CHAPTER II

LITERATURE REVIEW

1. Life cycle of filarial parasite

Lymphatic filariasis is a mosquito-borne disease caused by filarial parasites: *W. bancrofti*, *B. malayi* and *B. timori* (Micheal *et al.*, 1996). The disease remains one of the most important helminthic infection worldwide. More than 120 million people in 80 countries are infected with lymphatic filarial parasites *W. bancrofti* (90% of cases) and *B. malayi* (10% of cases) (Michael *et al.*, 1996). The disease is ranked by the World Health Organization (WHO) as the second leading cause of permanent and long-term disability. Not only does it lead to great personal suffering from its disabling and disfiguring lesions, but it is also a significant impediment to socioeconomic advancement, both locally and nationally (World health report, 1995). Moreover, it has been declared by the international task force to be eliminated by the year 2020 (Behbehani, 1998).

In Thailand, *W. bancrofti* and *B. malayi* continue to be prevalent in the Thai-Myanmar and Thai-Malaysia borders with an estimated three million people exposed to the risk (Filariasis Division, 2002). Filariasis control is undertaken by the Department of Communicable Diseases Control, with plans to take up mass chemotherapy and morbidity control. The major constraints are inadequate staff, low coverage in mass chemotherapy due to side effects of DEC, slackening of carein morbidity control, and lacunae in knowledge on the possibility of zoonotic reservoir.

The mammalian hosts acquire the infection from mosquitoes carrying the infective third stage larvae (L3s), which are transmitted via a mosquito bite. The L3s are carried to the lymphatic system where they develop into L4s and adults. After mating, the adult females produce an abundance of first stage larvae or microfilariae

(Mf) which circulate in to the blood stream of infected hosts. When the microfilariae are ingested by a susceptible mosquito vector, they migrate to the thoracic muscles and develop to L3s, after 2 moulting (**Figure 2**).

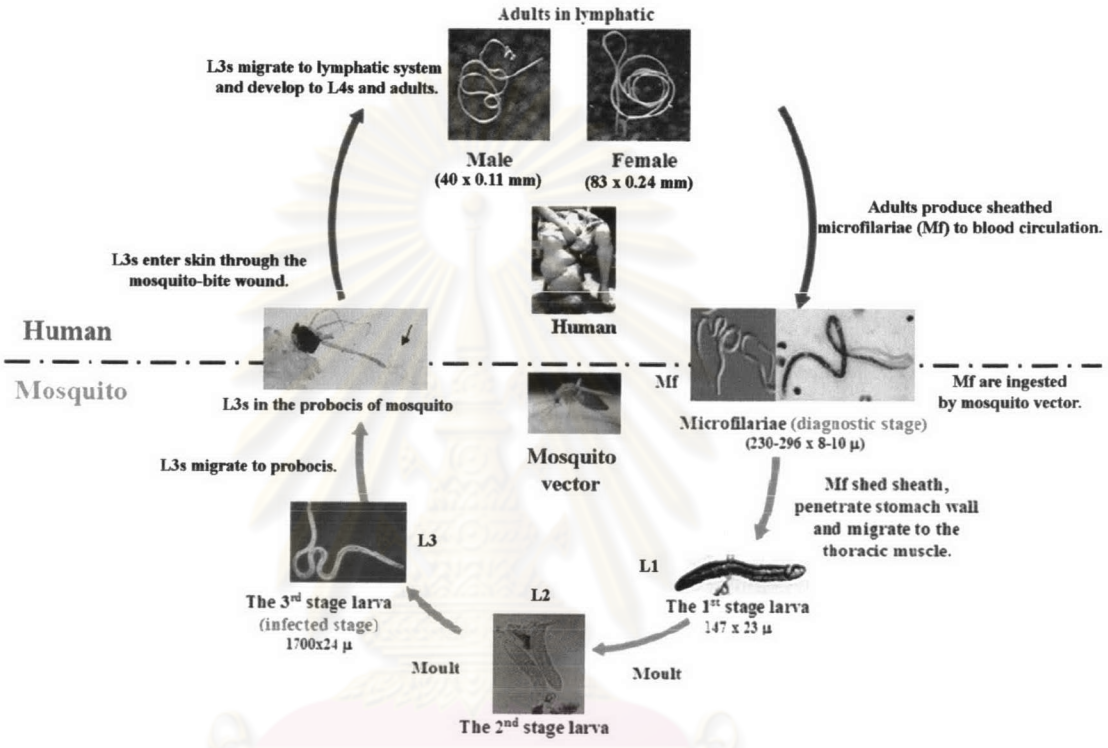

Figure 2 Life cycle of lymphatic filarial parasites.

