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Original Article

Infections and Epidemiology of *Cryptococcus* Species

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Abstract

Cryptococcus neoformans and *Cryptococcus gattii* are the major cause of cryptococcosis presenting primarily with meningitis and pneumonia. *Cryptococcus* spp. has been the most common cause of opportunistic fungal infections in HIV-infected individuals worldwide. With the introduction of highly active antiretroviral therapy (HAART) in the mid-1990s and the subsequent availability of azole drugs, the incidence of cryptococcal meningitis has decreased dramatically. In Taiwan, approximately 600~800 cryptococcal cases occurred annually during 2002-2011. In 1999, the occurrence of an outbreak of *C. gattii* in North America has raised worldwide concern. *Cryptococcus* spp. is ubiquitous in the environment. *Cryptococcus* spp. infections can infect both immunocompetent and immunosuppressed people. Climate change due to global warming may facilitate the spread of *Cryptococcus* spp. Therefore, enhanced diagnosis and surveillance is essential.

Keywords: *Cryptococcus neoformans, Cryptococcus gattii*, diagnosis, epidemiology, HIV

Introduction

Cryptococcosis commonly manifesting meningitis and pneumonia are majorly caused by *Cryptococcus neoformans* and *Cryptococcus gattii*. *Cryptococcus* spp. infections can occur in both immunocompetent and immunosuppressed individuals. In the past, due to the global AIDS epidemic as well as an overall increase of immunosuppression therapy, cryptococcosis cases had increased dramatically. Globally, *Cryptococcus* spp. infects almost one million people annually resulting in over 620,000 attributable mortalities [1]. In the pre-HAART era , the prevalence of cryptococcosis among HIV patients ranges from 2% to 10% in Western Europe and the United States and are more than 15% in Africa [2]. Since 1990, significant improvements in HIV care and treatment, especially the development of HAART together with the availability of azoles, have decreased the incidence of cryptococcal meningitis.

From1993 to 2000, a population-based active surveillance revealed that the annual incidence of cryptococcosis decreased from 4 cases in 1993 to 0.4 cases in 2000 per 100,000 population in the Houston metropolitan area of the United States [3]. The diagnosis of cryptococcosis is difficult and the mortality rate of disseminated cryptococcosis is 70-80%, which can be reduced to 10-20% with prompt diagnosis and early treatment [4]. Since 1999, an outbreak of cryptococcosis in humans and animals caused by *C. gattii* VGII occurred in Canada and subsequently expanded to Pacific Northwest. Statistic data during 2004-2010 showed that the mortality rate for the *C. gattii* was up to 25% with the majority of the patients being immunocompetent [5] , which has raised global public health concern.

Pathogens

The genus *Cryptococcus* includes around 37 species. *C. neoformans* and *C. gattii* are the major pathogenic species. Previously they were all named *C. neoformans*. Recently, *C. gattii* (previously *C. neoformans* var. *gattii*) has been reclassified as a separate species from *C. neoformans*. Based on capsular agglutination and biochemical properties, they can be further divided into five serotypes: A, B, C, D, and a rare AD hybrid (hybrids between serotypes A and D). *C. neoformans* comprises 2 subspecies: *C. neoformans* variety *grubii* (serotypes A, and three molecular types designated VNI, VNII, and VNB) and *C. neoformans* variety *neoformans* (serotypes D, and molecular types designated VNIV). The serotypes AD hybrid (molecular types designated VNIII) is more closely related to serotypes A. *C.gattii* can be subdivided into two serotypes (B and C), and four molecular types designated VGI, VGII, VGIII, and VGIV. The major molecular types of serotype B is VGI, and VGII; while for serotype C is VGIII, and VGIV. VGII genotype can be further subdivided into VGIIa , VGIIb, and VGIIc subtypes [6] (Figure 1) .

