CHAPTER 6

Conclusion

This study was conducted to investigate the life history of *F. gigantica*, the prevalence of infection of both adult and larval stages of *F. gigantica*. While, the molecular biological study was used to develop a specific primer based on HAT-RAPD technique for *F. gigantica* detection and the phylogenetic relationship of *F. gigantica* in Chiang Mai province and other related trematodes based on ITS2 sequences was determined.

The present study is the first investigation of life history completely study of *F. gigantica* in experimental hosts in Thailand, which revealed that the larval stages of *F. gigantica* can be produced in snail hosts, *L. auriculalia rubiginosa*, and well encysted on rice plants. The adult worms can be allowed to developed mature in the experimental definitive host, dwarf hamster and albino mice. It is indicated that *F. gigantica* can be successfully developed to mature in other hosts besides ruminants and humans.

The infection of *F. gigantica* in domestic cattle from slaughterhouses in Chiang Mai province indicates that the infection of *F. gigantica* in *Bubalus bubalis* was higher than *Bos taurus*. The epidemiology of larval stages in snail intermediate hosts revealed the 8 types of cercariae as parapleurolophocercous cercaria, pleurolophocercous cercaria, monostome cercaria, distome cercaria, xiphidiocercaria, echinostome cercaria, tranversotremacercaria and furcocercous cercaria were recoded, which has not found the type of *F. gigantica* cercariae as gymnocephalus cercariae.

A developed specific primer from HAT-RAPD marker can be used to detection the adult and larval stages of *F. gigantica*. The specific primer was designed as: FG-F (5'-

TCC GTT CGT TTT CCC CTC TG-3') and FG-R (5'-GGG TTT CGC CCA TAC AGG AT-3') with yielding a product size of 235 bp. This specific primer pair provides beneficial values for the detection of both adult and larval stage of *F. gigantica*. Using specific primer for detect the *F. gigantica* cercariae in field-collected snails was showed negative result. Thus, the developed specific primer pair is usefulness for the prevention and epidemiological control program of *F. gigantica*.

The phylogenetic analysis by using nucleotide sequence of ITS-2 region was performed by maximum likelihood (ML) and UPGMA methods and the genetic diversity analysis by using the four times rule for speciation By basing on ML and UPGMA methods can generated 4 groups of trematodes; first *F. gigantica* group including specimen of Chiang Mai, second 2 samples of *F. hepatica*, third group of 3 rumen flukes; *Orthocoelium streptocoelium*, *Fischoederius elongatus* and *Paramphistomum epliclitum* and fourth group of 3 minute intestinal flukes; *Haplorchis taichui*, *Stellantchasmus* sp., *Haplorchoides* sp. and liver fluke; *Opisthorchis viverrini*. These results can be confirmed the giant liver fluke which mainly caused fascioliasis in Chiang Mai was identified as *F. gigantica* and specimens were the same as those of *F. gigantica* recorded in other different countries. Nucleotide sequence of ITS-2 region has been proven as effective diagnostic tool for the identification of *F. gigantica*. The four times rule for speciation indicates that *F. gigantica* in this study are same species with other countries, while, *F. gigantica* difference from *F. hepatica*.