Review of Literature
2.1 Historical perspective

Filariasis is one of the oldest diseases and its history is as old as the history of human civilization itself. The dramatic symptoms caused by the infection of *W. bancrofti*, especially the enormous swelling of the legs or scrotum were recorded in the 18th century medical literature of India, Persia, China, and Japan. Several indications on the occurrence of elephantiasis are available from museums in Egypt; one such indication comes from the statue of king “Menchuhotep” in Egypt dating back to 2133-1992 B.C. Also, in a museum in Cairo, the statue of an Egyptian Pharaoh that depicts the signs of elephantiasis (Fig. 2) is seen even today (Dean, 2001). The mummified body of Natsef-Amun, a priest at Karnak in the time of Rameses XI during 1113-1085 B.C., was proven by autopsy to have lymphatic filarial worms in the groin even after 3,000 years (Fig. 3) and is still preserved in Leeds museum (Dean, 2001). Historical records of this disease seen in 7-8 A.D. is given as an illustration in ‘Yamai zoshi’ of elephantiasis leg in a woman (Fig. 4) which is preserved in the Tokyo National museum (Tada et al., 1999). People with elephantiasis were excluded from the Buddhist priesthood during 600-250 B.C. (Laurence, 1967). The Operational Manual on National Filaria Control Programme, published in 1995 by the National Malaria Eradication Programme (NMEP), India, reports that Susruta, a physician, mentioned about the disease as ‘Sleepad’ as early as 600 B.C. Later in A.D. 70, Madhavakara, a pathologist, in his book “Madhava Nidan” described the signs and symptoms of this disease (Sharma et al., 1995). The signs and symptoms of this disease stand true even today. NMEP (1995) mentions a description of elephantoid legs in Cochin as ‘Malabar Legs’. During 16th century, elephantiasis of the leg was attributed to the curse of Saint Thomas, in Kerala (South-West India), and was portrayed by Linschoten. This appears to be certainly the *B. malayi* infection (Fig. 5) (Laurence, 1989). A Jesuit missionary in Pondicherry, Southeast India, portrayed the elephantiasis of the scrotum (Fig. 6) and published in Paris, possibly *W. bancrofti* infection, which indicates
Fig. 2. A Pharaoh’s Affliction Cairo Museum: Statue of an Egyptian Pharaoh depicting Possible signs of elephantiasis

Fig. 3. The mummified body of Natsef-Amun, a priest at Karnak in the time of Pharaoh Rameses XI (1113-1085 B.C) proven after 3,000 years by autopsy to have Ly worms in the groin.  
Source: Leeds Museum

Fig. 4. Yamai zoshi of elephantiasis leg in woman

Fig. 5. Elephantiasis of the leg attributed to the curse of saint Thomas, Kerala, SW India, 16th century, as portrayed by Linschoten
the possible existence of the disease in Pondicherry (Laurence, 1989), at least in historical point of view.

Partick Manson in the year 1877 first identified the blood-sucking mosquito as the key agent in the transmission of lymphatic filariasis; he is internationally recognised as the father of Tropical medicine. He was the first to identify the non-periodic form of filariasis. However, microfilariae were first described from hydrocele fluid (Fig. 7) by Demarquay (1863) in Paris and from blood in India by Lewis in the year 1872. Manson in the year 1896 examined the blood films collected in 1884 by Davis in Samoa, who had also reported mf from the blood of persons in Polynesia. Subsequently, he described the localization of mf in lungs and cardiac muscles during the daytime.

Manson was acquainted with the periodic type of filariasis, so he assumed that the mf from Samoa to be nocturnal type. Thorpe (1896) observed that the mf from South Pacific were present in the daytime as well as at night and established the existence of non-periodic form of filariasis, which is known to be prevalent in the other islands of the region viz., Fiji, Caledonia, the Loyalty Islands and Polynesia. O'Connor (1923) showed that this form of filariasis was endemic to the islands of Western Pacific. The age specific analysis showed that mf and disease rate in persons aged above 16 years was comparatively more than those under 16 years. The clinico-epidemiological profile of this form of filariasis in this region showed that episodes of filarial fevers associated with lymphangitis and lymphadenitis were the commonly reported signs. Elephantiasis (Fig. 8) associated with hydrocele was known to manifest later in life (Weller et al., 1982).

In 1868, Wucherer described a species of worm in the urine of a patient with tropical haematuria in Brazil and clearly distinguished it from *Bilharzia haematoium*. In 1870, he noted that the chylous urine invariably contained nematoid worm. After this Manson found the same worm in the blood of a patient (1899). Subsequently in 1872, Lewis discovered a dead mf in the peripheral blood and four live female worms. Cobbold proposed the
Fig. 6. Elephantiasis of the scrotum, portrayed by a Jesuit missionary in Pondicherry, SE India, 18th century, and published in Paris. Possibly *W. bancrofti* infection.

Fig. 7. Hydrocele is the common manifestation of bancroftian filariasis among males (www.pon.nic.in/fil-free/chrc).

Fig. 8. A flower vendor in India with elephantiasis. ©WHO/TDR/Chandran (www.filariasis.org/index.pl?iid=1766).
scientific name “Filarial bancrofti” and Da Silva araujo proposed the generic name Wuchereria in 1877. Later, Manson (1879) found the two characteristics of filarial parasite, i.e., mosquito transmits it and has nocturnal periodicity. After the World War II remarkable contributions were made to the epidemiology and control of filariasis. Hewitt et al., discovered the filaricide Diethyl carbamazine (DEC) in 1947 that became the milestone in the history of the anti-filariasis campaign. In 1957, Kessel generated the first set of the result in the control of filariasis by mass administration of DEC (Sasa, 1976).

It was Manson (1877), who found mf in the blood of the patients with elephantiasis and associated this and other clinical signs of lymphatic obstruction with the infection (Manson, 1878). He also showed the development of the parasite in the female mosquito after the uptake of mf during its blood meal but wrongly concluded on the mode of transmission of these larvae to man. He postulated that when infected mosquitoes died in water, the filarial larvae were released and the swallowing of these larvae infected man. Low (1900), on the basis of histological studies on infected mosquitoes, was the first to suggest that human infection took place during the blood meal of an infective mosquito.

