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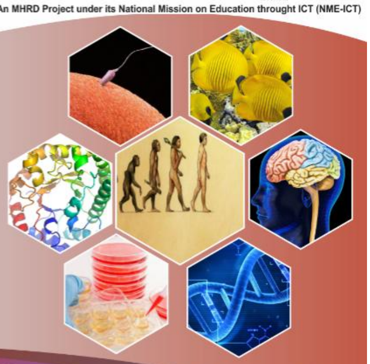

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Paper No. : 08 Biology of Parasitism

Module : M24 Morphology, Life cycles, Mode of entry of *Brugia*

ज्ञान-विज्ञान विमुक्तये

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Description of Module	
Subject Name	ZOOLOGY
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Keywords	Filariasis, nematodes, *Brugia malayi*, *B. timori*, *Mansonia*, *Aedes*, *Anopheles*

Contents:

1. Learning Outcomes

After studying this module, you shall be able to**:**

- ✓ Know various filarial parasites which infects human.
- ✓ Learn the habitat and morphology of human parasite *Brugia*.
- ✓ understand the periodicity shown by microfilariae of *B. malai*.
- ✓ Understand the life cycle of *Brugia malai*.
- ✓ Understand the mode of entry of filarial parasite.

2. Introduction

Nematodes are commonly referred as nonsegmented round worms, threadworms or pinworms. Previously they were considered as a Class under the Phylum Aschelminthes. However, due to differences in the characteristics and relationships among various classes in the Aschelminthes, nematodes are now placed in a separate phylum Nematoda. There are two classes of Phylum Nematoda, the Secernentea (Phasmidia) and the Adenophorea (Aphasmidia) having several orders with various parasitic worms. Class Secernentea has seven orders including Ascaridida which consists of human intestinal ascaris worms and Spirurida, having the dracunculid guinea worm and various filarial worms. The filarial parasites, *Wuchereria* and *Brugia* belong to this Class and the classification is as follows:

Phylum: Nematoda
Class: Secernentea
Order: Spirurida
Suborder: Spirurina
Superfamily: Filariidae
Family: Onchocercidae
Subfamily: Onchocercinae
Genera: Wuchereria/Brugia

There are various filarial parasites which infects human. Their adults are tissue specific and larval forms are called as microfilariae which are present in the blood stream or skin of the host. The most common parasites which cause human lymphatic filariasis are *W. bancrofti*, *B. malayi* and *Brugia timori* (Table 1). Their adults live in the lymphatic vessels and lymph nodes and microfilariae are sheathed and found in the blood.

Table.1: Most common filarial nematodes infecting human.

Parasite	Geographical distribution	Vector involved
Wuchereria bancrofti	Africa, Pacifica, Asia, America	*Culex quinquefasciatus, Anopheles spp., Aedes spp.*
Brugia malayi	Asia	*Mansonia spp.*
Brugia timori	Indonesia	*Anopheles barbirostris*
Onchocerca volvulus	Africa, South and Central America, Yemen	*Simulium spp.*
Loa-loa	Africa	*Tabanus spp.*

The lymphatic filariasis is considered as a neglected tropical disease with significant economic and social consequences on the individuals, families and communities affecting from the disease. The worst symptoms of the disease generally appear more often in male adults than in female. The filarial disease damages the lymphatic system, limbs or genitals and causes severe pain. It is also responsible for the loss of productivity as well as social exclusion.

The filarial worm *Brugia* usually lives in the lymphatic vessels of their hosts primarily in tropical countries and like many other nematodes, give birth to young. During development, a transparent sheath is formed around the embryo and well developed young or microfilariae are born. A mosquito vector is involved in the life cycle of *Brugia*. As in the case of other nematodes, there are also different strains of *Brugia* distributed in different parts of the world. *B. malayi* is the cause of widespread filarial disease of human in the far-east from Korea south through China, the Philippines, Indonesia, Malaysia and New Guinea, Sri Lanka and

India. It is transmitted by mosquitos of the genera *Mansonia* and *Anopheles* and occurs also in monkeys and cats. Adult female worm measures 23 x 0.09 mm.

