito vectors collected from the treated locality may aid in tracing microfilaria-positive individuals. The presence of human filarial L_3 larvae in these mosquitos indicates a need for examination of blood samples from that locality for case detection.

Immunodiagnostic tests

None of the immunodiagnostic tests currently available is able to define accurately the presence of infection, either because of lack of specificity or because of inability to discriminate between present and past infection. However, the absence of antifilarial antibodies in a patient residing in a nonfilarial endemic area excludes the possibility of a filarial etiology.

Recent advances in immunodiagnosis offer encouraging leads, and it is to be hoped that in the future these problems will be solved. Reagents (antibody and antigen) with high degrees of stage- and species-specificity are being sought and developed to detect the presence of active infection, and to determine the intensity of infection (worm burden), to discriminate between present and past infection, to assess the effects of antiparasite control measures, to detect previous exposure to infective larvae, and to solve other currently insoluble diagnostic problems.

8. Treatment

Treatment of individual patients

The objective of treatment is to eliminate the parasite in order to reduce or prevent morbidity. Diethylcarbamazine citrate (DEC) is currently the only drug available for the treatment of lymphatic filiarisis that is effective, safe and relatively cheap. It kills almost all the microfilariae and a good proportion of adult worms. It is probably also effective against the L_3 and L_4 larval stages. DEC is generally very safe, but it is advisable not to give the drug to pregnant women; care should also be taken when treating people with chronic kidney or cardiac disorders.

Wuchereria bancrofti

Dosage. The recommended dose of DEC is 6 mg/kg of body weight daily for 12 consecutive days (total dose, 72 mg/kg). Repeated courses of treatment may be necessary to achieve radical cure. The drug is rapidly excreted from the body, mainly through the kidneys and the possibility of a cumulative effect is minimal. A repeat course may be initiated approximately 2 weeks after the last dose of the previous course.

The excretion of DEC in urine is highly pH-dependent. Under acid conditions (pH 5), some 70% of the given dose of DEC is excreted unchanged in the urine, while under alkaline conditions (pH 7.5 and above) only 5% of DEC is eliminated.

Side-reactions. There are two groups of side-reactions, general and local, both with or without fever. It is generally agreed that side-reactions are less likely to occur and are less severe in bancroftian filariasis than in brugian filariasis. General reactions, in more or less decreasing order of frequency, are: headache, body ache, dizziness, decreased appetite, malaise, nausea, urticaria, vomiting, and sometimes bronchial asthma. Local reactions are: lymphadenitis, funiculitis, epididymitis, orchitis, lymphangitis, abscess formation, ulceration and transient lymphoedema.

General reactions and fever are positively associated with microfilaraemia and the density of microfilariae. They occur early during the treatment and generally do not last more than 3 days. Local reactions occur mainly in people who have histories of adenolymphangitis. They tend to occur later and may last longer.

Both general and local reactions will disappear spontaneously and usually it is not necessary to interrupt treatment. Symptomatic treatment of side-reactions with antihistamines and analgesics may be helpful.

Results of treatment. The following results can be expected from treatment with DEC:

(*a*) elimination of, or great reduction in, the level of microfilaraemia;

(*b*) reduction in the frequency of, or complete prevention of, further attacks of acute filariasis;

(*c*) reversal of early lymphoedema, some early chyluria, some early hydrocele and some chronic lymphoedema.

Brugia malayi and B. timori

Treatment is similar to that for *W. bancrofti* but with the following differences.

(*a*) Treatment is associated with more severe side-reactions.

(*b*) There is little consistency in the daily and total doses of DEC commonly used in different countries where *B. malayi* is endemic. The daily dose ranges from 3 to 6 mg/kg of body weight and the total dose from 18 to 72 mg/kg of body weight.

Treatment of communities

Objectives of treatment

The target population and the objective of treatment should be clearly defined. The objective may be any of the following:

(*a*) to reduce morbidity by treating clinical cases of filariasis;

(*b*) to reduce transmission and consequently to reduce morbidity by treating people with microfilaraemia;

(*c*) a combination of (*a*) and (*b*);

(*d*) to interrupt transmission and thus, in favourable circumstances, to eliminate the parasite from the human population.

Approach to community treatment

There is a basic difference in approach between individual and community treatment. In the first case, it is usually the patient who comes to the doctor or to the community health worker. The patient realizes that he or she is ill and needs help, and is therefore more likely to comply with the treatment. This is particularly true in patients suffering from an acute attack of adenolymphangitis, who are likely to accept treatment with DEC willingly, and in whom the treatment may be expected to prevent or delay the development of chronic disfiguring or disabling lesions and to reduce the chances of recurrent acute attacks in the future. In the community as a whole, on the other hand, usually only a few people will ever have had

clinical filariasis. Furthermore, at the time of mass or large-scale treatment, not all of this group will actually be suffering from the disease. It follows that the desire for help may be less.

Motivation to increase cooperation

The key to success is the ability of the team undertaking treatment to communicate effectively with the community. Influential people in the community should be approached for assistance. The help of local authorities and local health personnel is essential. They should all be involved from the beginning. The general health needs of the community should be identified and help given as far as possible. Once mutual understanding is established, the objectives of treatment and the nature of anticipated side-reactions should be explained.

The people should be taught to recognize acute and chronic signs of filariasis by being shown actual cases selected from among the community. Living microfilariae from night-blood samples of known carriers should be demonstrated under a microscope. The significance of night-blood collection and the association between disease manifestations and the causative agent should be explained. Infective stage larvae from experimentally infected mosquitos should be shown and the role of mosquitos as transmitters emphasized. The community should be involved as much as possible in the treatment programme as a form of self-help.

Choice of treatment

(a) Mass treatment. This can be given as part of a vertically organized filariasis control campaign, with a single full course of DEC being given to all members of the community. Alternatively, repeated small doses of DEC may be handed out by chosen villagers to the remainder of the community. DEC can also be distributed in medicated salt in a mass treatment regimen.

(b) Selective treatment. Only individuals with clinical manifestations and/or microfilaraemia are treated. If the objective is to reduce morbidity by treating all those with acute or acute-on-chronic attacks of adeno-lymphangitis and/or filarial fever, this can be affected on a permanent horizontal basis via the primary health care system. Each community health worker is given a 2-day course of training in the recognition and treatment of these symptoms and is 'provided with a supply of DEC to treat all such cases as they present themselves. Experience in India has shown that a 6-day course of 500 mg of DEC per day is acceptable and effective in these circumstances.

If the objective is to select and treat all microfilaria-positive individuals in a community by means of a vertically organized campaign, then the most sensitive technique for detection of microfilariae that is practicable in the prevailing local conditions should be used. The microfilaria-positive cases should then receive a full course of DEC.

Dosage

The total dose of DEC per person is similar to that given for individual treatment. Whether the drug is given daily, weekly or monthly, similar results can be expected as long as the total dosage over the whole period is the same.

PART 2. CONTROL OF LYMPHATIC FILARIASIS

PART 2

Control of lymphatic filariasis

9. Objective of control

The objective of control should be clearly defined. It may be any of the following, in increasing order of difficulty:

(a) reduction of morbidity;
(b) reduction of transmission;
(c) reduction of morbidity and transmission;
(d) interruption of transmission.

Reduction of morbidity

If the objective is merely to reduce the existing filarial morbidity in the community there may be no need for blood examination or determination of microfilaria indices. What is needed is a team whose members are knowledgeable in the clinical manifestations and treatment of filariasis. The problem with this approach is that many early infections that will eventually lead to morbidity may be missed.

In order to determine which strategy should be employed to achieve the objective, as well as for subsequent evaluation of the campaign, it will usually be necessary to make observations on the prevalence of micro-filaraemia as well as of clinical manifestations. However, if this is not feasible, a good practical solution is to make DEC available, through the primary health care system, to all those suffering from an acute or acute-on-chronic attack of filarial adenolymphangitis or fever.

Reduction of transmission

Transmission can be reduced by decreasing the microfilaria rate, the vector density, or the contact between the vector and the human host; or by any combination of these methods. Reducing transmission will also bring about a decrease in the disease rate. The amount of transmission may be assessed by studying both the numbers of infective larvae of

human lymphatic filarial parasites in the mosquito population, and by measuring the prevalence and intensity of microfilaraemia in the human population.

Reduction of morbidity and transmission

This is the general approach to filariasis control in most endemic areas. There are no definite criteria as to the level to which the morbidity, the microfilaria rate and density, or the vector density and infectivity rate should be decreased in order for filariasis to cease to be a public health problem.

Interruption of transmission

Ideally this is the ultimate objective. However, owing to the longevity of the adult worms, and the limited resources of manpower and finance in most endemic countries, it is usually somewhat unrealistic to aim for elimination of filariasis. So far only a few countries have achieved this goal. However, once it is accomplished, and provided there is no risk of reimportation or zoonotic transmission, there will be no need for continued costly maintenance or evaluation of the control programme.

10. Methods for the control of lymphatic filariasis

Control of any mosquito-borne disease should be directed against both the parasite and the mosquito. The life cycle of the filarial parasite is relatively long. In contrast to the malaria parasite, it does not multiply in the mosquito vector, nor do the infective L_3 larvae multiply in the human host, where each develops to a single adult worm, male or female. The parasite, therefore, does not cause explosive epidemics of disease, and control is thus relatively easy to achieve.

Parasite control

Parasite control with diethylcarbamazine citrate (DEC) is relatively cheap. The drug is safe and effective for human lymphatic filariasis. Results are usually apparent within a relatively short period. Unfortunately, when given in standard doses, DEC quite often produces side-reactions—especially in brugian filariasis—which are frequently unacceptable to the affected communities (see page 27).

It should be remembered that people who do not complete the entire course of DEC will have their microfilarial densities substantially lowered,

even if they have only received one or two doses of the drug, and will therefore be less dangerous to the community. On the other hand, those who are not covered during the distribution of DEC may have high microfilarial densities and remain a potential source of reinfection for the community.

There are several ways of distributing DEC to the population: mass treatment with standard dosage; selective treatment with standard dosage; intermittent low-dose treatment (selective or mass); or use of DEC-medicated salt.

Since in many areas the elimination of the parasite from the community is unlikely to be achieved in the foreseeable future, treatment campaigns may have to be repeated. The selection of treatment regimen may also change during a control programme, the change being based upon the community's acceptance of a specific regimen, the degree of success obtained, and the cost-effectiveness of the regimen adopted.

Mass treatment with DEC

The concept behind this strategy is that, in an area of high endemicity, everyone is more or less equally exposed to the infective bites of the vector, and available methods are not sufficiently sensitive to diagnose subpatent or subclinical infections. Therefore, in mass treatment, DEC is given to almost everyone in the community irrespective of whether or not they have microfilaraemia or disease manifestations. However, the drug should not be given to infants, pregnant women, the elderly, or those with obvious debilitating disorders, especially those with cardiac or kidney disease.

The advantages of mass treatment are as follows:

(a) It is not necessary to examine every member of the community. If the target population is large, examination of a statistically appropriate sample will give a valid estimate of the filarial endemicity of the community.

(b) It is not necessary to use a highly sensitive method to detect microfilaria-positive individuals in the community.

(c) There is no need to worry about false negative results, which may be due to the insensitivity of the method, inappropriate blood collection or processing, or technical errors.

(d) In mass treatment, the chance of not giving DEC to a person occurs only once, i.e., during the distribution of the drug. In selective treatment, the chance of missing a person occurs twice: once during selection of diseased and microfilaria-positive individuals and again during the distribution of the drug.

(e) Mass treatment avoids the problems of discrimination whereby some microfilaria carriers, feeling themselves to be in perfect health, may wonder why they have been selected for treatment, while amicrofilaraemic people may wonder why they are excluded from receiving the medicine.

Selective treatment with DEC

In selective treatment, microfilaria carriers and/or people with clinical manifestations of filariasis are first selected by large-scale screening of the community. Subsequently DEC is given only to those who are microfilaria-positive. For reasons of economy and acceptability selective treatment is more suitable in areas of low endemicity. The decision as to the minimum level of microfilaria prevalence that justifies mass, as opposed to selective, treatment has to be an empirical one based on the objectives and the cost of the operation.

DEC-medicated salt

Common salt medicated with 1–3 g of DEC per kg can also be used for filariasis control, and is particularly useful after an initial reduction in prevalence has been achieved by mass DEC treatment or by large-scale selective treatment of microfilaria carriers using the usual recommended doses of the drug. This form of treatment is only feasible in filariasis areas where adequate control of the salt supply can be achieved, and where the people will accept medicated salt. Medicated salt treatment should be continued for at least 6–9 months.

Low-dose DEC treatment given "by the people for the people"

The principle of distribution of DEC for low-dose treatment "by the people for the people" is based on the following concepts.

(a) The use of medicated salt has shown that low-dose DEC schedules are an effective means of controlling or even eradicating filariasis.

(b) All members of the affected community are involved, encouraging them to participate in health care through informal health education.

