CHAPTER I

INTRODUCTION

1. BACKGROUND AND RATIONALE

Lymphatic filariasis is a debilitating and disfiguring disease, caused by filarial nematodes, *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*. The disease is widespread throughout tropical and sub-tropical regions worldwide (WHO, 1992; Michael *et al.*, 1996) (figure 1). Ninety percent of the infections are caused by *W. bancrofti*; most of the rest by *B. malayi* or *B. timori* (Ottesen *et al.*, 1997). It is estimated that 1.1 billion people, 20% of the world population, in more than 80 countries are at risk of acquiring the infection, whereas over 120 million have already been infected (WHO, 2000). Though the disease is not fatal, it is a critical cause of acute and chronic illness that affects people of both genders and at all walks of life. More than 40 million people are disabled and deformed by the infection (Ottesen *et al.*, 1997). It is ranked by the World Health Organization (WHO) as the second leading cause of permanent and long-term disability (World Health Report, 1995). It causes enlargement of the limbs, reproductive organs, pneumonitis and pathology of the renal function (Dreyer *et al.*, 1992). Moreover, the overt abnormalities lead to psychological consequences and the loss of labor, which can be severely devastating to household affairs and destructive to local and national economies (Ramaiah *et al.*, 2000). Because lymphatic filariasis is one of only seven infectious diseases; polio, leprosy, dracunculiasis, **filariasis**, onchocerciasis, measles and Chagas' disease (WHO, 2000), considered eradicable or potentially eradicable, the disease has been targeted by WHO to be eliminated by the year 2020 (Behbehani, 1998).

The prevalence of lymphatic filariasis in Thailand is estimated to be 0.99 cases/100,000 (Filariasis Division, 1999). Presently, the endemic areas located in 5 out of 76 provinces: Mae Hong-Son, Tak, Kanjanaburi, Surat-thani and Narathiwat. Lymphatic filariasis, caused by *B. malayi* nocturnal subperiodic type, is endemic in southern of Thailand, mainly in Narathiwat province. The main mosquito vector of brugian filariasis disease is *Mansonia sp. B. malayi*, diurnal subperiodic type, is found

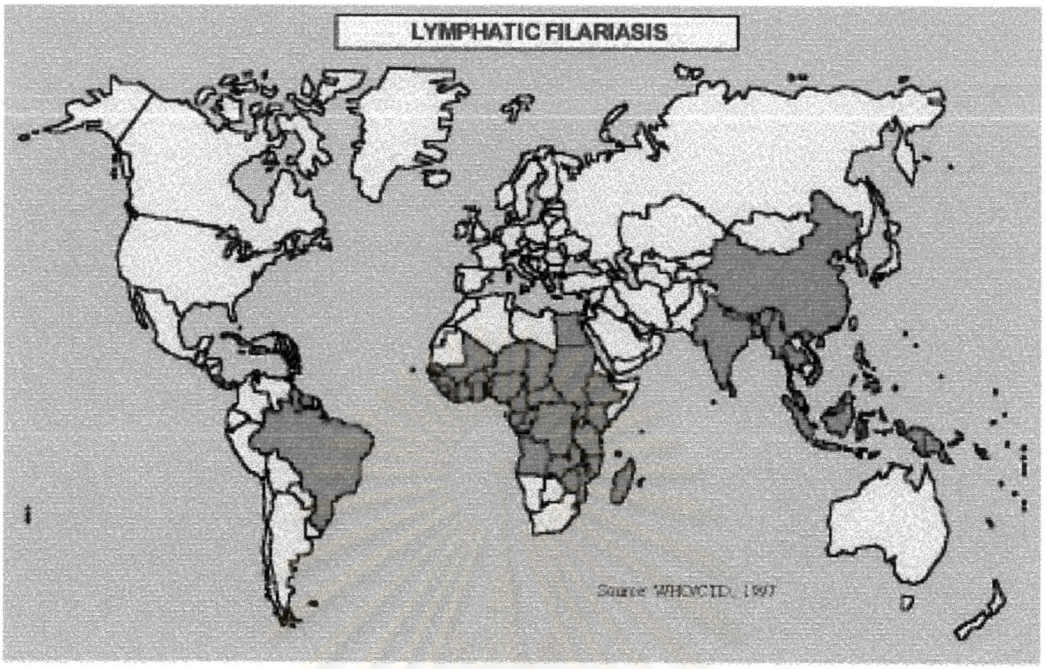

Figure 1 Distribution of lymphatic filariasis. The endemic areas of lymphatic filariasis are shown in red.

in a few cases in Surat-thani (Filariasis Division, 1998). In Thailand, *B. malayi* has domestic cats as animal reserviors (Phantana *et al.*, 1987).