2.2 Global distribution of lymphatic filariasis

Over 120 million people are affected in 80 countries, with 20% of the world’s population living at the risk of the disease (WHO, 1997a). The nematode parasite W. bancrofti is the major cause of this disease accounting for 90% of the cases. India alone contributes about 40% of the total global burden of this disease (Ramaiah et al., 2000) and there are approximately 21 million people with symptomatic filariasis and 27 million who have asymptomatic microfilaraemia (Sabesan et al., 2000; Das and Ramaiah, 2002). It has also been estimated that economic loss to India is to the tune of US $ 840 million (Ramaiah et al., 2000). In India W. bancrofti and B. malayi are the only two filarial species causing lymphatic filariasis and the former being the major contributor and the latter is endemic to a few parts of Kerala,
Tamil Nadu, Andhra Pradesh, Orissa, West Bengal, Assam, and Madhya Pradesh (Raina et al., 1995). A total of 289 districts in India were surveyed for filariasis until 1995; out of which 257 were found to be endemic. In India, a total of 553 million people are at risk of infection. *W. bancrofti* accounts for about 98% of the national burden, and is widely distributed in 17 states and union territories. An overview of the traditional endemic foci shows concentration of infection mainly around river basins, and eastern and western coastal parts of India (Das et al., 2002). In addition, approximately 16 million have lymphoedema or elephantiasis along with the recurrent episodes of acute adenolymphangitis (ADL). Lastly, a million individuals have cryptic infections resulting in conditions such as tropical pulmonary eosinophilia (TPE) (WHO, 2002). Fig. 9 shows the distribution of filariasis in India based on the filarial endemicty rates ranging from non-endemic to highly endemic areas (Sabesan et al., 2000).

### 2.3 Parasite species, morphology and races

#### 2.3.1 Filarial parasites

Nematode parasites causing LF belongs to:

- **Kingdom**: Animalia
- **Phylum**: Nematoda
- **Class**: Secernentea
- **Order**: Spirurida
- **Sub-Order**: Spirurina
- **Family**: Filarioidea
- **Genus**: *Wuchereria*

Filaria is widely distributed in the tropical and subtropical regions of Africa, Asia, Northern South America, Western Pacific and Eastern Mediterranean region. Humans and animals are infected by nematode parasites called "Filariae" which includes several hundred species of worms that are slender, elongated and parasitic in tissues of various vertebrate hosts. In this family, there are about 500 species occurring in humans,
Fig. 9. Human Lymphatic Filariasis distribution in India, based on historical data (1960-1995). Source: Sabesan et al., 2000
domestic animals, rodents, monkeys, lizards etc. Among these parasites, genera *Wuchereria*, *Brugia*, *Onchocerca*, *Dipetalonema*, *Mansonella*, and *Loa* are infectious to humans. They reside in lymphatics or muscles, connective tissues, body cavities of the vertebrate hosts. They are classified into three main groups by the habitat of the adult worm: (a) cutaneous group, (b) lymphatic group and (c) body cavity group. Based on the habitat of the adult worms, few of the filarial species infecting man and the disease caused by them with their intermediate hosts are listed in Table 1. However, only two genera, *Wuchereria* and *Brugia*, are mainly responsible for human lymphatic filariasis in India and neighbouring countries. There are two species of *Wuchereria* and nine species of *Brugia* (Table 2). Common animal parasites are *Setaria digitata*, *S. cervi* (bovine), *Dirofilaria immitis* (dog), *D. uniformis* (rabbit), *Litomosoides cami*, *Dipetalonema viteae* (gerbil), *Brugia pahangi* (cat) and *Acanthocheilonema viteae* (jird).

The three lymphatic dwelling parasites *W. bancrofti*, *B. malayi* and *B. timori* are vectored by haematophagus arthropods. The most important arthropod involved in the blood-sucking habits of ectoparasites is mosquitoes, bedbugs and fleas. These pathogenic/parasitic transmitters, cause transmission of several debilitating diseases such as filariasis, and sometimes fatal diseases such as malaria, dengue, Japanese encephalitis, plague, typhus, yellow fever etc. The filarial infection is transmitted by intermediate hosts which are always blood-sucking arthropods of the order Diptera, belonging to the four families (Fig. 10b). Some of the known vectors of human filariae are given in Table 3.

### 2.3.2 Morphology of filarial parasites

Adult *W. bancrofti* and *B. malayi* are minute, thread like and have a smooth cuticle. Adult males measure 40 mm in length and 0.1 mm in diameter, whereas females are 80-100 mm in length and 0.24-0.30 mm in diameter (Namduri and Kazura, 1989). The mf of both *W. bancrofti* and *B. malayi* are sheathed. The mf of *W. bancrofti* ranges from 244-296 μm in
### Table 1. Human filarial parasite species and the habitat of adult worms

<table>
<thead>
<tr>
<th>Species</th>
<th>Habitat</th>
<th>Intermediate host</th>
<th>Disease caused</th>
</tr>
</thead>
<tbody>
<tr>
<td>W. bancrofti</td>
<td>Lymphatics</td>
<td>Mosquito sp.</td>
<td>Bancroftian filariasis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Elephantiasis)</td>
</tr>
<tr>
<td>B. malayi</td>
<td>Lymphatics</td>
<td>Mosquito sp.</td>
<td>Malayan filariasis</td>
</tr>
<tr>
<td>B. timori</td>
<td>Lymphatics</td>
<td>Mosquito sp.</td>
<td>Timor fever</td>
</tr>
<tr>
<td>Loa loa</td>
<td>Connective tissue</td>
<td>Chrysopsis sp.</td>
<td>Loasis</td>
</tr>
<tr>
<td>Mansonella ozzardi</td>
<td>Serous membranes</td>
<td>Culicoides sp.</td>
<td>Ozzard's filarial</td>
</tr>
<tr>
<td>Onchocerca volvulus</td>
<td>Skin</td>
<td>Simulium sp.</td>
<td>Onchocerciasis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(River blindness)</td>
</tr>
<tr>
<td>Order</td>
<td>Family</td>
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<td>---------</td>
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<td></td>
</tr>
<tr>
<td>Diptera</td>
<td>Culicidae – Mosquitoes</td>
<td>Cx. quinquefasciatus</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mansonia sp.</td>
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<tr>
<td></td>
<td></td>
<td>A. barbirostris</td>
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</tr>
<tr>
<td></td>
<td>Simulidae – Black flies</td>
<td>Simulium damnosum</td>
<td></td>
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<tr>
<td></td>
<td>Certopogonidae – Biting</td>
<td>Culicoides furens</td>
<td></td>
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<tr>
<td></td>
<td>midges</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Tabanidae – Horse flies</td>
<td>Chrysops dimidiata</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Vectors of human filariae**
| Genus *Brugia*  
| (Buckley, 1960) | Genus *Wuchereria*  
| (Silva Aranjo, 1877) |
|------------------|-------------------|
| *B. malayi* (Brug, 1927) | *W. bancrofti* (Cobbold, 1877); |
| *B. pahangi* (Buckley and Edeson, 1956) | *W. kalimantani* (Palmieri et al., 1980) |
| *B. patei* (Buckley et al., 1958) | |
| *B. Brugiella buckleyi* (Dissanaike and Paramanathan, 1961) | |
| *B. ceylonensis* (Jayewardene, 1962) | |
| *B. guyanensis* (Orihel, 1964) | |
| *B. beaveri* (Ash and Little, 1964) | |
| *B. tupaiae* (Orihel, 1966) | |
| *B. timori* (Partono et al., 1977) | |

