CHAPTER 1

INTRODUCTION

Histoplasmosis is a systemic mycosis caused by the dimorphic fungus *Histoplasma capsulatum* (*H. capsulatum* var. *capsulatum*). The etiologic agent grows as a saprophyte in nature and is acquired by inhalation of airborne conidia. Most infections are mild or subclinical but positive for histoplasmin skin test. Acute pneumonia may result from exposures to inhaled dust containing high concentrations of this fungus. Clinically detectable dissemination is rare but occurs in immunocompromised host. The filamentous mold form is found in the environment and can be cultured in conditions below 35ºC. When it is present in tissue or grown in cultures, using brain heart infusion agar at temperatures greater than 35ºC, it transforms into budding yeasts. Histoplasmosis has a worldwide distribution, however, the central and eastern states of U.S.A. especially Mississippi-Ohio River Valley is recognized as a major endemic area (Edwards et al., 1969).

The diagnosis of histoplasmosis is usually made by the laboratory culture or a blood test. A skin test is available but is useful only for outbreak or investigations, not for diagnosis. Anti-*Histoplasma* antibodies may be detected in the serum of 90% of patients with histoplasmosis (Wheat, 1984; Wheat, 1982). However, false-positive results have been reported in patients with blastomycosis, paracoccidioidomycosis, and coccidioidomycosis. Histopathology using methinamine silver or periodic acid-Schiff stains of tissue permits rapid diagnosis but the low sensitivity (Sathapatayavongs et al., 1983). The gold standard for diagnosis of histoplasmosis remains isolation of the organisms in culture. Confirmation of the organism as

H. capsulatum requires conversion of the characteristic mold to the yeast-form at 37°C on blood agar plates (Wheat, 2003). However, *H. capsulatum* is a slow-growing in culture media and may take up to 4 weeks to grow (range, 1 to 4 weeks). In addition, identification of the mycelial form of *H. capsulatum* requires a biosafety level 3 laboratory.

The natural habitat of *Histoplasma capsulatum* is the soil with high nitrogen content, generally associated with the bird and bat droppings. It has been recovered from bat cave, pigeon roosts, chicken houses. The currently method for the isolation of *H. capsulatum* in environmental samples makes use of an indirect method, inoculating mice with soil suspension, followed by culturing portions of their organs. Unfortunately, the procedure is expensive and slow to confirm the presence of the fungus in the sample. Polymerase chain reaction (PCR) has become a powerful tool for the detection of microorganism. A nested PCR was followed employing both fungal-specific primers and second round PCR primers specific for the internal transcribed spacer (ITS) region of rRNA gene of *H. capsulatum*. The limit of detection of this method is 10 spores (Reid and Schafer, 1999). On the other hand, ribosomal genes are conserved regions bearing the risk of nonspecific amplifications. A two-stage PCR assay specific for a 100 kDa-like protein gene was developed for the detection and identification of *H. capsulatum* in human tissue samples. The unique 100 kDa-like protein gene amplified by nested PCR showed a specificity of 100% identical to *H.capsulatum* (Bialek et al., 2002).

In Thailand, from September 1984 to March 2010, the number of disseminated histoplasmosis cases among HIV-infected patients was 1,253 (Data of

Epidemiological Information Section Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand). Disseminated histoplasmosis has been found every year in Maharaj Nakorn Chiang Mai Hospital. The number of patients from February 2000 to March 2009 was 32 cases (average 3-4 cases/year). A survey on natural sources of *H. capsulatum* had been carried out in Thailand 1966, more than 1,000 soil samples were negative for the isolation of this fungus. Moreover, a total of 84 soil samples from several area of Thailand have been processed for the recovery of *H. capsulatum*. Mouse inoculation failed to recover *H. capsulatum* from any of these specimens including those collected in caves (Taylor, 1968). Until now, there has never been reported of the environmental sources of this fungus in Thailand.

Other than histoplasmosis, in Thailand cryptococcosis was the third (13.49%) most common opportunistic infection among patients with HIV infection after tuberculosis (30.12%) and *Pneumocystis carinii* (*Pneumocystis jeroveci*) (19.68%) (http://epid.moph.go.th/). This disease is also one of the most life-threatening fungal infections, especially in AIDS patients. Cryptococcosis, an opportunistic disease commonly associated with meningoencephalitis in human, is caused by the yeast *C. neoformans*. It has been classified into three varieties and four major serotypes on the basic of phenotypic, biochemical, serological and genetic differences, namely *C. neoformans var. grubii* (serotype A), *C. neoformans var. neoformans* (serotype D) and *C. neoformans var. gattii* (serotype B and C). Recently, *C. neoformans* var. *gattii* has been proposed into a new species, *C. gattii* based on the differences in its morphology, antigenic structure, virulence profile, epidemiology, ecology, and geographic distribution (Kwon-Chung et al., 2002). *C. neoformans* serotype A and D

have been isolated from various environmental sources including peach juice, fruit, milk, vegetables but the major saprophytic source is avian droppings, particularly pigeon excreta (Littman, 1968). In contrast, *C. gattii* has a more restricted geographical distribution, being prevalent in tropical and subtropical areas where it has been isolated from different species of Eucalyptus (*Eucalyptus camaldulensis, E. tereticornis, E. blakelyi, E. rudis and E. gomphocephala*) and other trees (Sorrell, 2001; Chakrabarti et al., 1997; Chen et al., 1997).

The previous study in Chiang Mai, Thailand revealed that almost clinical and environmental isolates of *C. neoformans* in this area belong to serotype A and natural habitats are bird droppings including dove (*Streptopelia chinensis, S. decaocta and Geopelia striata*) and pigeon excreta (*Columba livia*) (Sriburee et al., 2004; Keerativasee et al., 2008). Moreover, this organism has also been isolated from chicken excreta on rare occasions in this area (0.5%) (Keerativasee et al., 2008). The reason why *C. neoformans* was rarely isolated from the chicken dropping may be due to the combination of high pH and the presence of thermostable low molecular weight substances to inhibit its growth (Walter and Yee, 1968).

Isolation of C. *neoformans* is established easily by culture and confirmed by India ink preparation, culture, and biochemical tests. Recently, molecular biological tools like PCR assays have been introduced successfully to diagnose cryptococcal disease (Bialek et al., 2002b). Specificity testing was performed with strains of phylogenetically related fungal species, some of which might occur in clinical specimens either as pathogens or as contaminants. There is a little information in the detection of *C. neoformans* by PCR from natural environments.

Therefore, the nested PCR may be useful and sensitive for detections of *C. neoformans* and *H. capsulatum* in environmental sources. The aim of the study is to detect the possible environmental sources of *H.capsulatum* and *C. neoformans* in soil contaminated with bat and avian droppings by using nested PCR instead of mice inoculation and culture methods. Histoplasmosis and cryptococcosis cause important health problems worldwide and also in Thailand. Determination of the saprophytic sources of both fungi will be the useful information of both diseases especially for histoplasmosis that has never been reported of natural reservoirs of *H. capsulatum* anywhere else in Thailand.