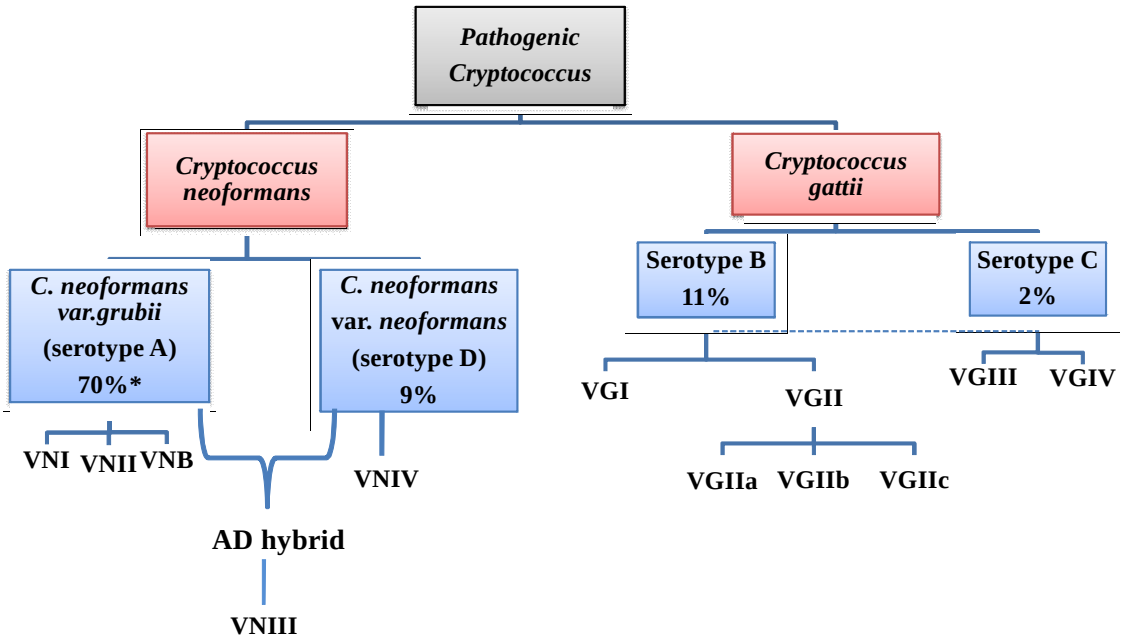

Figure 1. Five serotypes (A, B, C, D, and AD) of the pathogenic *Cryptococcus spp.* and their respective prevalence rate [7].

Cryptococcus spp. is a basidiomycete fungus. It is elliptical or round in shape and 4-6 μm in diameter. A characteristic polysaccharide capsule of variable thickness surrounds the cells. *Cryptococcus* spp. can reproduce both sexually and asexually. It reproduces primarily asexually by budding and pseudohyphae may form. The sexual phase is quite rare. *Cryptococcus* spp. has two mating types: **a** or α. The α mating type is the predominant mating type found in nature and is more virulent.

Transmission and pathogenicity

Cryptococcus spp. is commonly found in soil, decaying wood, and pigeon feces. *C. neoformans* are often found in soil, especially in soils having high nitrogen content and contaminated with pigeon feces. *C. neoformans* var. *grubii* has a world-wide distribution, while *C. neoformans* var. *neoformans* is more confined to Europe. *C. gattii* is often associated with *Eucalyptus* trees and decaying organic matters. *Cryptococcus* spp. spread through the air. People become infected when breathe in dust or desiccated pigeon feces containing cryptoccocal conidia.

There are four major virulence factor of *Cryptococcus* spp.: melanin production, polysaccharide capsule, growth at 37°C, and being α mating type. Melanin production can protect the *Cryptococcus* spp. by increasing resistance to phagocytosis by macrophages. Polysaccharide capsule is the most important virulence factor, which also increase resistance to phagocytosis by macrophages. Tolerance to human body temperature 37°C is a precondition for invasion into the central nervous system. α mating type strains with *STE*12 alpha gene which mediate the expression of several virulence-related genes, such as genes involved in polysaccharide capsule and melanin production [8].