2. Malayan filariasis

Lymphatic filariasis due to *B. malayi* is still a public health problem in many countries of Asia, particularly in India, Indonesia, Malaysia, Philippines, Sri Lanka and Thailand (WHO, 1992). So far, at least four physiological types have been declared, ie the nocturnally periodic, nocturnally subperiodic, diurnally subperiodic and non-periodic (Wilson *et al.*, 1958; Sudjadi *et al.*, 1984). In Thailand, only two types (the nocturnally subperiodic and diurnally subperiodic types) have been identified (Filariasis Division, 1998). In Southern Thailand, Surat-thani and Narathiwat provinces are endemic areas for malayan filariasis (Filariasis Division, 2001). This may be a result from suitable mosquito breeding places, many large areas of swamps, and the existence of animal reservoir hosts especially domestic cats.

In the domestic environment, it is clear that the mosquito vectors of filarial worms of domestic animals usually have greater chances of biting man than those that usually feed on the forest animals. Cats in endemic areas are reservoir hosts of *B. malayi* and/or *B. pahangi*. Control of malayan filariasis is therefore difficult since animal to man transmission continues, even after the human reservoir has been greatly reduced. The zoonotic transmission is responsible for the inefficiency of mass chemotherapy with diethylcarbamazine (DEC) in the endemic area in Malaysia (Lim and Mak, 1983).

3. Zoonotic filariae

Human infections with animal filariae, referred to as zoonotic filarisis, occur worldwide. The numbers of cases and parasite species involved have steadily increased. Many widely different species of filariae such as *B. malayi*, *B. pahangi*, *D. immitis* and *D. repens* has been identified as agents of infections (Mak *et al.*, 1980; Orihel and Eberhard, 1998).

3.1 *Brugia malayi*

Subperiodic *B. malayi* has become an important zoonotic filariae occuring in Indonesia (Java, Kalimantan, and Sumatra), Malaysia (Peninsular Malaysia), Philippines, Pacific islands, and Thailand (Dissanaike, 1979). Transmission from man to man, man to animal, or animal to man, by forest dwelling species of *Mansonia* takes place mostly in the swamp forests, or nearby villages, both indoor and outdoor. Human and domestic animals are particular exposed to bites of mosquito from the forest areas.

Epidemiologically, subperiodic *B. malayi* shows little host-specificity. It has been reported in leaf-monkeys (*Presbytis cristatus*), macaques monkeys (*Macaca irus*), palm civet cats (*Paradoxurus hermaphroditus*), wild cats (*Felis bengalensis*), the pangolin (*Manis javanica*), and domestic cats (*Felis catus*) (Laing *et al.*, 1960). Subperiodic *B. malayi* has been experimentally transmitted from man to cats and to other animals, including monkeys and slow loris (*Nycticebus coucang*), civets, dog (*Canis familaris*), hamsters, and white rats (*Rattus* spp.) and others rodents (Laing *et al.*, 1960). *B. malayi* causes the lymph thrombi within affected afferent lymphatic vessels of cats infected. It can naturally and experimentally transmit from cats to man. (Buckley, 1958; Edeson *et al.*, 1960a; Dondero *et al.*, 1972). The ability to develop in such wide range of vertebrates shows its remarkable adaptability.

3.2 *Brugia pahangi*

Althouh *B. pahangi* has been found occasionally in leaf-monkeys and slow loris, it is mainly a parasite of domestic cats, dogs, and of wild carnivores. In this respect, *B. pahangi* appears to be more a parasite of primates. In a long-term infection with *B. pahangi*, the affected afferent lymphatic vessels and popliteal nodes of infected cats are almost obliterated by fibrous tissue, such nodes often become non-functional, and by-passed by new lymphatic vessels, but an inguinal lymph node complex usually develop to replace lymphatic filtration in the limb (Rogers *et al.*, 1975).

