Introduction
Introduction:

Lymphatic filariasis (LF) is a parasitic disease caused by mosquito-borne filarial worms of the species *Wuchereria bancrofti*, *Brugia malayi* or *B. timori*. They inhabit in the lymphatic’s or lymph nodes of the human host. These tissue dwelling worms belong to the phylum Nematoda, class Secernentea, order Spirurida and family Filaridae (WHO, 1997a). Infection of the human host occurs when third-stage larvae (L₃) of the parasite enter the body following the bite of an infected vector mosquito. These infectious larvae mature over a period of months into lymphatic-dwelling adult worms that later release immature larvae called microfilariae into the host’s bloodstream. Lymphangitis, hydrocele, and elephantiasis are the pathological symptoms caused by *W. bancrofti* and *Brugia* spp. Bancroftian filariasis caused by *W. bancrofti* is responsible for 90% of lymphatic filariasis cases throughout the tropics and in some sub-tropical areas. On the other hand, *Brugia malayi* is confined to Southeast and Eastern Asia and *Brugia timori* is found only in Timor and its adjacent islands (McMahon and Simonsen, 1996; Roberts and Janovy, 1996). The disease is spread worldwide and 120 million people (about one fifth of world’s population) are estimated to harbour lymphatic filarial infections. Adult *W. bancrofti* is restricted to man, while *Brugia malayi* and *B. timori* are found in all classes of vertebrates including domestic and wild animals except fish (Roberts and Janovy, 1996). Transmission of *W. bancrofti* occurs by mosquito vectors *Culex*, *Anopheles* and *Aedes* spp., while *B. malayi* and *B. timori* are predominantly transmitted by *Mansonia* spp. Clinical manifestations of lymphatic filariasis are almost exclusively due to adult worms. The symptoms primarily include filarial fever and lymphangitis which later gives rise to recurring lymphoedema (Palumbo, 2008) and ultimately culminating into elephantiasis. High adult worm load, and consequently high microfilarial density, favours the development of lymphangitis and elephantiasis. In year 1998, Global Alliance to Eliminate Lymphatic Filariasis (GELF), a partnership and control program of WHO, was created to raise political, financial, and technical support for LF which includes distribution of anti-filarial medications (albendazole and ivermectin or albendazole and diethylcarbamizine) and its elimination as a public health problem by the year 2020 (WHO, 2000; Ottesen, 2000; Das et al., 2002). This treatment restores filaria antigen-specific immune responsiveness which is usually down regulated during infection (Tish et al, 2005). In addition, the medication also kills microfilariae, the sheathed offspring produced by adult female worms. This is vital in preventing transmission, which is dependent on the ingestion of microfilariae by mosquito vectors during feeding. The chemotherapy has been successful
in some areas but minimal in others because of compliance issues in endemic communities (Kazura, 2002).

Recent completion of \textit{B. malayi} genome revealed that its nuclear genome is of 80 to 100 megabase (Mb) which is organized into five chromosomes including an XY sex determination pair. The sequencing of \textit{B. malayi} nuclear genome was performed by whole-genome shotgun (WGS) sequencing approach (Elodie et al, 2007). The genome size of closely related free-living nematode \textit{Caenorhabditis elegans} genome is 100 Mb and the \textit{C. briggsae} genome is 104 Mb which is almost similar to \textit{B. malayi}. The overall G + C content of \textit{B. malayi} (30.5%) are lower than of \textit{C. elegans} (35.4%) or \textit{C. briggsae} (37.4%). \textit{B. malayi} has about 14,500-17,800 protein-coding genes which is lower than the 19,762 (WormBase data release WS133) and 19,507 genes reported for \textit{C. elegans} and \textit{C. briggsae}, respectively, which suggests that parasitic nematode genomes have fewer genes than their free-living counterparts, echoing a pattern observed in bacterial pathogens. Operons are a common form of gene organization in bacteria and some protozoa, but in Metazoa, operons have been identified only in nematodes, platyhelminths, and urochordates. About 838 potential operons,

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Rhythm</th>
<th>Reservoir</th>
<th>Main vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{W. bancrofti}</td>
<td>Periodic</td>
<td>Humans</td>
<td>\textit{Culex, Anopheles}</td>
</tr>
<tr>
<td>\textit{W. bancrofti}</td>
<td>Subperiodic</td>
<td>Humans</td>
<td>\textit{Aedes}</td>
</tr>
<tr>
<td>\textit{B. malayi}</td>
<td>Periodic</td>
<td>Humans</td>
<td>\textit{Anopheles}</td>
</tr>
<tr>
<td>\textit{B. malayi}</td>
<td>Subperiodic</td>
<td>Humans, monkeys, cats</td>
<td>\textit{Mansonha}</td>
</tr>
<tr>
<td>\textit{B. timori}</td>
<td>Periodic</td>
<td>Humans</td>
<td>\textit{Anopheles}</td>
</tr>
</tbody>
</table>

