CHAPTER I

INTRODUCTION

1. Backgrounds and Rationale

Lymphatic filariasis, mainly caused by filarial nematodes *Wuchereria bancrofti* and *Brugia malayi*, is a major debilitating and disfiguring disease. It is estimated that 1.1 billion people, 20% of the world population, in more than 73 countries, are at risk of acquiring the infection, while over 120 million individuals have already been infected (WHO, 2000). In Thailand, *W. bancrofti* and *B. malayi* are the causative agents for lymphatic filariasis in humans. Although filariasis control program has been performed succesfully, bancroftian filariasis is still a problem in endemic areas of Thailand. This is mainly due to difficulty to access health care system. Lymphatic filariasis caused by subperiodic *B. malayi* is prevalent mainly in Narathiwat province and Surat-thani province in Southern Thailand (Filariasis Division, 2001; Triteeraprapab *et al.*, 2001) (**Figure 1**). This is due to many suitable mosquito breeding sites, large swamp areas, and the existence of animal reservoir hosts, especially domestic cats (Harinasuta *et al.*, 1971a; b; Guptavanij *et al.*, 1971a, b; Phantana *et al.*, 1987; Kanjanopas *et al.*, 2001; Lek-Uthai *et al.*, 2001; Chansiri *et al.*, 2002). Using conventional Giemsa stain technique, *B. malayi* has been found in domestic cats in Southern Thailand with the infection rates ranged from 1.59 % to 3.01% (Filariasis Division, 1998; 2001). Subperiodic *B. malayi* has become an important zoonosis filariae occurring not only in Thailand, but also in Indonesia (Java, Kalimantan, and Sumatra), Malaysia (Peninsular Malaysia), Philippines, and Pacific islands (Dissanaike, 1979).