Experimentally, *B. pahangi* can be transmitted from cats to man (Edeson *et al.*, 1960a), in whom low level of microfilaremia can be detected. Clinical manifestations, similar to tropical eosinophilia syndrome, have been reported in a human volunteer experimentally exposed to *B. pahangi* (Buckley, 1958). In Indonesia, humans could be infected by both *B. malayi* and *B. pahangi* (Palmerie *et al.*, 1985).

3.3 Dirofilariasis

Human dirofilariasis is zoonosis due to infection with *D. immitis* and *D. repens*. These parasites are mosquito-transmitted. Human are accidental hosts for the filaria in whom the life cycle cannot be completed (Disssanaike, 1979).

D. immitis (heartworm) is a common parasite of dogs and other canids in many regions of the world, and on occasion has been identified in other animals such as seals, beavers, horses, domestic and wild cats, bears, nutrias, and muskrats. The adult worms live in the right side of the heart and produce microfilariae, which circulate in the peripheral blood. The large number of mosquito species that can transmit the parasite, and the shared environment of humans and dogs, probably accounts for the frequency with which humans are infected. Human infections with *D. immitis* have been reported in many parts of the world, and are found wherever the parasite is enzootic (Muro *et al.*, 1999; Pampiglione *et al.*, 1991; 2001).

Although adult *D. immitis* worms have been found on several occasions in the heart and major vessels of humans at necropsy (Faust *et al.*, 1941; Abadie *et al.*, 1965; Goldstein and Smith, 1985), the usual finding is immature worms located in partially or completely occluded small pulmonary arteries, where the obstruction has produced an infarct and eventually a well-circumscribed, granulomatous, coin lesion containing the worm (Beaver and Orihel, 1965). The lesions, which measure about 1 to 3 cm in diameter, are frequently found on routine chest X-rays. Because these lesions mimic neoplasms and other pathologic conditions (including tuberculosis, fungal infections, and hamartomas), they typically receive immediate medical

attention. As often as not, individuals with coin lesions in the lung are completely asymptomatic. When symptoms are associated with the lesions, they include cough, chest pains, moderate eosinophilia, and occasionally hemoptysis and fever (Boreham, 1988).

D. repens is a parasite of subcutaneous tissue of domestic and wild carnivores such as cats, dogs and foxes (Disssanaike, 1979; Smith, 1995). The filaria is prevalence in Europe, mainly in Mediterranean basin, sub-Saharan Africa, and southern Asia (Settnes and Engebjerg, 1991; Hira *et al.*, 1994). An increasing number of zoonotic infections with the filaria from different parts of the world have been reported (Disssanaike, 1979; Pampiglione *et al.*, 1995). As the filariae die in the human tissue, local imflammatory responses manifest as subcutaneous nodule, and it has often been confused with tumor (Disssanaike, 1979; Settnes and Engebjerg, 1991; Latifoglu *et al.*, 2002).

4. Filarial nematodes in animal reservoirs in Thailand

Blood examination of cats, dogs, monkeys, rats and other rodents have been carried out in several surveys in the endemic areas in the southern part of Thailand. In 1970, a study on malayan filariasis was made in Chumporn, a province in southern Thailand (Figure 1). The blood samples from 110 cats, 98 dogs and 5 monkeys were examined. Larvae of *D. repens* and *D. immitis* were found in 9 cats (8.18%) and 50 dogs (51.02%) respectively. However, *B. malayi*-like microfilariae were found in only 2 cats (0.90%). One filarial worm resembling female *B. malayi* was recovered from an inguinal lymph gland of a cat. *B. malayi*-like microfilariae were also found in 8 of 289 cats (2.77%) in Chumphorn province in 1971 (Guptavanij *et al.*, 1971a, b).

In 1986, the species identification of parasite was made by Phantana *et al.* using Geimsa stain. In this report, thick blood films of cats from endemic area in Surat-thani province were stained and the Innenkorper of microfilariae were measured

to confirm the species of *B. malayi*. In 1987, blood examination of both stray and domestic cats in Narathiwat province revealed that 4.13% were positive for *B. malayi*-like microfilariae (Phantana *et al.*, 1987).

In 1999, blood examinations of 294 domestic cats in Surat-thani province showed that 7 (2.4%), 1 (0.3%) and 4 (1.3%) cats were positive for *Brugia* sp., *D. immitis* and *D. repens*, respectively (Filariasis Division, 2001). Blood examinations of domestic cats in the same area were under taken, that revealed 8 of 265 cats (3.0%) were positive for *Brugia* sp in 2000, 1 of 63 cats (1.6%) were positive for *Brugia* sp. in 2001, while no *D. immitis* and *D. repens* were detected (Filariasis Division, 2001).

