J Parasit Dis (June & December 2009) 33(1&2):3–12

REVIEW ARTICLE

J P D

A review of the complexity of biology of lymphatic filarial parasites

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Received: 17 November 2009 / Accepted: 01 December 2009

Abstract There are about five more common, including *Wuchereria bancrofti* and *Brugia malayi*, and four less common filarial parasites infecting human. Genetic analysis of *W. bancrofti* populations in India showed that two strains of the species are prevalent in the country. The adult filarial parasites are tissue specific in the human host and their embryonic stage, called microfilariae (mf), are found in the blood or skin of the host, depending upon the species of the parasite. Three genetically determined physiological races exist in *W. bancrofti* and *B. malayi*, based on the microfilarial periodicity. They are the nocturnally periodic, nocturnally subperiodic and diurnally subperiodic forms. The susceptibility of a mosquito species to filarial infection depends on various factors, which could be genetic, physiological or physical. Survival analysis of *Culex quinquefasciatus* infected with *W. bancrofti* showed that the parasite load in the mosquito is a risk factor of vector survival. The extrinsic life cycle of the parasite is initiated when the mf are ingested by a mosquito vector during feeding on the host blood. On maturity, most of the infective L3 stage larvae migrate to the head and proboscis of the mosquito to get transmitted to the mammalian host during subsequent feeding. They develop to the adult L5 stage and the period of development and the longevity of the parasites varies according to the species of the nematode and the mammalian host. The rate of production of mf by the adult female was found to be stable at least for a period of five years. The life span of the mf has some influence on the dynamics of transmission of filariasis. Recent studies show that the endosymbiont, *Wolbachia*, plays an important role in the survival of filarial parasites. The possibility of *in vitro* and *in vivo* culture of filarial parasites is also reviewed.

Keywords Lymphatic filariasis, Parasite, Life cycle, Microfilariae, Distinguishing characters, Periodicity, Vector, susceptibility, Endosymbiont, animal models

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Introduction

Nematodes are appendageless, cylindrical, non-segmented invertebrate worms possessing a body cavity and a complete digestive tract with tri-radiate pharynx. The body is covered with a cuticle and has four main longitudinal hypodermal chords. They are bilaterally symmetrical and have well developed nervous, excretory and reproductive systems, but lack specialized respiratory and circulatory systems. Over 15,000 nematode species have been described so far and their size vary from the smallest being 82 μm in length to the largest reaching over 8 meter. Most of the nematodes are marine or fresh water and soil inhabiting, but a few of them parasitize crops, insects, livestock and human.

There are many theories on the origin of nematodes. One of them postulate that they have evolved from arthropods by degeneration and retention of juvenile characters (Poinar 1983). Originally, nematodes were free-living microbotrophic, feeding on fungi. Eventually they found their way into the intestines of both invertebrates and vertebrates. The most successful ones invaded the body tissues of invertebrates and vertebrates (Clark 1994). The best example of this is the filarial worms which invade the short lived arthropod host, such as mosquitoes, for their relatively shorter duration of larval development and transmission

and the longer lived vertebrate hosts for their time taking process of maturation into adult and reproduction.

The first recorded nematodes were human parasites such as round worms and guinea worms. It was Sir Patrick Manson in 1878, made the discovery that mosquitoes transmitted the nematodes, which caused filariasis. Since then, the life cycles of most of the common vertebrate parasitic nematodes were understood and are being elucidated in detail (Anderson 2000).

Taxonomic position of different species

Nematodes have long been considered as a Class under the Phylum Aschelminthes. However, since those characteristics used to show relationships among various classes in the Aschelminthes are now questionable, nematodes are now placed in a separate phylum Nematoda (Inglis 1983). Under this phylum, there are two classes, the Secernentea (Phasmidia) and the Adenophorea (Aphasmidia). Both these classes of Nematoda have several orders with vertebrate parasitic worms (Anderson et al. 1983). Class Secernentea has seven orders, which include Ascaridida, containing the human intestinal ascaris worms, and Spirurida, containing the dracunculid guinea worm and various filarial worms. The human lymphatic filarial parasites, *Wuchereria bancrofti* and *Brugia malayi*, also belongs 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

As in the case of any other nematodes, occurrence of various strains are possible among the filarial nematodes. The recent development in molecular tools could help, up to certain extent, in differentiating strains of one of the major filarial parasite like *W. bancrofti* (Kumar et al. 2002). Genetic analysis of *W. bancrofti* populations in India showed that two strains of the species are prevalent in the country, one in the eastern side and the other on the western side of the Western ghats. A highly significant genetic differentiation was estimated between these two strains (Thangadurai et al. 2006).