Clinical manifestation and treatment

Cryptococcus spp. infects primarily immunocompromised patients. In immunocompetent patients, inhalation of airborne cryptococcal propagules from the environmental usually results in colonization of the host respiratory tract without producing any symptom or causing only self-limiting lung diseases. In immunocopromised host, disseminated cryptococcosis may occur with very bad prognosis. Early symptoms may be mild and non-specific. Thus, early diagnosis is difficult. Common symptoms include fever, headache, nausea, chest pain, cognitive impairment, alteration in consciousness and mild cough .The clinical symptoms vary depending on the invasion of different tissues and organs. *C. neoformans* can directly invade the blood–brain barrier (BBB) causing cryptococcal meningitis which may lead to permanent damage of the central nervous system. Less commonly, it can cause pulmonary infection resulting in pneumonia-like illness. In disseminated cases, nodular and ulcerative skin lesions may occur.

Amphotericin B and flucytosine are used for treatment of cryptococcal infection in the initial therapy phase and after a few weeks azole drugs (fluconazole or itraconazole) are used for treatment.

Clinical symptoms of *C. neoformans* and *C. gattii* are quite similar. However, *C. gattii* is significantly more common in immunocompetent patients and more likely to cause serious neurological sequelae. Clinical isolates of *C. gattii* had significantly higher MICs to amphotericin B, flucytosine, and triazoles than *C. neoformans* variety *grubii*. Among different genotypes within *C. gattii*, VGII had significantly higher MICs to flucytosine and triazoles than VGI and VGIII [9]. Therefore, a more judicious approach taking genotypes differences into consideration is warranted for treatment of cryptococcal infection.

Laboratory diagnosis

1. Microscopy examination: The India ink stain can be used to visualize the thick polysaccharide capsule of *Cryptococcus* in specimens (CSF, sputum, urine and serum). Mucicarmine stain and melanin stain are used to stain tissue sections.
2. Culture: *C. neoformans* can grow rapidly under Potato Dextrose or Sabouraud's Dextrose Agar (without cycloheximide) at 37℃. *C. neoformans* can produce melanin on Birdseed (Niger Seed) Agar, Cornmeal Tween 80 and Caffeic acid media and appear as brown colony on agar plate.
3. Biochemical reactions: Vitek, API20C Aux or MicroScan Rapid Yeast Identification panel kits can be used for specific identification of *Cryptococcus*. Distinctive biochemistry character such as production of urease, laccase, phenoloxidase, and catecholamine can be employed for identification.
4. Antigen detection: Latex agglutination can be used to detect polysaccharide antigens in blood or CSF. A titer of latex aggregation greater than 1:8 is considered as acute infection. Antigen titer test may also help to follow the course of therapy. A new point-of-care (POC) assay for detection of cryptococcal antigen lateral flow assay (LFA) can be used for rapid diagnosis and screening of infection.
5. Molecular diagnosis: Nucleic acid detection methods such as polymerase chain reaction (PCR), real-time PCR and array technology using the ribosomal RNA Internal Transcribed Spacer (ITS) or URA5 gene as target sequence can be used for rapid and specific identification of *Cryptococcus* pathogen. MALDI-TOF MS provides another reliable alternative for species identification.
6. Molecular typing: M13 minisatellite, RFLP (URA5 and PLB1), AFLP, and MLST can be used to distinguish serotypes A, B, C, D, AD and VNI-IV, VGI-IV. MLST utilizing 7 housekeeping genes are used for genotyping both *C. neoformans var. grubii* and *C. gattii*, and allele numbers and sequence types can be obtained from ISHAM MLST Database website [10].

Epidemiology

Crypotococcosis is a zoonotic diseases affecting human, cats, dogs, horses, cattle, sheep, goats, koala and monkey, etc. In general, it is not transmitted from human-to-human or animal-to-human. Pigeons are resistant to cryptococcal infection, because their high body temperature of 42℃ can inhibit the growth of *C. neoformans*. Compared to other rapid transmitted diseases, its public health impact is lower. Therefore, in Taiwan, like in many other countries, such as USA, Canada, EU, Japan, and Australia, *C. neoformans* is not categorized as a notifiable disease. Consequently, precise statistics on incidence rates and disease burden are generally lacking.