(c) The people derive great satisfaction and pride from their achievements, which boost their confidence and self-reliance.

(d) Distribution of the drug itself is more efficient than when using DEC-medicated salt.

(e) Side-effects are less likely to occur than with the standard dose regimen.

The dose of DEC in the low-dose schedule is 25 mg for those below 10 years of age and 50 mg for those aged 10 years and above. This dose is

distributed on a weekly basis for at least 18 months. The total dose over the 18 months is about 3900 mg of DEC for an adult, which is more or less equivalent to the standard course of DEC treatment. The drug should be distributed by motivated people in the community (such as the village chief, schoolteachers, or people with elephantiasis) to the heads of all the families in the village, and proper recordings of the distribution are made. Each family head is in turn responsible for treating the members of his or her family. Certain people should be excluded from treatment, as given on page 27.

Annual single-dose treatment with DEC

Single doses of DEC of 6 mg/kg of body weight given once a year have recently proved acceptable and reasonably effective against diurnally-subperiodic *Wuchereria bancrofti* infections in lightly-infected communities in Samoa and Tahiti. The treatment reduced microfilaraemia prevalence by 50% and mean microfilarial density by 90% in treated microfilaria-positive cases. A large-scale trial with a population of 50 000 was also successful in Tahiti, bringing the microfilaria rates down from 4.4% to 1.9% after 4 years, the average dose received during the 4 years being 2.76 mg/kg of body weight. This regimen merits further investigation in other countries.

Vector control

Before vector control measures are started, the mosquito species responsible for transmission have to be determined and their breeding sites, feeding habits, biting densities, resting habits, and infection and infective rates investigated. Without confidence in the identification of the developing and infective larvae in the mosquitos, infection and infective rates are of no value for epidemiological studies. Since the various mosquito species involved in the transmission of different forms of filariasis have different breeding and feeding habits, different approaches will have to be made for their control.

The standard approaches are against the adults or the larvae of the mosquito vectors, or both. In general, antilarval measures are conducted in urban areas against *Culex quinquefasciatus*. Similarly, in rural areas, measures may be directed against the larval stages of the vector species (anophelines, certain species of *Aedes* such as *A. polynesiensis*, and *Mansonia* spp.), provided that their breeding places are accessible and relatively few in number, and that operations can be closely supervised.

Where there is little information about the breeding places of the vector mosquitos, or where they are very widespread or inaccessible, as often

happens in rural areas (with *Anopheles gambiae*, for instance), measures may have to be taken against adult mosquitos.

It should be remembered that the adult filarial worms have a long life-span. Even if vector control measures are successful, the microfilaria and disease rates may remain at the same level for several years in the absence of drug treatment.

Antilarval measures

Before carrying out antilarval measures, a map should be prepared showing all breeding places. The actual water surface area in each breeding site should be measured at least twice a year, once during the rainy season and once during the dry season.

Use of insecticide. As far as possible, breeding places should be cleared of scum and vegetation before the larvicide is applied, so as to maximize its efficiency. Larviciding is commonly used in urban areas, primarily against *C. quinquefasciatus.* This species is generally resistant to organo-chlorine insecticides. However, newer selective insecticides in the organo-phosphorus, carbamate, and pyrethroid groups can provide effective control for this vector.

Commonly used larvicides are the organophosphorus insecticides such as temephos, fenthion, fenitrothion, chlorpyrifos, and pirimiphos-methyl. Temephos is relatively non-toxic to mammals and rapidly biodegradable; it can therefore be used in mosquito breeding sites that are also sources of drinking-water. However, its residual effect is too short to be effective in polluted water. Chlorpyrifos is relatively toxic but it remains active in polluted water where *C. quinquefasciatus* normally breeds. Certain insect development inhibitors, such as methoprene, have also been used for *C. quinquefasciatus* control.

The following dosages have been used against *C. quinquefasciatus:*

(*a*) chlorpyrifos: dosage 0.1–1.0 mg of active ingredient per litre of water; remains highly active for 12–24 weeks;

(*b*) methoprene: dosage 1.0 mg of active ingredient per litre of water; inhibits emergence of adults for some 21 days.

Biological control.

(*a*) *Larvivorous fish.* Although larvivorous fish have been used as biological control agents for mosquitos for a long time, there has been no proper assessment of their efficacy. *Gambusia affinis* and *Poicilia reticulata* are the two fish most commonly used. *G. affinis*, being a temperate-climate species, cannot reproduce successfully at temperatures above 29 °C. Hence it is not suitable for use in most tropical environments. At present it is considered environmentally unacceptable to import

fish to any locality for mosquito control, and hence the use of local larvivorous fish is to be encouraged.

(b) *Microbial agents.* With the rapid development of microbial agents for mosquito control in general, these agents will soon become available for filariasis vector control, and it is anticipated that they will have an important role to play. Generally, *Bacillus thuringiensis* H-14, which produces a potent insecticidal exotoxin and is scarcely self-replicating in the field, has been shown to be effective against larvae of mosquitos of the genera *Aedes* and *Anopheles.* On the other hand, *Bacillus sphaericus* (strains 1593 and 2362), which is self-replicating in the field and produces an insecticidal endotoxin, is highly effective against *Culex* and *Mansonia* mosquitos.

Environmental modification. Most mosquitos are quite fastidious in their selection of breeding sites. They may prefer fresh water, polluted water, brackish water, lakes, rivers, rock pools, rice fields, a certain degree of shade and temperature, or particular velocities of flow, aquatic plants or other factors necessary for the development of the larvae. Once a species incriminated as a vector has been properly identified, and adequate knowledge of its bionomics is available, it may be possible to modify the environment so as to make it unsuitable for that species. This concept of "species sanitation" helps to narrow the public health objectives to attainable goals. Modifications may include the following.

(a) *Drainage*: good urban drainage systems help to control urban bancroftian filariasis transmitted by *C. quinquefasciatus.*

(b) *Reclamation of swamp land* and its conversion to rice fields achieves the following goals:

    (i)   more food production;

    (ii) reduction or elimination of breeding sites of *Mansonia* and consequently a decrease in the prevalence of brugian filariasis;

    (iii) creation of new breeding sites of other mosquito species, which may be less likely to transmit filariasis.

Destruction of swamp forest also eliminates the habitat of *Macaca* and *Presbytis* monkeys, which are reservoir hosts for subperiodic *Brugia malayi.*

(c) *Filling of land depressions* that contain standing water is one of the most satisfactory methods of eliminating mosquito breeding sites.

(d) *Community efforts* can reduce the number of man-made small-container breeding sites of *Aedes polynesiensis.*

(e) *Removal of aquatic plants* (*Pistia*, etc.) helps to control *Mansonia* spp.

(f) *Polystyrene balls* may be used in pit latrines, etc. to prevent access of *C. quinquefasciatus* to the water for oviposition, and to interfere with larval respiration and pupal emergence.

Anti-adult measures

Insecticides. The usefulness of insecticides in the control of filariasis is limited. This is because many important vectors do not rest indoors, and therefore cannot be controlled by house-spraying. In addition, vector control by insecticide spraying requires long and continuous effort and is less cost-beneficial against filariasis than against malaria.

It is therefore not surprising that the beneficial effects of insecticide spraying in the control of filariasis are seen mostly in conjunction with malaria control programmes, where the mosquito is a double vector for malaria and filariasis or where the vector of filariasis has the same habit of invading human dwellings as the vector of malaria.

As with malaria control, the selection of insecticide, dosage, and the scheduling of cycles and rounds should be developed from knowledge of vector bionomics, human habits, housing conditions, weather patterns, and the interrelationship of these variables with disease transmission by the vector. Indoor biters and indoor resters are most vulnerable to residual spraying. These include most of the anopheline vectors of filariasis.

Community measures. The frequency of human–vector contact can be reduced by: the construction of better housing, with mosquito screens and/or mosquito-proof bedrooms; the use of mosquito nets, with or without insecticide impregnation, repellents, mosquito coils and proper clothing; the use of latrines; and the covering of breeding sites. In rural areas cattle are often tethered in front of bedroom windows so as to attract the mosquitos away from the human inhabitants.

A simple method of adult vector control—and one suitable for community use—is the treatment of mosquito nets with a residual pyrethroid insecticide, such as permethrin or deltamethrin. The net is placed in a plastic bag containing a dilute solution of insecticide. The exact quantity of liquid needed to treat the net, with negligible run-off, should be used. The dosage applied is about 0.5 g of active ingredient/m^2. The treated net is allowed to dry and the residual effect of the insecticide can last for 6 months or more. Mosquitos seeking a blood-meal will come into contact with a lethal dose of insecticide while trying to pass through the net to feed on the person inside.

Obtaining the active participation of the community is one means of extending vector control coverage to communities that do not receive the benefit of a routine and organized vector control service. There are no hard and fast rules for effective development and implementation of community participation programmes in vector control. There are undoubtedly difficulties and obstacles to be overcome, but such programmes are a long-term process which can be expected to produce meaningful results as they progress year after year.

11. General guidelines for filariasis control programmes

The problems of filariasis control differ from one country to another and may not be similar even in different areas of the same country with the same species of parasite and vector. It is therefore impossible to lay down rigid guidelines for control that can be applied in all countries where the disease is endemic. The purpose of this section is to provide general guidelines to improve the approach to, and understanding of, the problem of filariasis, and to promote a systematic procedure for achieving filariasis control in the most practical, efficient and economical way.

General constraints

These are:

(*a*) lack of trained manpower;
(*b*) insufficient funds;
(*c*) inadequate infrastructure for health care delivery;
(*d*) lack of proper epidemiological data and knowledge of the distribution of filariasis and/or vectors;
(*e*) low priority of the disease compared with other health problems.

Systematic approach

Preliminary data collection

Data on prevalence of elephantiasis or hydrocele can be obtained from the following sources:
— *in urban areas*: from hospitals, private physicians and other health services;
— *in rural areas*: from health services, private physicians or village chiefs.

Spot surveys

On the basis of preliminary data on prevalence of elephantiasis and hydrocele, spot surveys should be undertaken in various areas where cases are prevalent in order to collect baseline data on:

(*a*) the characteristics of the human population;
(*b*) the prevalence of microfilariae and disease;
(*c*) the species of parasite;
(*d*) the periodicity of the parasite in order to determine the optimal time for blood collection, and the possibility of an animal reservoir in the case of *Brugia malayi*;

(e) the species, breeding sites and behaviour of the vectors;
(f) possible reservoir hosts;
(g) the characteristics of existing health care delivery services.

A good recording system is essential for epidemiological studies of filariasis.

Pilot control studies

These should be done on a small scale, using various strategies and treatment regimens, applying the most sensitive evaluation methods, even when they are expensive, and carefully evaluating the studies for a sufficient period of time. This may appear to be a costly investment but, in the long run, it will indicate the best and most economical approach to the control of filariasis in the prevailing circumstances.

Evaluation of control measures

The following are useful for evaluating control measures: microfilaria rate; microfilarial density; clinical assessment; and entomological assessment.

(a) Microfilaria rate

The microfilaria rate is expressed simply as the percentage of microfilaria-positive people among the total number of people examined. The people examined should be representative of the general population at risk. It is recommended that quantitative blood samples are taken in order to compare pre- and post-treatment values and to compare the results from different areas.

(b) Microfilarial density

The microfilarial density in the blood is the number of microfilariae per unit volume of blood sample. This is also called the microfilaria count, and the figures are usually analysed by means of a log-probit analysis or as geometric means.

Microfilarial density is important because it has been shown that in some instances it has a better association with disease rate than has the microfilaria rate. During the period after treatment of the population with diethylcarbamazine citrate (DEC), individuals with high microfilarial densities should be looked for particularly carefully, since they are most likely to be sources of reinfection for the treated population. A high number of such individuals during the post-treatment period may indicate inadequate coverage, drug refusals or new immigrants.

(c) Clinical assessment

When possible, a clinical assessment of filariasis should always be made. This includes the following observations on the acute and chronic manifestations of the disease.

> (i) *Adenolymphangitis rate.* This is expressed as the percentage of people with either a clinical history of adenolymphangitis in a defined period of time (usually 1 year) or with acute physical signs of filariasis at the time of physical examination among the total number of people examined. If possible, efforts should be made to record the duration of each attack of adenolymphanigitis and the frequency of attacks in the community. Knowing the total duration of the adenolymphangitis attacks that each person has experienced in a year and the total number of people with adenolymphangitis, the number of working days lost per year in the community on account of filariasis can be calculated.
>
> (ii) *Elephantiasis rate, chyluria rate and others.* These are calculated similarly to the adenolymphangitis rate.

The acute clinical manifestations should be the preferred parameter for the early clinical evaluation of a control programme. The prevalence of chronic manifestations takes a longer time to change and should only be used in longer-term evaluation.

