

## CHAPTER IV

### RESULTS

#### 1. Identification of filarial parasites

Blood films of all samples were stained and identified their species by Giemsa stain. It was found that both Thai-Karens and Myanmar workers, from endemic area in Tak province were infected with *W. bancrofti*. The microfilariae of all subjects from Narathiwat were identified as *B. malayi*. For *B. pahangi* from cat, the slide was confirmed from Parasitology Unit, Department of Pathology, Faculty of Veterinary, Chulalongkorn University. *D. immitis* were classified from all stray dogs. All specimens were also confirmed by histochemical stain for acid phosphatase activity.

#### 2. PCR products of ITS1 and ITS2

The first round PCR products of both ITS1 and ITS2 regions, from *D. immitis* were amplified, while there was no detectable bands from other tested filarial parasites; *W. bancrofti*, *B. malayi* and *B. pahangi*. Therefore, we designed the internal primers for semi-nested PCR of ITS1 and ITS2. On agarose gels, there were only slight size differences (approximately 10-15 bp) among some of the PCR products belonging to *W. bancrofti*, *B. malayi* and *B. pahangi*, while *D. immitis* showed distinct of ITS1 and ITS2. The PCR product of *D. immitis* ITS1 was 667 bp, bigger than those of *W. bancrofti* (~570 bp), *B. malayi* (~580 bp) and *B. pahangi* (~590 bp) (figure 6). The PCR product of *D. immitis* ITS2 was 570 bp, smaller than those of *W. bancrofti* (~660 bp), *B. malayi* (~650 bp) and *B. pahangi* (~620 bp) (figure 6). Although most species could not be distinguished reliably based solely on size of the ITS1 or ITS2 PCR products, *D. immitis* could be differentiate from the other filarial species tested. In order to rule out of any possible co-amplification of host DNA, a panel of host samples (human, dog and cat) was subjected to the same PCR amplification, as they were used for nematode DNA. Evidently, there were products in the host controls.

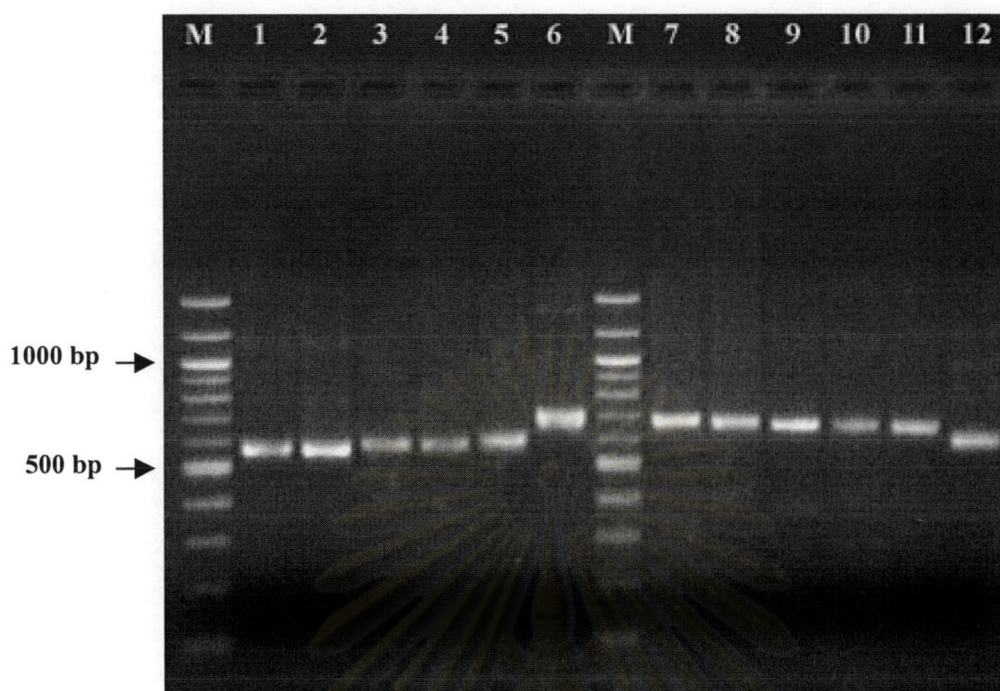


Figure 6 PCR products of filarial ITS1 (lanes 1-6) and ITS2 (lanes 7-12). Lane M, 100 bp ladder; lane 1, Thai-Karen *W. bancrofti* (~570 bp); lane 2, Myanmar *W. bancrofti* (~570 bp); lane 3, human *B. malayi* (~580 bp); lane 4, cat *B. malayi* (~580 bp); lane 5, cat *B. pahangi* (~590 bp); lane 6, dog *D. immitis* (667 bp); lane 7, Thai-Karen *W. bancrofti* (~660 bp); lane 8, Myanmar *W. bancrofti* (~660 bp); lane 9, Thai *B. malayi* (~650 bp); lane 10, cat *B. malayi* (~650 bp); lane 11, cat *B. pahangi* (~620 bp); lane 12, dog *D. immitis* (570 bp).

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