In 2001, *B. malayi*-like from an infected cat from Narathiwat province, was identified intensively by microfilarial morphometry, acid phosphatase activity, and adult morphology. The result indicated that both microfilarial and adult charactertics conformed to topotypic *B. malayi* (Karnjanopas *et al.*, 2001). In 2002, *B. malayi* was found in 7 domestic cats in Pra-sang subdistrict, Surat-thani province. Therefore, the cat plays an important role as the animal reservoir for *B. malayi* in the endemic areas of Thailand.

5. Species differentiation of filarial parasites

As noted above, the species differentiation between *B. malayi* and *B. pahangi* is rather difficult to achieve. Numerous methods have been described in attempts to differentiate microfilariae of subperiodic *B. malayi* from those of *B. pahangi*. Schacher (1962) discussed his own attempts and those of numerous other workers to separate the two species by standard measurement techniques. Other investigators have described differences in the length of the 'Innenkorper' (Sivanandam and Fredericks, 1966), varitions in the sheath-casting frequency (Sivanandam and Dondero, 1972), morphologic differences in rectal protrusions (Beckett and Macdonald, 1972). In addition, attempts have been made to separate the

two filarial species by biologic means using mosquitoes of the genus *Armigeres* which are very good vectors for *B. pahangi* but poor vectors for *B. malayi* (Edeson *et al.*, 1960b; Cheong *et al.*, 1965). All of these techniques, however, are time consuming, tedious, or not completely reliable for consistent differentiation.

Chalifoux and Hunt (1971) introduced a histochemical method for demonstrating differences between the microfilariae of two common canine filarial worms, *D. immits* and *Dipetalonema reconditum*, based on the specific distribution of acid phosphatase activity in these organisms. The same technique can be used to differentiate between microfilariae of *B. malayi* and of *B. pahangi* (Redington *et al.*, 1975). In *B. malayi*, the excretory vesicle and the anal vesicle, and to a lesser extent, the amphid and phasmid areas exhibit acid phosphatase activity with little such activity seen in the remainder of the body. The micrifilariae of *B. pahangi*, in contrast, show heavy diffuse acid phosphatase activity along their entire length. The excretory vesicle and the anal vesicle are still recognizable because of their more intense staining. This relatively simple histochemical method is more advantageous and reliable than the morphologic and biologic methods for differentiating these of two microfilarial species. However, this technique needs fresh samples to yield the best results and require expertise to identify and confirm the species.

6. Species differentiation by PCR-RFLP of ITS1 of ribosomal DNA

Ribosomal RNA gene (rDNA) family is a multigene family consisting of many copies (100- 500 in animals) of genes. Eukaryotic rDNA consists of tandemly repeated clusters of the 18S, 5.8S, and 28S genes separated by two internally transcribed spacers, ITS1 and ITS2 (Long & Dawid, 1980) (**Figure 3**). The multiple copies of the highly conserved rRNA genes are probably maintained because cells require large amounts of the particular product, which a single copy could not produce in appropriate time.

