CHAPTER II

LITERATURE REVIEW

Lymphatic Filariasis

The filarial nematodes of human; *W. bancrofti*, *B. malayi* and *B. timori* (Micheal *et al,* 1996), belong to the superfamily Filarioidea (Ubelaker, 1993). They are slender parasitic nematodes in the circulating and lymphatic systems, muscles, connective tissues, or serous cavities of the vertebrate. In Thailand, *W. bancrofti* and *B. malayi* are the causative agents for lymphatic filariasis in humans. The worms lodge in the lymphatic system. When an adult male worm mates an adult female worm, the female worms release a large number of microfilariae which circulate in the infected individuals' bloodstream. Both *W. bancrofti* and *B. malayi* microfilariae retain their egg membrane, referred to as a sheath. The disease is transmitted from person to person by various genera of mosquitoes. When the mosquitoes bite an infected human, they pick up the microfilariae from the bloodstream. Then the microfilariae develop in the mosquitoes into infective larval stage (L3). The larvae then migrate to the mosquitoes' mouths and can be transmitted to other hosts via mosquito bites. Having passed through the skin, the larvae travel to the lymphatic system where it take about one year to fully develop into adult worms (figure 3).

Signs and symptoms of lymphatic filariasis

Like many parasitic diseases, pathogenesis is driven by the strong host inflammatory response. Filariasis can be regarded as having 4 clinical manifestations: (1) Asymptomatic amicrofilaraemia, (2) Asymptomatic microfilaraemia, (3) Acute mansifestations, and (4) Chronic manifestations (WHO, 1992). The asymptomatic manifestation is the time during which the larvae complete their development. In this phase there is usually no or mild inflammatory symptoms. The infected individuals indeed have hidden lymphatic filariasis pathology as evidenced by lymphoscintigraphy (Freedman *et al.*, 1994; Dissanayake *et al.*, 1995) and kidney damage (Dreyer *et al.*, 1992). The asymptomatic microfilaraemic is indicated by the

