CHAPTER 7

SUMMARY

The aims of this study is to detect *Cryptococcus neoformans* and *Histoplasma capsulatum* in soil contaminated bat and avian excreta samples by using nested PCR method.

1. A total of 265 soil samples were collected, these including 88 samples from soil contaminated with bat droppings, bird droppings including white wagtail (15 samples), wagtail (17 samples), pigeon (21 samples), myna (30 samples), red whiskered Bulbul (3 samples) and chicken (91 samples). Samples were collected in Chiang Mai except some samples of soil contaminated with bat guano were from Nakornsawan and Mae Hong Son provinces. All soil samples were processed and each soil suspension was cultured on L-Dopa agar to isolate *C. neoformans*.

2. Genomic DNA was directly extracted from each soil suspension by phenol-chloroform-isoamyl alcohol method. Each extracted DNA sample was used to detect *C. neoformans* and *H.capsulatum* by nested PCR.

3. Universal fungal primers (FungusI, FungusII, amplified product of 429 bp) and primers specific for a gene encoding 18S rRNA of this fungus (CrypI,CrypII, amplified product of 278 bp) were used to detect *C. neoformans* by nested PCR. One sample of soil contaminated myna droppings was positive for *C. neoformans*. However, this soil sample and other all were negative for this fungus when isolating on L-dopa agar. The specificity of the PCR with CrypI and CrypII primers was confirmed by the total absence

of amplification products when genomic DNA from *Mycobacterium avium*, *Aspergillus fumigatus*, *Penicillium marneffei*, *Candida albicans*, *Candida dubliniensis*, *Candida krusei* and *Histoplasma capsulatum* were applied in the reaction. Detection limit of nested PCR assay for *C. neoformans* was $2x10^3$ cells per sample for both first round and second round.

4. The nested PCR was also used to detect *H. capsulatum* in each extracted soil sample. Using primers specific for a gene encoding 100 kDa-like protein of this fungus (Hc primers), PCR products of 391 and 210 bp were amplified with primers HcI, HcII and HcIII, HcIV, respectively. Seven of 88 (7.95 %) soil contaminated with bat guano, 1 of 21(4.76 %) soil contaminated with pigeon droppings samples and 10 of 91 (10.99 %) soil contaminated with chicken droppings samples were positive for *H. capsulatum*. Sequencing analyses of PCR products showed 94 to 97 % identity to nucleotide sequences of gene encoding 100kDa-like protein of *H. capsulatum*. The specificity of the PCR with HcI, HcII and HcIII, HcIV primers was confirmed by the total absence of amplification products when genomic DNA from *M. avium*, *A. fumigatus*, *P. marneffei*, *C. albicans*, *C. dubliniensis*, *C. krusei* and *C. neoformans* were applied in the reaction. Detection limit of nested PCR assay for *H. capsulatum* was $2x10^3$ cells per sample for both first round and second round. Positive soil contaminated with bat guano samples for *H. capsulatum* were collected from Chiang Dao and Pha Dang caves in Chiang Mai whereas positive soil contaminated with chicken and pigeon droppings were collected from amphoe San Pee Sua and amphoe Muang, respectively .

5. The nested PCR method will be a very useful alternative tool for the detection of fungi especially *H. capsulatum* in natural reservoirs. The method for the isolation of this fungus is difficult and not practical for general mycology laboratories since *H.*

capsulatum has been classified into the risk group level 3. This study indicates the association of bat and avian droppings and *H. capsulatum* in this area of Thailand. Soil contaminated with chicken droppings may be the possible important reservoir of this pathogenic fungus in Chiang Mai.