**Table 3.** Nematode parasites causing lymphatic filariasis: Recorded species of *Brugia* and *Wuchereria* parasites
length whereas the mf of *B. malayi* ranges from 177–230 μm in length (Fig. 10a). The distinctive features of these two parasites are, mf of *B. malayi* has two terminal nuclei distinctly separated from other nuclei, the last terminal nucleus is small and is at the tip of the tail, whereas the body nuclei of *W. bancrofti* mf do not extend to the tip of the tail. The ultra-structural morphology of cuticular surface membranes of adult *B. malayi* has been studied by freeze fracture electron microscopy (Smith et al., 1996). Localization and isolation of *W. bancrofti* adult worms by ultrasonography has facilitated the ultra-structural studies of the surface by scanning electron microscopy (Araujo et al., 1995).

### 2.3.3 Microfilarial periodicity

A circadian pattern in which the highest numbers of mf occur in the blood at night is known as nocturnal periodicity (Gupta et al., 1990). These parasites have a unique circadian periodicity and are found in the peripheral circulation during certain periods of the day (Edeson, 1955; Denham and Mc Greevy, 1977). Turner and Edeson (1957) first described the nocturnally periodic and nocturnally sub-periodic forms in man. Depending on the infecting species, mf exhibits periodicity in circulation. In most of the endemic areas, *W. bancrofti* mf are of periodic type and appear in circulation from 21.00 hrs to 23.00 hrs. The sub-periodic form of *W. bancrofti* present at pacific region shows mf all the time. There are two strains of *B. malayi*, one nocturnally periodic and the other nocturnally sub-periodic. Recently, a third form has been described which exhibits a diurnally sub-periodic pattern (Cabrera and Rozeboom, 1965). When not in circulation, mf generally resides in the capillaries and the blood vessels of the lung. The basis of filarial periodicity is unknown. However, it has been observed that the periodicity can be altered by reversing the working and sleeping habits of the host (Mark, 1987). The correlation between peak peripheral microfilaremia and peak biting time suggests that the filarial nematodes have adapted their periodicity.
Fig. 10a. Microfilaria of *W. bancrofti*. The microfilaria is sheathed, its body is gently curved, and the tail is tapered to a point. The nuclear column (the cells that constitute the body of the microfilaria) is loosely packed, the nuclei can be visualized individually and do not extend to the tip of the tail (Haematoxylin stained x400) (www.dpd.cdc.gov)

Fig. 10b. A *Culex quinquefasciatus* mosquito on a human finger. The *Culex quinquefasciatus* mosquito is proven to be a vector of *W bancrofti* (www.waterandhealth.org/images/mos.jpg)
patterns to their vector periodicity patterns, which facilitates their transmission (Wharton, 1963).

In Andaman Nicobar islands, mf of diurnally sub-periodic *W. bancrofti* were detected in the peripheral blood throughout the 24 hours period with a peak at 18:00hrs. Overall studies undertaken so far in these islands report high microfilaremia rates and a very low disease rate. The incidence of microfilaremia rates was very low in children and increased with increasing age with the highest rate in the 41-50 age group (Russel et al., 1975), characteristic of areas with low force of infection (Sasa, 1976) and is frequently associated with diurnally sub-periodic *W. bancrofti* in French Polynesia (Cartel et al., 1992), Samoa (Murray, 1948) and other South Pacific island groups also (Kessel, 1960). This is in contrast to the age distribution of periodic *W. bancrofti* on the mainland India, which increased until about 20 years of age, followed by a decline to about 40 years, after which the microfilaremia levels stabilized (Rajagopalan et al., 1981a).

2.4 Life history

Lymphatic filarial parasites alternate between two hosts: (1) the definitive host, either man or vertebrate animal (e.g. monkey, cat, rat, and jirds), and (2) the intermediate host, being mosquito to complete its life cycle (Fig. 11). The life cycle of the lymphatic filarial parasites, *Wuchereria* and *Brugia* is essentially similar. Adult filarial worms normally dwell in the lymph canals and nodes. The female worms in the mammalian host mate with males and produce embryos called the microfilaria (mf), which appears in the circulating blood, or occasionally in hydrocele fluid or chylus urine. When the mf are ingested by mosquito (intermediate host) they lose their sheaths in the mosquito midgut and migrate to the thoracic muscles where they develop to first stage larvae (L1), then to the second stage larvae (L2), and finally to the infective or third stage larvae (L3) in about 2 weeks. The L3 then migrate to the head of the mosquito and get transmitted to the mammalian hosts during the subsequent feeding (Sasa, 1976). During subsequent feeding on the
Fig. 11. Life cycle of *W. bancrofti*
human host, L3 are deposited on the skin surface near the site of the puncture wound made by the biting mosquito and actively migrate into the puncture wound and then into the blood followed by lymphatic system (Mak, 1983). The L3 larvae develop into adult worms within the human body and the estimates have shown that it takes about one year for the larvae to grow into adult worms, mate, and produce mf in human hosts, which then circulate in blood to be picked up by the vector again (Sasa, 1976).

