CHAPTER I

INTRODUCTION

Cryptococcus neoformans is the agent of cryptococcosis and cryptococcal meningitis, which are the prevalent infections in immunosuppressed patients. The important of *C. neoformans* as an opportunistic pathogenic fungus has considerably increased over the last two decades, owing to the intensive chemotherapy of cancer patients, and the spread of AIDS epidemics (1, 2). It was estimated that 5-10% of AIDS patients were infected with *C. neoformans* (3). In France, for instance, the number of case of cryptococcosis since 1985 has constantly increased, in parallel with the number of case of HIV-infected patients (1). Prior to the AIDS epidemic, the true prevalence of cryptococcal infection in the United States was unknown because reporting was not required. However, incidence had been estimated to be of one to two cases per 1 million people. Following the emergence of the HIV-related immunodeficiency, the cryptococcal meningitis is rapidly recognized among the most frequent infections in both the United States and rest of world (4). The first report case of cryptococcosis in Thailand was recorded in 1960. Among AIDS patients in Thailand, cryptococcosis is the second most common opportunistic infection after tuberculosis. Most of these patients are in the late stage of HIV infection (5). Epidemiological studies in AIDS patients in Thailand revealed that infections caused by *C. neoformans* var. *grubii* (serotype A) isolates were predominant. The isolation of serotype D strains from AIDS patients has also been reported in France, Germany, and USA. In Brazil, it was estimated that 43% of AIDS patients were cryptococcosis (6).

C. neoformans var. *neoformans* is the worldwide variety, its main habitats are pigeon droppings and soil contaminated with decaying pigeons or chicken droppings. Not part of the normal microbial flora of humans, it is generally accepted that organism enters the host by the respiratory route in the form of dehydrated haploid yeasts or as basidiospores. After some times in the lung, the organism hematogenously spreads to extrapulmonary tissues; since it has a predilection for the

brain, resulting as meningoencephalitis (7). Minimally encapsulated, desiccated yeast cells have long been claimed to be the infectious propagules of *C. neoformans*, but penetration to the lung parenchyma requires particles of less than 2 μm in diameter. Yeast diameter, however, is in the 4-8 μm range, and desiccation markedly reduces the viability of this form. Basidiospores, which are 1.8-2 μm in diameter, are small enough to penetrate alveoli, easily aerosolized, and like many other spores, which are very resistant to desiccation. These features appear to favor the basidiospore rather than the yeast cell as the infectious propagule of *Cryptococcus*. Wickes *et al* (1996) (1) had shown that *C. neoformans* is able to produce hyphae and basidiospores in the absence of mating and this process is associated with the α-mating type. The haploid α-mating type of *C. neoformans*, unlike the a-mating cells, can form monokaryotic hyphae under appropriate conditions, with development of basidia and variable basidiospores. Clinical and environmental isolates are predominantly of α-mating type, varying from 40:1 (α:a) for environmental isolates to 30:1 for clinical isolates. The predominance of the α-mating type and the fact that it can generate an asexual basidiospore through haploid fruiting, in addition to some properties of the basidiospore, such as resistance to desiccation and cell size, further support the concept that the basidiospore is indeed the infectious propagule of *Cryptococcus*.

The major environmental source of *C. neoformans* throughout the world is bird excreta especially pigeon droppings and eucalyptus trees (8). The association between *C. neoformans* and pigeon droppings has been confirmed in several studies. It is difficult to determine the origin of infection and the route of transmission in patients with cryptococcosis. Serotyping of *C. neoformans* and Random Amplified Polymorphic DNA (RAPD) analysis are available for epidemiological surveys. In addition, Restriction Fragment Length Polymorphism (RFLP) and Pulsed Field Gel Electrophoresis (PFGE) have allowed fine epidemiological studies (9). The epidemiological survey of serotype and molecular type of environmental isolates has been studies in worldwide.