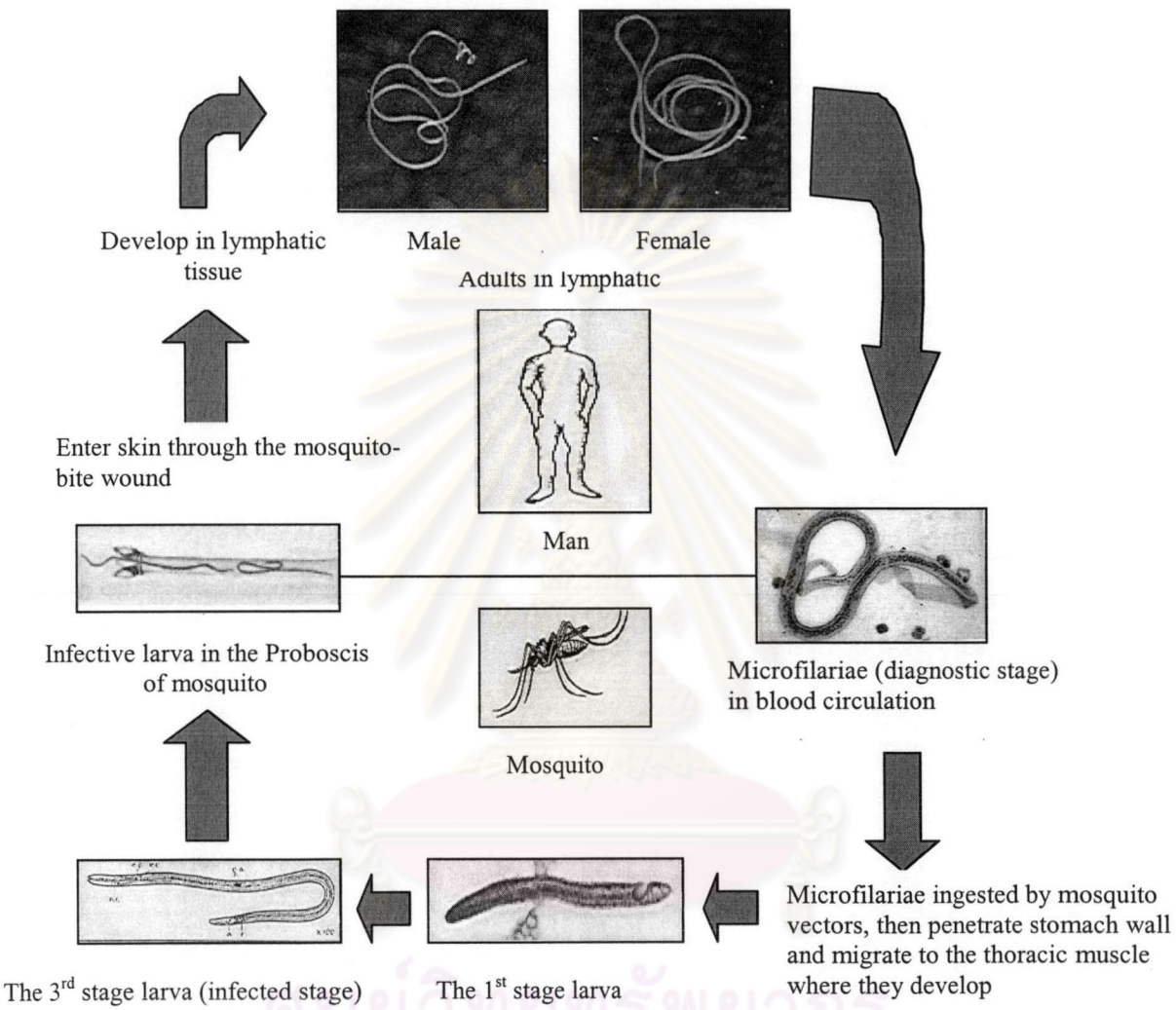

Figure 3 Life cycle of lymphatic filarial parasites.

presence of microfilariae in the blood circulation, and adult worms can be located in the lymphatic system. In case of no microfilariae presence in blood stream, the specific antigen (Rocha *et al.*, 1996; Weil *et al.*, 1996) or ultrasound (Dreyer *et al.*, 1996) were used to identify the disease.

During the acute phase, when the adult females are releasing large numbers of microfilariae, there is intense lymphatic inflammation accompanied by chills, fever and toxemia (Ramaiah *et al.*, 1996). Swollen lymph nodes can be very painful and the skin overlaying the lymph nodes is often inflamed. In males, hydrocoele (fluid-filled balloon-like enlargement of the sacs around the testes) always occur (WHO, 1999).

At the cellular level, there are inflammatory cells, particularly polymorphs eosinophils and lymphocytes infiltrate the lymph node linings (Jungmann *et al.*, 1992). Adult worms are mostly killed within the abscesses forming around them, many of which develop into the bacterial infections (Olszewski *et al.*, 1997).

The obstructive phase is so far most debilitating. The blockage of the lymphatic ducts causes back up of the lymphatic fluid, which distends the lymphatic vessels. The lymph passes into the surrounding tissue causing distension of the scrotum, hydrocoele, and forces lymph into the urine, causing chyluria. The affected areas become progressively fibrotic which leads to scar tissue formation which is not reversible after treatment of the infection. The excess tissue can only be removed by surgery. The repeated cycles of lymphatic fluid infiltration followed by fibrosis can lead to very large disfiguring limbs. Often the skin becomes dry, crack and easily contaminated by secondary infection of fungi and bacteria. Many symptoms associated with lymphatic filariasis are also related to secondary bacterial infections which allow it to be simply treated with antibiotics (Olszewski *et al.*, 1997), improvement of hygiene and exercise to increase lymph flow (WHO, 1992). In the endemic areas, chronic and acute manifestations of filariasis tend to develop faster and more frequent among the refugees or newcomers than among local residents who are continually exposed to the infection.