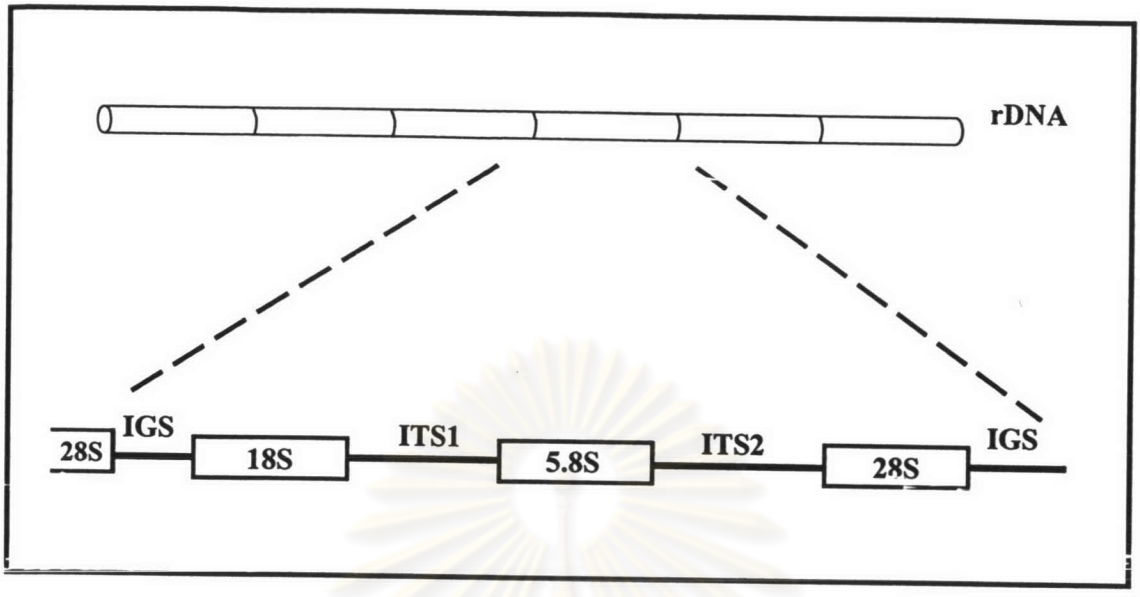

Figure 3 Diagram of a eukaryotic ribosomal RNA gene family in animals, which consisting of tandem repeats of ribosomal genes and spacers: IGS, is the intergenic spacer located between each set of ribosomal genes. The regions coding for the 5.8S, 18S, and 28S subunits of rDNA are shown by bars. ITS1 locate between 18S and 5.8S rDNA and ITS2 locate between and 5.8S and 28S rDNA.

The rDNA evolve cohesively within a single species and exhibit only limited sequence divergence among rDNA copies within single individual (Arnheim *et al.*, 1980). In contrast, comparisons between species show normal levels of sequence divergence. Combination of these two observations is referred to as concerted evolution (Dover, 1982; Elder and Turner, 1995). The mechanisms driving concerted evolution are unequal crossing over and gene conversion. Irrespective of the exact mechanism, the degree of homogenization is a result of the interplay between homogenization mechanism and mutation process (Schlotterer and Tautz, 1994).

Highly conserved regions in the ribosomal repeat array can be used for study of relationships across phyla. More variable regions can be used at lower taxonomic levels. The ITS region does not encode for any product, permitting it to evolve at a faster rate than the ribosomal coding regions. The level of variation in this region makes it suitable for detecting genetic variation among genera, species and within species (Dlauchy *et al.*, 1999; Gondim *et al.*, 2004).

The advent of DNA technology has provided alternative approach for the identification of parasites (Callaghan and Beh, 1994; Christensen et el., 1994). The rDNA are among the most useful targets because they evolve in a 'concerted fashion', that is, the DNA sequence of an individual is uaually reperesentative of a species (Brown *et al.*, 1972). Ribosomal genes are abundant in each organism (Hillis and Dixon, 1991), making it feasible to develop highly sensitive diagnostic techniques. The use of polymerase chain reaction (PCR) employing 'conserve' oligonucleotide primers has made it possible to characterize a broad range of parasitic organisms from minute quantities of materials.

The ITS regions of rDNA contain reliable genetic markers to distinguish closely related species of protozoan, trematodes, cestodes, arthropods and nematodes (Wesson *et al.*, 1992; Adlard *et al.*, 1993; Bowles and McManus, 1993; Morgan and Blair, 1995), whereas sequences of rDNA coding regions appear to be less

useful for species identification due to a low level of sequence divergence (Zarlenga *et al.*, 1994).

PCR-RFLP have been employed to define genetic marker in the ITS for identification parasite nematodes to the species level. For example, *Ascaris lumbricoides* from humans and *A. suum* from pigs are difficult to be differentiated by morphology. However, *Ascaris* species from different hosts can be distinguished by PCR-RFLP (Zhu *et al.*, 1998). The ITS provides genetic markers for identification strongylid nematodes to species (Chilton *et al.*, 1995; Gasser *et al.*, 1994; Gasser and Hoste, 1995; Stevenson *et al.*, 1996; Hung *et al.*, 1996). For filarial nematodes, the PCR-RFLP of ITS1, digested with *Ase* I, can be used to differentiate *B. malayi*, *B. pahangi*, and *D. immitis* (Nuchprayoon *et al.*, 2003).