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REVIEW

Cryptococcus gattii: An emerging global mycotic pathogen of humans and animals

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The pathogenic *Cryptococcus* species consisting of *Cryptococcus neoformans* and *Cryptococcus gattii* are the principal cause of cryptococcosis which is a highly infectious global fungal disease of humans as well as animals. Cryptococcosis once considered as a sleeping giant has become an awakening giant after the discovery of human immune deficiency virus. It constitutes a major public health problem both in developed as well as developing countries. Globally, one million cases of cryptococcal meningitis occur every year in HIV/AIDS patients resulting in nearly 625,000 deaths. *Cryptococcus gattii* has emerged as an important mycotic agent which causes a significant burden on human and animal health. Global warming may be one of the important factors associated with the emergence of *Cryptococcus gattii* in some regions of the world. The pathogen, *Cryptococcus gattii*, has a propensity to infect mainly the immunocompetent subjects. It is found in the soil, air, water and trees. The source of infection is exogenous and the respiratory tract is recognized as the principal portal of entry of *Cryptococcus gattii*. The humans and animals may acquire the infection from the saprobic reservoirs. Both the fungi can be easily isolated from clinical and environmental samples on Pal sunflower seed medium. However, differentiation of *Cryptococcus gattii* from *Cryptococcus neoformans* requires L-canavaline-glycine- bromothymol blue agar. Molecular techniques are useful to identify the several genotypes of *Cryptococcus gattii*. A number of drugs such as amphotericin B, flucytosine, fluconazole and itraconazole are tried for the treatment of cryptococcal infection. Multidisciplinary approach is imperative to understand the dynamics of *Cryptococcus gattii* infection. Further studies on the ecologic niches, risk factors and therapeutic agents will be fruitful. Since animal infections due to *Cryptococcus gattii* are often a sentinel for human infection, the veterinarians are advised to immediately report the incident of cryptococcal disease.

Key words: *Cryptococcus gattii*, emerging pathogen, public health, saprobic environment

INTRODUCTION

There are a number of infectious diseases with multiple etiologies which pose serious threats to the health of humans and animals. Among the many microbial pathogens causing infectious diseases worldwide, fungi represent one important major class (Pal, 2007). In many cases, the incidence of fungal diseases is increasing due to the rise in susceptible hosts. Several fungal species frequently cause infections in immunocompromised hosts, while others are known to produce disease in apparently healthy individuals (Pal, 2007).The pathogenic *Cryptococcus* species complex, consisting of *Cryptococcus gattii* and *C. neoformans*, cause diseases in both of these populations. This is particularly evident with *C. gattii*, which is endemic in many tropical and sub-tropical regions and has been associated with outbreaks in humans and a wide range of mammals, making it a significant

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emerging mycotic zoonotic pathogen of global public health interest (Sorrell, 2001). The humans and animals infections due to *C. gattii* have been described from many countries of the world (Galanis and McDougall, 2010; Springer and Chaturvedi, 2010; Lester *et al.*, 2011; Patil *et al.*, 2013).Globally, *C. neoformans* and *C. gattii* affect approximately 1000,000 individuals annually with over 620,000 fatalities and account for about one third of all HIV/AIDS associated deaths, surpassing tuberculosis mortality in Africa (Park *et al.*, 2009).

Although humans and animals infections due to *C. neoformans* are reported earlier from many countries including India (Pal and Dave, 2006; Pal, 2007), in recent years *Cryptococcus gattii* has emerged as an important cause of cryptococcosis on Vancouver Island, British Columbia, Canada in 1999 causing epidemics of humans and animals infections and death (Lester *et al.*,2004;Galanis and McDougall, 2010; Lester *et al.*, 2011).Global warming may have been a factor for the emergence of *C. gattii* in British Columbia as long term changes in climate have created pockets of warm temperature which allows the fungus to grow and spread (Bartlett *et al.*, 2008). *Cryptococcus gattii* has been first described in Africa in a Congolese Bantu boy and the organism has revealed the unusual presence of elongated and cigar-shaped yeast morphology in cerebrospinal fluid (Springer, and Chaturvedi, 2010).