2.5 Bacterial endosymbionts (Wolbachia) of filarial parasites

Recently, the discovery of the presence of rickettsia like endosymbionts in filarial parasites has evoked a lot of interest on these organisms, their biology, pathogenesis and control (Taylor et al., 2005). Studies have shown that the nematodes are highly dependent upon these symbiotic intracellular bacteria, Wolbachia (Fig. 12a-d), as they are essential for normal development and fertility. Advances in the understanding of the symbiotic relationship of Wolbachia bacteria with filarial nematodes have made rapid progress in recent years, especially the evolution of the symbiotic association together with insights into the functional basis of the interaction derived from genomic analysis. They have also been known to contribute to inflammatory-mediated pathogenesis and adverse reactions to anti-filarial drugs. Recent field trials have shown the use of antibiotics as a promising new tool for the treatment of filarial infection and disease.

2.6 Clinical manifestations

Lymphatic filariasis is characterized by broad range of clinical manifestations. Although the entire population living in an endemic area is exposed to the parasite, a large group of individuals show no signs of infections, either parasitological or clinical and are classified as endemic normals (EN). The other group, though normal by clinical criteria harbor circulating mf in their blood circulation, is known as asymptomatic microfilaremics (MF). The clinically affected groups suffer from either acute
Fig. 12. *Wolbachia* – The bacterial endosymbionts of filarial parasites (Mark et al., 2001; Helen et al., 2004; Wieslaw, 2005).

(a) Distribution of *Wolbachia* in adult *B. malayi*. Cross section (A) and longitudinal section (B) of adult female *B. malayi* stained with anti-WSP antibody. *Wolbachia* (red) occur in the lateral hypodermal cords (LC) and ovaries (OV). Note the "halo" of staining on the surface of bacterial cells. C, cuticle and G, gut. Magnification of A, 1000x and magnification of B, 1500x.

(b) *Wolbachia* in human lymphatic filarial nematodes. (a) Bacteria (arrows) in the lateral cord of *B. malayi*.

(c) Aggregate of *Wolbachia* in the lateral chord of *D. immitis* female. Scale bar = 0.5 μm

(d) *Wolbachia* (arrow) dividing by binary fission. Scale bar = 0.5 μm.
attacks of lymphatic inflammation such as lymphadenitis, lymphanginitis or from chronic obstructive form of the disease characterized by elephantiasis legs, hydrocele or chyluria and are classified as chronic pathology (CP). The other extreme case of the infection is manifested as tropical pulmonary eosinophilia (TPE) that causes cough dyspnoe and nocturnal wheezing. These individuals are characterized by elevated levels of eosinophils and parasite antigen specific IgE (Ottesen and Nutman, 1992). It is postulated that these varied disease manifestations are caused by differential host immune responses. An understanding of molecular basis of the clinical spectrum of the disease would facilitate effective treatment and control of the disease (Partono, 1987). The clinical manifestations of the infection are swelling of the limbs (lymphoedema) and genitals (hydrocele), with elephantiasis as the most severe forms are often preceded and accompanied by acute attacks of filarial fevers (adenolymphanigitis, ADL). Development of severe morbidity is most likely after multiple infections (cumulative exposure to worms), although it is believed that even a single infection may cause disease (Hairston and de Meillon, 1968).

The clinical manifestations of lymphatic filariasis vary from one endemic area to another and also differ to some extent based on the species of the parasite that is involved (Sasa, 1976). In India both hydrocele and lymphoedema are seen with almost equivalent frequency (Pani et al., 1991). The range of clinical manifestations of lymphatic filariasis is broad and the diversity of clinical responses to filarial infection is considered to reflect the intensity and type of immune response to the parasite or parasite products. These include 'filarial fevers', the syndrome of tropical pulmonary eosinophilia, a condition in which individuals are entirely asymptomatic yet have persistent mf and lymphatic pathology.

2 6.1 The microfilaraemic stage

In a W. bancrofti endemic area the overwhelming majority of infected individuals have few overt clinical manifestations of filariasis despite the
presence of large numbers of circulating mf in the peripheral blood. The prevalence of microfilaremia increases with age during childhood and usually reaches a plateau between 20-30 years of age; during the childbearing years, the prevalence tends to be higher among men than among women (Brabin, 1990).

2.6.2 Filarial fever

Filarial fever is an acute recurrent fever with headache, malaise, chills, rigors and sweating and it may closely resemble malaria. It occurs irregularly often continuing for many years after leaving the endemic area and may come on for the first time as long as 20 years after leaving the tropics. It is usually accompanied by symptomatic lymphangitis and other early signs of filariasis may occur as fever alone.

2.6.3 Asymptomatic microfilaraemia

Individuals with microfilaremia but who are asymptomatic and without acute or chronic lymphatic involvement form the group that is immunologically least reactive (Ottesen, 1984). Their lymphocytes generally fail to respond significantly to filarial antigens in vitro (Ottesen et al., 1977) and their levels of serum antibody to both adult and mf antigens are minimal or absent (Mcgreevy et al., 1980; Ottesen et al., 1982).

2.6.4 Tropical pulmonary eosinophilia

Tropical pulmonary eosinophilia (TPE), a syndrome characterized by symptoms of bronchial asthma with paroxysmal nocturnal cough and anorexia, is a relatively unusual manifestation of infection with the filariae W. bancrofti or B. malayi. The disease is more common in men than in women; it is practically nonexistent in young children. Patients usually have peripheral hypereosinophilia and high levels of serum IgE, and of antifilarial IgG4 and IgE antibodies. The chest X-ray can be normal or show diffuse
pulmonary infiltrates. Pulmonary function tests reveal restrictive lung disease; some patients may also have obstructive disease.

Tropical pulmonary eosinophilia individuals appear entirely distinct from the other groups of patients and they comprise less than 1% of all filarial patients. This clinical syndrome is now generally regarded as a form of 'Occult filariasis' in which the absence of circulating mf reflects an immunological hyper-responsiveness on the part of the host that leads to very effective clearance of these parasites from the blood. The chronic phase of this syndrome is marked by pulmonary fibrosis and restrictive lung disease (Udwadia, 1975) which may have resulted from more tissue damage induced by the heightened or inadequately suppressed pulmonary lymphocyte or eosinophil responsiveness (Pinkston et al., 1983).

2.6.5 Lymphatic pathology (chronic pathology)

The immune mechanism that initiates the lymphatic pathology is entirely unclear, but the importance of this inflammation to the development of the lymphatic pathology and damage that characterize chronic filariasis cannot be over estimated.