Table 1 Filarial nematodes infecting human

Parasite	Geographical distribution	Adult dwelling place in the body	Mf presence	Vector Involved	Disease symptoms
Wuchereria bancrofti	Africa, Pacifica, Asia, Americas	Lymphatics	Blood	*Cx. quinquefasciatus, Anopheles spp., Aedes spp.*	Lymphangitis Elephantiasis
Brugia malayi	Asia	Lymphatics	Blood	*Mansonia spp.*	Lymphangitis, Elephantiasis
Brugia timori	Indonesia	Lymphatics	Blood	*An. Barbirostris*	Lymphangitis, Elephantiasis
Onchocerca volvulus	Africa, Central & South America, Yemen	Skin nodules, eye	Skin	*Simulium spp.*	Dermatitis, ocular lesions, blindness
Loa loa	Africa	Subcutaneous tisues	Blood	*Tabanus spp.*	Calabar swellings, eye irritations
*Mansonella ozzardi**	Central & South America	Mesentery of abdominal wall	Blood, skin	*Culicoides spp.*	Lymph node swelling, joint pain
*Mansonella perstans**	Africa, America	Perirenal tissues, pleural cavity, pericardium	Blood	*Culicoides spp.*	Skin itch, joint pain
*Mansonella streptocerca**	Africa	Subcutaneous tissues	Blood skin	*Culicoides spp.*	Skin itch, joint pain
*Brugia pahangi**	Indonesia	Lymphatics	Blood	*Aedes spp.*	Lymphangitis, Elephantiasis

*Less common unnatural infections transmitted from animals (Zoonotic)

Table 2 Identification of *W. bancrofti, B. malayi* and *B. timori* mf based on measurements of body characters

Body characters	*Wuchereria bancrofti*	*Brugia malayi*	*Brugia 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
Nerve ring	49.39 μm	48.30 μm	63.80 μm
Excretory pore	93.00 μm	67.00 μm	84.40 μm
Innenkorper	33.05 μm	30.70 μm	60.00 μm
Anal pore	253.40 μm	181.60 μm	238.00 μm

Human filarial parasites and their distinguishing characters

There are about five more common and four less common filarial parasites infecting human (Table 1). The adults of these parasites are tissue specific in the human host and their embryonic stage, called mf, are found in the blood or skin of the host, depending upon the species of the parasite. Human lymphatic filariasis is caused by the filarial parasites, *W. bancrofti* (Cobbold 1877), *B. malayi* (Lichtenstein 1927) and *Brugia timori* (David and Edeson 1965). The adults of these parasites live in the lymphatic system, especially the lymphatic vessels and the nodes, and the mf, which are sheathed, are found in the blood. *W. bancrofti* is prevalent in tropical Africa, South East Asia, Papua New Guinea, Philippines, Candelonia and Thailand. *B. malayi* is spread over South and South East Asia. *B. timori* is localized in Lesser Sunda Islands of Eastern Indonesia (WHO 1992).

Since *W. bancrofti* and *B. malayi* co-exist in many places (Raina et al. 1990; Rajendran et al. 1997), their identification to species level is very important in diagnosis as well as epidemiological surveys. These species could be differentiated based on the measurements of various body characters of the mf (Table 2).

The infective L3 stage of these species, by dissecting out from mosquito vectors, can be differentiated by examining the caudal papillae (Fig. 1). There are three caudal papillae, two lateral and one terminal. All the three caudal papillae are distinctly protruding in *W. bancrofti* as compared to *B. malayi*. The lateral papillae of *B. malayi*, under electron microscopy, show a gutter-like indentation around their bases and this character is absent in *W. bancrofti* (Zaman and Narayanan 1986). Apart from these, mf of various species could be distinguished by examining the morphological features (Fig. 2). 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 (Fig. 3).

