Module 4

Filaria – Vectors

Learning Objectives :

At the end of the module, the participant will be able to understand:

- Disease Transmission
- Vectors of Lymphatic Filariasis
- Vector Biology
- Life Cycle
- Entomological Indices and its significance
- Vector Control

Disease

Lymphatic filariasis, commonly known as elephantiasis, is a neglected tropical disease. It is caused by infection with the mosquito-borne, thread-like, parasitic filarial worms Wuchereria bancrofti, Brugia malayi and B. timori. It is an ancient disease, with significant social and economic consequences for affected individuals, families and communities. The worst symptoms of chronic disease generally appear in adults, in men more often than in women, and include damage to the lymphatic system, arms, legs or genitals, which causes significant pain, wide-scale loss of productivity and social exclusion [1]. In 2020, 863 million people in 50 countries were living in areas that require preventive chemotherapy to stop the spread of infection. The global baseline estimate of people affected by lymphatic filariasis was 25 million men with hydrocele and over 15 million people with lymphoedema [2]. The microfilariae of W.bancrofti and B.malayi exhibit periodicity (Nocturnally periodic and subperiodic). A wide range of mosquitoes (Culex, Anopheles, Aedes and Mansonia) can transmit the parasite, depending on the parasite species, geographic area and microfilariae periodicity. B. malayi nocturnal periodic infection is prevalent in the states of Kerala, Tamil Nadu, Andhra Pradesh, Orissa, Madhya Pradesh, Assam and West Bengal. The infection in the other six states is confined to a few villages only.

Filariasis has been a major public health problem in India. The disease was recorded in India as early as the 6th century B.C. by the famous Indian physician, Susruta in his book 'Susruta Samhita' [3]. In India, over 670 million people are at risk of infection by W.bancrofti and B.malayi parasites in 272 districts. Infection with W.bancrofti is widespread in 16 states and 5 Union Territories, contributing to about 99.4 % of the total cases. The remaining cases are due to B. malayi and are confined to the Western coast of Kerala and a few pockets in Andhra Pradesh, Assam, Orissa, Madhya Pradesh, Tamil Nadu and West Bengal.

In 2004, the National Campaign of the Mass Drug Administration (MDA) with DEC - was launched in 202 districts and thereafter, National Task Force recommended DEC+Albendazole in the country. The MDA programme was further scaled up in 2007 to cover all 256 districts in 2013, validation started through Transmission Assessment Survey (TAS). Triple Drug Therapy (IDA) i.e. DEC + Albendazole + Ivermectin has been introduced in 2018 in the Arwal district of Bihar and extended to 21 districts as of 2021 in a phased manner.

Vectors

Several species of four genera of mosquitoes - *Anopheles*, *Culex*, *Aedes* and *Mansonia*-play major roles in the transmission of lymphatic filariasis in different endemic regions globally. In India, *W. bancrofti* is nocturnally periodic in the entire

mainland and is transmitted by the ubiquitous mosquito *Culex quinquefasciatus*, which breeds in polluted water. In the Car Nicobar group of islands, *W.bancrofti* is subperiodic and is transmitted by Downsiomyia (Aedes) nivea, which breeds in tree holes and is a day biter.

B. malayi in India is nocturnally periodic and is transmitted by Mansonia annulifera, *Ma. uniformis* and Ma. indiana mosquitoes, which breed in water containing floating aquatic vegetation such as Pistia, Salvinia, Eichornia, etc. *Ma. anulifera* is the principal vector and *Ma. uniformis* and *Ma. indiana* are the secondary vectors.

Culex quinquefasciatus

Mansonia annulifera

Mansonia uniformis

Downsiomyia (Aedes) nivea

Vector Identification and Bionomics

Culex quiquefasicatus

It is a medium-sized mosquito, recognizable by dark tarsi and rounded abdominal bands. Head: Vertex and nape covered with narrow golden brown scales and scattered upright scales. Palpi brown, about 1/6th length of the proboscis. Proboscis: dark brown usually with an indistinct pale area in the middle especially on the underside and sides. Legs brown, dark brown or nearly black, abdominal tergite I almost entirely covered with long yellow scales, a patch of dark scales on the apical border in middle, abdominal tergites II-VII dark brown or black with ochreous (not white) basal bands, a little wider in middle. This species is distributed throughout the tropics and subtropics.