Diagnosis of lymphatic filariasis

The standard technique employed to diagnose lymphatic filariasis is microscopic examination. Morphological identification of microfilariae such as pattern, numbers of nuclei, shedding of sheaths which circulate in blood requires specialized expertise and is extremely time-consuming. The diagnostic methods were improved to facilitate surveillance activities and to monitor control effort. Since the development of serodiagnostic tests, antigen and antibody detections had been used to identify the infection; the antibody detection assay does not discriminate between current and previous infections (Chanteau *et al.*, 1994; Triteeraprapab *et al.*, 2001a). The ideal diagnostic test in field study should be sensitive for active infection. Specific antigen detection has been in focus. Although the detection of specific antigen is more effective than specific antibodies (Chanteau *et al.*, 1994; Triteeraprapab *et al.*, 1998; Triteeraprapab *et al.*, 2001a), specific antigen detection may be missed in case of co-infection and zoonotic infection. In order to overcome some of these problems, DNA-based methods have been developed for parasites detection. Polymerase Chain Reaction (PCR) is a method for detecting species-specific DNA segments, *Ssp* I repeated sequences (Zhong *et al.*, 1996; Williams *et al.*, 1996; Nuchprayoon *et al.*, 2001) which found in *W. bancrofti* and *Hha* I repeated sequences in *Brugia sp.* (Lizotte *et al.*, 1994; Triteeraprapab *et al.*, 2001b). The sensitivity and specificity of the PCR method were applied to identify the mosquitoes harboring filarial larvae, which is more effective than the traditional dissection technique (Chanteau *et al.*, 1994; Ramzy *et al.*, 1997; Triteeraprapab *et al.*, 2000). Recently, rDNA has received much attention for species differentiation in nematodes. They are versatile genetic markers in many organisms, as diverse as protozoa (Morgan *et al.*, 1997), plants (Siboe *et al.*, 2000) and fungi (Kumeda & Asao, 1996). Non-transcribed spacer regions might be exploited in the evolutionary studies.

Ribosomal RNA Genes in organisms

Eukaryotic nuclear rDNA is a multigene family consisting of tandem repeat of genes interspersed with transcribed and non-transcribed spacers (Long & Dawid, 1980). Ribosomal DNA present patterns of concerted evolution, which has been

defined as the non-independent evolution of repetitive sequences, resulting in sequence similarity which is greater within a species than between species (Elder & Turner, 1995). The cluster of rRNA genes is identified in the chromosomal site called nucleolar organizer (Wallace & Birnstiel, 1966). These genes are transcribed by RNA polymerase I (Chambon, 1975) and then processed in several steps to yield mature rRNA (Perry, 1976). 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. Large variations in gene numbers exist not only between closely related species but also between different strains of the same species, as described in *Drosophila melanogaster* (Ritossa & Scala, 1969; Spear & Gall, 1973). In addition, it is likely that the ribosomal gene number is polymorphic in any population (Long & Dawid, 1980). Ribosomal genes are usually found on homologous chromosomes in closely-related species, such as chimpanzees and humans (Tantravahi *et al.*, 1976). This section of genome includes the 18S, 5.8S and 28S gene, which code for ribosomal RNA (rRNA) (figure 4). It also includes the variable DNA sequence areas of the intervening internal transcribed spacer (ITS) regions called ITS1 and ITS2. Although the ITS regions were not translated into proteins, they have a critical role in the development of functional rRNA (Atanas *et al.*, 2000; Abeyrathne *et al.*, 2002). Some of these extra RNA sequences are thought to play a transient part in ribosome assembly (Musters *et al.*, 1990).

Species differentiation with rDNA

Because the ITS regions of the nuclear rDNA are highly variable, they can be used as the ideal marker for discriminating species. Along with the high level of variation but also the conserved rRNA genes flanked in this region makes them suitable for detecting genetic variation among genera, species and within species. Many parasite species have been characterized using these regions and the results show that ITS could distinguish the variation. The majority of the studies have focused upon human and animal parasites; Studies in Strongylid nematodes have demonstrated that the ITS2 region is valuable region for species identification (Hoste