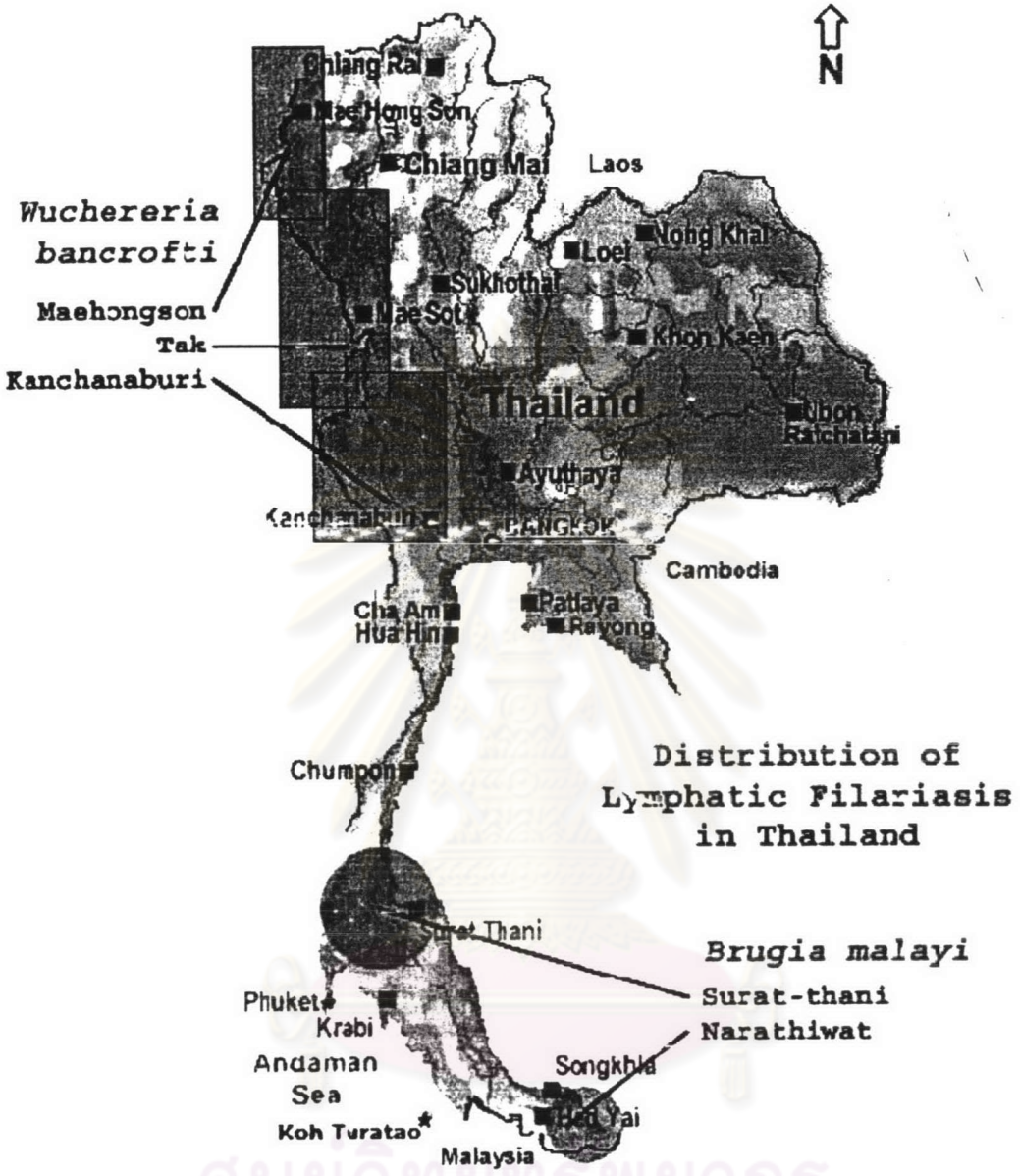


Figure 1 Endemic areas of lymphatic filariasis in Thailand.

Generally, *B. malayi* can be transmitted from man to man, or man to animal, or animal to man, by *Mansonia* mosquito vectors (Dissanaike, 1979). Control of human infection is therefore difficult, since animal to man transmission continues even after the human reservoir has been greatly reduced. It has been suggested that zoonotic transmission is responsible for the inefficiency of mass chemotherapy with diethylcarbamazine (DEC) in the endemic areas in Malaysia (Lim and Mak, 1983). Therefore, besides chemotherapy and vector control, the success of lymphatic filariasis control program should also cover the control of reservoir hosts. However, not only *B. malayi*, but also another filarial species, including *B. pahangi*, *D. immitis* and *D. repens* can infect domestic cats (Nithiuthai and Chungpivat, 1992; Chungpivat and Sucharit, 1993; Chansiri *et al.*, 2002). Currently, it is still difficult to differentiate the filarial species by the conventional microscopic method.

In endemic areas, the routine method for identification of microfilaria species is microscopic examination, based on the delineation of particular morphological features using Giemsa stain. Nevertheless, the technique is difficult to discriminate clearly between closely related species such as *B. malayi* and *B. pahangi*, as well as *D. immitis* and *D. repens*. Histochemical staining, to detect acid phosphatase activity, could overcome the problem (Chalifoux, 1971; Yen and Mak, 1978; Chungpivat *et al.*, 1990; Nithiuthai and Chungpivat, 1992; Chungpivat and Sucharit, 1993). However, this technique requires fresh samples in order to yield the best results. Furthermore, both staining methods need expertise to clearly identify and confirm the species.

Recently the advent of DNA technology has provided an alternative approach for the identification of parasites (Christensen *et el.*, 1994; Nuchprayoon *et al.*, 2001; 2003; Triteeraprapab *et al.*, 2001). Ribosomal genes (rDNA) are among the most useful targets because they evolve in a 'concerted fashion'. This means the rDNA sequence of is usually representative of a species (Brown *et al.*, 1972). Moreover, 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 the 'conserve' oligonucleotide primers has made it possible to characterize a broad range of parasitic organism from minute quantities of material. Many studies have shown that the internal transcribed spacer (ITS) 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; Zhu *et al.*, 1998; Almeyda-Artigas *et al.*, 2000; Conole *et al.*, 2001; Nuchprayoon *et al.*, 2003). Studies of PCR-linked restriction fragment length polymorphism (PCR-RFLP) profiles of the nematodes' ITS regions have provided the data on nematode diversity, as well as the critical taxonomic character useful for species comparison and identification (Gasser *et al.*, 1994, 1996; Nuchprayoon *et al.*, 2003).