Cryptococcus generally infect immunocompromised patients, such as patients with AIDS, malignant tumor, hepatitis C, organ transplant, steroid therapy, uremia, diabetes, rheumatoid arthritis, liver cirrhosis, or leucopenia. Furthermore, a study on adult-onset immunodeficiency in Taiwan and Thailand has shown that severe disseminated opportunistic infections including cryptococcosis is strongly associated with high-titer autoantibodies to interferon-γ[11]. A 2007 estimation, in Africa, 80-90% of AIDS patients were infected with cryptococcosis, and over 50% in USA [12]. *C. neoformans* infection was documented in 2.8% of the organ transplant recipients. However, in China, druing1980-2006, 71% of cryptococcosis patients are not immunosuppressed, and only 8.5% are AIDS patients [13]. This reinforces that epidemiology may vary according to the geographic region.

C. neoformans var. *grubii* (serotype A) is distributed all around the world. It consists of more than 70% of clinical cases. The pathogen exists in guano of pigeon or other birds (sparrow and parrot guano). The rate of pathogen isolation is higher in the dry feces than in the wet ones [14], because the high concentration ammonia in fresh wet feces may inhibit the growth of *C. neoformans*. The isolation rate of birds feces ranges from 17% to 43% [15]. The majority of cryptococcosis patients in Taiwan are infected by *C neoformans var. grubii* (serotype A/VNI/VN6) and mating typeα. A study in Taiwan in 2009 has shown the isolation rate from pigeon feces is 6.54% (74/1118) and 5.0% (3/60) in other birds. All of these 77 isolates belong to sereotype A and VNI, which is the same as clinical isolates from humans [16].

C. neoformans var. *grubii* (serotype D) constitutes 9% of cryptococcosis in the world, and is mainly distributed in North Europe. Clinical symptom is acute with more fever and systemic infection easily, and few meningitis.

C. gattii (serotype B and C) is prevalent in tropical and subtropical regions, especially where Eucalyptus trees are grown, including *Eucalyptus camaldlensis, E. tereticornis* and *E. citriodora,* etc. *E. camalculensis* Dehn is the most relevant species. *C. gattii* was isolated from *E. camalculensis* Dehn in Australia as well as California, Italy and northern India. The subtropical and tropical climate in Taiwan is suitable for growing Eucalyptus. The *Eucalyptus* is introduced from Australia in 1986 into Taiwan, since then it has become one

of the major species for afforestation in Taiwan due to its rapid growth and high economic value and provides major raw material for pulp factory. Currently, the most common *Eucalyptus* species in Taiwan are *E. longifolia* , *E. globulus E. robusta* and *E. citriodora,* etc. Whether their vegetation distribution and flowering season are correlated with the geographical and temporal distribution of *C. gattii* infection deserve further investigation. Furthermore, soil- and air-borne conidia of *C. gattii* can be widely disseminated through human activity and traffic. Epidemiology of cryptococcal infection has changed, which may partially due to the worldwide climate change caused by global warming. In the past *C. gattii* is restricted to tropical and subtropical region. In early 1999, a cryptococcal outbreak occurred in human and animal in Vancouver Island, Canada in temperate region, and subsequent spread to British Columbia inland [17]. After 2004, it further expanded to other temperate region such as the Pacific of Northwest of USA (Oregon, Washington, California and Idaho). In Europe, the first *C. gattii* environmental isolate was discovered from the Mediterranean coast of Italy, and then spread to regions of Northern Europe [17]. Statistic data from 1999 to 2007 indicated a mortality rate of 8.7% (19/218). Statistic data indicate high mortality rates of 8.7% (19/218) to 25% (15/60) [5]. The newly emerging *C. gattii* deserves attention in light of its ability to cause high mortality rate, prevalence in immunocompetent patients, higher resistance to antifungals than *C. neoformans* and tendency to have adverse sequelae which necessitate surgery and longer therapy regimens. The outbreak in Vancouver Island and British Columbia is caused predominantly by VGIIa genotype and to a lesser extent by VGIIb genotype. In the USA, apart from VGIIa and VGIIb genotypes, VGIIc genotype was also discovered. In murine inhalation model, virulence of VGIIa and VGIIc genotypes are much higher than VGIIb. Therefore, it is pivotal to closely monitor the subsequent transmission of the high virulent genotypes of VGIIa and VGIIc [18]. Serotype B of *C. gattii* constitutes 11% of cryptococcosis in the world, while serotype C only accounts for 2%, 88% of serotype C occurs in Southern California.