Different strains based on periodicity of microfilariae

Three genetically determined physiological races exist in *W. bancrofti* and *B. malayi*, depending on the microfilarial periodicity (Sasa and Tanaka 1974; Tanaka 1981; Gupta et al. 1990; Tewari et al. 1995; Weerasooriya et al. 1998; Shriram et al. 2002; Pichon and Treuil 2004). They are the nocturnally periodic, nocturnally subperiodic and diurnally subperiodic forms (Table 3). In the Indian sub-continent, both the parasites exist as nocturnally periodic forms and the mf appear in the peripheral circulation at night. Diurnally subperiodic strain of *W. bancrofti* occurs in many islands in South Pacific, French Polynesia, Thailand and Nicobar island. The mf remain in the peripheral blood all the time with a peak density at 12–20 hours. The zoonotic *B. malayi* prevalent in Indonesia and Malaysia is nocturnally

Table 3 Different strains of filarial parasites based on periodicity of microfilariae

Periodic form of filarial parasite	Geographic distribution
Nocturnally periodic *W. bancrofti*	Asia, Africa, Malaysia, Philippines, PNG
Nocturnally sub-periodic *W. bancrofti*	Thailand
Diurnally sub-periodic *W. bancrofti*	South Pacific, Andaman & Nicobar Islands
Nocturnally periodic *B. malayi*	India, China, Malaysia, Indonesia, Philippines, Japan, Korea
Nocturnally sub-periodic *B. malayi*	Malaysia, Indonesia, Philippines

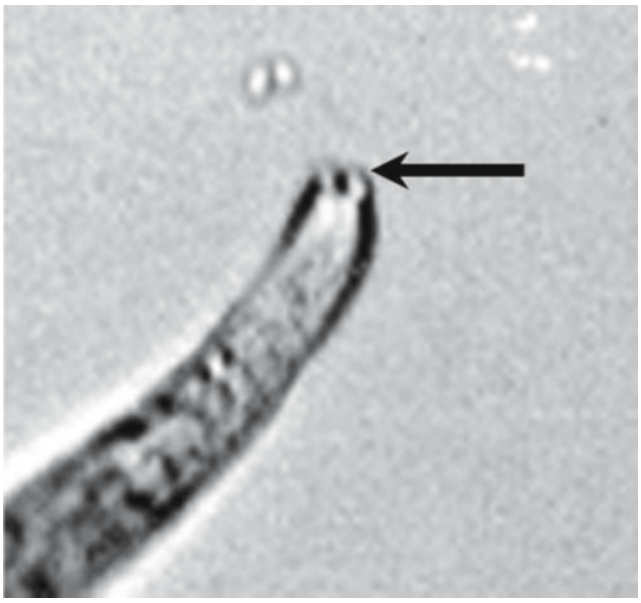

Fig. 1 Caudal papillae of L3 of *W. bancrofti*

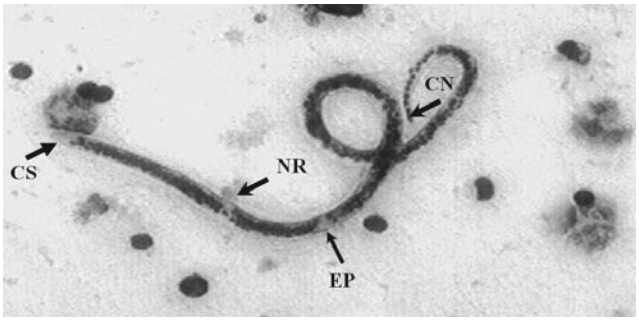

Fig. 2 Mf of W. bancrofti showing cephalic space (CS), nerve ring (NR), Excretory pore (EP), and caudal nucleus (CN)

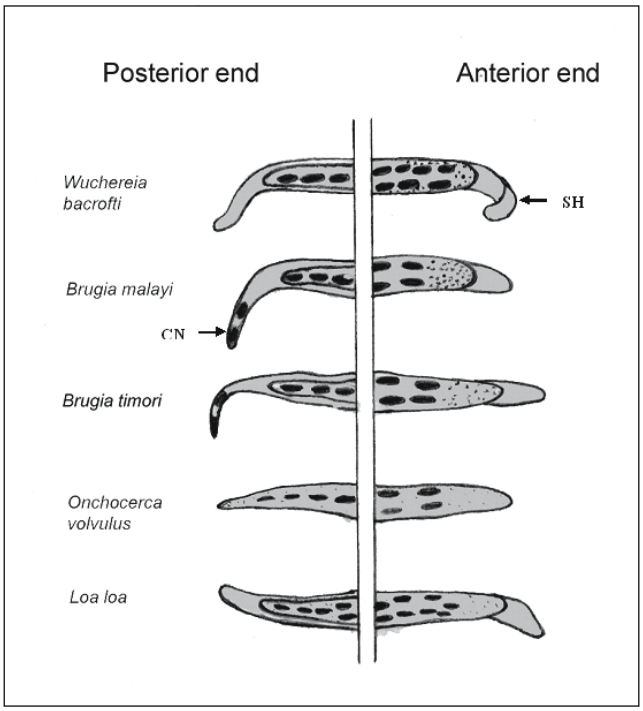

Fig. 3 Differentiation of species of microfilariae on the basis of presence or absence of caudal nuclei (CN), and the presence or absence of a sheath (SH)