(d) Entomological assessment

Having established the identity of the local vectors and their biting behaviour, and having acquired confidence in identifying infective larvae, evaluation of changes in transmission should be based on the annual transmission potential (ATP). The ATP is the total number of infective larvae of human lymphatic filarial parasites that are found in the number of mosquitos that would bite a person who was fully exposed during all hours of biting for a full year.[1] The ATP is usually calculated by extrapolation from data obtained during a number of mosquito catches made at a particular catching point over the year. It is an index that is useful for comparing the amount of transmission in different places, or in the same place before, during, and after control. It does not represent the actual degree of exposure to infective larvae undergone by any person in the place concerned. This would be much lower and is almost impossible to estimate.

It is recognized that it is not possible to distinguish the infective larvae of different species of *Brugia* using morphological criteria. This fact must be taken into account when assessing the infective rate of the vector

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[1] The number of bites that would be received by one fully exposed person in a year is called the annual biting rate.

mosquitos (or the ATP) in endemic areas of brugian filariasis when other *Brugia* species are present in domestic or peridomestic animals.

Large-scale control programmes

Objectives. The objectives of a control programme should be clearly defined and spelled out. These could be:

(*a*) reduction in disease rate;
(*b*) reduction in microfilaria rate;
(*c*) reduction in the annual transmission potential (ATP);
(*d*) a combination of (*a*), (*b*), and (*c*);
(*e*) elimination of the parasite from the human population.

Target population. If the control campaign does not include the whole country, and if there are no natural boundaries confining the area of disease transmission, consideration must be given to where the boundaries of the campaign should be set. Topographical boundaries should be preferred to administrative ones.

National strategy. Each country will have to develop its own national strategy to control filariasis based on:

(*a*) the magnitude of the public health problem presented by filariasis, the estimated population at risk, and the degree of endemicity based on microfilaria and disease rates;
(*b*) availability of resources;
(*c*) amenability to control.

Priorities should be set in relation to:

(*a*) other health problems in the country;
(*b*) the degree of endemicity that is considered as requiring immediate action;
(*c*) protection of groups of people at high risk of developing disease (e.g., immigrants to endemic areas).

Once the scope of the problem has been assessed and the target population delineated, a cost–benefit estimate should be made to determine whether a vertically organized filariasis control programme should be undertaken and, if so, what priority the programme should be given in relation to other health problems, and in which areas control should begin. Subsequently a cost-effectiveness analysis should be made, based on data from pilot control studies, to select the least costly method of achieving the central programme's objectives. Alternatively, it may be more expedient to set up a "horizontal" DEC delivery system, designed to reduce morbidity and/or microfilaraemia, through the primary health care system (see page 29).

The objectives of the programme should be clearly defined and general strategies should be developed to cope with various conditions and situations in different endemic areas.

A training programme should be developed to train existing health care personnel or, if necessary, to recruit and train new ones, at all levels and in all aspects of the programme.

Health education and community participation should be an integral part of the programme and gradual integration into the primary health care system should be the ultimate aim.

Factors to be considered

(*a*) The strategy used should be acceptable to the target population.

(*b*) Health education and community participation should be an integral part of the control programme.

(*c*) Regarding chemotherapy with DEC, the following points must be borne in mind:

(*i*) The cost of DEC is only a small fraction of the cost of drug delivery.

(*ii*) Side-reactions occur only at the start of DEC, treatment and are positively associated with the presence of microfilaraemia or disease manifestations. They are of short duration and self-limiting in nature. As the control programme proceeds, fewer people will have side-reactions.

(*iii*) DEC is a very effective microfilaricidal drug. Even a single dose produces a dramatic decrease in microfilarial density and consequently a potential decrease in transmission.

(*iv*) Whatever treatment regimen is adopted, people with acute clinical filariasis should be treated immediately with DEC by the local health personnel at village level or by motivated people in the community (see page 29).

(*v*) Efforts should be made to integrate the filariasis control programme into the primary health care system.

(*d*) The use of other control measures, e.g., vector control and reduction of human/mosquito contact, should be considered.

Suggested approaches to control

Where the available funds, manpower, and health system infrastructure are inadequate to support a large-scale control programme, and where epidemiological and pilot control data are not available, the following guidelines may be useful.

The target population should be divided into small sectors, and appropriate measures taken in each sector, step by step, with consolidation of the areas controlled. The definition of "small" cannot be given here as an

absolute number; it will depend on the ability of the filariasis control programme to deal with a population of a certain size using a specified set of strategies. It will therefore differ from one country to another, depending upon the prevailing circumstances and resources.

The following strategies may be adopted in particular circumstances.

"Small" population with high endemicity (microfilaria rate >10%)

(*a*) Do a total population survey.

(*b*) Give mass treatment with DEC.

(*c*) Follow by yearly re-treatment for two consecutive years of:
  — newcomers;
  — people who had microfilaraemia before mass treatment; and
  — people still suffering from acute filariasis during the post-treatment period.

(*d*) In the third year, give a second mass treatment with DEC.

(*e*) 5–10 years after the first mass treatment, make a filariasis survey of the population for evaluation purposes and plan the future strategy on the basis of the results.

Alternative procedures might be as follows:

(*f*) Do a total population survey.

(*g*) Follow by treatment with DEC-medicated salt for 6–9 months.

(*h*) As in (*c*)–(*e*) above.

or

(*i*) Do a total population survey.

(*j*) Give low-dosage DEC "by the people for the people" for at least 18 months.

(*k*) As in (*c*)–(*e*) above.

"Small" population with low endemicity (microfilaria rate <5%)

The same methods and strategies may be used as for small populations with high endemicity, with two exceptions: (1) the second mass treatment is unnecessary; and (2) re-evaluation of the control programme may be delayed until 10–15 years after the first mass treatment.

Alternatively, where the microfilaria rate is low, as in areas that have been treated in the past, a filariasis survey of the whole population may be done, followed by selective treatment of microfilaria carriers and people with acute lesions.

"Small" population with medium endemicity (microfilaria rate 5–10%)

The procedures given above may be used, depending on resources and the objectives of the programme.

"Large" population with high endemicity (microfilaria rate >10%)

The following approaches may be considered.

1. (*a*) Divide the population into small sectors.

(*b*) Follow the same approach as for a "small" population with high endemicity (page 46).

2. (*a*) Do a sample survey.

(*b*) Follow by two consecutive mass treatments with DEC, 3–5 years apart.

(*c*) Re-evaluate the programme 10–15 years later.

3. (*a*) Do a sample survey.

(*b*) Follow by treatment with DEC-medicated salt for 6–9 months.

(*c*) Repeat treatment with DEC-medicated salt twice at intervals of 3–5 years; or give mass treatment with standard dosage at the same intervals.

(*d*) Re-evaluate the programme 10–15 years later.

4. (*a*) Do a sample survey.

(*b*) Give low-dosage DEC "by the people for the people", for at least 18 months.

(*c*) Repeat low-dosage DEC twice at intervals of 3–5 years; or give mass treatment with standard dosage at the same intervals.

(*d*) Re-evaluate the programme 10–15 years later.

"Large" population with low endemicity (microfilaria rate <5%)

The following approaches may be considered.

1. (*a*) Divide the population into small sectors.

(*b*) Follow the same approach as for a "small" population with low endemicity (page 46).

2. (*a*) Do a sample survey.

(*b*) Follow this by one mass treatment.

(*c*) Re-evaluate the programme 10–15 years later.

3. (*a*) Do a sample survey.

(*b*) Follow by treatment with DEC-medicated salt for 6–9 months.

(*c*) Repeat treatment 3–5 years later, either with DEC-medicated salt or by mass treatment with standard dosage.

(*d*) Re-evaluate the programme 10–15 years later.

4. (*a*) Do a sample survey.

(*b*) Give low-dosage DEC "by the people for the people" for at least 18 months. Repeat treatment with low-dosage DEC or give mass treatment with standard dosage, at intervals of 3–5 years.

(*c*) Re-evaluate the programme 10–15 years later.

5. (a) Do a full population filariasis survey.

(b) Treat all microfilaria carriers and people with active lesions with standard DEC dosage.

(c) Re-evaluate the programme 10–15 years later.

Other effective measures (see also page 37).

Besides parasite control using DEC, other methods, based on vector control or reduction of human–mosquito contact, are known to be effective and may be used either alone or as a supplement to DEC treatment. Among these are the following:

(a) reclamation of swamp land suitable for agriculture with subsequent control of *Brugia malayi*;

(b) reduction in the number of breeding places for *Aedes polynesiensis* through community efforts to reduce man-made breeding sites;

(c) filling of land depressions for the permanent elimination of mosquito breeding sites;

(d) removal of aquatic plants to control *Mansonia* spp. and certain *Anopheles* mosquitos;

(e) construction of better housing with mosquito screens, or mosquito-proof bedrooms; proper clothing or the use of mosquito nets, with or without impregnation of insecticides (these measures are especially important where the vectors feed at night); provision of proper lighting and ventilation;

(f) good urban drainage, particularly important to decrease the vector population of *Culex quinquefasciatus*; protection of pit latrines from mosquito breeding by means of traps or treatment with insecticide.

PART 3. SURVEY METHODS

PART 3

Survey methods

12. Preparation and organization of a medical epidemiological baseline survey

This part is intended for field workers involved in surveys and control programmes for lymphatic filariasis. It describes simple and efficient survey methods for use in the field in order to obtain reliable results in a relatively short time. Elaborate techniques have been omitted.

Preparation and selection of study sites

Before initiating any programme, a study site has to be selected. This is usually done through a preliminary visit to known endemic areas of filariasis or through correspondence with local contacts who are well informed on the filariasis situation in the areas concerned. Once the site of operations has been selected, official letters have to be sent by the ministry of health, or its equivalent, informing the local administrative and health authorities about the scope and purpose of the programme and asking them to give permission for the programme and all available support. At least one person from the local administrative or health office should be appointed to serve as a local contact to help the programme team. This person should be assigned responsibility for helping to cope with local practical arrangements, such as finding transportation for the team, identifying local people who are able to serve as guides, field technicians or nurses, informing local communities about the scope and purpose of the programme, identifying motivated people at the village level to help the team, finding a suitable place for the team to stay, etc.

The significance of night-blood collection and the relationship between the parasite, the vector and the disease manifestations should be explained to the people. After each community has been well briefed and its cooperation with the team is assured, a date can be selected for the initiation of the programme in that community.

Sampling techniques and the selection of clusters as working units [1]

In some countries it may be essential and feasible to examine all people in the communities that will be subjected to filariasis control measures. In many cases, however, it will suffice to examine only a sample of the total community. Funds and manpower will thereby be conserved and a sufficiently precise estimate may be achieved. In many circumstances it may be necessary to obtain advice on the design of the sample.

Generally, some form of cluster sampling will be necessary. The basic sampling units, referred to here as clusters, should be villages, settlements, compounds, or blocks in urban areas, of about 300 inhabitants each. They may be chosen randomly, or they may be selected as giving adequate distributional coverage of the whole area or as providing examples of different conditions of transmission, etc. Every effort should be made to examine each person in the selected cluster for filariasis. If the village has more than 300 inhabitants, a cluster sample of approximately 300 inhabitants should be selected as a working unit.

Baseline data collection

Registration of the population

The registration of the population should be done by house-to-house visiting. Some person of dignity and authority, appointed by the village chief or local community leader, should be present and involved in the registration. Each house in the village should be numbered sequentially, starting with the house of the chief or community leader. A schematic map of the village should be drawn on the spot, showing the location of the houses and of all water bodies in the area.

All people living in each house are registered, starting with the head of the family, spouse, children, and other people living in the house. The numbering system has to be as simple as possible so as to avoid errors. As there will probably be several teams working simultaneously and independently, the simplest system is to number consecutively all communities to be examined. Then, within the cluster, the people to be examined are numbered consecutively. The census number of a person will thus consist of two parts, the first being the community number, and the second the personal number within the community (e.g., 7/133 indicates the 133rd person in community number 7).

The following information should be collected during the registration: name, age, sex, family relationship to the head of the household, place of origin, and duration of residency. Each registered person should receive a census card on which census number, name, age and sex are recorded. The

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[1] These theoretical guidelines should be adapted to local conditions.

census card should be kept by the individual while the team is present in the community and throughout the duration of the filariasis control programme.

The census registration form

Fig. 3 shows an example of a census registration form. At the top are recorded entries of a general nature on the family and its living conditions. This is followed by a list of the members of the household. Each sheet is designed to cover one household, with space for information on up to 20 people. If more people are present in one household, an additional form is used. If computer facilities are likely to be available, it is recommended that a data collection form like the one shown in Annex 2 be used, together with a codesheet.

Night-blood collection

In each village a suitable house should be selected where the people can come for blood collection. The timing of blood collection should be in accordance with the periodicity of the microfilariae. For the nocturnally periodic and nocturnally subperiodic forms of lymphatic filariasis blood collection should be after 20h00; for diurnally subperiodic parasites, blood may be taken at any time during daylight hours. With low-density carriers, it is preferable to take blood as close as possible to the peak hour.