In Taiwan, during 2007-2011, according to statistics of the Bureau of National Health Insurance the annual cryptococcal cases is about 600 to 800 and among them about 100 to 140 cases are meningitis [3]. A clinical study conducted by National Taiwan University Hospital during 1982 to 1997 discovered increased cases of *C. neoformans* var. *neoformans* with the increase of AIDS patients [19]. In Taiwan, during 1997–2010, isolates from 219 patients with proven cryptococcosis at 20 hospitals representative of all geographic areas showed 210 of 219 patients were infected by *C. neoformans* with the genotypes being VNI (206 isolates) and VNII (4 isolates). Only 9 of 219 patients were infected by *C. gattii* with the genotypes being VGI (3 isolates) and VGII (6 isolates).[20].

Cryptococcal infection is not uncommon in Taiwan, which indicates the wide distribution of Cryptococcus fungus in our environment. Cryptococcus should be considered in differential diagnosis of pneumonia or meningitis of patients living near pigeon house or with possible bird exposure.

Prevention

The pathogenic cryptococcus belongs to risk group 2 (RGII) human pathogens. Currently, there is no vaccine available. Cryptococcal infection is closely associated with pigeon excreta carrying cryptococcal pathogen. While pigeon itself will not be infected, droppings of pigeon or other poultry and soil contaminated with their excreta are suitable substrate for the propagation of Cryptococcus pathogen. The Cryptococcus fungus can survive and persist in desiccated pigeon droppings or soil for months to years which become aerosolized dust circulating in the air and infect people who breathe it in. Pigeon excreta should be disposed properly to avoid contamination of the environment.

Pigeon keeper or poultry workers with weakened immune systems are at elevated risk of infection and should avoid contact with pigeon or poultry droppings and contaminated soil. Immunocompetent people who live near pigeon houses or pass by are not especially at high risk of infection.

Frequent cleaning and disinfection of pigeon cages, keeping dry and well aerated, avoid over-crowding of pigeon population and proper handling and disposal of pigeon fecal matter by disinfection or fumigation are important to avoid infection. When entering pigeon house, wear dusk mask to avoid inhaling contaminated dust with pathogen. Before cleaning the pigeon house, wet with water to avoid dust formation and spray with disinfectants such as 1% sodium hypochlorite solution (5 times diluted household bleach). Protect skin wounds and wear gloves by cleaning.

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Outbreak Investigation Express

A Case Report of Neonatal Legionellosis in a Nursery in Central Taiwan

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Abstract

Legionnaires' disease is one of nosocomial infections. It is rarely occurred in infants. One 7-day-old healthy neonate developed pneumonia in a nursery room of an obstetrics and gynecology clinic. *Legionella pneumophila* serogroup 5 was isolated from his sputum sample. He recovered from the pneumonia episode after receiving antimicrobial medication against *Legionella pneumophila*. Parents of neonates born within three months of the neonate's Legionnaires' disease were telephone interviewed to investigate neonates' health condition after birth. Four neonates were hospitalized; none of them were positive for serological test against *Legionella pneumophila* serogroups 1-6.

One strain of *Legionella pneumophila* serogroup 5 was isolated from the water dispenser which is located in the room next to the nursery room which showed indistinguishable PFGE profile with that of the strain isolated from the patient. The water dispenser was used for formula preparation for the neonates in the nursery room. *Legionella pneumophila* of serogroups other than 5 was isolated from the tap water samples obtained from the nursery room. A new water dispenser was used for formula preparation. Filters were mounted to tap water sources in the nursery room. No further case of Legionnaires' disease was detected in the clinic. This report highlights the essentiality of nosocomial infection control in nursery rooms. *Legionella pneumophila* is one of the agents that causes neonatal pneumonia.

Keywords: neonate, Legionnaires' disease, nosocomial infection

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