subperiodic and the mf is present in the peripheral blood at all times, with a slight nocturnal rise. *B. timori* present in the Timor island is nocturnally periodic.

Various hypothesis have been postulated on the mechanism of microfilarial periodicity. As early as in 1951, Hawking and Thurston demonstrated that periodic fluctuation in the number of mf was due to their accumulation in the lungs during day and release to the circulating blood at night. Stimuli like body temperature are shown to have effect on the periodicity of mf of *W. bancrofti*, *B. malayi*, *B. ceylonensis* and *Dirofilaria repens* (Hawking et al. 1966). Later, it was postulated that accumulation in lungs is due to greater oxygen tension in the lungs during the day compared to that in the night when the host is under rest (Hawking and Clark 1967; Hawking et al. 1981). However, why and how mf remain in lungs during the day and the exact mechanism of mf periodicity as such is not yet fully understood. The periodicity of mf seems to be oriented to the 24 hours cycle of the host in which the circadian rhythm of the host could act as a cue to the control of the circadian rhythm of the mf. Whatever be the reason, the mf periodicity is in agreement with the feeding behavior of the vector mosquito (Vanamail and Ramaiah 1991; Weerasooriya et al. 1998) enabling the mosquito to ingest the mf in large numbers by presenting themselves in large numbers in the peripheral blood during the peak biting time of the mosquito. However, the mf need not be absent from the peripheral blood during the rest of the period unless there are some physiological factors which govern their appearance and disappearance. For example, there are several animal filariae such as the *Litomosoides carinii* and *Dipetalonema viteae*, which do not show any orientation to host circadian rhythm or vector feeding rhythm through mf periodicity (Reddy et al. 1984), and still have successful transmission.

Mosquito susceptibility and host range of different species/strains of filarial parasites

Many of the Anopheline, Mansonoid, Culex and Aedes mosquitoes have been incriminated as vectors of human

lymphatic filariasis. *C. quinquefasciatus*, *C. pipiens molestus*, *C. pipiens pipiens*, *Anopheles sinensis*, *A. gambiae*, *A. melas*, *A. merus* and *A. maculates* are natural vectors of periodic *W. bancrofti*. Subperiodic *W. bancrofti* is transmitted by *Ochlerotatus niveus*, *A. oceanicus*, *Aedes polynesiensis* and *A. pseudoscutellaris*. In the case of *B. malayi* periodic form, *Mansonia annulifera*, *M. uniformis*, *A. nigerrimus*, *A. umbroses*, *A. barbirostris* and *A. melas* have been incriminated as natural vectors. *M. annulata*, *M. bonneae* and *M. dives* are the known vectors of *B. malayi* subperiodic form. *A. barbirostris* is the vector of *B. timori* (WHO 1989). In India, the vector of *W. bancrofti* is *C. quinquefasciatus* (Das 1976; Rajagopalan et al. 1977; Rajagopalan and Das 1987). Even when different species of mosquitoes were fed artificially on heparinized microfilaraemic blood, *C. quinquefasciatus* showed the highest percentage of infection by *W. bancrofti* (Paily et al. 2006). The vectors of *B. malayi* are *M. annulifera*, *M. uniformis* and *M. indiana* (Sabesan et al. 1991). The subperiodic *W. bancrofti* prevalent in the Andaman and Nicobar islands of India is transmitted by *Ochlerotatus niveus* (Tewari et al. 1995).