Closely related to *B. malayi* is *B. pahangi*, which is found principally in carnivores, but also in many other mammals including primates. It has been found in Malaysia and East Pakistan and can infect human experimentally. The vectors are *Mansonia*, *Aedes* and *Armigeres*.

Brugia timori is closely related to *Brugia malayi* distributed in Timor and neighbouring islands of Indonesia. It is restricted to the eastern islands of the lesser Sunda archipelago (Nusa Tenggara Timur), where it replaces *B. malayi*. The differentiating feature of *B. malai* and *B.* timori is present in table 2. The only known natural vector of *B. timori* is Anopheles barbirostris but the culicine mosquito species such as *Aedes togoi* is also found to support development of the nematode under experimental conditions.

Table.2: Differentiating features of *Brugia malayi* and *Brugia timori*.

Characters	*Brugia malayi*	*Brugia timori*
Microfilariae		
Mean length	220 μm	310 μm
Cephalic space	Length to width ratio = 2:1	Length to width ratio = 3:1
Sheath	Stained pink with Giemsa	Stained pale pink with Giemsa
Terminal nuclei	4-5 in a single row	5-8 in a single row
Adult worms		
Ecology	Various ecotypes transmitted by *Anopheles* or *Mansonia* spp., some strains with animal reservoirs, nocturnally periodic or subperiodic	One ecotype (anthropophilic), transmitted by *Anopheles*, no animal reservoir known, nocturnally periodic.
Geographic Distribution	India, South east Asia	Lesser Sunda archipelago of eastern Indonesia, East Timor.
Females	Body length to ovejector length ratio = 360:1	Body length to ovejector length ratio = 170:1
Male adanal papillae	3-4 on a side, regularly spaced.	3-5 on a side, irrregularly spaced.

3. Habitat and Morphology of the *Brugia*

Brugia malayi is a digenetic endoparasite using mosquitoes as its intermediate host and various mammals including human as definitive hosts. In open swamp, irrigated field and hill forest of South and East Asia, the mosquito vectors belong to the genera *Aedes*, *Mansonia*, *culex* and *Anophles*. In these intermediate host, *B. malayi* present in the proboscis, stomach, thorax and muscles. When a mosquito vector bites human or other definitive hosts, it enters the wound and then migrates to the lymphatic system of the host through the blood stream. It lives in the lymphatic system throughout its adult life.

Adults of *B. malayi* are long and slender covered with smooth cuticle, kinked and has a long cephalic space having a length and width ratio of about 2:1 (figure 1). The length of female adult ranges from 43-55 mm and width between 130-170 µm. The size of male adult measure between 13 mm to 23 mm in length and 70 µm to 80 µm in width. The microfilariae are ranging from 177-230 µm in length and 5-7 µm in width. The head of the adult is slightly swollen and bears two circles of well defined papillae. The tail of *B. malayi* is ventrally curved. Sexual dimorphism also exists within the adults.

Figure.1: Adult of *Brugia malayi*

The anatomical features of *microfilariae malayi* was first given by Brug in 1927 from Malay Archipelago. Later, Korke (1929) and Iyengar (1932) have also described the morphology of the microfilariae characters (figure 2):

1) **Sheath:** It is a delicate, closely fitted membrane which projects beyond head and tail of the microfilariae. The average length of the sheath is generally 268.48µ.
2) **Cuticle:** A thick cuticle surrounds the worm and the striation of the cuticle is very faint and which is almost not visible.
3) **Cephalic space:** The anterior portion of the worm is slightly longer than broad and devoid of nuclear mass. The average distance from anterior end to the beginning of first nucleus (BNC) in proportional to the length of the microfilariae is 3.38%.
4) **Nerve ring (NR):** It is a band like region which is most conspicuous and well marked and devoid of nuclear mass. It is present at 22.6% of the body length behind the end of the head region.