In Thailand, the endemic areas of *W. bancrofti* are adjacent to the Thailand-Myanmar border, Mae Hong-Son, Tak and Kanjanaburi provinces (figure 2). The strain of *W. bancrofti*, common among the Thai-Karens is the nocturnal sub-periodic form (rural type), and the main mosquito vectors belong to the *Aedes niveus* group (WHO, 1992); however, the migration of the Myanmar migrants to Thailand has jeopardized the overall public health of Thai people. The Myanmar migrants also carry *W. bancrofti* nocturnal periodic form (urban type), which has *Culex quinquefasciatus* as its main mosquito vector (WHO, 1992). Since *Cx. quinquefasciatus* is common in Thailand, and they can transmit filarial parasites under laboratory conditions (Kanjanopas, 1997; Triteeraprapab *et al.*, 2000). The migrant population from Myanmar could form a new and important reservoir of bancroftian filariasis in Thailand (Triteeraprapab *et al.*, 1999; Triteeraprapab *et al.*, 2000).

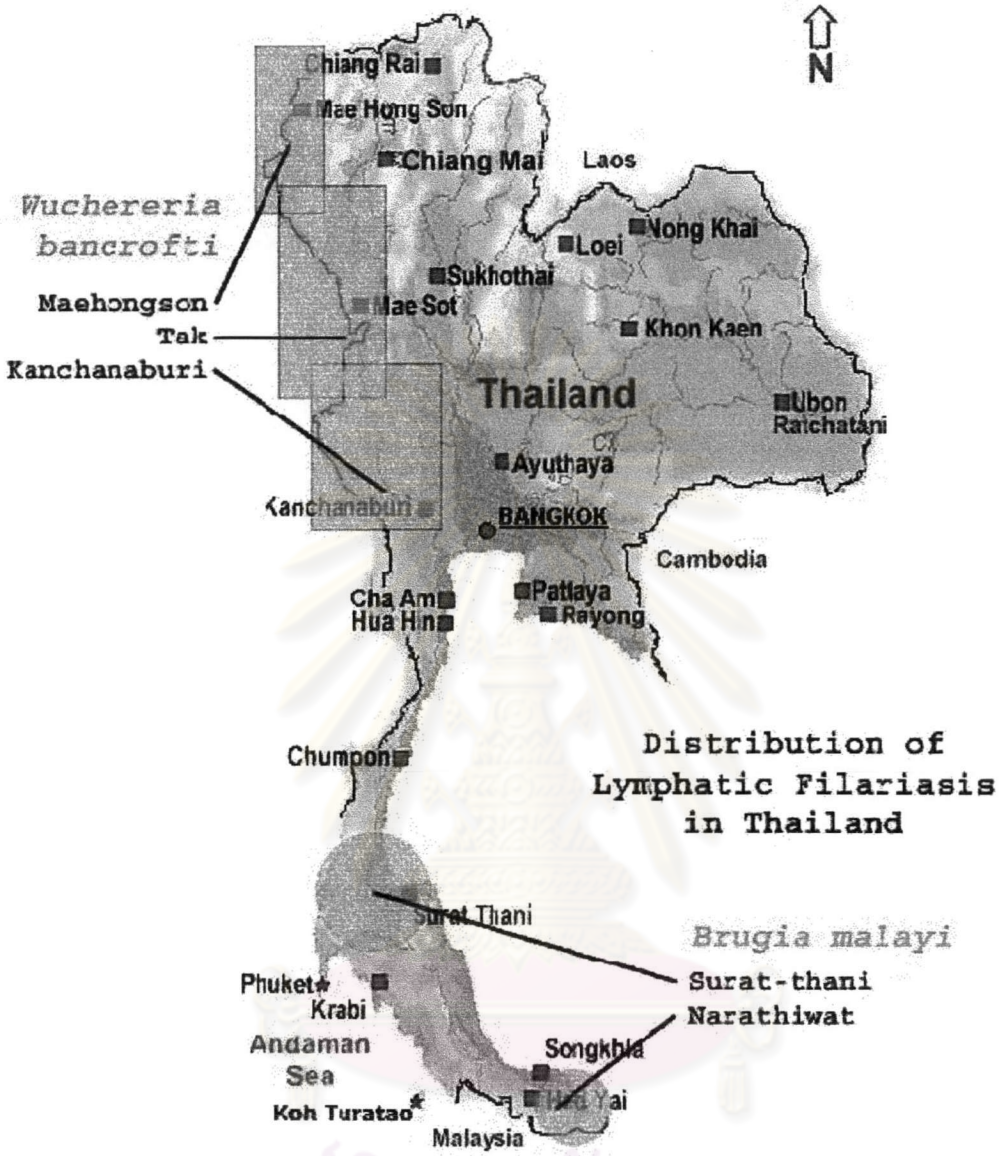

Figure 2 Geographic distribution of lymphatic filariasis in Thailand

□ Endemic areas of *W. bancrofti*: Mae Hong-Son, Tak and Kanjanaburi provinces

○ Endemic areas of *B. malayi*: Surat-thani and Narathiwat provinces

Since there are many filarial infected dogs and cats in Bangkok, Thailand. The filarial species that always infect dogs are *Dirofilaria immitis* and *B. pahangi*, for cats are *B. pahangi*. (Nithiuthai & Chungpivat, 1992; Chungpivat & Sucharit, 1993). Not only human microfilariae (*W. bancrofti, B. malayi and B. timori*), but also some animal filariae such as *D. immitis* had been found to affect human as zoonotic infections (Beaver *et al*, 1984; Theis *et al.*, 2001). These observations showed the possibility of cross-infection of human and animal parasites that may lead to unreliable diagnosis.

At present, the conventional method for species differentiation of microfilaria species is microscopic method based on the delineation of particular morphological features using Giemsa stain, and location where the specimens come from. Nevertheless, the technique is difficult and is unable to discriminate clearly between closely-related species such as *B. malayi* and *B. pahangi*. The histochemical staining, Acid phosphatase activity, could overcome the problem, but it needs fresh samples to yield the best results. Both staining methods require an expertise to identify and confirm the species. The molecular analysis has been also introduced as a new tool to determine the parasite species. Ribosomal DNA (rDNA) has provided valuable approaches to specify genetic markers for closely-related species identification among several eukaryotic organisms, including nematodes. The application of the ITS to identify the organism has received the most attention by nematologist during the last ten years (Gasser *et al.