\textbf{Lymphatic Filarial parasite species, periodicity and their vectors}

containing ~1800 genes (16% of the total; 2 to 5 genes per operon), were found in the assembled genome of \textit{B. malayi}. The completion of genome project of \textit{B. malayi} has generated sequences for tens of thousands of genes. Many of these genes have proved to be hard to annotate with functional descriptors, because they have limited similarity to genes from well-studied organisms. Even when genes can be annotated robustly through homology, it has been difficult to prove essential function in living parasites. A new reverse genetic technology called ‘RNA interference’ based on the discovery that double stranded RNA (dsRNA) molecules derived from the same sequence as a targeted mRNA can result in specific and significant enzymatic degradation of the mRNA, has the promise of overcoming
Introduction

the current impasse in functional analysis of unknown genes. Effective RNAi by soaking worms in double stranded RNA (dsRNA) has been demonstrated in B. malayi adults (Aboobaker et al, 2003) along with other parasitic nematodes. However, genome sequencing data revealed that some genes necessary for systemic RNAi in C. elegans were found to be absent from B. malayi, including sid-1, a membrane channel that transfers dsRNA molecule from a source cell to neighbouring cells; sid-2; sid-3; and rsd-6. The presence of a putative drsh-1 ortholog suggests that B. malayi is also capable of microRNA processing. The effectiveness of RNAi in B. malayi implies that either these genes are rapidly evolving or are not required in B. malayi or that alternate pathways for siRNA transfer exist. Improvement of RNAi protocols for filarial nematodes would offer an attractive testing platform for verifying candidate drug targets.

Despite of plethora of research and development going on around the world covering various facets of filarial parasite including genomics, proteomics and immunology, yet there is no absolute cure for this morbid disease.

Memoirs of Lymphatic Filariasis in human

Lymphatic filariasis or “Elephantiasis” has been known since times of yore (Sasa, 1976; Grove, 1990) and is recorded in the primal medical literature and art of China, Egypt, India, Japan and Persia (Neisius, 1927; Lawrence, 1967; Hoeppli, 1969; Zimmer, 1979; Price, 1989; Lawrence, 1990; Routh and Bhowmik, 1993). Indian mythology also contains the fable of “curse of St. Thomas” which states that swollen legs occur in the descendants of those who martyred him (Bowers and Carruba, 1970; Lawrence, 1970). Antediluvian Indian physicians described this disease by the name of “Sheelpad” which is synonymous to elephantiasis; shila means rock and pada means foot (foot heavy like rock) and chyluria by name of “Shuklameha”. The parasite W. bancrofti was discovered in 1862 by Jean-Nicholas Demarquay and Otto Wucherer, in hydrocele fluid and chylous urine (Demarquay, 1863; Wucherer, 1868) and their microfilariae were discovered in blood by Lewis in 1872. In 1876, Joseph Bancroft, physician and a parasitologist, discovered a female adult worm in an abscess on the arm of a butcher (Cobbold, 1877) and later Sibthorpe and Bourne discovered the adult male (Bourne, 1888; Sibthorpe, 1889). The name Wuchereria bancrofti was formally adopted in 1921 (Seurat, 1921). Microfilaria which was morphologically different from W. bancrofti was identified by Lichtenstein and Brug (Lichtenstein, 1927; Brug, 1928; Brug and de Rook, 1930) and a new genus was proposed for these worms and they were named Brugia malayi (Buckley, 1958; 1960). Edeson and Wharton, (1958) and Laing et al,
(1960) broadened the range of vertebrate host to include other monkey species, domestic cats and dogs. Partono infected Mongolian gerbils with the new species and obtained adult worms and confirmed that the new species belonged to the genus *Brugia* and called it *B. timori* (Partono et al., 1977). On August the 10th, 1887 Patrick Manson fed some mosquitoes on the blood of his microfilaraemic gardener Hin-Lo and was able to demonstrate the development of the larva within the insect (Manson, 1878a, 1878b, 1884), which was later confirmed by Lewis. The mosquitoes used by Manson in his initial experiment were later found to *Culex quiquefasciatis* (Manson-Bahr, 1961), which is still regarded as one of the most important vectors of filariasis. By 1976 other species of *Culex* and species of *Anopheles, Aedes* and *Mansonia* had been added and the list had grown to several hundred (Sasa, 1976; Grove, 1990).