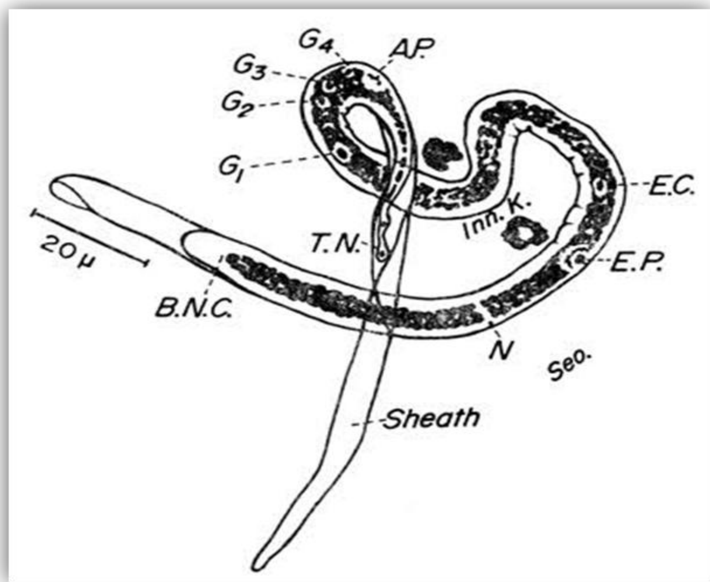

Figure.2: Morphology of *B. malayi* microfilariae.

5) **Excretory pore (EP):** It is markedly differentiated from the surrounding nuclear mass with conspicuous oval space. This is situated at 32.26% of the total length of the worm from anterior end. The average size of the pore is about 7.55 x 4.33µ.

6) **Excretory cell (EC):** It is present posterior to the excretory pore. It is elongated with large nucleus and rich in cytoplasm. It is situated at 38.8% of the whole body length from the anterior end. The average size of excretory pore is 7.99 x 3.99μ. The approximate distance between excretory pore and excretory cell is 13.32μ.
7) **Innen-körper:** It is a hyaline like region having few scattered nuclei. It starts anteriorly from 53.95% of the whole body length and ends posteriorly to 67.87%. The average length of Innen-körper is 9.99μ.
8) **G cells:** These cells are frequently hidden by other nuclei except the first G cell. It is large and elongate with prominent nucleus. The average size of first G cell is 5 x 3.33 μ which is twice larger as compare to other three indistinct G cells which are closely situated in a line.
9) **Anal pore (AP):** It is prominent as the excretory pore. It is large and oval shaped present more than half width of the microfilaria. It is situated at 82.94% of the body length from the anterior of the body.
10) **Terminal nuclei (TN):** The tail structure of the microfialriae is so much characteristic in swelling at the tip of the tail. There are one or two nuclei very often found in this region, one is present at the tip of the tail and other is situated in the middle point between the ends of the nuclear column. The tip of tail has the swelling at the corresponding places.

About 905 million people were at risk of lymphatic filariasis with 90.2 millions of victims worldwide in 1984 and the figures were 751.4 million and 78.6 million in 1992. At present, worldwide 1.3 billion people are at risk of lymphatic filariasis infection and about 120 million people are affected in 83 countries. Amongst them 45.5 million live in the Indian subcontinent and 40 million in Sub-Saharan Africa.

Some times *W. bancrofti* and *B. malayi* may co-exist in many places; therefore, it is very important to identify their differentiating characters for making diagnosis and epidemiological surveys (Table 3). These species could be differentiated based on the measurements of various body characters of the microfilarial forms such as the number and position of caudal nuclei, length and width of microfilariae, cephalic space and presence or absence of sheath.

Table.3: Body characters of *W. bancrofti, B. malayi* and *B. timori* microfilariae.

Body characters	***W. bancrofti***	***B. malayi***	***B. timori***
Length	309.2-346.8 μm	205-240 μm	265-323 μm
Width	5.30 μm	4.00 μm	4.40 μm
Cephalic space	4.10 μm	7.50 μm	13.00 μm
Length-width ratio	1:1	1.9:1	3:1

In addition, the infective third larval stage (L_3) stage of these species can be differentiated by investigative the caudal papillae (Figure 3). There are three caudal papillae in which two of them are located at lateral side and one is on terminal side. In *W. bancrofti,* all these caudal papillae are distinctly protruding as compared to *B. malayi*. Under electron microscope, these lateral papillae of *B. malayi* show a gutter-like indentation around their bases which is absent in *W. bancrofti*. Apart from these, microfilariae of various species could be distinguished by examining the morphological features. Major characters by which they can be differentiated are the number and position of caudal nuclei, cephalic space and/or the presence or absence of sheath.