The susceptibility of a mosquito species to filarial infection depends on various factors, which could be genetic, physiological or physical. Each step in the penetration process of mf in the mosquito host requires particular mechanism and the failure of any one would reduce invasiveness. The mf entering the mosquito gut through the blood meal has to overcome a series of barriers known as the gut barriers. First of these are the cibarial and pharyngeal armatures, which in some species of mosquitoes are well-built and might damage the sheath of mf (Coluzzi and Trabucchi 1968; Bryan et al. 1974). The speed of clotting of blood in the mosquito mid-gut varies from species to species and the mf might get trapped within the abdomen of the mosquito if the clotting occurs before the migration of mf to the thorax (Kartman 1953; Ewert 1965). The peritrophic membrane of certain species of mosquitoes also limit the penetration of mf through the gut wall (Esslinger 1962). The mid-gut wall of mosquitoes too provides a potential barrier to mf invasion because before penetration, the mf has to exsheath in the mid-gut of the mosquito host. It has been reported that, following exsheathment, mf of *Brugia* spp. rupture the mid-gut epithelium by means of cephalic hook, tearing the luminal surface and the basement lamina (Esslinger 1962). In anophelines, like *A. gambiae*, *A. arabiensis*, *A. melas* and *A. funestus*, the mf get damaged by the mosquito fore-gut armatures (Bryan and Southgate 1988). Limitation, facilitation and proportionality are the three types of relations

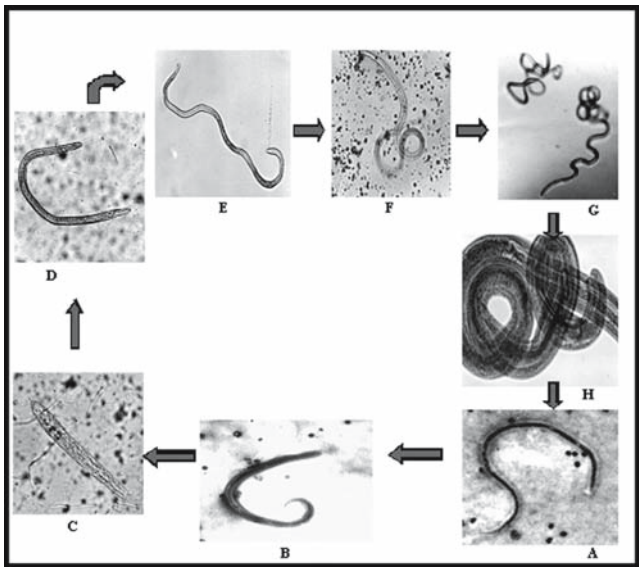

Fig. 4 Life-cycle of the lymphatic filarial parasite, Wuchereria bacrofti. A- microfilaria in human blood; B- microfilaria in mosquito vector; C- first stage larva (L1) in mosquito vector; D- second stage larva (L2) in mosquito vector; E- third or infective stage larva (L3) in mosquito vector; F- third or infective stage larva (L3) in human host; G- fourth stage larva (L4) in human host; H- adult worm in human host.i

observed in human filaria/mosquito couples depending upon the species of the anopheline that ingests the mf (Southgate and Bryan 1992). Survival analysis of *C. quinquefasciatus* infected with *W. bancrofti* showed that the parasite load in the mosquito is a risk factor of vector survival (Krishnamoorthy et al. 2004). Feeding of *C. quinquefasciatus* on *W. bancrofti* mf positive blood for a second time did not increase the burden of infective stage in the mosquitoes, above that found after a single feeding, indicating the operation of some sort of regulatory mechanism of the parasite burden due to their pre-exposure to the parasite infection (Paily et al. 1995a). This regulation could be due to the immune response of the pre-exposed mosquitoes as there are reports of visible changes in the hemocyte populations of *W. bancrofti* infected *C. quinquefasciatus* that lead to the production or up-regulation of immune molecules (Paily et al. 2005b; 2007).

Life cycle of lymphatic filarial parasites

Filarial parasites require two different host systems to complete its life cycle (Fig. 4). The definitive host is the man or some other vertebrate animal, depending upon the species of the infecting parasite, and the intermediate host is a blood sucking arthropod such as mosquitoes. Adults of human lymphatic filarial nematodes live in the lymphatic system of

man and are viviparous. Adult male and female mate and the gravid females release embryonic stages called mf into the lymphatic system, which migrate to the blood and circulate. The extrinsic life cycle of the parasite is initiated when the mf are ingested by a mosquito vector during feeding on the host blood. The mf in the mid-gut of the mosquito vector migrate through the gut wall to the hemocoel and subsequently to the thoracic muscles, within about 24 hours. Exsheathment of mf takes place both in the mid-gut as well as in the haemocoel of the mosquito host. Those mf migrating immediately after ingestion will exsheath in the hemocoel and those remain in the mid-gut for more than 2 hours cast their sheath in the mid-gut itself, and then migrate (Chen and Shih 1988). In thoracic muscles, they become shorter and thicker to develop into the sluggish first stage larvae (L1). At about 5th–7th day, the L1 moults to become the second stage (L2), which is more active, and finally by 10th–11th day they moults to become the infective stage larvae (L3). This stage is very active showing oscillatory pattern of movement between the head, thorax and abdomen of the mosquito. On maturity, most of the L3 migrates to the head and proboscis of the mosquito to get transmitted to the mammalian host during the subsequent feeding.