**Geographical Distribution of Lymphatic Filariasis**

The geographic distribution of various types of human filariasis is accounted for primarily by the existence of the obligate arthropod vector in sufficient numbers to maintain the parasite life cycle in the ecosystem. Improved sanitation conditions in developed countries of Europe and North America have reduced breeding sites for mosquitoes to a level which no longer sustains transmission of human lymphatic filaria. Conversely poor sanitation, increasing urbanization and a requirement of rural population in arid regions to live near rivers have fostered the spread of lymphatic filariasis and onchocerciasis in many developing countries (Hunter, 1992; Albuquer et al., 1995; WHO, 1998).

There have been several attempts to develop disease maps for lymphatic filariasis (Sasa, 1976, WHO, 1984, 1992), however, the major focus of this work has been restricted either to mapping the extent of the geographical spread of the infections, or in limited cases, to displaying spatial variation in infection prevalence at local geographical scales. Michael et al., (1996) after aggregating and projecting, prevalence data from over 120 published papers, and documents held by the WHO, to the national level, suggested that filariasis infects 120 million people or 2.0% of the world’s population. Modern surveys using antigen detection have shown that surveys of microfilaraemia alone can under-estimate the prevalence of filariasis by as much as 30% (Turner et al., 1993; Chanteau et al., 1995). According to latest reports it is estimated that 1.1 billion people (20% of the world population) in more than 83 countries are at risk of acquiring the infection, while more than 129 million individuals have already been infected (Keiser & Nutman 2002; Molyneux et al., 2003). Of these, 83 million
people have lymphatic disability, 23 million men have hydrocoeles and 15 million people have elephantiasis. Millions more suffer from debilitating acute attacks of filarial fevers and lymphadenitis (Ramchandran, 1997). Almost half of the 129 million estimated LF cases are in the South East Asia Region and another 34.1% of cases are in the African region; the rest are in the Western Pacific (16.1%), Eastern Mediterranean (0.3%) and America (0.3%). They include 83.63 million cases of microfilaria carriers, 16.02 million cases of lymphoedema, and 26.79 million cases of hydrocoeles (WHO, 2005).

**Distribution in India**

In India lymphatic filariasis is second only to malaria as the most important vector borne disease. The disease is endemic in 22 states and Union territories. Bancroftian filariasis in India is mostly found in Andhra Pradesh, Orissa, Assam, Madhya Pradesh, Maharashtra, West Bengal, Uttar Pradesh, Tamilnadu and Bihar contributing to 95% of total filariasis burden, whereas brugian filariasis is found mostly in Kerala (Reddy et al., 2000; Ramaiah et al., 2000a; Das et al., 2002). An estimated 450 million people living in 257 districts across 18 states and Union territories are at risk from infection. However, three relatively less-developed states (Uttar Pradesh, Bihar and Andhra Pradesh) alone account for 52% of the endemic population and 62% of the infected population (Das et al., 2001). Currently there may be up to 27.09 million people carrying microfilariae in their blood, 20.83 million cases of symptomatic filariasis, and about 429.32 million individuals (Figure 1) staying in filaria endemic region without showing any symptoms of the disease or any parasite and thus are potentially at risk of infection in the country. Probability mapping, based on data quantities, clearly indicates that the risk of filarial infection in India is not constant throughout the country but exhibits strong regional trends (Sabesan et al., 2000).