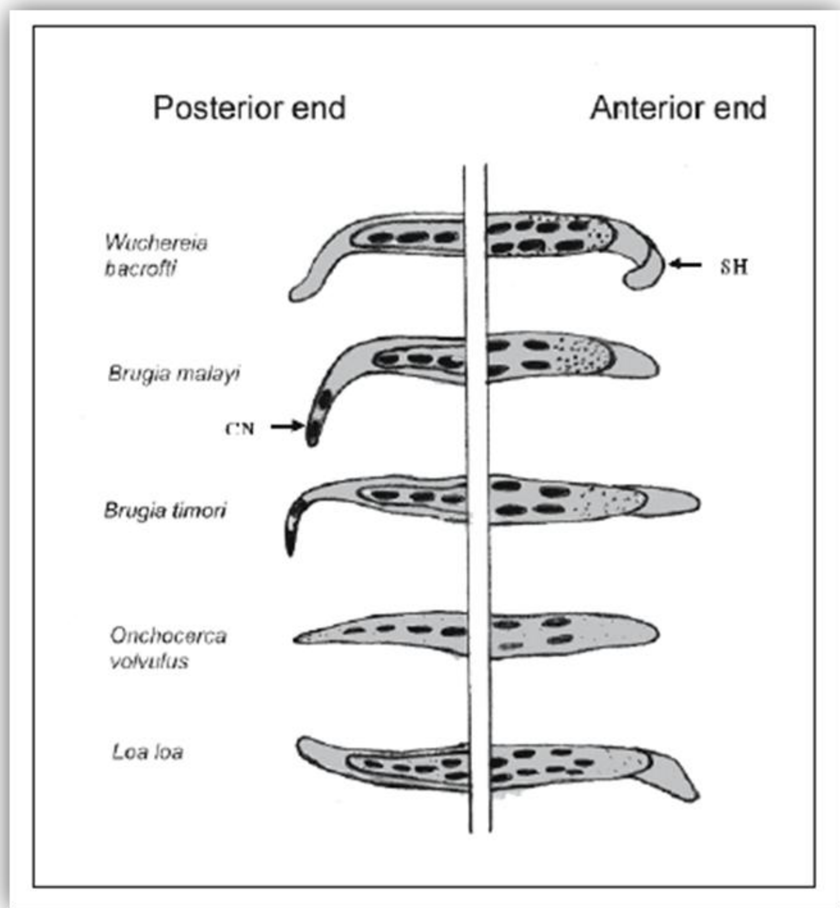

Figure.3: Differentiation of different species of microfilariae on the basis of presence or absence of caudal nuclei (CN) and sheath (SH).

4. Periodicity of microfilariae

Brugian filariasis occurs in two basic forms: nocturnal periodic, which shows a marked nocturnal periodicity and nocturnal sub-periodic in which a minor nocturnal peak occurs but the larvae are present in the peripheral blood continuously through each 24 hour periods. Anophelines and certain culicines (*Culex quinquefasciatus*, certain *Mansonia* and *Aedes* species) are the vectors of the nocturnal periodic form. Culicines are vectors of subperiodic forms.