Bionomics

Cx. quinquefasciatus is a domestic mosquito associated with human residence and activity throughout its range. A female lays an egg raft averaging 150-300 eggs during each gonotrophic cycle. Larval to adult development is dependent on temperature, nutrition and population density and can be as short as 7 days under optimal conditions. Larvae prefer slightly alkaline waters < pH 8 with high organic content. High densities of larvae may be found in sewage drains, cesspools and septic tanks. Larvae can also be found in ditches, ground pools, ponds, tree holes, rocky pools, stream margins, coconut husks, wells etc. Larvae are often found in association with *Aedes aegypti* and *Ae. albopictus* in domestic and peridomestic water containers also. Adults can feed on blood from a variety of vertebrates including wild and domestic birds and mammals as well as man. Females mate within 2-6 days of emergence and may begin to seek hosts within 48 hours of emergence. Since *Cx. quinquefasciatus* must acquire a blood meal for reproduction and does not undergo diapauses, this species is active and reproduces year-round. In India, it may complete 2-3 gonotrophic cycles in a lifetime during the hotter season and 4-8 cycles in the cooler season. Adult Cx. quinquefasciatus lives for an average of one month under normal conditions. It is a crepuscular to nocturnal mosquito, restricting adult emergence, mating and

oviposition to dusk and blood feeding to the night. Females are strong flyers and can be found 1-3 kilometres away from their preferred breeding habitats. Most *Cx. quinquefasciatus* rest indoors during the day.

Mosquito Life Cycle

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

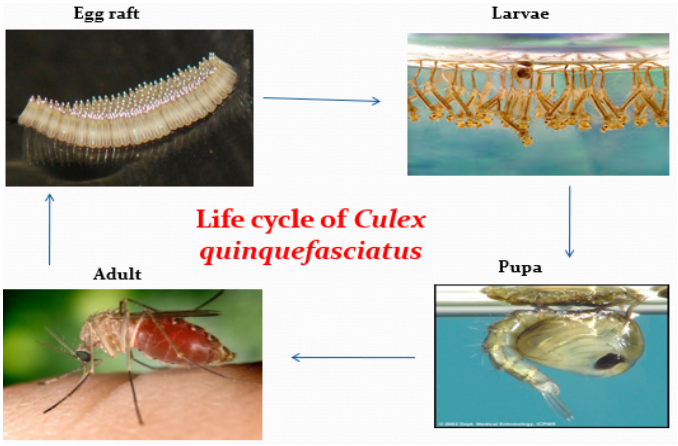


Figure 12.Life Cycle of *Culex Mosquito*

The simplest approach to the control of *Cx. quinquefasciatus* is a source of reduction of larval habitats. Most domestic habitats can be eliminated or modified to prevent access by mosquitoes while peridomestic habitats associated with agricultural practices may be modified or treated to reduce productivity. Fish especially the guppy *Poecilia reticulata*, have been used to successfully control *Cx. quinquefasciatus* in several larval habitats in India.

Mansonia

Size moderate and build robust, general colouration yellowish–brown or black, legs with many pale markings and white rings on tarsi, wings speckled with light and dark broad scales. Tergite VIII of a female with several chitinous hooks or teeth, the arrangement of which is of value in identification. Lobes of sternite VIII of characteristics shaped in different species.

Mansonia anulifera

Adult: Small yellowish-brown species. *Palpi*: about 1/3 length of proboscis, Yellow basally, broadly white apically, proboscis pale brown or yellow, usually darker at the base and on apical 1/3. *Thorax*: mesonotum with narrow yellow or golden scales, two conspicuous round white spots near the front margin, another pair posterior to these, and three other spots, less distinct, at about the level of wing roots. *Legs*: Yellowish, marked with numerous snowy white rings.

Mansonia uniformis

Adult: Brownish species, with less defined rings on the femora. *Palpi*: about 1/3rd length of proboscis, with scattered brown and yellow scales. *Proboscis*: Mainly yellow, with the apical 1/3 dark brown. *Thorax*: Scales of mesonotum light brown and greenish, later forming a pair of broad sub-lateral stripes from the front to above wing roots. *Legs*: four or five oblique pale markings on the outer side of the hind femur. *Abdomen*: Lateral chitinous hooks on tergite VIII curved and slightly separated forms the median series.