**Life-cycle of Brugia malayi**

Lymphatic filarial parasites *W. bancrofti* and *B. malayi* have a complicated life-cycle that requires two hosts, human and mosquito for transmission and propagation of infection. Human is a definitive host since it facilitates reproductive phase of the parasite while mosquitoes are the transitional host providing optimal milieu for advancement of mf into L3 stage (Figure 2). The infection commences when human host comes in contact of mosquito containing 1.2-1.6 mm long infective larvae and are bitten repeatedly. With the mosquito bite the infective larvae through the labium of mosquito creeps onto the skin of human host. If the parasite finds a portal of entry (e.g. the bite wound), it enters and is transported to human
lymphatic’s and lymph nodes where the adult male and female worms attain sexual maturity, mate and begin to produce tiny first stage larvae called ‘microfilariae’ (mf). Mf is 250 to 300 μm long and 8 μm wide and is surrounded by an egg membrane (sheath). Millions of mf, produced by viviparous reproduction, is passed into the lymphatic system which migrates to the peripheral blood and demonstrates either nocturnal or diurnal periodicity, coinciding with peak feeding times of the mosquito vector (Moulaia-Pelat et al., 1993; Nutman, 2000). In the mosquito, the microfilariae develop within 7-21 days into the filariform stage of the parasite known as infective larvae (L3) after undergoing two moults. The mf leaves the mid-gut to reach the hemocoel and then migrate into the flight muscles of the mosquito where they moult into L2 and thereafter L3. The mature L3 measuring around 1.2-1.6 mm migrates to the proboscis of the mosquito ready to infect a new host during next mosquito bite. Mosquitoes deposit infective the larvae on the host skin adjacent to the puncture site while feeding and the L3 migrate through the subcutaneous tissue to the nearest lymphatic vessel from where they reach the afferent vessel of the lymph node and undergo maturation by moulting first to fourth stage larvae at around 7 days post infection (p.i.) and then to L5 (pre-adult stage) reaching adulthood at around 30 days p.i. In adolescent and adult men, there is a preference for the lymphatics of the spermatic cord. In bancroftian filariasis, the time from infection to appearance of microfilariae is 6-12 months while in brugian filariasis it may be only 3½ months.

**Spectrum of Lymphatic Filariasis**

Infection with L3 is usually followed by a period of vigorous immune responses to the invading larvae. Failure of the immunity in clearing the invading larvae during this phase may lead to the development of various pathologies associated with filarial infection. The most common is hydrocele, with lymphoedema leading to enlargement of legs, arms, breast and the genitals. Other forms of the disease such as tropical pulmonary eosinophilia and chyluria occur less frequently. Hydrocele is not seen in areas affected by brugian filariasis. In filariasis the host tissue inflammation is substantially more complicated process than simple blockade of lymphatic vessels or the circumscribed reaction to dying intra-lymphatic adult worms (Freedman, 2002). The death of the adult worm triggers an acute inflammatory response, which progresses distally (retrograde) along the affected lymphatic vessel, usually in the limbs. The clinical manifestations can be broadly be due to two reasons, one caused due to adult worms and other is ‘occult filariasis’ due to hyper-responsiveness of the host against microfilariae. Lymphatic filariasis is typically spread over a wide spectrum, ranging
from individuals who appear to be free from disease, through asymptomatic microfilaria carriers, to patients with varying degree of clinical manifestations. Endemic normals are 'amicrofilaraemic' individuals under constant exposure to filariasis. They have circulating anti-filarial antibodies in their blood but show no clinical evidence of disease (Ottesen, 1989; Weil et al., 1996). They are negative for circulating filarial adult (CFA) parasite antigen when measured by Og4C3 ELISA (More and Copeman, 1990; Malla et al., 2007) and low parasite specific IgG4 titres. The asymptomatic microfilaraemic individuals acts as parasite reservoir and hence also termed as 'carriers'. Subclinical lymphatic dilatation and dysfunction are the common clinical manifestations of this group of individuals. They also have very high parasite specific IgG4 titres. The immune response to parasite antigens in these individuals appears to be suppressed and hence regarded as hyporesponsives (Nutman et al., 1987a). Acute bancroftian filariasis is primarily associated with lymphadenitis in the groin or armpit and a typical retrograde lymphangitis. The acute attack may last for 3-15 days, and may occur several times (5-12) a year in the same individual (Huntington Jr, 1944; King, 1944; Bean et al., 1992; Shenoy et al., 1995; Shenoy, 2008). At this stage, there is often lymphoedema of the foot and ankle. In most cases, the lymphadenitis occurs in the inguinal region on one side, and there is lymphangitis on the medial side of the limb and foot of the same side. Repeated episodes of lymphangitis or acute manifestations lead to the formation of fibrous and calcified tissues in and around the lymphatic vessels (Olszewski et al., 1997, 1999; Dreyer et al., 1999a). The repeated attacks of adenolymphangitis precede the development of chronic lymphatic pathology of filariasis and these often continue for many years (Pani et al., 1994). Patients with elephantiasis show tortuosity, dermal back-flow, obstruction, stasis and poor regional node visualisation (Witte et al., 1993; Azoubel, 1996; Shenoy, 2008). Lymphoedema is frequently present during the episodes, but usually subsides after acute stage. Some times lymphoedema does not subside and lead to chronic changes (Figure 3a). Chyluria is another incurable form of the chronic filarial syndromes, is caused by the intermittent discharge of intestinal lymph (chyle) into the renal pelvis and subsequently into the urine (Figure 3b). Chyluria was associated with increased vascular endothelial growth factor (VEGF) levels, whereas elephantiasis presents a high endothelin-1 (ET-1) profile (Debrah et al., 2007). Symptoms due B. malayi infection are largely same as that due to W. bancrofti. However, fever associated with lymphangitis in patients from endemic areas of B. malayi is more common than in those of W. bancrofti. Lymphoedema and elephantiasis of legs and arms occurs but the absence of involvement of the genito-urinary organs is a characteristic feature of brugian filariasis (Figure 3a). Legs are more frequently affected than the upper extremities.
Secondary bacterial infections, inflammatory reactions and immune response to parasite antigens add to the morbidity of the disease (Shenoy et al., 1999). Tropical Pulmonary Eosinophilia or Weingarten's syndrome is caused due to immunologic hyper-responsiveness to filarial infections (Dreyer et al., 2000) and is characterized by nocturnal paroxysmal cough, hyper-eosinophilia, elevated erythrocyte sedimentation rate (ESR), diffused miliary lesions or increased bronchovascular abnormalities especially at the bases of the lungs, and extremely high parasite specific IgE. This condition responds well to DEC treatment.