2.6.6 Hydrocele

Recurrent attacks of filarial infection sooner or later lead to hydrocele. The hydrocele fluid is clear, straw coloured and may contain mf. In most endemic areas, such as east and west tropical Africa, Egypt, the northern states of Uttar Pradesh and Bihar in India, and Indonesia hydrocele is the most common sign of filariasis.

2.6.7 Elephantiasis

Not all elephantiasis patients may be currently infected with filarial parasites. Recent studies have shown that filarial infection can result in lymphatic impairment permitting opportunistic bacterial infection. These
secondary infections can in turn lead to more extensive pathology. Elephantiasis begins as lymphoedema. The leg(s), scrotum, arm(s), are affected usually in that order of decreasing frequency. In most countries males are affected more than females. In Africa, India and Sri Lanka the swelling may remain below the knee (WHO, 1984). In the initial stage the swelling can be observed around the ankles, from which it gradually spreads to the back of the foot, the leg, and the thigh. The affected limb may increase to more than three times its original size.

2.6.8 Chyluria

The prevalence of chyluria is usually very low. Chyluria occurs when dilated lymphatic vessels of the urinary excretory system rupture, causing leaking of lymphatic fluid and chyle into the urine. The urine may be milky white in color, particularly after a fatty meal. This results from rupture of obstructed and dilated small intestinal lymphatics into the urinary tract so that chyle no longer returns to the blood via the thoracic duct. More advanced cases may develop weakness and loss of weight resulting from the loss of fat and protein from the body.

2.7 Chemotherapy

Many new and old drugs were tried/used in the treatment of the filarial disease. Several drugs were synthesized and tested for antifilarial activity, during early 20th century and most of them where found to have either low efficacy or high toxicity beyond acceptable level or both. With the discovery of diethylcarbamazine (DEC) in 1947, a new tool to treat the lymphatic filariasis caused by both \textit{W. bancrofti} and \textit{B. malayi} was made available (Hewitt et al., 1947). DEC, a Piperazine derivative is usually produced as a citrate salt and the conventional dose of this drug is 6 mg/per kg body weight administered for 12 days. It is primarily microfilaricidal and has also been reported to have partial macro-filaricidal activity (Ottesen, 1985). It is also known for adverse reactions such as mild to severe drowsiness, nausea and
gastrointestinal upset (Ottesen, 1985). Even after a half century of its discovery, DEC remains the treatment of choice for LF infections and there are no reports of resistance of parasites till date.

A macrolide antibiotic, ivermectin is another antifilarial drug that has shown promise, especially for the control of onchocerciasis and is administered in a single 200-400 mg/kg dose. Though the drug is not commercially available in India, a few field trials have been carried out and found to be efficacious (Ramaiah, et al., 2002). Diethylcarbamazine (DEC) is the only chemotherapeutic tool currently available for controlling this disease, although Ivermectin is in advanced stage of field trials. Chemotherapeutic trials have shown that a significant proportion of microfilariae remained unaffected by the DEC treatment in some individuals, indicating that the parasite population in such individuals probably is not susceptible to the drug (Kimura and Mataika, 1996). It is essential to identify and characterize such population and subsequently investigate their prevalence among the population and also to determine their susceptibility to other drugs in order to rationalize the use of drug administration. However, mechanisms that confer susceptibility/resistance and drug response to DEC are not yet clearly known. The only known mechanism for DEC till date is that this acts through human host immune system by modulating the arachidonic acid metabolic pathway (Maizels and Denham, 1992). It is surprising to note that while for Ivermectin and Albendazole, which are late entrants, have some information on their mode of action, for DEC very little information is available. Major problem being faced in the GPELF (Global Programme for Elimination of Lymphatic Filariasis) is clearing the entire mf populations from the people with infection (mf), which calls for effective microfilaricidal drug that is effective against adult parasites.

2.8 Diagnosis

For laboratory diagnosis, the blood samples are collected on a glass slide, stained, observed under microscope and the concentration of mf is
determined. While the blood smear is a convenient and is a widely available technique, it is generally considered to be of insufficient sensitivity to be a reliable diagnostic technique. There is evidence that the concentration of mf in capillary blood collected by finger prick is higher than in blood collected by venous puncture (McMahon et al., 1979). In 1939, Knott developed the first technical method for the detection of mf, which is used till today. Bell (1967) developed the filtration method to identify and separate the mf from the sample. Dickerson et al. (1990) improved the method for preservation of unfiltered blood sample, which facilitates large-scale screening of field samples. Diagnostic aspiration of hydrocele or breast lump has also been resorted to by some investigators to detect microfilaria. Eberhard and Lammie (1991) have described protocols for preparation, staining and microscopic examination of the specimens.

The DEC provocation test, as devised by Sullivan and Hembree (1970) and later modified by different groups of scientists, involves the administration of a single, provocative dose of DEC and subsequent examination of the blood sample drawn about 15 to 90 minutes later. But this technique is unhelpful in sub-periodic bancroftian filariasis, where levels of mf rapidly fall following DEC therapy.

2.8.1 Molecular diagnosis

The identification of parasite DNA within the individual DNA using molecular tools has been verified as the most sensitive and specific method in the laboratory diagnosis of lymphatic filariasis for detection of both infection and infectivity. The high sensitivity and specificity of these methods facilitates detection of even a single microfilaria in samples. The identification of repetitive non-coding sequences from B. malayi and W. bancrofti has enabled the development of DNA-based techniques for identification of parasites. Highly sensitive and specific polymerase chain reaction (PCR) assays have been developed recently for the diagnosis of W. bancrofti and B. malayi infection. Further refinement of PCR methods to permit ELISA-based
detection of PCR products has also been made by researchers (Nutman et al., 1994).