Mansonia Indiana

Adult: Very similar to Mansonia uniformis but differs in the absence of greenish stripes on the mesonotum. The scaling of mesonotum is usually almost uniformly dark brown, in some specimens, there are some pale scales towards the

lateral margins of mesonotum, and these may show up as faint light spots, but there are no definite white spots as in Mansonia annulifera. Chitinous hooks on tergite VIII of females widely spaced and curved, without a definite gap between these and median teeth.

Bionomics of *Mansonia* mosquitoes

Female *Mansoni*a lay their eggs in star-shaped clusters on the underside of floating leaves, using specifically adapted hook-like setae on their abdomens. Typical immature habitats include large, permanent water bodies- ponds, canals and lakes with dense amounts of floating vegetation such as water lettuce (*Pistia stratiotes*), water hyacinth (*Eichornia crassipes*), *Salvinia molesta*, etc. Immatures attach to and respire through the roots and stems of these aquatic plants using modified siphons. As the larvae and pupae are closely linked to their host plants, manual removal of the host plants or herbicidal treatments on the vegetation is often the most effective method of *Mansonia* population control.

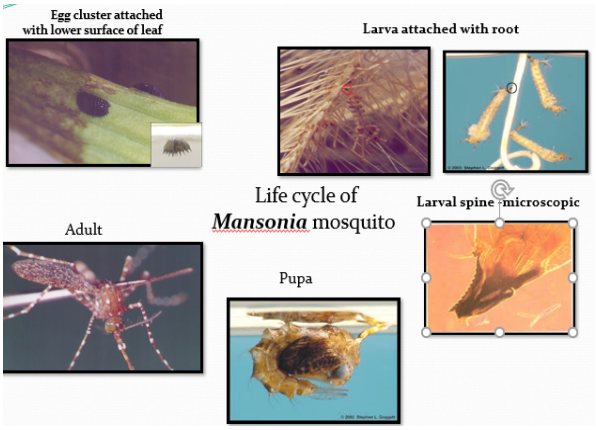


Figure 13.Life cycle of *Mansonia* Mosquito

Mansonia females are strong flyers and can be found several kilometres away from their preferred immature habitats seeking blood meals at dawn and dusk from a variety of animals including man. Some species are fearsome biters and can become a local nuisance.

Host Preference: *Mansonia* is catholic in feeding habits and indicates that man is not the preferred host and they are attracted to a wide range of hosts. *Ma.annulifera* is more anthropophilic than other species. *Ma.uniformis* is strongly attracted to cattle. They are available throughout the year, however, they are more abundant during monsoon and post-monsoon periods (July to September and December- January) coinciding with rainfall pattern.

Biting Activity: *Mansonia* mosquitoes bite shortly after dusk and a wave of biting has been observed with regularity, this biting continued through the night till morning. It is also known that *Mansonia* bites during the day in shaded places. *Mansonia spp*. are shown to bite outdoors more than inside houses.

Resting Places: Most *Mansonia* rests outdoors during the day. However, *Ma.annulifera* is more endophilic compared to other species.

Downsiomyia (Aedes) nivea

Thorax: paratergite without scales; postnotum without broad white scales; scutum with anterior half conspicuously pale-scaled. Wings entirely dark scaled, hind leg, except proximal part of femur dark scaled.

Bionomics: Downsiomyia (*Aedes*) nivea is a forest mosquito. Immature stages normally inhabit water in tree holes. The biting activity was seen throughout the day, exhibiting a bimodal peak, the first at dawn and the other towards dusk.

Vector control measures are very difficult owing to their exophily and diurnal feeding behaviour. Further, the larvae are not amenable to larvicidal control because of many scattered peculiar and inaccessible breeding habitats. Personal protection measures (use of repellent creams) may be useful for protecting from the risk of disease transmission.

Microfilarial Periodicity and Mosquito Biting Behaviour

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

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

Resting Behaviour

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

Gonotrophic Cycle and Lymphatic Filariasis Transmission

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

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

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

Mosquito Collection

From each sentinel and spot check site, entomological data collection should be made from 10 catching stations spending 15 minutes in each catching station using a flashlight and aspirator tube in the early morning between 6 a.m. and 10 a.m. All the female *Culex quinquefasciatus* shall be dissected to find out the filarial infection.