Motivated people in the community should be assigned the responsibility for organizing the villagers in such a way that blood samples can be collected in an orderly manner. Two members of the team should be assigned to collect blood samples, each one assisted by one or two villagers. Usually, one team member is able to collect approximately 150 blood samples per night, so that the whole collection from a cluster of 300 people can be carried out in one night.

A measured volume of fingerprick blood is collected from each person to make a thick smear on a glass slide. Each blood film is labelled with the corresponding census number using a diamond pencil (or a black pencil when frosted-end microscope slides are used). The blood films should be dried in a horizontal position and then stored in a plastic slide box.

For certain purposes, venous blood may have to be collected using a disposable syringe. The blood sample may be divided into three portions. The first portion is used to make the standard thick smear; the second may be mixed with anticoagulant in a vacuum tube for subsequent examination by a filtration method; and the last portion may be drawn into a plain vacuum tube for collection of serum. Care should be taken that the second portion of the blood sample is mixed well with the anticoagulant. This will prevent clot formation which may otherwise interfere with subsequent filtration. The tube containing the blood sample with anti-

Fig. 3. Sample census registration form and coding instructions
(Adapted, with permission, from: SASA, M. *Human filariasis: a global survey of epidemiology and control.* Baltimore, London, Tokyo, University Park Press, 1976.)

Community .... No: .... Section no.: .... Date: ....

Investigator: ....

Household no.: .... Type of housing: ....

Protection against mosquitos: ....

Source of water: .... Sewage disposal: .... Latrines: ....

Number	Name	Sex	Age	Relation	Status	Profession	Migrations
	1						
	2						
	3						
	4						
	5						
	6						
	7						
	8						
	9						
	10						
	11						
	12						
	13						
	14						
	15						
	16						
	17						
	18						
	19						
	20						

coagulant should be tightly fixed to the corresponding disposable syringe with a rubber band, in order to ensure that the same syringe is used for the filtration (see below). Each blood sample should be labelled with the corresponding census number.

Processing blood samples

The blood samples should be processed the following morning. Blood samples for filtration should be processed first, using the corresponding syringe to filter the blood sample through a 3–5-μm Nuclepore filter (see page 62). The same syringe should not be used for filtering more than one blood sample, otherwise there is a risk of contaminating the filters with microfilariae from other carriers, thereby creating false positives. If possible, the filters should be stained with Giemsa stain the same day. If time is short, the filters may be fixed with pure methanol after they are properly dry and stored in a plastic slide box.

By the time all the blood samples have been filtered, the blood films should be dry enough for further processing (see also page 58). It is always advisable to dehaemoglobinize one blood film first, by itself, to test whether it is dry enough. Half-dried blood films may dissolve in the water, and there is a danger of losing precious specimens. Blood films should be processed in lots of 50–100 at a time, making every effort to screen them on the same day for the following reasons: (i) to identify people with microfilariae; further blood samples can be taken from them later if it is desired to demonstrate living microfilariae under a microscope to the village people; (ii) to select suitable carriers for any periodicity studies that may be needed; and (iii) to identify microfilaria carriers who would be suitable as volunteers for entomological studies. Blood samples for serum collection should also be processed on the same day.

Clinical examination

The village people should be requested to come for clinical examination on the morning following night-blood collection. It is recommended that a polyclinic be opened providing free medication to the village people for simple and common health problems. In most communities, this may be the only incentive that will motivate the people to cooperate at all stages of the programme. Demonstration of living microfilariae may also be done at this stage.

To obtain reliable results, clinical examination should always be done in a well-lit room. The following information should be collected from each person.

Adenolymphangitis. Recurrent attacks of lymphadenitis, most frequently in the inguinal region, and at times associated with a retrograde

lymphangitis, *without* any concomitant injury or wound on the corresponding foot, are typical of the history of actute filariasis.

The number of attacks and the duration of each attack over the past year should be recorded. In many communities where filariasis is endemic, the village people are familiar with this clinical entity. An enlarged lymph node by itself is not a good indicator of filariasis, since barefoot communities are constantly exposed to mechanical trauma that may precipitate lymph node enlargement.

For longitudinal studies, motivated people in the community should be assigned the responsability for recording all cases of adenolymphangitis in the community and for treating them immediately with diethylcarbamazine citrate (DEC).

Scarring. The presence of scar tissue, around the groin or elbow resulting from ruptured abscesses, is a reliable sign supporting the accuracy of the clinical history. It should therefore be looked for carefully, the patient being examined in a well-lit room. Once formed, the scar will remain visible for a long time.

Chyluria. Chyluria (the presence of chyle in the urine, giving it a milky appearance) is more frequently observed after a heavy meal. It should be differentiated from pyuria (pus in the urine) by the absence of pain on urinating and the absence of white blood cells from the sediment of the urine.

Hydrocele. Small hydroceles are difficult to diagnose. Transillumination with a pen-torch in a dark room is characteristic and quite often very helpful. It enables a distinction to be made from chyloceles which do not give a positive transillumination test, but on palpation give a positive ballottement test. It should be noted that the two testicles are seldom equal in size; one may be considerably larger than the other, simulating a small hydrocele.

For field purposes, the following grading is recommended for hydroceles:

grade I   — smaller than the patient's fist;
grade II   — in between grades grades I and III;
grade III — bigger than the patient's head.

Lymphoedema and/or elephantiasis. Clinical differentiation between lymphoedema and elephantiasis is not easy (see page 23). For practical reasons the condition of the affected extremities should be observed carefully. When pitting oedema is present, the affected extremity has a better chance of returning to its original size when treated with DEC, while those with gross deformities are less likely to benefit from any conventional treatment procedures. The following grading system is recommended:

grade I   — lymphoedema with slight increase in limb size; mostly pitting oedema; some fibrosis; spontaneously reversible on elevation;

grade II   — lymphoedema with obvious increase in limb size but without deformities; mostly non-pitting oedema; much fibrosis; not spontaneously reversible on elevation;

grade III   — elephantiasis, the affected limb being deformed, with local bulging and with chronic skin abnormalities; *or* grade II lymphoedema with much dermatosclerosis.

Potential and natural vectors of filariasis

Entomological surveys involving precise assessment of the seasonal abundance, and feeding, resting, and breeding habits of vectors are described in detail on pages 69-72. The objective of this section is to describe a simple way of determining the potential and natural vectors of filariasis in any area within a relatively short period.

Potential vectors. Microfilaria carriers, identified through screening of blood films for microfilariae, can be asked to volunteer for vector studies. The more carriers who participate, the greater the chance of success. Each carrier is requested to sleep under a mosquito net, held partially open. All blood-fed mosquitos resting inside the net are collected the following morning and placed in clean paper cups, each covered with a piece of gauze, in lots of 25-30. The cups containing the mosquitos should be kept in an insulated box along with a bottle of sugar solution for feeding. The mosquitos should be carefully maintained alive for 10-14 days in the box, well protected against the extremes of ambient temperature and humidity. The mosquito boxes should be kept closed most of the time to minimize evaporation, and must be protected from ants, cockroaches, etc.

The mosquitos should be allowed to feed on a cotton wool pad placed on the gauze and partially soaked with 50-100 g/litre sugar solution. The sugar solution should be renewed twice a day and the cotton pad changed for a fresh one once every 2-3 days. This will prevent fermentation and mould formation, which may kill the mosquitos.

After 10-14 days, depending upon the species of the parasite and the temperature, the surviving mosquitos should be killed, identified by species, and dissected for filarial larvae. All species of mosquitos capable of supporting the development of the ingested microfilariae to the infective stage can be considered to be potential vectors. The living infective larvae should be shown to the village people and the role of mosquitos as transmitters emphasized.

Natural vectors. Once the potential vectors have been identified, efforts should be concentrated on collecting as many specimens of these species

as possible, making use of light traps, hand or spray capture of resting mosquitos, and human or animal baits. Often children in the village are very keen and interested in helping with the collection of mosquitos, and many of them can quickly be taught to capture mosquitos effectively. All the captured mosquitos should be killed on the day of capture, separated into species, and the potential vectors should then be examined for infective larvae using a mass-dissection method. The infective larvae should be preserved and correctly identified.

For comparison of catches before and after a control programme, it is necessary to standardize the sampling procedures by having: (a) fixed catching stations; (b) fixed numbers of collectors; (c) fixed intervals of time over which each collection is made; and (d) a fixed frequency of collection.

Reservoir hosts and filarial parasites in animals

Efforts should be made to examine domestic and peridomestic animals for filarial parasites for the following reasons.

(a) There is a need to identify reservoir hosts which, in some areas, may have epidemiological significance in subsequent control of filariasis.

(b) Animal filarial parasites can be transmitted by the same mosquito species that bite human subjects. Therefore, findings on the infected and infective rates of the local vectors have to be evaluated with care.

(c) Humans may have been infected with filarial larvae of animal origin that may provoke immune responses in the human host. Seroepidemiological surveys in such an area have to be evaluated with care.

13. Recommended techniques for field surveys

Parasitological techniques

Blood films

The examination of blood films is the method most widely used for field surveys. If properly done, it is a reliable method for qualitative and quantitative microfilarial studies. It is recommended that a measured amount of blood (at least 20 µl and preferably 60 µl, to be smeared in 3 rows of 20 µl each on the same slide) be used.

Cleaning the slides
(a) Wash with a watery solution of detergent or soap.
(b) Rinse thoroughly in running water.
(c) Immerse in 96% alcohol.

(*d*) Dry and polish with a clean, grease-free cloth.

(*e*) Store in a plastic slide box.

Note: All microscope slides should be thoroughly cleaned before use, including factory "pre-cleaned" slides. Any dust, grease, detergent or cotton threads on the slide may cause part or all of the blood film, and the microfilariae it contains, to float off during subsequent dehaemoglobinization.

Making blood films

(*a*) Clean the pulp of the finger (or the lobe of the ear) with a cotton wool ball, soaked in 70% alcohol.

(*b*) Dry the surface of the skin by allowing the excess alcohol to evaporate.

(*c*) If blood is to be taken from the finger, use a sterile lancet to make a small cut perpendicular to the finger lines, with the patient's finger tip held firmly between the examiner's thumb and index finger. Alternatively, prick the ear-lobe with a sterile lancet. A separate lancet should be used for each person.

(*d*) Allow the blood to ooze freely.

(*e*) Draw the required quantity of blood into a dry haemoglobin pipette or into a disposable calibrated capillary tube.

(*f*) Expel the blood on to a microscope slide with the tip of the capillary tube resting on the surface of the slide until most of the contents have been expelled. Then lift the tip from the surface and blow the remaining blood on to the slide. This last procedure will prevent bubble formation. Make a rectangular smear of uniform thickness and dimensions of about 5×1.5 cm; this can be examined qualitatively or quantitatively.

(*g*) Label the slide with a diamond pencil (or with a black pencil when frosted-end microscope slides are used).

(*h*) Allow the slide to dry at room temperature in a horizontal position.

(*i*) Store in a plastic slide box.

Note the following points:

(*i*) Any excess alcohol left on the surface of the skin may partially fix the blood sample.

(*ii*) Too much squeezing of the finger (or ear lobe) may dilute the blood sample with tissue fluids.

(*iii*) Bubbles in the blood film may interfere with absorption of the stain.

(*iv*) Rectangular blood films are easier to read but slightly more difficult to make. Linear blood films tend to dry very quickly and may prevent sheath shedding in periodic *Brugia malayi* microfilariae.

(*v*) If the blood film is smeared too thickly, the microfilariae tend to float off during subsequent dehaemoglobinization, and the density of the stained film will be such that it is difficult to see the microfilariae. Also the sheaths of the microfilariae may not take up the stain, thus creating the false impression that the microfilariae are sheathless, or have shed their sheaths.

(*vi*) Fumes from recently varnished wooden slide boxes may partially fix blood films and interfere with subsequent dehaemoglobinization.

(*vii*) Blood films should be thoroughly dried before dehaemoglobinization. Partially dried blood films tend to float off into the water. The process of drying may take up to 12 hours in the dry season, but 24–48 hours may be needed in the rainy season. Blood films collected with a heparinized capillary tube or from blood samples mixed with anticoagulant need at least 48 hours to dry. Most of the losses of microfilariae from blood films are due to dirty slides, insufficiently dried blood films, making the smear too thick or, especially, from using blood that has been mixed with anticoagulant.

Giemsa stain

(*a*) Place the blood films in lots of 50–100 on a stainless steel staining rack and immerse them in a pail filled with clean water.

(*b*) Wait until the haemoglobin seeps out and the blood films become pale in colour (2–5 min).

(*c*) Dry in air.

(*d*) Fix with pure methyl alcohol (methanol) for 30–60 seconds.

(*e*) Dry in air.