The genus *Cryptococcus* contains 37 species of which *C. neoformans* and *C. gattii* are more pathogenic and are implicated in most cases of human and animal cryptococcosis (Gupta and Fries, 2010). Both the fungi occur as saprobe in a variety of environment materials. *C .neoformans* is mainly recovered from avian droppings particularly the pigeon's excreta (Pal, 1997; Pal, 2007) where as *C. gattii* is isolated primarily from several trees species (Chowdhary *et al.*, 2012).Both the pathogens occur in yeast form and reproduce by budding. The mating produces the perfect (sexual) stage of the fungus. *C gattii* is an anamorph (asexual) and the teleomporh (sexual) state is *Filobasidiella bacillispora* which results from mating between *C. gattii* (Pal and Dave, 2006; Pal, 2007).*C. neoformans* and *C. gattii* exist in two mating types of "α" and "alpha" (Pal,1986; Pal and Dave, 2006).Several studies have indicated that "alpha" mating type is more frequent as compared to "α" mating types (Pal,1986; Pal *et al*,1991; Pal,1993; Pal,1997;Pal and Dave,2006). *C. gattii* has two serotypes: B and C. Based on a large global molecular epidemiologic survey, *C. gattii* could be divided into four major genotypes: VGI, VGII, VGIII, and VGIV using orotidine monophosphate pyrophosphorylase (URA5) gene restriction fragment length polymorphism (RFLP) analysis and M13 polymerase chain reaction (PCR) fingerprinting (Tseng *et al.*, 2013).The present paper highlights the growing significance of *C .gattii* as an emerging global human and animal mycotic pathogen.

HOST

Cryptococcus gattii infection is reported in humans and also in wide range of domestic, terrestrial and marine animals from many countries of the world (Torres-Rodrigues *et al.*, 2006; Brynes *et al.*, 2011).This pathogenic basidiomycetes yeast is known to produce natural infection in many species of animals which include alpaca, cat, cockatoo, dog, dolphin, echidna, ferret, goat, horse, kiwis, koala, llmas, porpoise, sheep, squirrel, and tapir (Sorrell,2001; Miller *et al.*, 2002; Krockenberger *et al.*, 2003; Lester *et al.*, 2004; Duncan *et al.*,2006; Torres-Rodrigues *et al.*, 2006; Bowles and Fry, 2009;Lester *et al.*, 2011;Norman *et al.*, 2011).Stephen and co-workers (2002) have investigated multispecies outbreak of cryptococcosis due to *C .gattii* in Southern Vancouver Island, British Columbia. Subclinical infection due to *C. gattii* has been described in cats and dogs (Duncan *et al.*, 2005). Outbreaks of disease have been observed in goats (Torres-Rodregues *et al.*,2006).In India, infection due to *C. gattii* has been documented in humans (Nagarathna *et al.*,2010;Patil *et al.*,2013).However, the infections due to *C. gattii* in domestic animals such as cats, dogs, goats and horses are common in Australia, Brazil ,Canada and New Zealand (Sorrell,2001;Duncan *et al.*, 2006; Bowles and Fry, 2009).Though animal infections due to *C. neoformans* are reported from India (Pal and Dave,2006;Pal,2007), there appears to be no documented case of *C. gattii* infection in animal host. Hence, it is important to mention that the role of *C. gattii* in various clinical disorders of animals should be studied in India and also in other countries.

GEOGRAPHIC DISTRIBUTION

The initial isolation of *Cryptococcus gattii* from hu-

man clinical samples in the tropical and subtropical regions suggested that *C. gattii* is a tropical or subtropical pathogen (Kwon-Chung and Bennett, 1984). More recently, however, clinical isolations from temperate regions in the United States, Canada, Europe, and Asia have widely expanded the prevalence areas of *C. gattii* (Sorrell, 2001). Accordingly, *C. gattii* has been reported from such diverse countries as Argentina, Austria, Canada, China, Congo, India, Italy, Japan, South Korea, the Netherlands, Spain, South Africa, United Kingdom, and United States (Datta *et al.*,2009;Nagarathna *et al.*,2010;Springer and Chaturvedi, 2010; Pail *et al.*, 2013;Tseng *et al.*, 2013).