**Diagnosis of Lymphatic Filariasis**

Diagnostic tools are an essential component for the successful elimination of lymphatic filariasis worldwide. Significant advancements have occurred in recent years providing rapid and accurate diagnostics for lymphatic filariasis allowing easy on-site testing with rapid and simple detection of results. The traditional diagnosis of lymphatic filariasis has been dependent upon the detection of microfilariae in blood. The simplest method is a thick blood film of capillary blood stained with Giemsa stain (Khamboonruang et al., 1987; Sabry, 1992; Knott, 1935 Melrose et al., 2000b). Another widely used concentration method is the membrane filter technique whereby diluted blood is passed through a filter, from where trapped microfilariae are recovered, stained and counted (Bell, 1967; Chulerek and Desowitz, 1970; Nathan et al., 1982; Moulia-Pelat et al., 1992). This requires immediate processing of blood sample and relatively expensive filter apparatus and filters are used. Alternatively, capillary blood collected by finger-prick is used to make a thick film and stained with Giemsa (Khamboonruang et al., 1989; Sabry, 1992). The disadvantage of the thick blood film is underestimation of microfilaraemia prevalence if mf densities are low (Panicker et al., 1991; Turner et al., 1993). It is still a useful and cheap technique for field work where other more sensitive techniques are too expensive (Moulia-Pelat et al., 1992).

Immunodiagnosis is based on antigen or antibody reaction. Antibody based method depends upon the detection of antibody against filarial antigens. A wide range of crude filarial parasite antigens have been utilized for this purpose such as *Setaria digitata* (Dissanayake and Ismail, 1980), *Litomosoides carinii* (Rajasekariah et al., 1986), *Acanthocheilonema viteae* (Rajasekariah et al., 1986), *Setaria cervi* (Almeida et al., 1990), *Dirofilaria immitis* (Turner et al., 1993), *B. malayi* (Terhell et al., 1996) and *B. pahangi* (Simonsen et al., 1996). To avoid cross reactivity due to the crude nature of antigens, purified native antigens (Cheirmaraj et
al., 1992; Ramaprasad and Harinath, 1995) and recombinant antigens were later employed (Dissanayake et al., 1992; Ramzy et al., 1995; Burkit, et al., 1996; Langy et al., 1998, Rao et al., 2000; Lalitha et al., 2002; Janardhan et al., 2007). A few recombinant antigens e.g. Bm14, WbSXP1, BmSXP-1 and BmR1 have been shown to detect IgG4 antibodies and demonstrated good sensitivity of the test for field use (Dissanayake et al., 1992; Chandrashekar et al., 1994; Rahmah et al., 2001, 2003; Lalitha et al., 2002) and several test trials are underway. Antigen based detection method has been used for detection of LF to overcome the problems of antibody based assay (Itoh et al., 2001; Weerasooriya 2003; Rocha et al., 2004; Patil et al., 2005; Seybolt et al 2006; Dixit et al., 2007). The commercial ELISA (Trop Bio Og4C3 Antigen Test, produced by Trop Bio Pty Ltd., Townsville, Australia) is based on the assay developed by More and Copeman (1990, 1991) and is a very good marker of active filarial infection with adult worms (Chanteau et al., 1994; Rocha et al., 2004) and shows strong specificity for W. bancrofti antigen also. The majority of the field studies have shown the sensitivity of the Og4C3 ELISA to be of 94–100% (Freedman et al., 1994). In 1997, there was introduction of a lateral flow, rapid-format card test for detecting filarial antigenaemia (the ICT Filariasis Test; ICT Diagnostics, Sydney, Australia) based on immuno-chromatographic technique that used specific monoclonal and polyclonal antibodies attached to colloidal gold (Weil et al., 1997). The ICT test was recommended by international authorities as the diagnostic method of choice in year 2000 for mapping the distribution of bancroftian filariasis. A sandwich ELISA based on detection of circulating filarial antigen using monoclonal antibodies against recombinant antigens WbSXP-1 and Bm-SXP-1 (Rao et al., 2000; Lalitha et al., 2002) have been introduced and technical scale production of rWbSXP-1 for future industrial application have been done (Janardhan et al., 2007). Kit based on WbSXP-1 and Bm-SXP-1 has been recently commercialized by M/s Span Diagnostics, Surat, Gujarat. Although, these assays are sensitive for infection but they lack in specificity, as they cannot distinguish current infection from past infection or exposure to the parasite and due to some degree of cross-reactivity with other helminth infections.