(*f*) Immerse the blood films in lots of 50–100 on a stainless steel staining rack in a staining jar filled with approximately 400 ml of diluted Giemsa stain for 15 minutes. (One part of Giemsa stock solution in 14 parts of buffer solution, pH 7.2.) One such batch of diluted Giemsa stain can be used to stain three racks of blood films. After staining each rack of blood films, 2 ml of Giemsa stock solution should be added to the diluted Giemsa stain.

(*g*) Rinse thoroughly in a gentle flow of clean running water, or immerse the staining rack with the stained blood films in a large pail, filled with clean water.

(*h*) Dry in air.

Note: If the blood films are correctly stained with Giemsa, the morphological features of the microfilariae can be easily studied. To check whether microfilariae have floated off during dehaemoglobinization, the water used for dehaemoglobinization may be filtered through a Nuclepore filter and examined for microfilariae.

Examination and counting. Thick films may be examined dry, or with a drop of xylol under a coverslip, or with immersion oil. The microfilariae seen have to be identified as to species (see page 72) and counted. Counting is done by scanning to and fro across the film in lines of one microscope field in breadth with the aid of a moveable stage. An eye-piece with the aperture squared off makes this process more accurate, and a hand tally-counter may help in recording counts. Provided that the species of the microfilariae has been identified, and there is no question of confusion with microfilariae of another species of similar size, the counting

can usually be done accurately and rapidly with a low magnification (× 25 to × 50).

Standardization of blood-film examination. Technicians employed in filariasis surveys should be regularly tested as to their skill in comparison with a senior member of the staff who has great experience, or with each other. The same blood films with microfilariae are examined by each person separately. Qualitative results, i.e., the diagnosis as to whether microfilariae are present or absent, can then be entered in a 2×2 contingency table (see Fig. 4). Sensitivity (correct identification of negative films) with regard to the "reference" senior technician can be expressed as a percentage, as can repeatability when one technician is tested against another.

The planners of the programme will have to decide what degree of achievement is required. Under field conditions, sensitivity and

Fig. 4. 2 × 2 Contingency table for use in standardization of blood-film examination

(Reproduced, by permission, from: SASA, M. *Human filariasis : a global survey of epidemiology and control.* Baltimore, London, Tokyo, University Park Press, 1976).

	A		
	Positive	Negative	Total
B	a	b	
	c	d	
Total			

Repeatability: $\dfrac{a}{a+b+c} \times 100\,(\%)$

If A is the reference (senior technician)

Sensitivity: $\dfrac{a}{a+c} \times 100\,(\%)$

Specificity: $\dfrac{d}{b+d} \times 100\,(\%)$

Fig. 5. Comparison of microfilaria counts (for explanation, see text)
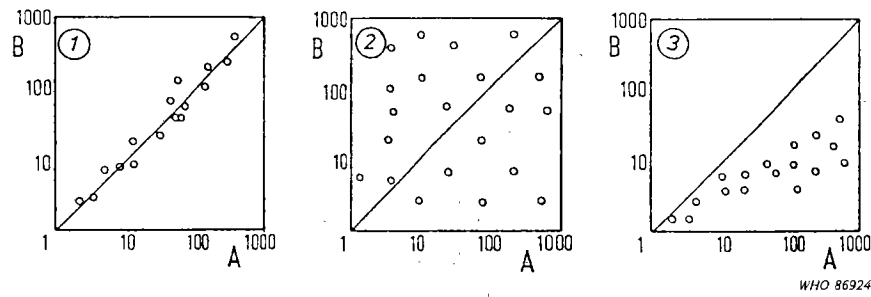
repeatability above 80% are good. Specificity (correct identification of positive films) should be higher than that—it is possible to achieve figures of 95% and above.

When the numerical values of microfilarial counts are to be compared, they should be plotted on a graph (Fig. 5). The horizontal or x-axis is used for the "reference" senior technician. It is practical to divide the axes logarithmically or to use double logarithmic paper with each scale going up to 10^3. This allows one to see almost at a glance whether all points lie on the diagonal from 0/0 to 1000/1000 (good agreement) (Fig. 5 (1)), or whether they are equally distributed on either side (no systematic difference) (Fig. 5 (2)). If they lie mostly below the diagonal, the counts of the technician recorded on the x-axis were consistently higher (Fig. 5 (3)). If this was a senior technician it probably means that the microscopist whose counts were recorded on the vertical or y-axis always misses some microfilariae.

Tests of this kind should be used not only in the initial training but also in the later phases of the programme as a means of supervision. Whenever possible, tests should be administered under actual working conditions, otherwise their results may not be very meaningful.

Nuclepore filtration technique[1]

(a) Insert a Nuclepore membrane filter with a pore size of 3–5 μm into a circular Swinnex filter-holder of 25-mm diameter. It is easier to align the membrane correctly if the filter-holder is wet. The rubber sealing ring must also be correctly positioned.

(b) Draw the plunger out of the syringe and insert the tip of the syringe into the filter-holder. Make sure that the syringe and the filter-holder are tightly fitted together.

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[1] Nuclepore membrane filters are available, with full instructions for use, from Nuclepore Inc., Pleasanton, CA, USA.

(c) Shake the blood sample (usually 3 ml) gently and pour it into the syringe.

(d) Add pre-filtered water to the syringe to lyse the blood cells.

(e) Insert the plunger into the syringe and express the blood through the filter.

(f) Disconnect the syringe, pull the plunger out, refit the syringe to the filter-holder, and pour some more pre-filtered water into the syringe. Express it through the filter again.

(g) Repeat the procedure several times until no more blood is seen in the washing. Air is then blown through the filter-holder.

(h) Remove the filter by hand and put it on a microscope slide with the side containing the microfilariae facing upwards.

(i) Label the slide and dry the filter in air.

(j) Fix with pure methanol in a horizontal position for 30–60 seconds.

(k) Dry in air.

(l) Stain with diluted Giemsa solution or Field's stain for 15 minutes in a horizontal position (the same dilution as with blood films).

(m) Finally, examine the dried filter under a microscope, with or without immersion in oil.

Note: Always dislodge the syringe from the filter-holder before pulling the plunger out. This prevents the filter being sucked upwards, which may cause leakage and subsequent loss of microfilariae through the washing process.

The use of Nuclepore membrane filters in field surveys. Nuclepore membrane filters are expensive and should only be used in the field in pilot control areas to assess the prevalence of carriers with low-density microfilaraemia, particularly at the end of mass DEC campaigns. In pretreatment surveys, it is sufficient to take 1 ml of venous blood. The counting of microfilariae on pretreatment filters is not recommended as the numbers are usually too high to permit accurate counting. After one or more courses of DEC, the number of microfilariae will be low enough for counts to be made easily and correctly. As the DEC control programme progresses, it is recommended that samples of 3 ml of blood be taken, to increase the sensitivity of the evaluation.

Entomological techniques

In order to determine which species are the local vectors of lymphatic filariasis or, subsequently, to measure the amount of transmission that is occurring, it is necessary to dissect mosquitos and examine them for filarial larvae.

Individual dissection

(*a*) Kill the mosquitos by mechanical shaking, or with chloroform vapour, or by freezing.

(*b*) Place each specimen on a microscope slide after removing wings and legs. (Removal of wings and legs may be omitted in order to save time but, if so, extra care must be taken with the subsequent examination for parasites, making sure that these are not hidden by bits of leg or wing.)

(*c*) To hold the specimen, pierce the thorax with a dissecting needle under a dissecting microscope, and, with another dissecting needle, divide the specimen into head, thorax and abdomen in separate drops of normal saline solution.

(*d*) Tease the head and abdomen open. The mature larvae usually wriggle out actively from the tissue of the insect.

(*e*) The thorax should be teased more carefully into small pieces, covered with a coverslip and examined under a low-power compound microscope.

Note: First-stage larvae are small, motionless, and have different sizes and shapes, so that they are very easily missed when examining with a dissecting microscope, especially when the tissue is not covered with a coverslip.

Periodicity of the microfilariae

If the periodicity of the microfilariae of the local species or strain of parasite is not known, it will be necessary to determine this by observation. This should be done on volunteers with microfilaraemia, preferably those with high counts. If a peripheral health service facility or a local hospital is available, it is recommended that carriers be taken there for periodicity studies. From each volunteer a 20–40-µl blood film of finger-prick blood is made every 2 hours throughout one complete 24-hour cycle. The blood films are processed and stained with Giemsa and the microfilariae counted. The counts are plotted on a graph which will demonstrate the periodicity. Examples of the different periodicities likely to be encountered are shown in Fig. 6.

Mass dissection

The mass dissection method is useful since it enables large numbers of mosquitos of the same species to be examined rapidly and easily to determine the numbers of infective larvae they were harbouring, and thus to estimate the amount of transmission that is occurring.

(*a*) Kill the mosquitos by mechanical shaking, or with chloroform vapour, or by freezing.

Fig. 6. Comparison of observed and theoretical periodicity curves of various filarial forms

——— observed
——— theoretical

WHO 86925
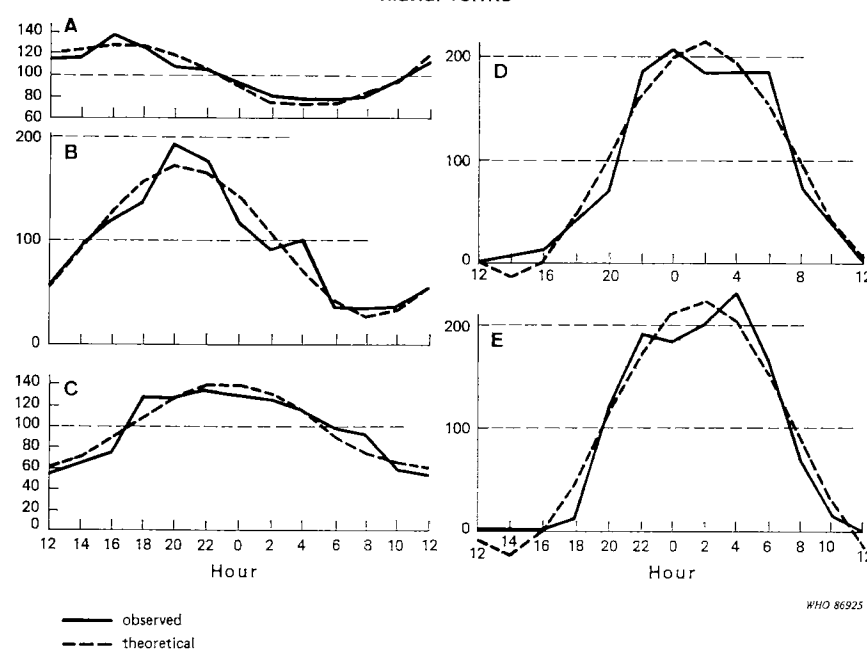
A. Diurnally subperiodic *W. bancrofti* in the South Pacific: ROSEN, L. *American journal of hygiene,* **61**: 219 (1955).
B. Nocturnally subperiodic *W. bancrofti* in west Thailand: HARINASUTA, C. ET AL. *South-east Asian journal of tropical medicine and public health,* 1: 152-154 (1970).
C. Nocturnally subperiodic *B. malayi* in Ralawan, Philippines: CABRERA, B.D. & ROZEBOOM, L.E. *American journal of epidemiology,* **81**, 192 (1965).
D. Nocturnally periodic *B. malayi* in Penang. Malaysia: TURNER, L.H. & EDESON, J.F.B. *Anuals of tropical medicine and parasitology,* **51**: 271 (1957).
E. Nocturnally periodic *W. bancrofti* in Bukit Lanjan, Malaysia: RAMACHANDRAN, C.P. ET AL. *Medical journal of Malaya,* **18**: 193 (1964).

(*b*) Place them in a Petri dish of 15-cm diameter and add 2-5 ml of normal saline.

(*c*) Crush the mosquitos by rolling a test-tube over them.

(*d*) Place the crushed mosquitos on a sieve suspended in a funnel filled with normal saline at 40 °C. The motile infective larvae leave the crushed mosquitos, drop through the mesh of the sieve, and sink to the bottom of the funnel pipe which is closed by a tap (Fig. 7).

(*e*) After 30-60 minutes, open the tap and collect the larvae in a small Petri dish. If there are few larvae, it may be better to collect in a sedimentation tube, centifuge, and examine the sediment on a slide.
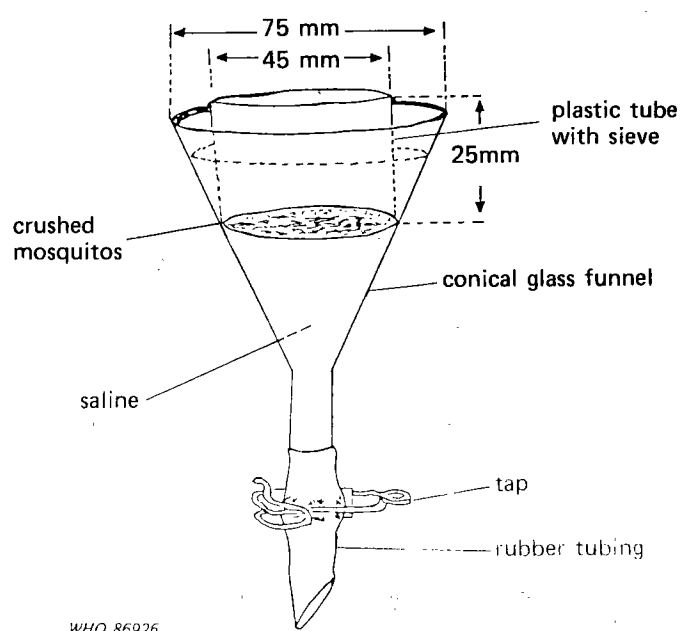
(*f*) Count the larvae under a dissecting microscope.