The first PCR-based assay for detection of DNA from a human filarial parasite was developed for the detection of the *B. malayi* Hhal repeats DNA (Lizotte et al., 1994). This assay was tested successfully on human blood samples collected in Indonesia (Lizotte et al., 1994; Fischer et al., 2000; Kluber et al., 2001). The SspI PCR assay for the detection of *W. bancrofti* DNA was also developed (Zhong et al., 1996) and was first tested on blood samples collected in French Polynesia (Williams et al., 1996), India (McCarthy et al., 1996) and Egypt (Ramzy et al., 1997). The SspI PCR assay was then adapted for use on pools of mosquitoes (Chanteau et al., 1994). It was then improved and field-tested on pools of field-collected mosquitoes in Egypt (Ramzy et al., 1997). Since then, other laboratories have been successfully adopting the PCR assay for mosquitoes in different field situations (Bockarie et al., 2000; Farid et al., 2001; Hoti et al., 2001; Kamal et al., 2001). However, these techniques required standardization prior to their application as a routine monitoring tool. This assay could detect 0.1 pg of *W. bancrofti* genomic DNA, representing approximately less than 1% of the DNA found in one mf (Zhong et al., 1996) or one infective mosquito vector (Chanteau et al., 1994; Nicolas et al., 1996) and availability for processing mosquitoes in pools of 10-100 (Hoti et al., 2001).

2.9 Genetic polymorphism

2.9.1 Biological diversity

The two closely related filarial parasites, *B. malayi* and *W. bancrofti* can be easily distinguished by the cell nuclei at the caudal tip. They are visible in *B. malayi* but not in *W. bancrofti*. The adults differ in external morphology, such as in the number of papillae, while the primary distinction is in the size. The adults of *W. bancrofti* are larger in size than the adults of *B. malayi*. In addition to these two major species, there are many other species in each genus. *B. timori*, a human parasite, causing diseases similar to *B. malayi*. 
B. pahangi, the animal filarial parasite is considered to be non-infective to humans, known to form fertile hybrids with B. malayi. These three Brugia species show extensive antigenic similarities (Maizels et al., 1983). In addition, there are at least 6 species of Brugia (Sasa, 1976), and one known species of Wuchereria (W. kalimantani), found in the silvered leaf monkey Presbytis cristatus (Palmieri et al., 1980). Within individual species, the adult male worms of B. malayi show significant morphological variation with respect to their posterior cuticular ornamentation, and these features distinguish isolates from China, India, Indonesia, Malaysia and Korea (Bain et al., 1989). The most remarkable variation within the species of both B. malayi and W. bancrofti is those observed with respect to periodicity, as described earlier. This remarkable feature describes the circadian rhythm with which microfilarial numbers rise and fall in the peripheral blood, in apparent synchrony with the biting behaviour of the local mosquito species (Hawking, 1975).

Filarial literature still follows the periodic and sub-periodic subdivision as described earlier. The periodicity and subdivision of B. malayi into biological types was reexamined by Partono and Purnomo (1987) and they identified several problems. First, the periodicity indices drew an arbitrary borderline between the 'periodic' and 'subperiodic' types. Second, some isolates like East Kalimantan were in fact aperiodic, though in all other respects such as infectivity to non-human hosts were like the subperiodic. Third, isolates such as Pekan Baru showed nocturnal periodicity but were otherwise 'subperiodic' with features such as Mansonla, transmission and infectivity to laboratory animals. Partono and Purnomo reviewed the biological properties of 10 isolates from diverse localities in Indonesia and concluded that the key differentiating factor was infectivity to animals, thus reclassifying the 'periodic' type as anthropophilic and the 'subperiodic' as zoophilic.

Most B. malayi across South and East Asia is the periodic or anthropophilic type and the zoophilic type is restricted to Indonesia, Malaysia and Phillipines (Sasa, 1976). A variant of the anthropophilic type (rocky beach
strain) described as being Aedes-transmitted in Korea and Japan, but has not been further characterized (Sasa, 1976). The strain of *B. malayi* used in most laboratories is zoophilic type, originating in Pahang in West Malaysia (Edeson and Wharton, 1958). This strain is now propagated by TRS Labs Inc. of Georgia, USA and it is named as the TRS strain. There is no explicit indication of any differential pathology between the different strains of *B. malayi*, in the manner distinguishing Bancroftian filariasis from the Brugian form of the disease (Partono, 1987). Molecular polymorphisms among the *B. malayi* strains have been demonstrated by Underwood *et al.*, (2000) using two microsatellite markers. BMsat1 showed that several zoophilic isolates from Indonesia have a microsatellite allele, which is 2 bp longer than the allele from the anthropophilic parasite tested, and 2 bp shorter than the allele present in the TRS strain. One Indonesian isolates also showed 6 bp longer form of the microsatellite BMsat2. Fascinatingly, this microsatellite is suggested to reside within a novel protein coding sequence, which is maintained in-frame by the 6 bp insertion.

2.9.2 Gene diversity

Studies on polymorphism of genes of lymphatic filarial parasites are scanty. A recent study analyzed the polymorphism of the 18S rRNA gene in *W. bancrofti* mf collected from three different zones in India by PCR and restriction fragment length polymorphism (RFLP) (Bhandari *et al.*, 2005). The RFLPs of the amplified products obtained after digestion with restriction enzymes *Ssp I*, *Msp I* and *Hha I* showed no difference in the banding patterns among the mf isolates from different endemic zones. Further the sequencing of PCR products did not show any difference in the nucleotide sequence either. The phylogenetic analysis of the sequences of *W. bancrofti* mf isolates from different endemic zones has shown branching with the earlier reported sequences of *W. bancrofti* and its close relative *B. malayi*. 


2.9.3 Antigenic diversity

Antigenic variation or diversity is an evolutionary strategy for pathogens to escape the effects of a specific immune response mediated by antibodies and will decide the suitability of antigens as vaccine targets. Surface antigens are the key tools that the parasites utilize for immune evasion. An inverse relationship has been found between detectable antibody binding to the surface of mf, and the presence of mf themselves (McGreevy et al., 1980) suggesting that antibodies mediate clearance of mf. In this case, the antigens on the external sheath of mf may be expected to vary within one filarial species. Whether mf surface antigens do indeed vary has been directly addressed in one study (Ravindran et al., 1994). The target antigens of this reaction have yet to be defined. The mf sheath is composed of a tightly cross-linked set of repeat-rich proteins, together with some carbohydrate structures (Hirzmann et al., 1995; Zahner, Hobom et al., 1995). The key proteins (SHP-1, -2, -3 and variants thereof) have all been defined, but the accessibility of antibodies to each protein on acetone-fixed mf has not been established. Interestingly, Ravindran and colleagues found that antibody binding was not sensitive to protease treatment of the mf. This does not necessarily indicate a carbohydrate target, as the dense cross-linking of sheath proteins is likely to protect them from proteolysis. Molecular polymorphisms in filarial antigens have not yet been carried out at the molecular level, but interesting data have already emerged as a result of studies executed for other purposes. For example, the Filarial Genome Project has provided partial cDNA sequences from some 22,000 cDNA clones from *B. malayi* (The Filarial Genome Project, 1999) and 6000 cDNA clones from *W. bancrofti* (http://www.ajtmh.org/cgi/content/full/71/5_suppl/37). All these clones are derived from the TRS strain, and yet minor sequence variants representing putative alleles can be observed within this one dataset. These presumably correspond to polymorphisms, which have survived repeated passage through laboratory animals.
2.9.4 Diversity of Onchocerca parasites (river blindness)