The detection method based on molecular technique has been successfully used for W. bancrofti DNA in blood, plasma, paraffin embedded tissue sections (McCarthy et al., 1996) and sputum (Abbasi et al., 1999) and B. malayi DNA in blood (Lizotte et al., 1994; Rahmah et al., 1998) and urine (Lucena et al., 1998). Recent report on detection of B. malayi parasite DNA in human blood by real-time PCR (Rao et al., 2006) have replaced conventional PCR techniques for technical reasons (improved sensitivity) and practical reasons (faster results
with less labour). However, PCR is expensive and facilities are often not available in filaria-endemic areas.

Non-invasive techniques like ultra-sonography can be used to detect adult worms in the scrotum and breast (the 'filarial dance sign') of bancroftian patients (Dreyer et al., 1996; Simonsen et al., 1997; Suresh et al., 1997; Faris et al., 1998) and has detected viable worms in children (Dreyer et al., 1999b). Nuclear magnetic resonance (NMR) spectroscopy has also been used in vitro as well as in situ for the detection of human filarial parasite, Brugia malayi (Shukla- Dave et al., 1999). Filaria-specific enzymes viz. acetylcholinesterase (Misra et al., 1993), glutathione binding proteins, glutathione S-transferase, proteases and superoxide dismutase (Beuria et al., 1995; Bal and Das, 1995, 1996) have been characterized and show diagnostic potential either as antigens for antibody assays or by the detection of the enzyme itself. The best of these assays are sensitive for infection but are non-specific, as they cannot distinguish current infection from past infection or exposure to the parasite and due to cross-reactivity with other helminth infections.

Programmes for Elimination of Lymphatic Filariasis

There are several approaches to controlling vector-borne parasitic diseases viz; vector control, breaking of vector–host contact by use of repellent or bed nets, vaccination and chemotherapy. Vector control can be very successful in situations where malaria and filariasis have common vectors (Webber, 1991). However, development of insecticide resistance in vectors makes these campaigns less effective (Feng et al., 2002). Diethylcarbamazine (DEC) a piperazine derivative has been used to treat filariasis since its discovery in 1947 (Hewitt et al., 1947; Santiago-Stevenson et al., 1947) and still is the most widely used anti-filarial. DEC is an inhibitor of arachidonic acid metabolism and makes the microfilariae more susceptible to immune attack (Piessens and Beldekas, 1979). DEC is given orally and is rapidly absorbed reaching peak blood levels one or two hours after ingestion. The plasma half life of DEC varies from 6.1 to 8.1 hours. Excretion is mainly renal and the blood concentration reaches zero within 48 hours (Mitsui and Aoki, 1998). DEC has dramatic effect on circulating microfilariae and it has been shown to reduce microfilarial prevalence by 74.9% in the annual treatments and 90% in the biannual treatments. Attack of filarial fever and incidences of acute oedema cases also reduce to some extent by this drug (Panicker et al., 1991). It is a very effective microfilaricide but has a limited amount of macrofilaricidal activity (Weil et al., 1988). Delayed reappearance of microfilaraemia after
DEC therapy suggests the effect of this drug on the reproductive potential of female adult parasites.