The nocturnal periodic form is generally a rural disease. It occurs throughout the major part of Asia, southern India, Korea, Viet Nam, West Malaysia, China, Indonesia and most part of

Japan. In the nocturnal periodic form, most of the microfilariae during the day circulate in the blood vessels which supply blood to the lungs. During the middle of the night, microfilariae migrate to the peripheral blood system and lymph vessels. Because of this marked 24 hours periodicity of microfilariae, these are ingested mainly by the mosquitoes bite during night such as *Anopheles, Mansonia.*

The Anophelines involved in the filariasis transmission differ according to the area, but many of the principal malaria vectors are also important filarial vectors such as *Anopheles anthropophagus, An. campestris, An. sinensis* and *An. barbirostris.*

The *Mansonia* species includes *Ma. annulata, Ma. annulifera* and *Ma. uniformis.* These species mainly breed in more or less permanent water with floating or rooted vegetation. Adult mosquitoes bite and rest mainly outdoors but in some area they may also bite and rest indoors. In China, Korea and Japan *Aedes togoi* is vector of *B. malayi* nocturnal periodic form.

Brugia timori is known only from the small Indonesian islands of Timor, Alor, Flores and Roti. Its microfilariae are nocturnally periodic and are transmitted by *An. barbirostris* and possibly other anopheline species. There are no known animal reservoirs.

The nocturnal subperiodic form of *B. malayi* occurs in west Malaysia, Indonesia, Philippines and Thiland. It is transmitted by *Mansonia* species such as *Ma. Annulata*, *Ma. dives*, *Ma. bonneae* and *Ma. uniformis*. The species occurs in much vegetation such as swampy forests. The adult mosquitoes mainly bite during night but also during day time. The subperiodic form of *B. malayi* is essentially a parasite of swamp monkey. Humans get infected when they live at the edge of these areas. Other reservoir includes *Macaca* monkey, domestic and wild cats.

Several hypotheses have been proposed to explain the mechanism of microfilarial periodicity. In the early 1951, Hawking and Thurston verified that periodic variation in the number of microfilariae occurs because of their accumulation in the lungs during day time and their release into the circulating blood at night. It was also found that the body temperature acts as stimulus which affects the periodicity of *B. malayi* microfilariae. Later, it was further explained that accumulation of microfilariae in lungs during day time occur due to higher oxygen tension in the lungs as compared to night when host is under rest. However, the exact mechanism of microfilarial periodicity is not yet fully understood. The periodicity of microfilariae appears to be depends upon the circadian rhythm of the host which acts as a cue to regulate the circadian rhythm of the microfilariae. Whatever may be the reason of the periodicity, but this periodicity is in agreement with the feeding behaviour of the mosquito to ingest large number of microfilariae present at the peripheral blood during the peak time of the mosquito biting. It increases the chances of survival of these microfilariae.

The susceptibility of a mosquito species to infect with microfilariae depends upon genetic, physical and physiological factors. The penetration of microfilarial larvae in the mosquito tissue requires a specific mechanism and failure of which will reduce invasiveness. The microfilariae entering into mosquito gut during blood meal has to overcome a series of barriers in the gut of the vector.

The cibarial and pharyngeal armatures acts as one of the barrier which in some species of mosquitoes are well- developed and may damage the sheath of the larvae. The blood clotting time in the mosquito’s mid-gut differs in different vector species and if the blood clots before the migration of microfilariae to the thorax, they trapped within the abdomen of the mosquito. The peritrophic membrane in certain vectors also checks the penetration of microfilariae through the gut wall. The wall of mosquito mid-gut also acts as a potential barrier to parasite larvae as they have to exsheath in the mid gut of the mosquito before penetration. It has been reported that after exsheathment, microfilariae of *Brugia* spp. rupture the epithelium of mosquito mid-gut by cephalic hook and damage the luminal surface and basement lamina. Facilitation, limitation and proportionality are the main relations which are observed in human filaria/mosquito couples depending upon the species of the mosquito that ingests the microfilariae.

5. Life cycle of *Brugia malayi*

The life cycle of filarial parasites completed in two different host, intermediate host and definitive host. The definitive host is human and intermediate host is mosquito which sucks blood from definitive host. The adults of *B. malayi* live in the lymphatic system of human. They are viviparous nematodes which produce young offspring called microfilariae. Adult male and female *B.malayi* mate and the gravid females release microfilariae into the lymphatic system of human which then migrate and circulate into the blood system.