Dissection to Determine Filariae in Mosquitoes

Gently anesthetise the female mosquitoes, few at a time, with ether or chloroform. Identify and record the species. Remove the wings and legs of mosquitoes. Separate the body of the mosquito into three parts; head, thorax and abdomen. Each part is placed in a separate drop of saline on a side. Carefully dissect the thorax and abdomen by tearing all tissues apart with dissecting needle to examine the developing filarial larvae. Carefully dissect the proboscis and head for the presence of infective larvae. Keep a coverslip over each part. Examine under the low and high power of the compound microscope. If found positive, count the number of larvae and record.

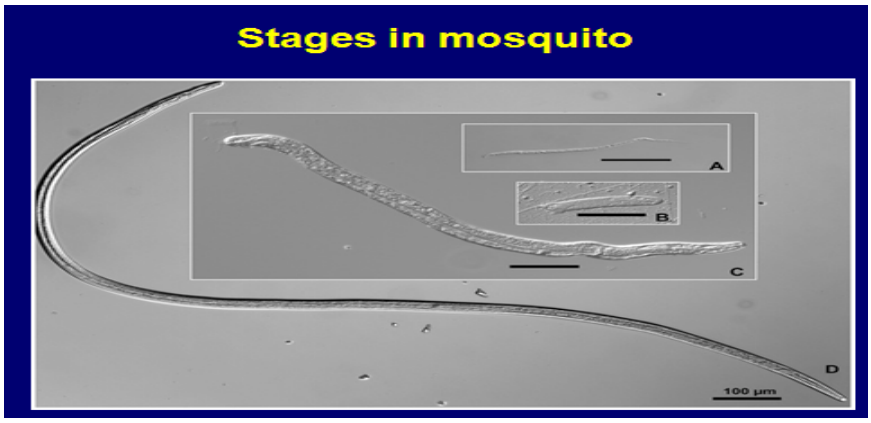
Stages of filarial larvae in mosquitoes:

1. **Mf Stage:** This stage can be found in the stomach of the mosquito soon after the blood meal.

1. **1st Stage Larvae:** This larval stage is a thick and short, sausage-shaped form with a short spiky tail. It possesses a rudimentary digestive tract and is immobile. It is confined to the thoracic muscle of the mosquito.
2. **2nd Stage Larvae**: This is a long sausage-shaped stage with a developing intestinal canal. In Wuchereria and Brugia, larvae were found in the thoracic muscles showing slight movement.
3. **3rd Stage or Infective Larvae:** This is the final stage of the development of filarial larva in the mosquito. It is very active and can be found in any part of the mosquito. This stage is filariform larva and its digestive system, body cavity and genital organs are fully developed.

Microfilaria (A)

L1: Stage 1 larva or sausage stage larva (B)
L2: Stage 2 larva or pre-infective stage larva (C)
L3: Stage 3 larva or infective stage larva (D)



Calculation of Entomological Indices

Ten Man-hour Vector Density:

$$\frac{\text{No. of female } \textit{C. quinquefasciatus} \text{ collected}}{\text{No. of man-hours spent on mosquito collection}} \times 10$$

Infection rate (%):

$$\frac{\text{No. of mosquitoes +ve for any stage (L1/L2/L3 stages) of the parasite}}{\text{No. of female vector mosquitoes dissected}} \times 100$$

Infectivity rate (%):

$$\frac{\text{No. of mosquitoes +ve for infective larvae (L3 alone)}}{\text{No. of female vector mosquitoes dissected}}$$

Mean number of L3/ infective mosquito:

$$\frac{\text{No. of infective larvae (L3) found}}{\text{No. of infective mosquitoes}}$$

Vector Control: Though, Lymphatic Filariasis Elimination Programme is comprised of only two main pillars of its strategy i.e. Mass drug administration (MDA) and Lymphoedema Management and the component of vector control has not been included in the Global and National Strategy. However, vector control for Filaria Vector is being supplemented under the IVM strategy undertaken in the Urban Towns, where the larviciding is being undertaken for the control of Malaria and Filaria Vectors besides source reduction and environmental modifications/ manipulations.