The adult male of *W. bancrofti* has a length of 23.8–30.6 mm and width of 90–120 µm. The length and width of adult female is respectively, 42.2–46.3 mm and 160–188 µm. Mf is of 309–346.8 µm in length and 5.3 µm in width. The L3 is of the size of 1.2–1.6 mm in length and 18–23 µm in width. The L3 can remain alive and active for about 46–50 days, or as long as the mosquito survives (Paily et al. 1995c). When the mosquito feeds on the host, L3 are deposited on the skin surface and after withdrawal of the proboscis, they get into the wound and travel to the efferent lymphatics and sub-capsular sinus. Approximately 9–10 days after entry, the L3 moults to become the fourth stage larva (L4). The L4 stage undergoes developmental process over several days to months, depending upon the species, to undergo the final moult and become adult (L5). The period of development and the longevity of the parasites vary according to the species of the nematode and the mammalian host (WHO 1984). Estimation based on deterministic model showed that the life span of *W. bancrofti* adult female is 10.2 years. The rate of production of mf by the adult female was found to be stable at least for a period of 5 years (Vanamail et al. 1990). In a 10-year observation on experimental infection of periodic *B. malayi* in man, microfilariae were first detected at 41 and 46 weeks after inoculation in two subjects and remained detectable until 8–8.5 years after infection indicating an adult reproductive period of 8–9 years in the human body (Wang et al. 1994).

Mf longevity and virulence

The life span of the mf has some influence on the dynamics of transmission of filariasis (Narasimham et al. 1984). In order to study the longevity of mf in the human host, they have transfused *W. bancrofti* microfilaraemic human blood to amicrofilaraemic individuals. The number of mf that was transfused did not correlate with the subsequent counts in the recipients. However, the duration of microfilaremia in the recipients was found to vary from 42 to 133 days. In an earlier study on the longevity of mf of *W. bancrofti*, Carme and Laigret (1979) showed that people could remain carriers of mf for many years without re-infection and the mf have a longevity of 4–6 months. However, in the absence of immunological studies on the recipients before and after transfusion, no explanation could be offered for the variations in the mf counts as well as their longevity. As such, no information is available on the exact life span of mf or more importantly, their age-dependent viability in the human host. Information on viability or virulence is more important as it is a matter of the ability of the aged mf to develop to the infective stage in a suitable mosquito host, rather than just being motile (Goonerathnam and Sen 1970; Obiamiwe and Mac Donald 1971).

Endosymbiont of filarial nematodes

The reckettsial endosymbiont, *Wolbachia*, have been found to be widespread among filarial parasites of human as well as animals. As many as 10 filarial nematode species are known to harbour *Wolbachia* (Bandi et al. 1998; Genchi et al. 1998; Henkle-Duhrsen et al. 1998). Electron microscopic observations as well as PCR amplification and sequencing have shown that *W. bancrofti*, *L. sigmodontis*, *Mansonella ozzardi, O. volvulus, O. ochengi* and all the species examined in the genera *Dirofilaria*, *Onchocerca* and *Brugia* harbor *Wolbachia* (Sironi et al. 1995; Bandi et al. 1998; Casiraghi 2001). Immunohistochemical staining studies (Kozek 1977; Henkle-Duhrsen et al. 1998; Hoerauf et al. 1999) have shown that *Wolbachia* occur intracellular throughout the syncytial hypodermal lateral cord cells of both male and female parasites. Inside the cells their location is membrane-bound vacuoles derived from the host, as also in the cytoplasm in some cases. Among the developmental stages of filarial parasites, their presence has been found in mf, second, third

and fourth stage larvae (Kozek 1977; McGarry et al. 2003). Opinion regarding the relationship between the intracellular *Wolbachia* and filarial nematodes as mutualistic and necessary for the normal growth, development, and fertility of filarial worms is building up but still is debatable (Rao et al. 2002; Fenn and Blaxter 2004).