The standard DEC treatment regimen is 6 mg/kg body weight per day over a 10–21 day period (WHO, 1994). This is suitable for treating isolated cases but it is not a suitable regimen for community-wide mass treatment programs because it is labour-intensive and incurs high costs (Michael et al., 1996). Common cooking salt medicated with DEC (0.1 to 0.6%) (WHO, 1994) been used effectively in mass treatment programs for bancroftian filariasis in India (Narasimham et al., 1989), China (Shaoqing et al., 1994) and Tanzania (Meyrowitsch, 1996) and has been shown to be effective in brugian filariasis (Shenoy et al., 1998). Adverse reactions of DEC in persons with filarial infections can be either localized (associated with death of the adult worm) or systemic (associated with death of microfilariae). In areas where there is co-endemicity with onchocerciasis and/or loaisis, diethylcarbamazine is not used because of the possibility of severe reactions. Side effects of DEC are mild when drug is given in daily doses of 6mg/kg (Sasa, 1976).

Apart from DEC, another drug ivermectin has been proved to be very effective producing long-term suppression of microfilaraemia in bancroftian and brugian lymphatic filariasis in a number of countries (Ottesen et al., 1990; Ismail et al., 1991; Cao et al., 1997). Ivermectin is a potent macrocyclic lactone which belongs to the class of compounds known as avermectins (deSilva et al., 1997). Since its introduction for human use in 1981, ivermectin has been used almost exclusively for the treatment of Onchocerciasis (Whitworth, 1994). It is available for human use in tablets of 6 mg, for oral dose. In contrast to DEC, ivermectin has slow and delayed effect on microfilaraemia and it has been shown to remove 100% of microfilariae at a single dose of 400 μg/kg and irreversibly reduce the fecundity of the adult female worm by at least 65% (Plaisier et al., 1999) and is also found to effectively reduce the frequency of adenolymphangitis attacks in brugian filariasis (Shenoy et al., 1998b). As with DEC, the macrofilaricidal action of ivermectin is debatable (Ismail et al., 1996). Ivermectin is relatively well tolerated however; adverse effects include fever, itching, dizziness, oedema, mild mazzotti reaction and minimal ocular inflammation in patients with eye involvement. The adverse effects usually occur during the first 3 days after treatment and are dose dependent (deSilva et al., 1997). Despite these side effects ivermectin is widely used for onchocerciasis control. Two programmes, the Onchocerciasis Elimination Programme for the Americans (OEPA) and the African Programme for Onchocerciasis Control (APOC) have been implemented based on mass distribution of ivermectin, either semiannually or annually.
Recently identified drug resistance to ivermectin (Bourguinat et al., 2007; Lustigman and McCarter, 2007) and severe side reactions to DEC therapy appears to be the issue of concern. Apart from synthetic compounds it was recently discovered that although treatments with DEC or ivermectin were efficacious in reducing microfilarial rates, combinations of these drugs have been found to be significantly more effective than each drug alone. Moreover, albendazole has also recently been found to be an efficient macrofilaricidal drug when used in combination with ivermectin or DEC. However, a few reports (Rao et al., 1981; Narasimham et al., 1989; Shenoy et al., 1998) on effective clearance of microfilaraemia, but unsuccessful eradication of lymphatic filariasis by daily use of (DEC)-fortified salt for six months, together with a single dose of ivermectin+DEC and reported teratogenicity of albendazole suggests re-evaluation of the combination therapy regimen for global elimination of lymphatic filariasis. Another novel approach to filariasis control is to target the endosymbiont bacteria, *Wolbachia* which live intracellularly within filariids, and appear to be essential to healthy growth and development of the parasite (Hoerauf et al., 2001). Depletion of *Wolbachia* via few anti-rickettsial drugs has resulted in macrofilaricidal or long term sterilizing activity (Taylor et al., 2000).