Previous studies (Nuchprayoon *et al.*, 2003) reported that PCR-RFLP of ITS1 digested with *Ase* I could differentiate *B. malayi*, *B. pahangi* and *D. immitis*. This sensitive and specific PCR-RFLP technique will be useful for the lymphatic control program in monitoring and evaluation of the animal reservoirs. Since, the identification of zoonotic *B. malayi* in domestic cats of the endemic areas in Thailand was unclear, this molecular technique will facilitate epidemiological assessment and precise identification of *Brugia* species in reservoir hosts. The real prevalence of the disease is necessary in helping on-going lymphatic filariasis control program.

The objective of this thesis was to study the prevalence of filarial nematodes in domestic cats in an endemic area in Pra-sang district, Surat-thani, a province in Southern Thailand, by using PCR-RFLP technique compared to morphological studies by Giemsa and acid phosphatase stained blood films.

2. Research Question

What is the prevalence of filarial nematodes in domestic cats in Prasang District, Surat-thani province, Thailand?

3. Objective of This Research

To study the prevalence of filarial nematode parasites in domestic cats in Pra-sang District, Surat-thani province, Thailand.

4. Hypothesis

1. Domestic cats in Pra sang District, Surat-thani province, Thailand are infected with filarial parasites.

2. In addition to microscopic methods, the PCR-RFLP of ITS1 region could identify filarial nematodes in domestic cats.

5. Keywords

Domestic cats

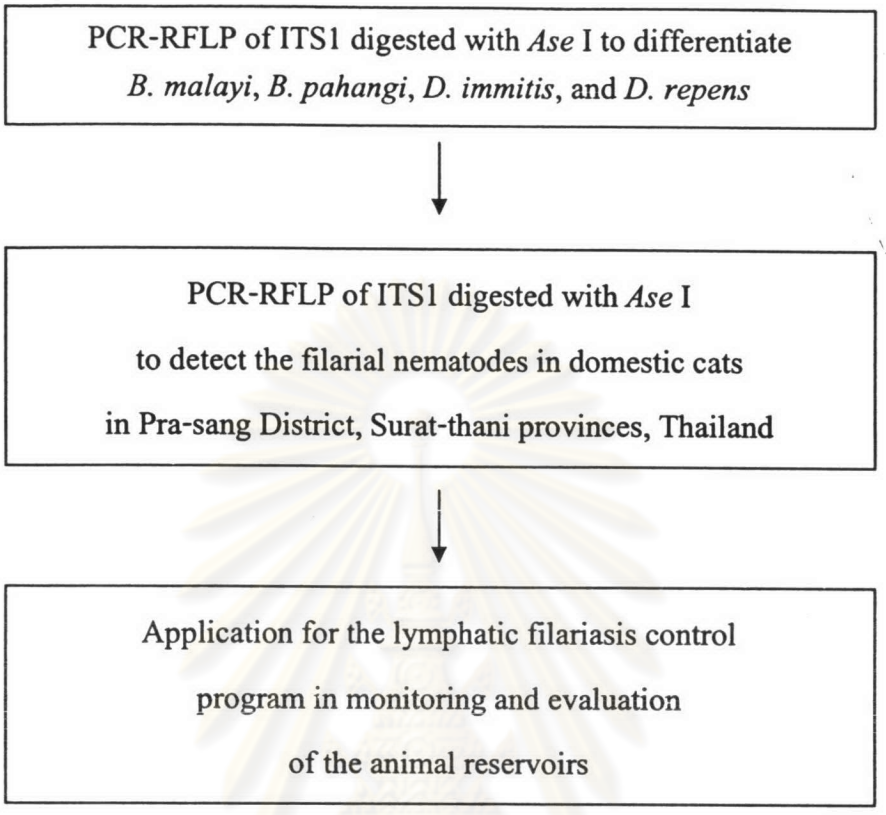
Filarial nematodes

ITS1

PCR-RFLP

Thailand

6. Conceptual Framework



7. Expected Benefit & Application

1. The real prevalence of filarial nematodes in domestic cats in Pra-sang District, Surat-thani province, Thailand will be established.

2. The data will be useful for on-going control program of lymphatic filariasis.

3. Development of a specificity and sensitivity method for identification of filarial nematodes in cat reservoir hosts, and can be applied for identification of filarial nematodes in human and mosquito vectors.