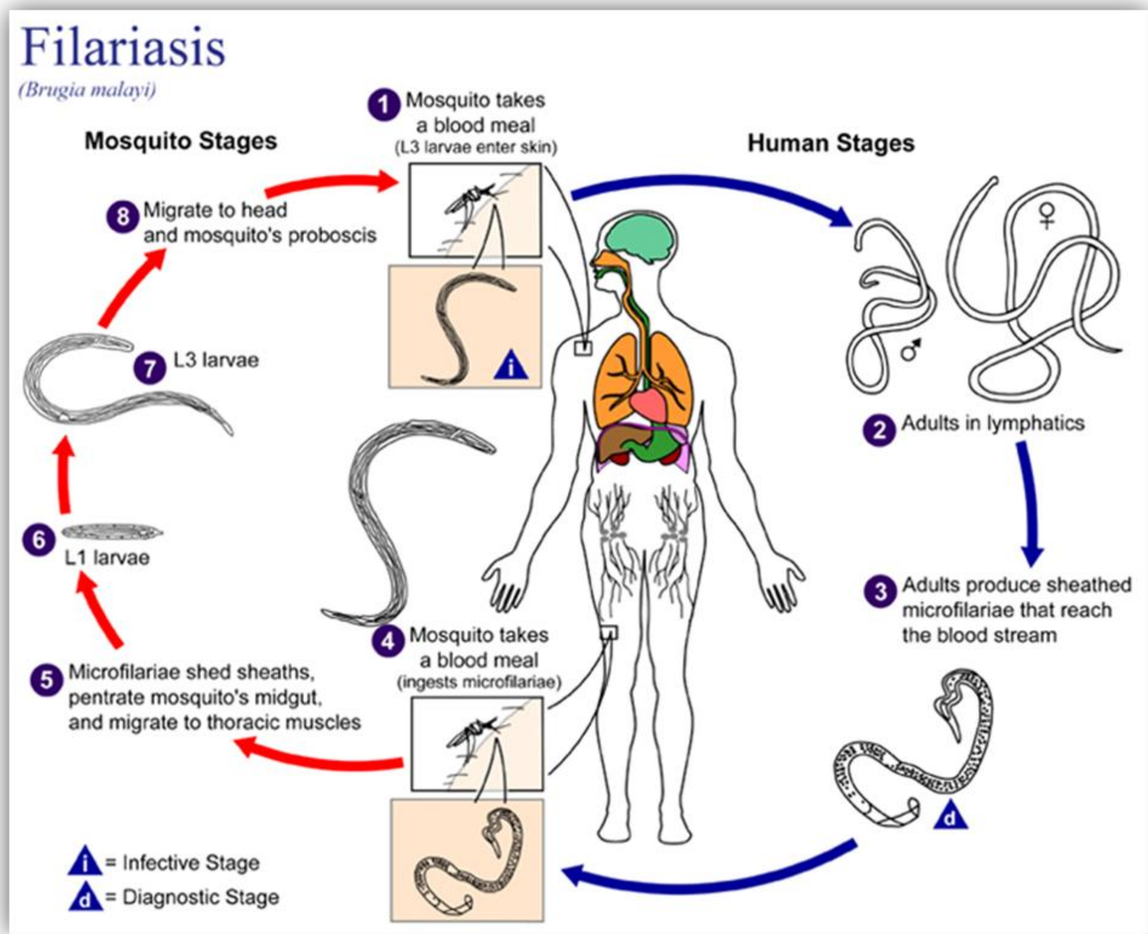

Figure.3: Life cycle of *Brugia malayi* in mosquito vector and human.