In 1997, a ‘global alliance’ consisting of the WHO, the World Bank, UNICEF, The Carter Centre, Glaxo Smith Kline, Merck and Co., academic institutions and other non-government agencies has been formed to help facilitate the elimination of lymphatic filariasis (WHO, 1997). The strategies underpinning the GPELF (Global programme for elimination of lymphatic filariasis) are (i) MDA with single-dose albendazole and ivermectin (Mectizan) tablets in areas, where LF is co-endemic with onchocerciasis, annually for 4–6 years, (ii) single dose albendazole and diethylcarbamazine (DEC) in areas, where onchocerciasis is not endemic, annually for 4–6 years, (iii) exclusive use of DEC-fortified tablet or cooking salt for 1–2 years in some settings, (iv) vector control measures, especially the use of insecticide-treated materials, where appropriate and cost-effective, (v) home-based self-management of lymphoedema and elephantiasis for affected individuals and (vi) improved access to surgical intervention for men with hydrocoele. National eradication programs are already underway in several localities in various endemic countries. Despite the available controlling measures the uncertainties regarding operational issues, such as the required duration of treatment and the optimal drug coverage within the prescribed time-frame still exist and needs to be conquered.
Preface to the work embodied in the present study

Recent clinical trials with a combination of DEC or ivermectin with albendazole are although producing promising results in ongoing limited clinical trials (Ismail et al., 2001), development of a macrofilaricidal drug is urgently required for long-term effects and reduction of filarial pathology. All the strategies advocated till date for the effective control of lymphatic filariasis are based on interrupting parasite transmission (Ottesen et al., 1999). Current treatment is done with diethylcarbamazine (DEC) or ivermectin, which principally act on circulating microfilariae resulting into reappearance of microfilaraemia after withdrawal of the drug (Ottesen, 2002). This leaves us with unavailability of any potential drugs acting on adult parasites. Individuals living in endemic areas of filariasis require long-term chemotherapy in order to prevent re-infection and transmission. However, such strategies are difficult to implement on a permanent basis at the population level (Molyneux et al., 2005; Tisch et al., 2005). In this situation, identification of novel proteins which may be used as vaccine candidates or discovery of novel drug-targets against filariasis seems to be a promising approach. In the present study, the screening of larval stage cDNA library of B. malayi with resistant L3 serum raised in Mastomys coucha led to isolation of five highly immunoreactive cDNA clones encoding SXP antigen, Allergen ladder protein, DEAD box RNA Helicase, Ribosomal protein and an immunogenic antigen. Amongst these, ATPase dependent DEAD Box RNA Helicase (BmL3-Helicase) was the novel clone, not yet reported in any of the filarial species. The BmL3-Helicase clone showed about 58% similarity with C. elegans RNA Helicase, 78% with B. malayi RNA Helicase sequence generated from draft genome. It also contains a DEAD box conserved domain which is the signature sequence present in almost all RNA Helicases. The expression and purification of recombinant BmL3-Helicase was carried out using an E. coli expression system and the findings confirmed this protein to be an ATP-dependent RNA Helicase. The results have also shown that the ATPase activity of the recombinant protein was polynucleotide dependent and was markedly enhanced by dsRNA. Sero-reactivity with various categories of human bancroftian sera and stage-specificity of enzyme were observed. The three-dimensional model of the protein was designed to get an insight into the structure-function relationship of the enzyme. Protection studies were carried out in Mastomys coucha (a rodent filarial animal model) to see the protective efficacy of recombinant protein after challenge with infective larvae (L3). The immunological profile of enzyme was explored after vaccination with purified recombinant enzyme in Mastomys coucha with Freund’s adjuvant. The antibody titre, specificity,
isotyping and type of immune response against the recombinant BmL3-Helicase were observed. Further, siRNA mediated silencing of this gene was carried out in both adult parasites and their larval forms (microfilariae) to assess its functional role as also to validate it as a drug-target in case of filariasis. The enzyme was found to possess properties of both vaccine candidate as well as drug-target. The results obtained in the present thesis report on the functional characterization of a novel and unexplored protein of human lymphatic filarial parasite \textit{B. malayi} with a promising drug and vaccine target potential.
Lymphatic filariasis endemicity status in the countries of the South-East Asia programme review group, latest available
Global distribution of Lymphatic Filariasis
Infective stage L3

6

adult

-8 years

8 9

10

11

sexual

"macrofilaria"

ovoviviparous-

eggs hatch

intrauterine and

retain part of the

eggshell as a

sheath

Infective L3 emerging from
mosquito mouthparts

100 days

10 days

100 days

L3

L4

L2

L1

1

Microfilaria

arrest in

bloodstream

发育 in muscle
of mosquito vector

arrest in

vertebrate

-100 days

Life cycle of Lymphatic Filarial parasite* Brugia malayi (source: www)
Lymphoedema causing gross enlargement and fibrosis of the limbs, breasts or genitalia

Chyluria (left) caused by lymphatic filariasis