(*g*) The larvae can be transferred to a suitable medium for identification.

14. Other useful techniques

Parasitological techniques

Counting chamber (Fig. 8)

The counting chamber is prepared by marking the centre of a glass microscope slide (76 × 38 mm) with a grid of lines about 2 mm apart. Usually the lines are scratched on, using a diamond pencil. Strips of glass, which have been cut from another glass slide, are then placed on the marked slide so that a rectangular well is formed that measures either

Fig. 8. Counting chamber for microfilariae
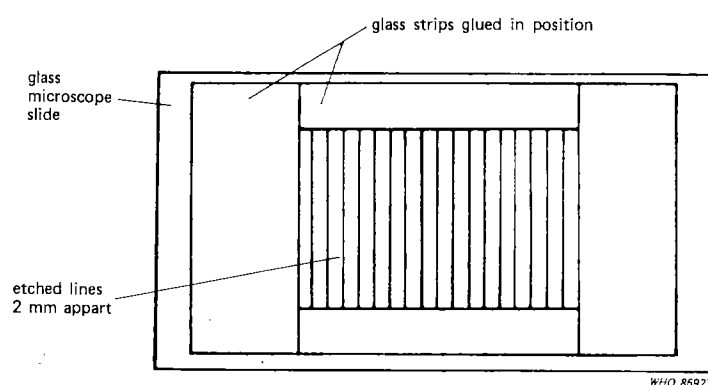
2×3 cm or 2×4.5 cm. The glass strips are glued on to the slide using DPX slide mountant, or Canada balsam, or some suitable glass glue. Once the strips are properly fixed and the glue has dried, the slides are cleaned and are then ready for use.

How to use the counting chamber

(a) Place a small volume of filtered water in the chamber.
(b) Draw a measured volume of blood (50–100 μl) into a pipette.
(c) Expel the blood into the chamber.
(d) Fill the chamber almost to the top with filtered water.
(e) Count the microfilariae.

Note: This method enables speedy and accurate counts of microfilariae to be made. However, it is not possible to make a species diagnosis and the specimens cannot be kept for records. Sufficient water must be added to the chamber to prevent the formation of a large meniscus which may otherwise obscure the microfilariae at the edges of the chamber. A coverslip should not be used.

If time is too short for immediate examination on the spot, it is possible to store the blood samples in 1 ml vials containing 0.5–0.9 ml of acetic acid (30 ml/litre) for later examination.

Haematoxylin stain

(a) Blood films should be prepared, dehaemoglobinized, and fixed in a similar way as before staining with Giemsa stain (see pages 58-60).
(b) Stain with Mayer's acid haemalum for 15 minutes at 70 °C.
(c) Immerse in water for 15 minutes.
(d) Rinse with a gentle flow of running water.

(*e*) Dip in 200 g/l alcohol containing 2 g/litre hydrochloric acid for 1–2 seconds.

(*f*) Rinse again.

(*g*) Immerse in water for 15 minutes to "blue" the smear.

(*h*) Dry in air.

Haematoxylin gives an excellent morphological picture of the microfilariae and stains the sheaths well.

Millipore membrane filtration technique[1]

(*a*) Place a 25-mm circular filter pad into a similar-sized circular Swinnex filter-holder.

(*b*) Place a similar-sized Millipore membrane filter with a pore size of 5 μm on top of the filter pad. The filter should be labelled in pencil at the edge. Position the rubber sealing ring correctly and screw up the filter-holder tightly.

(*c*) Draw 1 ml of venous blood into a 10-ml syringe and then draw 2 ml of sodium citrate anticoagulant.

(*d*) Draw up to 7 ml of a 100 ml/litre solution of sulfestol (Teepol) in normal saline into the same syringe and shake gently to haemolyse the blood.

(*e*) Pass the blood through the filter.

(*f*) Wash three times with normal saline.

(*g*) Fix with 10 ml of formalin–saline.

(*h*) Wash twice with distilled water.

(*i*) Blow air through the filter. (The dried filters can be stored unstained for several days in small Petri dishes or paper bags.)

(*j*) Immerse in hot but not boiling Harris' haematoxylin for 5 minutes.

(*k*) "Blue" briefly in gently running water.

(*l*) Dry on a clean microscope slide.

(*m*)Cover with immersion oil and a coverslip.

Note: Do not draw the plunger out of the syringe while the syringe is still attached to the filter-holder, as this may suck up the filter and cause subsequent leakage, as with the Nuclepore filter. Millipore filters are not transparent; they have to be cleared with immersion oil before examination. For field work the Nuclepore filter is easier to use.

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[1] Millipore filters are available from Millipore Corp., Bedford, MA, USA.

Knott's concentration technique

(*a*) Mix 1 ml of venous blood with 10 ml of dilute formalin (20 ml/litre) in a conical centrifuge tube.

(*b*) Leave the tube in an upright position for 12 hours or centrifuge for 5 minutes at 350 *g*.

(*c*) Pour off the supernatant.

(*d*) Examine the sediment for microfilariae. A few drops of 1% methylene blue may help in showing up the microfilariae.

Note: A disadvantage of this technique is that the precipitate has a jelly-like consistency which sometimes makes further examination difficult.

Microfilariae in hydrocele fluid or urine

(*a*) Pour 15 ml of hydrocele fluid or urine into a conical centrifuge tube.

(*b*) Centrifuge for 5 minutes at 350 *g* (higher speeds if possible).

(*c*) Pour off the supernatant.

(*d*) Examine the sediment for microfilariae.

Entomological techniques

Larval sampling methods

Prior to sampling larvae, all actual and potential breeding places should be surveyed, recorded and mapped. Surveys should be carried out at intervals throughout the year to record seasonal variations in mosquito density. Larval surveys should not be done immediately after a period of heavy rain, since rain may flush out the larvae. Quantitative sampling of larval densities is far from accurate, since larvae are not distributed at random in the breeding sites, but are often crowded in certain areas. Generally only rough estimates are obtained. If more precise estimates are required, reasonably standardized samples must be obtained. The number of dips should be standardized to 10 or multiples of 10. The following methods are commonly used to collect larvae.

(a) Dipping

Depending on the size of the breeding places an enamel bowl or, better still, a ladle about 5–10 cm in diameter, should be used. The collecting instrument should be immersed in the water of the breeding place at an

angle of about 45° so that the surface water runs into the cavity but taking care that it is not filled to overflowing. The site should be approached carefully because the larvae are often disturbed by a collector moving or casting a shadow. There should be an interval of 2–3 minutes before a second dip is made to allow the larvae to return to the surface. After removal from the water, the sampling device is examined and larvae are collected with a pipette or a small nylon palette brush into a large bottle half full of water from the breeding site.

(b) Netting

Larvae can be collected from long stretches of water along the edge of streams, wells or other situations. A ring of iron wire 20–25 cm in diameter can be attached to a nylon bag with the upper part reinforced up to about 10 cm. The lower part of the net is attached to a transparent plastic cylinder (3 × 10 cm). A long cane or bamboo is attached to the ring and the net is skimmed through the surface water near the emerging or floating vegetation.

(c) Pipetting

Small pipettes with rubber teats may be used for collecting larvae from various breeding places.

(d) Procedures for special habitats

To collect larvae from tree holes, bamboo holes, crab holes, plant axils and other small-container breeding sites, specially made sucking devices should be used, e.g., a pipette 20–25 cm long or a length of plastic tubing attached to a 50-ml rubber suction bulb.

(e) Mansonia larvae

Mansonia larvae are difficult to sample by ordinary methods because they remain submerged and attached to the roots of aquatic plants. Plants can be uprooted and vigorously shaken in a bowl or a bucket of water; however not all the larvae will be dislodged using this method. Alternatively, the plant may be immersed in a 50 g/litre solution of sodium hydroxide until all the larvae become detached and rise to the surface.

(f) Transport of larvae and pupae

Larvae can be killed with boiling water and preserved in fixative solution (95 ml of alcohol + 5 ml of glycerol; or 94 ml of water + 6 ml of neutralized formalin + 0.5 g of borax).

Adult sampling methods

As it is not possible to examine the entire mosquito population in a large area, a representative sample has to be collected over a fixed period of time. The main objective is to obtain the most accurate information possible with the resources available.

(a) Indoor resting density

Mosquitos are collected from the walls of houses, hanging objects, the undersides of beds, empty boxes, empty drums, buckets, cellars, or other shelters, using a mosquito sucking tube or a test-tube. The methods commonly used are hand-capture with test-tubes or aspirators and spray capture. Other types of device are available for collecting resting mosquitos, such as electric aspirators. Sampling procedures can be standardized by having fixed catching stations, fixed numbers of collectors, fixed intervals of time over which the collections are made, and fixed frequency of collection. The data obtained can be expressed in terms of numbers collected per man-hour of effort (or per man-day, man-month or man-year).

Pyrethrum spray catches are very often used to collect endophilic mosquitos from sleeping rooms. Conventional "Flit" sprayers or aerosol dispensers can be used for this purpose. Spraying should be carried out in the early morning. White cotton sheets are spread on the floor and the mosquitos are knocked down by a pyrethrum spray. This provides a rough quantitative estimate of the mosquito resting densities. The data can be expressed as the number of mosquitos per room.

(b) Mosquito catches using human or animal bait

In order to obtain the precise human–mosquito contact or human-biting index, the method commonly adopted is to use people as bait, indoors and outdoors, and to collect mosquitos from their bodies. However, in areas where there is a risk of disease transmission by mosquitos such methods cannot be recommended and mosquito catches in traps using human bait have to be substituted. One such trap consists of a large mosquito net (about $3 \times 2 \times 2$ m) having a flap on each side which can be rolled up, leaving an opening about 1 m wide. The mosquito collector sleeps inside this, protected by another smaller mosquito net, so that he or she is not bitten by mosquitos. At certain intervals, usually every one or two hours, the main mosquito net is closed by lowering the flaps, and all mosquitos resting on the inside of the larger outer net are collected.

There is a variety of traps in which animals are used as bait and which employ the same principle as the human-bait trap.

The accuracy of these methods depends on the sample size, the size of the mosquito population, the degree of supervision, and the skill of the mosquito collectors. Generally the sensitivity of the methods is higher when mosquito densities are high.

Blood-meal identification

It is useful to know whether the mosquitos collected have fed on people or other animals. Information on collection and sorting of blood-meal samples for blood-meal identification is given in Annex 3. Samples may be sent to the Institute for Medical Research, Kuala Lumpur, as indicated in Annex 3.

Dissection of animals for adult worms

(*a*) Kill the animal, preferably with ether or pentobarbital.

(*b*) Dissect the testicles and lymph nodes and vessels from the popliteal, inguinal, cubital, axillary, cervical, retroperitoneal and thoracic sites. Put all tissues in a Petri dish filled with physiological saline solution.

(*c*) Dissect the intestine, liver, spleen and kidneys.

(*d*) Dissect the heart, lungs and great vessels.

(*e*) Dissect the skin from the carcass.

(*f*) Each organ, and the carcass, should be examined for adult filarial worms in a Petri dish filled with physiological saline solution under a low-power dissecting microscope.

(*g*) Collect the worms in phosphate buffered saline solution to relax them.

(*h*) Fix with hot (about 80 °C) diluted formalin (20–100 ml/litre) or hot ethanol (700 ml/litre).