The extrinsic life cycle of the *B. malayi* parasite begins when microfilariae are ingested by a mosquito during feeding of host blood. Microfilariae ingested with the blood meal pass into the stomach of the mosquito (in some vector like Anopheles most of the microfilariae are destroyed during their movement through oesophagus). They exsheath and then penetrate the stomach wall and pass into the haemocoel and subsequently they migrate to the thoracic muscles of the mosquito within about 24 hours. The exsheathment of microfilariae can occur in the mid-gut or in the haemocoel of the mosquito. Those microfilariae which migrate immediately after ingestion, usually exsheath in the haemocoel and those microfilariae which stay in the mid-gut for more than 2 hours exsheath in the mid-gut of the mosquito and then they migrate. In the thorax, the small larvae become more or less inactive which grow shorter and considerably fatter to develop into the sluggish sausage shaped first stage larvae (L1) after two days. After 5th to 7th day, the L1 moults into second stage (L2) larva which is more active and after 10th to 11th day, L2 moults into infective third larval stage (L3). This stage is very active and show oscillatory pattern of movement which leave the thoracic muscle and migrate through the head and down the fleshy labium of the proboscis. This is an infective stage and is formed in ten or more days after the microfilariae have been ingested by mosquito with the blood meal. When mosquito takes further blood-meal, the infective larvae rupture the skin of labella of the labium and crawl on the surface of the host skin. Several infective larvae may be liberated onto the skin of the host when a vector is biting. Most of them die and only few are able to find the abrasion or small lesion caused by mosquito bite and migrate to the efferent lymphatics and subcapsular sinus of the host. The L3 stage in the vector can remain alive and active for 46 to 50 days or as long as the vector survives. It is also important to note that the salivary gland of the mosquito is not involved in the transmission of filariasis as well as it is also not involved in multiplication cycle or sexual reproduction of the parasite.

After 9–10 days of entry, the L3 stage of parasite moults to become the fourth larval stage (L4). It undergoes developmental process for several days to months depending upon the type of species and finally moults to become adult. The total developmental period and the longevity of the parasites depend upon the type of species and the mammalian host. The production of microfilariae by the adult female was continues for at least for a period of five years. In an experiment, *B. malayi* was inoculated in the man and the observation was done for 10 year. It was found that the microfilariae were first detected after 41 and 46 weeks of infection in the two subjects and remained up to 8 to 8.5 years indicating that an adult can reproduce for a period of 8 to 9 years in the human body.

The presence of the filarial worm in the thoracic muscle of the mosquito or infective worm in the proboscis does not necessarily means that the mosquito is a vector of *Brugian* filariasis. This is because; there are several other filariae which can be transmitted by various mosquito species. For example, various *Setaria* species infect cattles, *Dirofilaria repens* and *D. immitis* infect dogs and various other species of *Brugia* like *B. patei* in Africa and *B. pahangi* in Asia, infects animals but not the humans. Therefore, careful examination is important to identify the filarial parasite which can infect the humans.

The reckettsial endosymbiont, *Wolbachia* is also associated among several species of filarial parasites of human and other animals. There are as many as 10 filarial nematode species which are known to associate with *Wolbachia* such as *L. sigmodontis*, *W. bancrofti*, *Mansonella ozzardi*, *Onchocerca*, *Dirofilaria*, *Brugia* and many more. The *Wolbachia* is found in the second, third and fourth developing stages of the filarial parasites. The mutualistic relationship of *Wolbachia* and filarial nematodes for the normal growth, development and fertility of nematode is building up but it is still debatable.

Some evidences are also present which indicate that the filarial worms associated with *Wolbachia* need them for their normal growth and survival. The process of co-evolution results in the co-adaptation and reciprocal dependence which provide additional support for the obligate symbiosis. The presence of *Wolbachia* in filarial parasites is fixed and the nematodes have evolved themselves in such a way that they dependent on the bacteria for a large number of biological processes.

6. Mode of entry of *Brugia*

When female mosquitoes (*Aedes*, *Anopheles)* bite to an infected individual for getting the blood, the microfilariae present in peripheral circulation of the patient enter the gut of the mosquito along with the sucked blood. Ingested microfilariae penetrate the gut wall of mosquito vector (*Aedes, Anopheles*). Inside the gut, microfilariae shed its outer transparent sheath and eventually reach the thoracic musculature or fatty tissues of the invertebrate host, where they shorten by metamorphosis into sausage-shaped bodies 240 to 250 µm in length and the first true molt occurs. A second molt follows and the infective larvae at this stage are slender and measure 1.4 to 2 mm in length. The time period from ingestion of microfilariae to maturation of infective larvae is 10 to 14 days for lymphatic filariae. Near the end of the residence of microfilariae in the insect, these third-stage larvae move to the labium or proboscis sheath of the mosquito where they are in the position to penetrate the skin or wound when the insect bites another person. The worm larvae travel through the wound made by the insect into the lymphatic vessel. They grow and moult into fourth-stage larvae, and eventually develop into sexually mature adult worms.