The adults of the major filarial parasite, *Onchocerca volvulus* occupies a subcutaneous niche in humans, causing 'river blindness', mainly in African countries. Microfilariae infest the skin, from where a black fly (vector) uptakes the parasites to continue its transmission. Two biological types have been described: a less malignant, non-blinding forest form, and a more pathogenic, blinding savanna biotype (Duke, 1980; McMahon *et al.*, 1988). Latin American *O. volvulus* is thought to have been imported from Africa in recent centuries, although it displays distinct preferences for local *Simulium* species over the African vector (Romeo De Leon and Duke, 1966). In the Upper Orinoco region of Venezuela, it has been suggested that a distinct biotype exists, defined by mf morphology, isozyme pattern, and a high level of blood micro-filaraemia (Botto *et al.*, 1984). Most molecular studies such as isoenzyme analyses revealed a number of polymorphic loci, but no alleles unique to either type. DNA probe based on repeat sequence has been developed that hybridises only to the forest form (Erttmann *et al.*, 1987), although success depends on conditions which discriminate between different copy numbers of similar repeat sequences in the two strains (Harnett *et al.*, 1989; Meredith *et al.*, 1989). Keddie *et al.*, (1999) used sequence polymorphism of 4 antigens namely calreticulin, protein disulphide isomerase, RAL-2 and aspartyl protease inhibitor (Ov-API-1 or Ov33-3) for comparing savanna/blinding (Ghana and Mali) and forest/non-blinding (Cote d'Ivoire and Liberia) strains. It was found that there is no coding difference, and the rate of synonymous substitution varied from 0 to 2±4 per 1000 nucleotides (Keddie *et al.*, 1999). These findings of only limited diversity have been used to argue that *O. volvulus* has emerged from a recent bottleneck (Unnasch and Williams, 2000). It would be interesting, therefore, to compare sequence diversity of the homologous genes, especially those that are drug/vaccine targets.

2.10 Genome organization

The genome size is one of the fundamental characteristics that has to be known for any molecular biology investigation as it is an important indicator
of the size of the libraries to be generated and is useful to estimate the number of various gene products. By conventional karyotyping it was found that *B. malayi* has 10 diploid chromosomes. Male worms contain 8 small, one large and one medium element, whereas females contain 8 small and two large (Sim et al., 1987). By the technique of rehybridization kinetics, the haploid genome size of *B. malayi* was estimated to be $10^8$ bp with G+C content of 28% (Rothstein et al., 1988). In general intron sequences are AT rich whereas in protein coding regions average G+C content ranges from 40 to 60% (Hammond and Bianco, 1992). The genome consists of *B. malayi* 320 bp repetitive DNA element, "Hha I repeat". By DNA renaturation kinetics it has been estimated that this repeat element is present in 30,000 copies per genome and occupies 12% of the genome (McReynolds et al., 1986). It is estimated that there are around 20,000 expressed genes. In the case of *W. bancrofti*, more than 4880 ESTs were known which includes 2680 ESTs of L3, more than 70 protein sequences of *wb* are existing in the protein data bank. Number of completely annotated sequences are 5 as on 24th October 2006. (http://www.expasy.org/cgi-bin/sprot-search-de?wuchereria%20bancrofti).

2.11 Evolution

Bancroftian filariasis appears to have moved around the world by the mass migration of the human host. Sufficient numbers of infected human hosts had to migrate in order for the parasite to overcome the difficulties of establishing new infections in new geographic regions where different species of mosquito were available as potential vectors. This means that many infected human hosts migrated at the same time. Originally, a parasite of nomadic man in South-east Asia, where it is transmitted by forest *Anopheles* and *Aedes* mosquitoes, sea-faring people took the parasite to Polynesia and probably also across the Indian Ocean. The recorded movement of the human host within historic times, some of it enforced, accounts for the presence of the parasite in the New World and on formerly uninhabited islands. It is doubtful that trace, other than that in other living human persons, can carry enough infected persons to a new geographic region to establish
the infection. This appears to be the reason why *B. malayi* has not spread far out from the mainland of South-east Asia. No mass migrations took place across the seas from the mainland (Laurence, 1989).

Molecular analysis of 5S rDNA of 16 species belonging to 6 genera of filarial parasites (Xie *et al.*, 1994) appears to be first such attempt to understand the molecular phylogeny of these parasites. These studies indicated that the filarial parasites formed four clades; the *Brugia* clade; the *Wuchereria* clade; the *Brugia-Wuchereria* clade and the *Onchocerca* clade. *Litomosoides sigmodontis* and *Acanthocheilonema vitæae* were found to be the most primitive among the 16 species and that the filarial parasites were recently derived probably after the spiruridae went through a bottleneck in the cretaceous disaster about 60 million years ago (Maggenti, 1981).

Recent analysis of phylogenies of the intracellular bacteria belonging to the genus *Wolbachia* identified six major clades, termed 'supergroups', but the branching order of these supergroups remains unresolved (Casiraghi *et al.*, 2005). Supergroups A, B and E include most of the wolbachiae found thus far in arthropods, while supergroups C and D include most of those found in filarial nematodes. Members of supergroup F have been found in arthropods (i.e. termites), and have previously been detected in the nematode *Mansonella ozzardi*, a causative agent of human filariasis. The authors generated new DNA sequences of the *Wolbachia* genes encoding citrate synthase (gltA), heat-shock protein 60 (groEL), and the cell division protein ftsZ. Phylogenetic analysis data generated did not permit clear resolution of the root of the global *Wolbachia* tree; the results suggest that the transfer of *Wolbachia* spp. from arthropods to nematodes (or vice versa) probably occurred more than once. However, the evolutionary history of filarial parasites needs to be worked out based on several neutral markers in order to derive robust hypothesis.