15. Identification of filarial worms

Human lymphatic filarial worms

Fig. 9 summarizes the main distinguishing features of the microfilariae of *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*, while Fig. 10 compares the larval stages of the three species in the mosquito vector. Fig. 11 shows the adult worms.
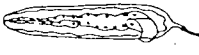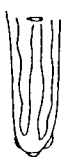
Fig. 9. Comparison of microfilariae of *Wuchereria bancrofti, Brugia malayi* and *B. timori*

	W. bancrofti	B. malayi	B. timori
Mean length (μm)	290	222	310
Cephalic space length : breadth	1 : 1	2 : 1	3 : 1
Sheath in Giemsa	Unstained	Pink	Unstained
Nuclei	Regularly spaced, separately situated	Irregularly spaced and overlapping	
Tail	Single row of nuclei do not reach the tail's end	Single row of nuclei reach the tail's end	
Terminal nuclei	None	2 nuclei, which bulge the cuticle conspicuously; closely spaced	2 nuclei, slightly bulging the cuticle; relatively far apart
Appearance in blood film	Smoothly curved	Kinky	Kinky
Innenkörper length (μm)	34	30.7	60

W. bancrofti
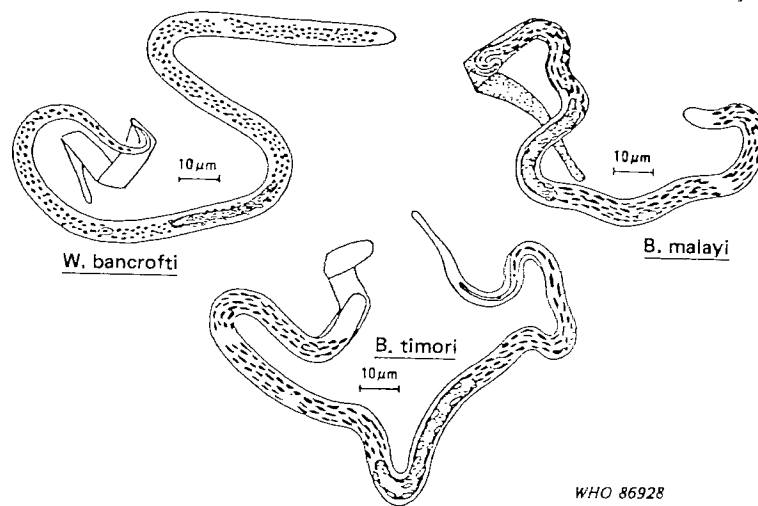
B. malayi

B. timori

WHO 86928

Fig. 10. Comparison of L_1, L_2 and L_3 larval stages of *Wuchereria bancrofti,*
Brugia malayi and *B. timori*

	W. bancrofti	B. malayi	B. timori
First stage (L_1):			
length (μm)	134-374	120-290	106-226
Second stage (L_2):			
length (μm)	318-1367	541-954	572-1352
Third stage (L_3):			
length (μm)	1049-2003	1162-1702	1474-1749

WHO 86929

Table 1 (page 76) compares the characteristic features of the micro-filariae of periodic and subperiodic forms of *B. malayi*.

Differentiation of human and animal lymphatic filarial worms

Brugia malayi and *B. pahangi*

B. pahangi is perhaps the most important of the animal *Brugia* species, as experimental human infection has been achieved and hence the possibility of natural human infection exists. Fig. 12 (page 77) shows the main differences between the two parasites, *B. malayi* and *B. pahangi*, in all stages.

The *innenkörper* in the microfilaria of *B. pahangi* is much longer than that of *B. malayi*, while the anal protrusion seen in the later L_1 and the L_2 larval stages is larger and nearer the tip of the tail in *B. malayi* than in *B. pahangi*.

Fig. 11. Comparison of adults of *Wuchereria bancrofti, Brugia malayi* and *B. timori*

	W. bancrofti	B. malayi	B. timori
Male:			
length (mm)	29	18	17
subventral adanal			
papillae (pairs)	8	3-4	4-5
spicule ratio	2.7 : 1	3.3 : 1	3.1 : 1
gubernaculum			
(lateral view) (μm)	28 × 5	19 × 4	19 × 4
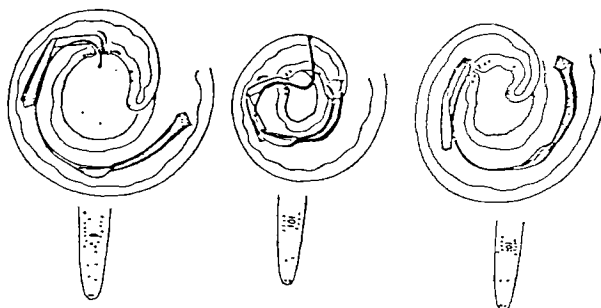
Female:			
length (mm)	61	48	27
ovejector length (μm)	190	106	160
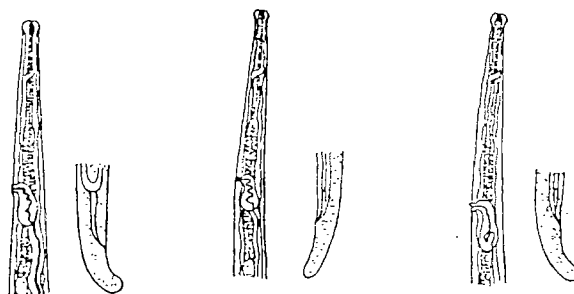
WHO 86930

Table 1. Differences between the periodic and subperiodic forms
of *Brugia malayi*[a]

Feature	Periodic form	Subperiodic form
Microfilarial periodicity	Markedly nocturnal: microfilariae rarely found during the day	Some nocturnal rise, but microfilariae readily found at all times
Microfilarial appearance in Giemsa-stained blood films	Empty sheaths common: few microfilariae still enclosed in sheath	Empty sheaths very rare: many microfilariae still enclosed in sheath
Microfilarial length in blood film	264 µm	247 µm
Experimental mosquito infections	Highly infective to *Anopheles barbirostris/ A. campestris*; does not develop readily in *Mansonia longipalpis*	Does not develop readily in *A. barbirostris*: highly infective to *M. longipalpis*
Natural vectors	See Annex 1	See Annex 1
Experimental infection in cats	Does not develop well, and microfilaria count remains low	Infection readily established, with high microfilaria counts
Natural infection in cats	Rare	Common
Terrain	Coastal rice fields and open swamp	Freshwater swamp forest

[a] Adapted from WILSON, T. ET AL. The occurrence of two forms of *Wuchereria malayi* in man. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **52**: 480–481 (1958). These data are applicable to Malaysia and some parts of Indonesia.
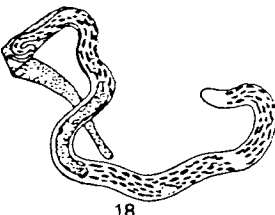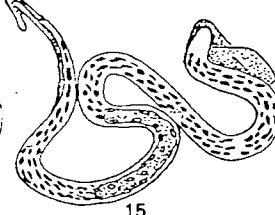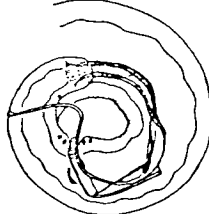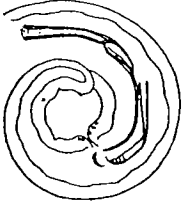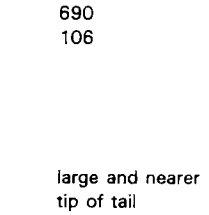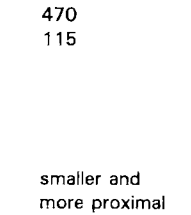
Brugia patei

This parasite is confined to Pate Island off the coast of Kenya and is found in cats, dogs and genet cats. The microfilariae are similar to those of *B. malayi* but have a short cephalic space (4.8 µm). The adult male has a broad cup-shaped base in its left spicule, which distinguishes it from all other species of *Brugia*.

Brugia ceylonensis

This is a parasite found in cats and dogs in India and Sri Lanka. The microfilariae are very similar to those of *B. malayi*, and the tip of the left spicule of the adult male has a spatulate appearance like that of *B. malayi*, which distinguishes it from *B. pahangi*.

Fig. 12. Comparison of *Brugia malayi* and *B. pahangi*

	B. malayi	B. pahangi
Microfilariae		
length (μm)	222	244
cephalic space (μm)	7.5	9.0
innenkörper (μm)	30.7	53.0

	B. malayi	B. pahangi
Adult males		
length (mm)	18	15
left spicule		
length (μm)	342	208
distal tip	spatulate	non-spatulate
right spicule		
length (μm)	106	83
spicule ratio	3.3 : 1	2.5 : 1

	B. malayi	B. pahangi
Adult females		
length (mm)	48	40
vulva (μm)	690	470
ovejector (μm)	106	115

	B. malayi	B. pahangi
L₁ and L₂ larvae		
anal protrusion	large and nearer tip of tail	smaller and more proximal

WHO 86931

Brugia buckleyi

This parasite, unlike other species of *Brugia*, is found in the right chambers of the heart and the pulmonary vessels of the wild hare in Sri Lanka. The adult worms are large, about double the length of those of *B. malayi*. The microfilariae have no special features but in the adult males the spicule ratio is small (2:1) and, in this respect, it differs from all other species except *B. tupaiae*.

Brugia tupaiae

This is one of the smallest *Brugia* species (male 10.3 mm, female 21 mm) and has the smallest spicules in the adult males (left 157 μm, right 76 μm) with a small spicule ratio (2:1). A characteristic feature of the adult female is the position of the vulva (anterior to the equatorial position of the oesophagus). The microfilaria has a relatively long *innenkörper* (50–60 μm) and there is no swelling or constriction between the last two sub-terminal tail nuclei.
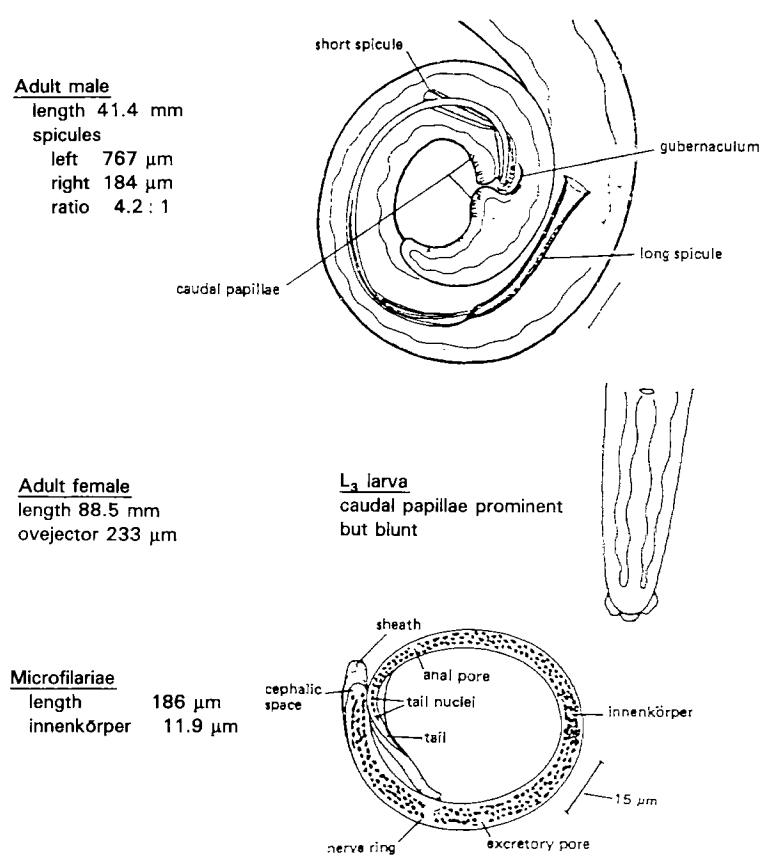
Wuchereria kalimantani (Fig. 13)

The only species of *Wuchereria* other than *W. bancrofti* is *W. kalimantani*, which has been reported from leaf monkeys *(Presbytis cristatus)* in South Kalimantan, Indonesia.

The microfilariae are smaller (186 μm) than those of *W. bancrofti* and have a very short *innenkörper* (12 μm). In addition the sheath stains reddish in Giemsa stain, unlike that of *W. bancrofti* which remains unstained.

The adults are larger than those of *W. bancrofti* (males 41.4 mm, females 88.5 mm). The left spicule of the male is the largest of all species of *Wuchereria* or *Brugia* and it has the largest spicule ratio (4.2:1; left spicule, 767 μm, right spicule, 184 μm). The ovejector in the female is also a little longer than that of *W. bancrofti* (225 μm).

The infective larva has three caudal papillae which are as prominent as in *W. bancrofti* but blunter and not bubble-like.

Fig. 13. Characteristic features of *Wuchereria kalimantani*

Adult male
length 41.4 mm
spicules
    left   767 µm
    right 184 µm
    ratio   4.2 : 1

short spicule

gubernaculum

long spicule

caudal papillae

Adult female
length 88.5 mm
ovejector 233 µm

L₃ larva
caudal papillae prominent
but blunt

Microfilariae
length   186 µm
innenkörper   11.9 µm

sheath
anal pore
cephalic
space
tail nuclei
tail
innenkörper
15 µm
nerve ring
excretory pore

WHO 86932

Other human microfilariae

Fig. 14 shows microfilariae of the other known human filarial para-
sites, which are found only in the African continent or in South America:
Onchocerca volvulus, Loa loa, Mansonella perstans, M. streptocerca and
M. ozzardi.