In any case, evidences available so far indicate that at least those filarial nematodes which harbor *Wolbachia* require them for their survival. The long-term co-evolution, which results in the co-adaptation and reciprocal dependence (Yamamura 1993) and 100% prevalence in the infected species provide additional support for the obligate symbiosis. As Bandi et al. (1998) reported, presence of *Wolbachia* within filarial parasites is fixed and the nematodes have evolved to become dependent on the bacteria for a diverse range of biological processes.

In vitro culture of filarial parasites

Filarial parasites cultured *in vitro* are useful for immunological studies, drug testing, etc. With respect to immunology, it could be a very good source of excretory, secretory and moulting antigens of worms, free of host immunological factors. Similarly, stage specific drug effect and mode of action of the drug on the worms could be determined, if they are cultured *in vitro*. Some species of animal filarial parasites, such as *Brugia pahangi* and *Dipetalonema vitae* have been cultivated successfully from third to fifth stage (Franke and Weinstein 1983; Mak et al. 1983). With respect to human lymphatic filarial parasites, a relatively recent report of *in vitro* cultivation of *B. malayi* subperiodic strain to sexually mature adult stages is a significant development (Riberu et al. 1990). With *W. bancrofti*, repeated attempts of *in vitro* culture could achieve only moulting of the L3 and limited growth of L4 (Franke et al. 1987, 1990; Hoti et al. 1994). *In vitro* development of this parasite to the adult stage is yet to be achieved.

Natural and experimental infection on animals

Since Poynton and Hodgkin (1939) first described *B. malayi* like mf from *Macaca fascicularis*, prevalence of *B. malayi* subperiodic infections on wild and domestic animals and their experimental transmission to man has been sufficiently demonstrated (Edeson and Buckley 1955; Edeson and Wharton 1957; Laing et al. 1960) and strengthened the belief that zoonotic infections of this strain can occur in nature. However, only the domestic cats and non-human primates are considered as important animal reservoirs of *B. malayi* subperiodic strain. Despite widespread and occasionally intensive blood surveys on a wide variety of domestic and peridomestic mammals, *W. bancrofti* has so far been found only in man.

With respect to the experimental infection of *B. malayi* subperiodic strain, the *Mongolian gerbil*, *Meriones unguiculatus*, and multimammate rat, *Mastomys coucha* are good animal models (Ash and Riley 1970; Patranyi and Mieth 1974; Sanger et al. 1981). After inoculation of infective larvae, the third moult occurred within 7–8 days and the final one by 29–35 days. The prepatent period in gerbil was 93 days as against 107 days in mastomys rat. Although these animal models are known to show patent infection of the parasite for periods beyond 6 months, they are in no way comparable to that of human lymphatic filariasis, as most of the worms are localized in different organs of the animals as against the filarial worm localization in the lymphatic system of human (Murthy et al. 1983; Athisaya Mary et al. 2006).

Various non-primate animals have been inoculated experimentally to find out an animal model for *W. bancrofti*, but were unsuccessful in getting the parasite development, except for partial development of the parasite to L4 in mongolian gerbil (Paily et al. 1999). However, limited number of parasites could develop to gravid females or adults in non-human primates such *Macaca cyclopis*, *Macaca fascicularis* and *Presbytis cristatus* (Cross et al. 1979; Dissanaike and Mak 1980). More recently, the Indian leaf monkey, *Presbytis entellus*, was found to develop the nocturnally periodic *W. bancrofti* leading to patent infection and the prepatent period varied between 195 and 240 days. Adult worm recovery was only 5–13% and the mf level was very low (Misra et al. 1997). Hence, it is proved that, though it is a poor model, the only available animal model for *W. bancrofti* is leaf-monkeys. However, the availability of this monkey species as well as the difficulties involved in working with a non-human primate makes it unsuitable for experimental infection of *W. bancrofti*.

Conclusions

With respect to the biology of human lymphatic filarial parasites, it is complex and several aspects are there to be studied. For example, complete information is lacking on aspects such as the longevity of the parasites and their stages in the mammalian host system, host parasite interaction, vector regulation of the parasite development,

etc. Similarly, successful animal models, which mimic the human diseases manifestation, as well as *in vitro* culture methods are also lacking. These information and facilities may help parasitological and drug screening works, as also genomic and molecular networking studies.

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