There are differences between serotypes and molecular types in various countries. The majority of North American isolates pooled from various geographic locations have been reported to be serotype A isolates (10). It has been reported that *C. neoformans* serotype A is distributed worldwide and is a major cause of all clinical

types of cryptococcosis (11). Epidemiological studies in AIDS patients in Thailand revealed that infections caused by serotype A isolates were predominant (6). Kuroki *et al* (2003) (12) isolated *C. neoformans* from chicken excreta in Phayao province. They found 36 of 150 samples (24%) of chicken excreta. All of them are serotype A. They suggested that *C. neoformans* in chicken excreta might cause cryptococcosis in AIDS patients living in northern Thailand. In 2004, Sriburee *et al* (13) studies *C. neoformans* in Chiang Mai province from clinical and environmental isolates. 385 environmental samples, of which 100 were dove droppings, 55 pigeon droppings and 230 eucalyptus flower, were collected from 7 Amphores in Chiang Mai. *C. neoformans* was isolated from 45 of 100 (45%) dove dropping samples, 9 of 55 (16.4%) pigeon dropping samples and 2 of 230 (0.9%) eucalyptus flower samples. Fifty-six environmental and 74 clinical isolates belonged to *C. neoformans* serotype A and only one clinical isolate belonged to *C. neoformans* serotype AD. PCR fingerprinting, using $(GACA)_4$ as a primer, 75 clinical and 54 environmental isolates were of group I and 2 environmental isolates were of group II. In Thailand, Cryptococcosis is among the most common AIDS-defining illness as well with the AIDS epidemic sweeping through other Southern Asia regions such as India, Vietnam, and Southern China. Cryptococcosis threatens become a major cause of morbidity and mortality in persons with AIDS in these regions, as well as in affected African countries (14).

Several typing approaches have been reported in epidemiological studies. These include Restriction Fragment Length Polymorphism (RFLP), Pulse Field Gel Electrophoresis (PFGE), and Randomly Amplified Polymorphic DNA (RAPD) analysis. Among them, the serotyping schemes have been found to be particularly important, because the association of cryptococcosis in AIDS patients with serotype A strain was confirmed by recent epidemiological and ecological studies. The suitability of RAPD analysis has been demonstrated by showing its ability to discriminate between, closely related isolates within restricted geographic areas (15). Yamamoto *et al* (1995) (9) studied *C. neoformans* from clinical and environmental isolates by RAPD analysis. Environmental isolates of *C. neoformans* were obtained from weathered pigeon excreta in hospitals, private houses and parks in Nagasaki Prefecture form 1993 to 1994. They suggested that there was relationship between clinical and environmental isolates and RAPD analysis is a useful method for

epidemiological surveillance. Meyer *et al* (2003) (16) studied *C. neoformans* from clinical an environmental isolates that were obtained from IberoAmerican countries by using M13 polymerase chain reaction-fingerprinting. They separated the Molecular types of *C. neoformans* by using M13 into 8 molecular types that were VNI (var. *grubii*, serotype A), VNII (var. *grubii*, serotype A), VNIII (AD hybrid), VNIV (var. *neoformans*, serotype D), and VGI, VGII, VGIII, VGIV (var. *gattii*, serotype B and C). The majority of the isolates, 68.2% (n=232) were VNI. Most of the cryptococcal isolates in his study were recovered from patients whose main rich factor was HIV infection. Overwhelming number of these isolates corresponded to molecular VNI; in accordance with another research paper by Casali (2003)(15)showed that isolates of this molecular type are the major source of infection in HIV-positive patients worldwide. They suggested that the similarity in the molecular type obtained from Spanish and Chilean isolates provides further evidence, that the cryptococcal strains present today in South America could be introduced during European colonization. This idea had been suggested by Franzot *et al* (1997) (17) when investigating isolates obtained from Brazil. The authors argue that the pigeon (*Columba livia*), thought to provide a major reservoir of *C. neoformans* in pigeon excreta, is believed to have originated in southern Europe and Northern Africa and has been dispensed worldwide by human travel.

Currently, there are many pigeons in the different geographical areas of Bangkok such as temples, public parks, private houses, etc. However, the epidemiological data about the ecology of *C. neoformans* in the Bangkok's environment and Cryptococcus meningitis patient are lacking. In the present study, the distribution of serotypes, molecular types and genetic diversity of pigeon droppings isolates of *C. neoformans* from different geographical areas of Bangkok were studied.