CHAPTER V

CONCLUSION AND DISCUSSION

The ITS1 of rDNA has been proved to be a valuable tool in species delineation. First, they are highly species specific. Second, they are flanked by conserved regions of rDNA permitting universal primers to be designed for amplificaion. Moreover, the ITS1 of rDNA transcriptional unit is repetitive in nature (Hillis and Dixon, 1991). It can be readily amplified from individual life cycle stage of nematodes (Gasser *et al.*, 1993; Campbell *et al.*, 1995; Stevenson *et al.*, 1995). Therefore, PCR-RFLP of ITS1 has considerable potential as a sensitive laboratory tool to identify organism, including filarial parasites.

We designed the new primers (ITS1-F and ITS1-R) for PCR-RFLP of ITS1 to differentiate filarial nematode species in human and animal reservoirs. The PCR-RFLP analysis demonstrated that *B. malayi*, *B. pahangi*, *D. immitis*, and *D. repens* could be specifically differentiated by the ITS1 restriction patterns using *Ase* I. The PCR-RFLP pattern of ITS1 using *Ase* I could easily distinguish filarial nematodes of pathogenic significance.

We could adjust the PCR reaction to detect as little as 10 pg of filarial parasite DNA. The PCR-RFLP provided more sensitivity than microscopic methods for differentiate filarial nematodes. The PCR showed high specificity that no amplicons were obtained from DNA of hosts. This molecular technique could be used for epidemiological investigations. The rapid and reliable epidemiological assessment and clear identification of filarial nematode species are necessary for control of the filarial parasites. The real prevalence is a valuable tool to design possible intervention and control strategies. Therefore, PCR-RFLP of ITS1 rDNA will be useful for the lymphatic control program in monitoring and evaluation the real situation of the disease.

The filarial nematodes in animal are not only the pathogens causing the problems in their animal hosts, but also serve as zoonotic problems in human, such as malayan filariasis and dirofilariasis. Treatment the infected animals are important in order to decrease the risk of human infection in the vicinity of the infected animals when suitable mosquito vectors are present (Baneth *et al.*, 2002).

Pra-sang district is an endemic area of both nocturnally subperiodic and diurnally subperiodic *B. malayi* (Shutidamrong and Phantana, 1986). Previous survey in Sin-jareon subdistrict, one subdistrict in Pra-sang district, identified *B. malayi* in 5 men and 7 domestic cats, respectively (Filariasis center, 2003). In this study, we surveyed the domestic cats in 2 villages in E-pun and Tri-khung subdistricts, an adjacent area to Sin-jareon subdistrict. Of the 52 cats examined, 7.6 % had *B. pahangi*, 1.9% had *D. immitis*. However, no *B. malayi* was identified. Our data suggest that the domestic cats are not an important host of *B. malayi* in this area. All *B. malayi* positive cases in both human and domestic cats in Sin-jareon subdistrict were treated with DEC and ivermectin, respectively, after *B. malayi* had been detected. Therefore, the finding that no *B. malayi* identified in study cats could imply the effective of the filariasis control program.

Accurate identification of filarial nematode species is necessary for control of zoonotic transmission. It is difficult to differentiate among filarial parasites morphologically, particularly the diagnostic stage, microfilariae. The diagnosis of most domestic cats is based on the detection of microfilariae in the peripheral blood. Although the Giemsa and acid phosphatase stained blood films are useful to differentiate filarial species, this technique is insensitive, time-consuming, and labour intensive. Furthermore, staining methods require expertise to identify and confirm the species. Our data showed that the specific PCR-RFLP provided more sensitivity than microscopic methods to detect filarial nematodes in cats.

The domestic cat is not only the reservoir host of its own filarial parasites, (*B. pahangi* and *D. repens*), but also plays an important role as a carrier of

sub-periodic *B. malayi* and *D. immitis* (Dissanaike, 1979; Chansiri *et al.*, 2002). Man is found to be accidentally infected with *B. pahangi* and *D. immitis* (Palmieri *et al.*, 1985). The role of domestic cats in maintaining zoonotic filariasis should be concerned. These findings warrant a thorough epidemiologic investigation in Pra-sang for the possility of these zoonotic filariae in human.