Most of the early theories of evolution were based on morphological and geographical variations between organisms. However, it is becoming
more and more evident that the techniques from molecular biology hold a promise of providing detailed information, than what we have been able to achieve in the past (Slatkin, 1987). Population structure can be estimated by several different methods. For molecular analyses using markers such as RAPDs or DNA fingerprinting, genetic differences between individuals are generated in the form of presence or absence data (i.e. non-allelic data). Population structure is then inferred when different sub-populations exhibit varying frequencies for the different variants that make up each individual's profile (e.g. bands produced by gel electrophoresis). Estimating population structure with such data requires just the right amount of genetic polymorphism: insufficient levels do not provide sufficient resolution, but it is also possible that highly variable markers will make all individuals appear equally distant from one another, regardless of their true relationship (Cornuet et al., 1999).

2.12 Nematode polyproteins

The nematode polyproteins are allergen/antigens (NPAs) with lipid-binding and transportation function (Kennedy, 2000b) found in many nematode species (Table 4) (e.g. Ostertagia ostertagi, Dictyocaulus viviparus, A. suum, D. immitis) (de Graaf, et al., 1995). They are synthesized in the gut of parasites from where they presumably transport small lipids to muscles and gonads via, coelomic fluid (Fig. 13) (Kennedy, 2000b). They are known to bind to haeme and divalent metal ions, arachidonic acid and its metabolites, lyso-platelet activating factor, lysophospholipids and retinoids, apart from sequestering pharmacologically active lipids (Kennedy, 2000b). Very importantly, nematode parasites are unable to synthesize their own complex lipids and derive them entirely from the hosts.

First characterized in A. suum and A. lumbricoides and named ABA-1 protein of Ascaris, they are found in worms belonging to numerous species since then and have no counterparts in mammals. NPAs are extra cellular in nature. They are produced as large precursor protein complexes, comprising
<table>
<thead>
<tr>
<th>Organism</th>
<th>Name of protein</th>
<th>New name of polyprotein</th>
<th>Gene</th>
</tr>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>Ascaris suum</td>
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<td>As-NPA</td>
<td>As-npa-l</td>
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<td>Ce-NPA</td>
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<tr>
<td>Caenorhabditis elegans</td>
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<tr>
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<td>Tc-npa-l</td>
</tr>
<tr>
<td>Ascarisidella galli</td>
<td>AgFABP</td>
<td>Ag-NPA</td>
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</tr>
<tr>
<td>Setaria cervi</td>
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<td>Sc-NPA</td>
<td>Sc-npa-l</td>
</tr>
<tr>
<td>Litomosoides carinii</td>
<td>Ladder protein</td>
<td>Lc-NPA</td>
<td>Lc-npa-l</td>
</tr>
<tr>
<td>Dirofilaria immitis</td>
<td>Neutrophil chemotactic factor; Cuticular antigen; Excretory/secretory antigen</td>
<td>Di-NPA</td>
<td>Di-npa-l</td>
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<tr>
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</tr>
</tbody>
</table>

The above list of NPAs is derived from a BLAST search using the amino acid sequence of the ABA-I protein. The individual NPA (nematode polyprotein allergens/antigens) units listed are merely illustrative examples of those that have been functionally tested.

**Table 4.** The nematode polyproteins are allergen/antigens (NPAs) with lipid-binding and transportation function (Kennedy, 2000) found in many nematode species.
Fig. 13. Site of production of nematode polyprotein antigen/allergen (NPAs). *Coenorhabditis elegans* was transformed with a plasmid containing the putative promoters sequences of Ce-npa-I fused to the gene encoding green fluorescent protein (GFP). The version of GFP used in engineered to contain a nuclear localization signal, causing GFP to locate to the nucleus. The bright-field image of an egg of a transformant (a) and a fluorescence image of the same egg (b) are shown. Fluorescence is confined to the nuclei of the gut cells. Although not shown, a similar expression pattern can be seen is the gut. Construction of the plasmid, transgenic strains and microscopy by Moira Watson and Iain Johnstone, Wellcome Centre for Molecular Parasitology, University of Glasgow. Scale Bars = 8.3 μm
of 10-50 tandemly repeated polypeptide units, depending upon the species and have a short hydrophobic leader sequence (Fig. 14). The repeat units get cleaved at cleavage sties (Arg-Arg-Lys-Arg) of subtilisin serine protease at the C-terminal into functionally similar repeat units of approximately 15 kDa. Secondary structural predictions about NPAs, and direct biophysical measurements, have demonstrated that the NPAs are rich in α-helix, with no β-structure either predicted from secondary structure prediction algorithms, or detected by circular dichroism (Kennedy et al., 1995). The predictions are that each individual NPA unit protein will fold into four main regions of helix, and it has been speculated that the tertiary structure is a four-bundle helix protein, similar to other invertebrate carrier proteins (Sheriff et al., 1987).

The allergen, found in all stages of the parasite, was located as the most abundant protein species in the body fluid of parasites and secreted into infected animals (Kennedy and Qureshi, 1986; Tomlinson et al., 1989; McGibbon et al., 1990; Christie et al., 1992; Spence et al., 1993). The ABA-1 protein of A. lumbricoides (of humans) and A. suum (of pigs) is abundant in the pseudocoelomic fluid of the parasites and also appears to be released by the tissue-parasitic larvae and the adult stages (Kennedy and Qureshi, 1986; Kennedy et al., 1987a, 1989).

ABA-1 appears to be Allergen A of A. suum (Ambler et al., 1973; Christie et al., 1990) and is the target of IgE antibody responses in both infected humans and rodents (Tomlinson et al., 1989; Christie et al., 1990; Kennedy, et al., 1991; Fraser, et al., 1993; McSharry et al., 1999). However, it is yet to be established if its allergenic activity is an intrinsic property of the protein or merely due to generalized IgE potentiation by the infection (Christie et al., 1992). A homologue from filarial nematodes is also associated with Th2/IgE responses (Paxton et al., 1993; Yazdanbakhsh et al., 1995; Allen et al., 1995). Immune response against this antigen appears to be genetically restricted as only a subset of Ascaris-infected people produce antibody to ABA-1 (Kennedy et al., 1990; McSharry et al., 1999).
Fig. 14. Organization of polyprotein of three species of nematode