Ellis and Pfeifer (1990) are credited to describe the first environmental isolation of *C. gattii* in 1990 in Australia from wood, bark, leaves, and plant debris of *Eucalyptus* trees. Now, *C. gattii* has been reported from 54 tree species and, evidently, *C. gattii* is established ecologically in trees other than *Eucalyptus* in several regions of the world, as supported by the isolations of *C. gattii* from the native trees in Argentina, Brazil, Canada, Colombia, Egypt, India, Italy, Mexico, and United States (Chowdhary *et al.* 2012).The recovery of *C. gattii* from several tree species especially from decayed hollows suggesting a possible ecologic niche of this pathogenic yeast (Springer and Chaturvedi, 2010).It is hoped that more countries may be included in the list every year.

TRANSMISSION

Cryptococcus gattii occurs as saprobe in the environment, usually in association with certain trees or soil around trees. Several activities as digging, cutting, hiking, construction and logging may increase the dispersion and concentration of *C .gattii* in the air. Historically, *Eucalyptus* trees have been implicated in the spread of *C. gattii* to different areas of the world. There are evidences to believe that humans as well animals may acquire the infection through the respiratory tract by inhaling the organisms from the saprobic reservoirs (Sorrell, 2001). In most cases, respiratory tract is considered as the prime portal of entry of pathogen. Occasionally, *C. gattii* can cause primary cutaneous cryptococcosis in an immunocompetent host who received traumatic injury while working with *Eucalyptus* trees (Perfect and Casadevell, 2002). Tranplacental transmission due to *C. gattii* has been recorded in a harbor porpoise (Norman *et al.*, 2011). Hitherto, there seems to be no authentic report of direct transmission of *C. gattii* infection from man to man, man to animal, and animal to man.

PATHOGENESIS

Humans and animals can become infected with *C. gattii* after inhaling airborne, dehydrated yeast cells or spores, which travel through the respiratory tract and enter the lungs of the host. The small size of the yeast and/or spores allows them to become lodged deep in the lung tissue. The environment inside the host body signals *C. gattii* to transform into its yeast form, and the cells grow thick capsules to protect themselves. The yeasts then divide and multiply by budding. After infecting the lungs, *C. gattii* cells can travel through the bloodstream, either on their own or within macrophage cells, to infect other areas of the body, typically the central nervous system (Sorrell, 2001; Perfect and Casadevell,2002;.Bartlett *et al.*, 2008;Pal *et al.*, 2012).

CLINICAL SPECTRUM

Cryptococcosis caused by the pathogenic species of *Cryptococcus* is a severe and life threatening mycotic disease of global importance and is associated with significant morbidity and mortality both in humans and animals (Pal *et al.*, 2012).The clinical course of infections are complex. It can present as meningoencephalitis, pneumonia and cryptococcemia in both immunocompetent and immunocompromised hosts (Tseng *et al.*, 2013).Some individuals with pulmonary infection are asymptomatic .The radiological findings of chest show lung cryptococcomas, infiltrates and cavitary lesions, and cryptococcomas are also detected in brain. Epidemiological studies suggest that patients infected with *C. gattii* mainly present with pulmonary disease, while those infected with *C. neoformans* commonly manifest meningoencephalitis (Sorrell, 2001; Perfect and Casedevell, 2002, Tseng *et al.*, 2013). Patients with severe disease frequently experienced headaches, altered mental status, fevers, nausea and vomiting. Coughing, shortness of breath, chest pain, night sweats, weight loss and seizures are also other clinical signs (Sorrell, 2001; Perfect and Casedevell, 2002; Tseng *et al,* 2013).

An unusual outbreak of cryptococcosis in cat, dog, ferret and bird has been investigated by Lester and co-workers (2004). In cats and dogs, the organs

affected due to *C.gattii* include the respiratory system, central nervous system and skin. Subclinical *C .gattii* infections are observed in cats and dogs (Duncan *et al.*, 2005). The clinical symptoms recorded by Torres-Rodriguez and co-investigators (2006) during outbreaks of cryptococcosis in goats due to *C. gattii* were nasal discharge, dysponea, cough, progressive cachexia, blindness, ataxia, paralysis and death.