Fig. 14. Microfilariae of other species

Onchocerca volvulus

Mansonella streptocerca

M. perstans

20 μm

M. ozzardi

Loa loa

WHO 86933
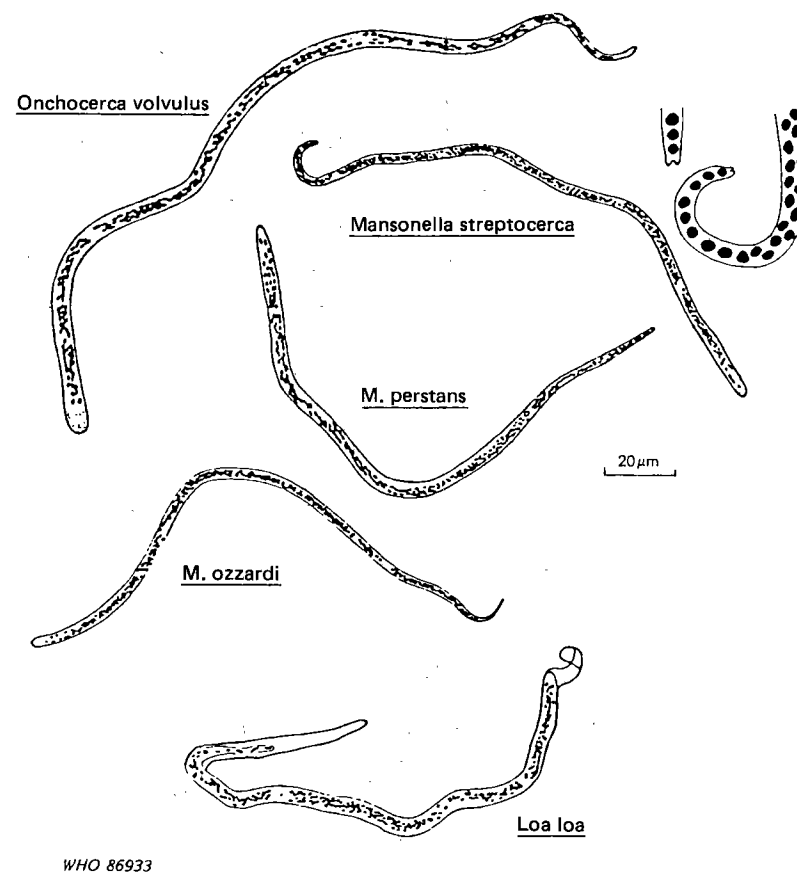
Suggestions for further reading

DENHAM, D.A. *Counting and identifying microfilariae. A laboratory manual.* London, London School of Hygiene and Tropical Medicine, 1979, 24 pages.

MAK, J.W., ed. *Filariasis. Bulletin No. 19,* Kuala Lumpur, Malaysia, Institute for Medical Research, 1983, 108 pages.

SASA, M. *Human filariasis. A global survey of epidemiology and control.* Baltimore, London and Tokyo, University Park Press, 1976, 819 pages.

SUZUKI, T. & SEREGEG, I.G. A mass dissection technique for determining infectivity rate of filariasis vectors. *Japanese journal of experimental medicine,* **49**: 117-121 (1979).

WHO Technical Report Series, No. 542, 1974 (Third report of the WHO Expert Committee on Filariasis), 54 pages.

WHO Technical Report Series, No. 702, 1984 (*Lymphatic filariasis*: fourth report of the WHO Expert Committee on Filariasis), 112 pages.

**Names of the principal (and subsidiary) mosquito vectors
of human lymphatic filarial parasites in the major endemic
zones of the world [1]**

Filaria species and type	Endemic area	Principal vectors (and subsidiary vectors)
Wuchereria bancrofti — periodic		
Zone 1	Tropical America	*Culex quinquefasciatus* *Anopheles darlingi* *(Aedes scapularis)* *(Aedes taeniorhynchus)* *(Anopheles albimanus)* *(Anopheles aquasalis)* *(Mansonia titillans)*
Zone 2	Tropical Africa	*Culex quinquefasciatus* [2] *Anopheles arabiensis* *Anopheles funestus* *Anopheles gambiae* *Anopheles melas* *Anopheles merus* *(Anopheles hancocki)* *(Anopheles nili)* *(Anopheles pauliani)* *(Anopheles wellcomei)* *(Culex antennatus)*
Zone 3	Middle East	*Culex molestus* *Culex quinquefasciatus* *(Culex antennatus)*
Zone 4	South Asia	*Culex quinquefasciatus* *Aedes poicilius* *Anopheles balabacensis* *Anopheles dirus* *Anopheles donaldi* *Anopheles flavirostris* *Anopheles candidiensis* *Anopheles anthropophagus* *Anopheles letifer* *Anopheles leucosphyrus* *Anopheles maculatus* *Anopheles minimus* *Anopheles sinensis* *Anopheles subpictus* *Anopheles vagus* *Anopheles whartoni* *(Aedes togoi)* *(Anopheles aconitus)*

[1] See map in Fig. 2, page 14, for outline of Zones 1–7.
[2] In continental Africa, only along the coast of the Indian Ocean (Kenya and the United Republic of Tanzania).

Filaria species and type	Endemic area	Principal vectors (and subsidiary vectors)
Zone 4 (continued)	South Asia	*(Anopheles barbirostris)* *(Anopheles kweiyangensis)* *(Anopheles nigerrimus)* *(Anopheles philippinensis)* *(Anopheles tessellatus)* *(Culex bitaeniorhynchus)* *(Culex sitiens)*
Zone 5	Far East	*Culex pipiens pallens* *Culex quinquefasciatus*
Zone 6	New Guinea	*Anopheles farauti* *Anopheles koliensis* *Anopheles punctulatus* *Culex quinquefasciatus* *(Aedes kochi)* *(Anopheles bancrofti)* *(Culex annulirostris)* *(Culex bitaeniorhynchus)* *(Mansonia uniformis)*

Wuchereria bancrofti — subperiodic

Zone 4	South Asia	*Aedes harinasutai* *Aedes niveus*
Zone 7	Polynesia	*Aedes cooki* *Aedes fijiensis* *Aedes kesseli* *Aedes oceanicus* *Aedes polynesiensis* *Aedes pseudoscutellaris* *Aedes samoanus* *Aedes tutuilae* *Aedes upolensis* *Aedes vigilax*

Brugia malayi — periodic	South Asia	*Anopheles anthropophagus* *Anopheles barbirostris* *Anopheles campestris* *Anopheles donaldi* *Anopheles kweiyangensis* *Anopheles sinensis* *Mansonia annulata* *Mansonia annulifera* *Mansonia uniformis* *(Anopheles nigerrimus)* *(Aedes kiangensis)* *(Aedes togoi)* *(Mansonia bonneae)* *(Mansonia dives)* *(Mansonia indiana)*

Filaria species and type	Endemic area	Principal vectors (and subsidiary vectors)
Brugia malayi — subperiodic	South Asia	*Mansonia annulata* *Mansonia bonneae* *Mansonia dives* *Mansonia uniformis* *(Coquillettida crassipes)*
Brugia timori — periodic	Flores, Timor	*Anopheles barbirostris*

Sample filariasis data collection sheet and codesheet

Data collection sheet

State _____   Village _____

District _____

Control status   1   [ ]   1 = precontrol
  2 = postcontrol
  9 = unknown

     2   3   4   5
Registration no. [ ][ ][ ][ ]

     6   7   8   9   10   11
Date [ ][ ] [ ][ ] [ ][ ]
     day   month   year

Name _____

    12   13   14
Age [ ][ ]   Sex [ ]   1 = male
       2 = female
       9 = unknown

Ethnicity _____

     15   16   17
Household no. [ ][ ][ ]

              18
Resident status [ ]   1 = in area
       2 = out of area
       9 = unknown

History of   19   1 = yes
filarial adenolymphangitis [ ]   2 = no
or abscess   9 = unknown

Number of attacks   20   0 = none
of either in [ ]   1-8 = no. of attacks
past year   9 = unknown

Number of malaria   21   0 = none
attacks in past year [ ]   1-8 = no. of attacks
         9 = unknown

Spleen size   22   [ ]   Hackett 0-5
       9 = unknown

Filarial scars   23   [ ]   1 = yes
       2 = no
       9 = unknown

Hydrocele   24   [ ]   1 = yes
       2 = no
       9 = unknown

Other genital lesion   25   [ ]   1 = yes
       2 = no
       9 = unknown

Elephantiasis   26   [ ]   1 = yes
       2 = no
       9 = unknown

Amount of blood   27   28   00 = none
examined (µl) [ ][ ]   99 = unknown

Number of   29   30   31   000 = none
microfilariae [ ][ ][ ]   999 = unknown
     Wuchereria

     32   33   34
[ ][ ][ ]
     Brugia

Body weight (kg)   35   36   37
[ ][ ][ ]

Single dose of DEC (mg)   38   39   40
[ ][ ][ ]

Number of doses of DEC   41   42
[ ][ ]

Generalized reaction   43   [ ]   1 = yes
       2 = no
       9 = unknown

Localized reaction   44   [ ]   1 = yes
       2 = no
       9 = unknown

Filariasis codesheet

Column	Variable and Instructions	Code Name
1	Control status (1 = pre, 2 = post, 9 = unknown)	STAT
2–5	Registration no. (1001 – open)	REGNO
6–11	Date	DATE
12, 13	Age in years (00–90, 99 = unknown)	AGE
15–17	Household no. (001 – open)	HSENO
18	Resident status (1 = in area, 2 = out of area, 9 = unknown)	RES
19	History of adenolymphangitis or abscess (1 = yes, 2 = no, 9 = unknown)	ADENO
20	Number of attacks of either in past year (0 = none, 1–8 = number of attacks, 9 = unknown)	ATFIL
21	Number of malaria attacks in past year	ATMAL
22	Spleen size, Hackett (0–5 = size, 9 = unknown)	SPSIZ
23	Filarial scars (1 = yes, 2 = no, 9 = unknown)	SCAR
24	Hydrocele (1 = yes, 2 = no, 9 = unknown)	HYDRO
25	Other genital lesion (1 = yes, 2 = no, 9 = unknown)	OTM
26	Elephantiasis (1 = yes, 2 = no, 9 = unknown)	ELE
27, 28	Amount of blood examined	BLOOD
29–31	Number of microfilariae (*Wuchereria*)	WUCH
32–34	Number of microfilariae (*Brugia*)	BRUG
35–37	Body weight (kg) (000–120, 999 = unknown)	WT
38–40	Single dose DEC in mg (000–998, 999 = unknown)	DEC
41, 42	Number of doses of DEC	NDOSE
43	Generalized reaction (1 = yes, 2 = no, 9 = unknown)	GEN
44	Localized reaction (1 = yes, 2 = no, 9 = unknown)	LOC

Collection and sending of blood-meal samples
## for identification

Data required

Each batch of blood-meal samples sent for testing should be accompanied by the following information:

- locality/site of collection;
- mosquito species in relation to filter paper samples;
- animal hosts available at the site of collection;
- date of collection.

Collection and sending of blood-meals

Each filter paper is divided into 16 segments, each segment containing 1 blood-meal sample (see Fig. 15).

Fig. 15. Filter paper used for collection of blood-meal samples

Filter paper No:
Locality:
Date of collection:

WHO 86934
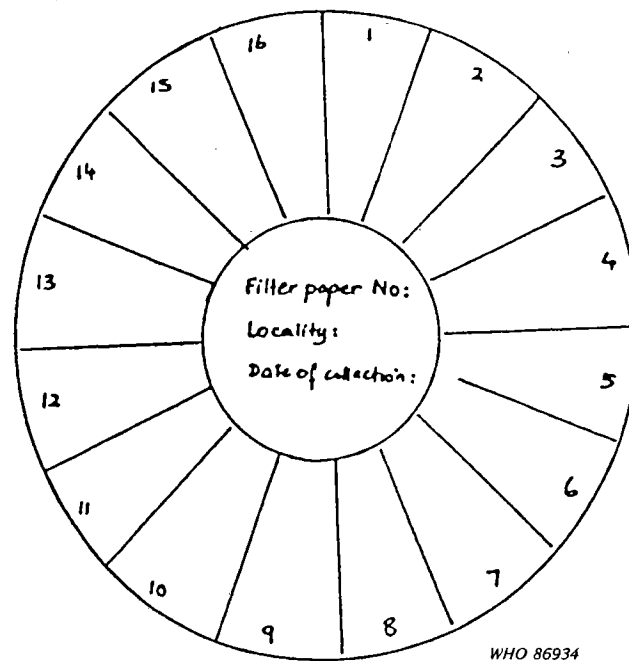
Each filter paper must be numbered, as must each segment.

Air-dry the filter papers thoroughly before packing.

For sending, the filter papers should be interspaced with greaseproof paper to prevent contamination of blood-meals. They should be sent in sealed plastic bags to:

> Head
> Malaria and Filariasis Division
> Institute for Medical Research
> Jalan Pahang
> Kuala Lumpur
> Malaysia