*, 1994; Gasser *et al.*, 1996; Zhu *et al.*, 1998; Almeyda-Artigas *et al.*, 2000; Conole *et al.*, 2001). The recent studies using PCR-linked restriction fragment length polymorphism (PCR-RFLP) profiles of the internal transcribed spacers (ITS) from nematodes are one way to assess nematode diversity, as well as to provide critical taxonomic character useful for species comparison and identification (Gasser *et al.*, 1994; Gasser *et al.*, 1996). In this study, individual species of human filarial nematodes, *W. bancrofti* and *B. malayi*, had been characterized by PCR-RFLP. In addition, the filarial nematodes from cats (*B. malayi* and *B. pahangi*) and dogs (*D. immitis*) were also characterized.

2. RESEARCH QUESTIONS

Primary Question

Can ITS1 and ITS2 regions be used as a marker to identify some human and animal filarial nematodes?

Secondary Question

What are the patterns of digested PCR product of ITS1 and ITS2 among the filarial nematodes tested (*W. bancrofti*, *B. malayi*, *B. pahangi* and *D. immitis*)?

3. LIMITATION OF THE STUDY

Most of the infected individuals stay at remote areas; therefore, we had the problems concerning the specimen conditions for histochemical study.

4. OBJECTIVE OF THIS RESEARCH

The aim of the study is to differentiate filarial nematodes species by PCR-RFLP of the ITS1 and ITS2 regions.

5. KEYWORDS

ribosomal DNA
filarial nematodes
PCR-RFLP

6. EXPECTED BENEFITS & APPLICATIONS

From our finding, its can be expected as follows:-

1. Establish a novel molecular marker to identify human filarial nematodes.
2. Identify the difference of PCR and digested PCR products of ITS1 and ITS2 regions among filarial nematodes tested.
3. Study the epidemiology of filarial parasites

7. RESEARCH METHODOLOGY

- Blood collection from:

  W. bancrofti from infected individuals in Tak province.

  B. malayi from infected individuals in Narathiwat province.

  B. malayi from infected cats in Narathiwat province.

  B. pahangi from infected cat at Parasitology Unit, Department of Pathology, Faculty of Veterinary, Chulalongkorn University

  D. immitis from infected stray dogs in Bangkok.

- Study Process

Step 1. DNA extraction from blood samples

Step 2. DNA purification

Step 3. DNA amplification

Step 4. PCR product precipitaion

Step 5. Digestion of PCR products with restriction enzymes

Step 6. Agarose gel electrophoresis

8. ADMINISTRATION AND TIME SCHEDULE

Phase	Process	Time schedule (months)								
		4	8	12	16	20	24	28	32	36
1	Preparation phase	●	●							
2	Sample collection		●	—	—	—	—	—	●	
3	Data analysis						●	—	—	●
4	Report								●	●