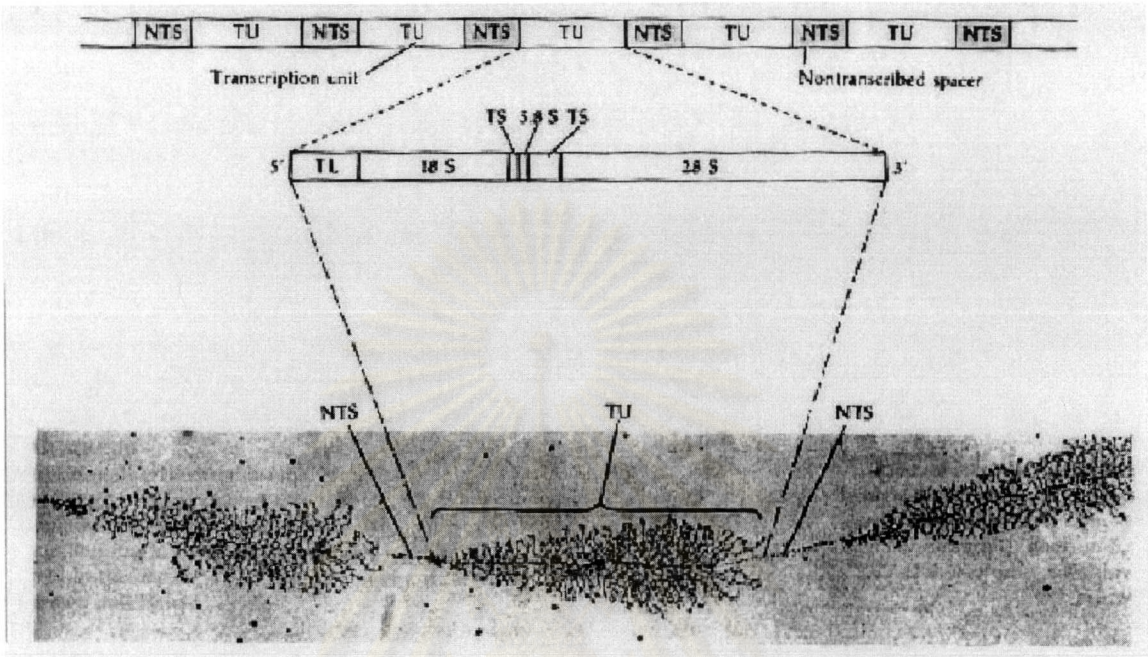

Figure 4 Organization of the genes for 18S, 5.8S, and 28S ribosomal RNA. rRNA present as tandem repeats in every animal genomes. ITS1 and ITS2 locate between 18S-5.8S and 5.8S-28S rRNA, respectively. Each unit of rRNA is transcribed separately as seen from the figure.

et al., 1993; Campbell *et al.*, 1995; Hoste *et al.*, 1995; Stevenson *et al.*, 1995). Ramachandran and his group delineated *Strongyloides stercoralis* from human and animal isolates using ITS regions (Ramachandran *et al.*, 1997). The ITS regions were also successful in differentiating filarial parasite species between *Litomosoides galizai* and *L. sigmodontis* (Gasser *et al.*, 1996) and between *Onchocerca volvulus* and *Mansonella ozzrdi* (Morales-Hojas *et al.*, 2001). *Ascaris lumbricoides* from humans and *A. suum* from pigs are difficult to differentiate by morphology. Zhu and colleagues have characterized individual *Ascaris* species from different hosts by PCR-RFLP (Zhu *et al.*, 1999). The ITS2 region was also used to delineate *Oesophagostomum bifurcum* from *Necator americanus*, human hookworm, in feces which the parasites co-exist in the northern of Togo and Ghana (Romstad *et al.*, 1997; Verweij *et al.*, 2000). Not only in nematodes, the ITS regions were also used to study nematodes and in the other parasites and organisms such as the differentiation of *Aedes triseriatus*, vector of LaCrosse virus, from *Ae. hensersoni* (Reno *et al.*, 2000). The previous studies could discriminate among the isolate of *Cryptosporidium* from a range of hosts using ITS regions (Morgan *et al.*, 1997; Morgan *et al.*, 1999).

Polymorphism detection assays

There are many PCR-based DNA analysis methods that are used to study the genetic variation in parasites (McKeand, 1998; Gasser & Zhu, 1999). A mutation detection technique, single-strand conformation polymorphism (SSCP), is a very effective technique for identification parasite species and in different developmental stages of hookworm (Gasser *et al.*, 1998). This technique relies on the physical properties of DNA molecules (Gasser, 1997) but it can produce false-positive results (Gasser & Zhu, 1999). Direct sequencing of PCR products can detect all variable positions but PCR artifacts can lead to incorrect data. PCR-RFLP using endonucleases is also useful because it is an uncomplicated method and has cost-associated advantage over direct sequencing. The variations in the length of DNA fragments are generated by digestion of restriction endonucleases. These variations are consequences of natural sequence differences between individuals. Most of the known RFLP patterns occur as a result of point mutations or single-nucleotide variations in DNA sequence

(Singh, 1997). Not all natural variants can be detected by RFLP analysis, as many do not result in a change in the length of restriction fragments generated with known enzymes.