DIAGNOSIS

Diagnosis of human and animal cryptococcosis frequently relies on direct microscopy, culture of clinical samples, or detection of cryptococcal antigen in body fluids using either latex agglutination (LA) or enzyme immunoassay (EIA) (Datta *et al.,* 2009). Culturing of clinical specimens like sputum, bronchoalveolar lavage (BAL), cerebrospinal fluid (CSF), tissue biopsies etc. is still considered the gold standard diagnostic method which can be easily attempted in most of the laboratories in the world. Antigen tests such as LA and EIA are performed on cerebrospinal fluid and serum, and are highly sensitive and specific diagnostic options. Molecular tests are employed for genotyping of *C. gattii* isolates (Tseng *et al.*, 2013). However, these methods require refrigeration, a cold chain for specimen transport, and technical expertise; therefore, they are often performed only in reference/diagnostic laboratories far from patients, potentially limiting their clinical utility. In addition, the costs of these tests are not affordable for many clinics. As a result, cryptococcosis often goes undiagnosed in many resource-limited countries. Moreover,experience of working with human and animal patients have shown that clinicians are not interested in species identification. Hence, it is advised to use easily available and less expensive sunflower seed agar (Pal medium) in all public health and microbiology laboratories for rapid isolation of *C. neoformans* / *C. gattii* from a variety of clinical material. This medium was developed by Pal in 1980 for the laboratory diagnosis and epidemiological investigation of cryptococcosis; and subsequent studies confirmed the overwhelming superiority of this medium with other media (Pal, 2007) .Both species of *Cryptococcus* imparts light to dark brown colonies on Pal medium. However, the differentiation of both species can be done on L-canavanine-glycine-bromothymol blue agar, D-tryptophan and also by D-proline assimilation test (Pal and Dave, 2006; Pal, 2007; Pal *et al.*, 2012).Sunflower seed medium was modified by Pal in 1986 at the Institute of Tropical Medicine, Antwerp, Belgium for studying sexual reproduction in *C. neoformans*. In vitro genetic crossing experiments are performed on modified Pal medium for indicating the mating types (Pal, 1986; Pal *et al*,1991;Pal,1993; Pal,1997).The morphology of the fungal cultures is studied in newly developed Narayan stain (Pal, 2004).

MANAGEMENT

The treatment of cryptococcosis is challenging as it is very expensive and requires usually a longer course of therapy. In human patients, at least 6 months of antifungal medication is advised. Amphotericin B often in combination with flucytosine is recommended for the treatment of severe cryptococcal infections including those with central nervous system involvement (Pal and Dave, 2006). The patients suffering with mild-to-moderate cryptococcosis can be treated with fluconazole or itraconazole (Pal *et al.*, 2012). Fluconazole can also be used for maintenance therapy in HIV-infected patients with cryptococcal meningoencephalitis (Pal, 2007). The same treatment regime is recommended for human and animal cryptococcosis. *C. gattii* infection requires more prolonged and invasive therapy (Sorrell, 2001). It is emphasized that the clinical efficacy of newly introduced antifungal drugs such as isavuconazole, posaconazole and voriconazole should be studied both in humans as well as in animals. Presently, there is no vaccine for *C. gattii* infection. However, one can take precautions when working with saprobic environment particularly with the trees. If symptoms develop, immediate therapy is highly imperative to avoid the further complications. Health education of the public is a critical component of the management of an emerging infectious disease.

CONCLUSION

Recently, *Cryptococcus gattii* has emerged as a zoonotic fungal pathogen causing pulmonary and systemic cryptococcosis mainly in apparently immunocompetent hosts. *Cryptococcus gattii* infection is of public health and economic importance. The infection has been mostly limited to tropical and subtropical areas. Currently, however, epidemiologic studies have shown that *C. gattii* occurs in areas other than tropical or subtropical zones.

The pathogen has been reported from 54 tree species in many parts of the world including India. Infection with *C. gattii* can present as meningoencephalitis, pneumonia and cryptococcemia. However, studies have suggested that patients infected with *C. gattii* mainly present with pulmonary disease, while those infected with *C. neoformans* commonly manifest meningoencephalitis. Diagnosis of cryptococcosis relies on direct microscopy in India ink, culture of clinical samples on Pal sunflower seed medium, or detection of cryptococcal antigen in body fluids using latex agglutination. Treatment of cryptococcosis usually takes long time. An early diagnosis and prompt therapy can reduce the morbidity and mortality of *C. gattii* infection. The complex interactions between the agent, host and environment need to be elucidated. More studies are required to investigate the role of *C .gattii* in immunocompetent as well in immune-suppressed subjects.

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