Type of behaviour of microfilariae in the peripheral blood of human follow a specific pattern. Microfilarial periodicity depends on alternate accumulation of microfilariae in the lung capillaries usually by day and approximately even distribution throughout all the circulating blood usually by night. This periodicity is oriented to the established circadian cycle of the host (Sleep-wake habit) instead of day and night as such. The period of circadian cycle approximate that of earth's rotation i.e. 24 hours. These rhythms are ubiquitous in living organisms. The microfilareae have their own endogenous rhythm, synchronized with the cycle of the host. The periodicity appears to be related to the difference in oxygen tension between venous and arterial blood during day and night. The circadian rhythm of body temperature probable also plays a part in regulating this periodicity. Another theory is that microfilariae with numerous granules become irritate under sunlight and migrate to the skin capillaries only after sundown.

The pre-patent period (the time from infection to detection of microfilariae released by fecund adult female worms) is approximately 1 year for the major human filarial parasites. This period is considerably shortened (10 to 12 weeks) in rodents such as jirds (Meriones unguiculatus) experimentally infected with *B. malayi*.

7. Summary

- Nematodes are commonly referred as nonsegmented round worms, threadworms or pinworms. They are divided into two classes, the Secernentea and the Adenophorea having several orders with various parasitic worms.
- There are several filarial parasites which infects human and the most common parasites are *W. bancrofti*, *B. malayi* and *Brugia timori*.
- The filarial worm *Brugia* usually lives in the lymphatic vessels of their hosts primarily in tropical countries and like many other nematodes, give birth to young. A mosquito vector is involved in the life cycle of *Brugia*.

- *Brugia malayi* is a digenetic endoparasite using mosquitoes as its intermediate host and various mammals including human as definitive hosts.
- Adults of *B. malayi* are long and slender covered with smooth cuticle. The length of female adult ranges from 43 mm to 55 mm and width between 130 µm to170 µm. The size of adult male measures between 13 mm to 23 mm in length and 70 µm to 80 µm in width.
- Brugian filariasis occurs in two basic forms: nocturnal periodic, which shows a marked nocturnal periodicity and nocturnal subperiodic in which a minor nocturnal peak occurs.
- Hawking and Thurston proposed that periodic variation in the number of microfilariae occurs because of their accumulation in the lungs during day time and their release into the circulating blood at night.
- The life cycle of filarial parasite completed in two different hosts, the definitive host is human and intermediate host is mosquito which sucks blood from definitive host.
- The reproductive cycle of *B. malayi* begins when a mosquito vector bites a human host and acquires the microfilarial parasite in its blood meal.
- These parasites lose their sheath and penetrate the gut wall of the mosquito and migrate into thoracic muscles.
- After 10 to 20 days, they molt twice to reach third infective larval stage. When third larval stage is completed, it then migrates to the proboscis of the mosquito.
- When mosquito bites to healthy host, the larvae enter into the human body through abrasion or the wound formed due to mosquito bites.
- The larvae then migrate through the subcutaneous tissue of the host into the lymphatic vessels where they live and grow into adult stage.
- The male and female worms copulate and produce sheathed microfilariae which circulate in the blood stream and allow the intermediate host (mosquito) to acquire the microfilaria repeating the cycle again.
- The reckettsial endosymbiont, *Wolbachia* is also associated among several species of filarial parasites. These nematodes have evolved themselves in such a way that they dependent on the bacteria for a large number of biological processes.

- The microfilariae enter into the human host when infected female mosquitoe bite to human host
- The worm larvae travel through the wound made by the insect into the lymphatic vessel. They grow and molt into fourth-stage larvae, and eventually develop into sexually mature adult worms.