INTRODUCTION — Cryptococcosis is an invasive fungal infection due to Cryptococcus neoformans which has become increasingly prevalent in immunocompromised patients. The microbiology and epidemiology of Cryptococcus neoformans and cryptococcosis will be reviewed here. The clinical features of cryptococcal infections are discussed separately.

MICROBIOLOGY

Serotypes — Cryptococcus neoformans is a basidiomycetous, encapsulated yeast. C. neoformans can be subclassified into four serotypes and two varieties. The serotypes are based upon capsular agglutination reactions and are designated A, B, C, or D. Serotype A and D cryptococci were previously classified under the variety neoformans. However, it has been proposed that the serotype A cryptococci be considered as a separate variety based upon genotypic differences [1] . Serotype A cryptococci are now considered variety grubii; serotype D are classified under the variety neoformans. Serotypes B and C are now considered as a separate species called Cryptococcus gattii [2] .

The significant differences in the ecology and epidemiology of the two varieties are discussed below. The clinical presentation of cryptococcosis due to the two varieties is generally indistinguishable, although one study suggests some possible distinctive features which are described below [3] .

Life cycle — The life cycle of C. neoformans involves asexual and sexual forms. The asexual form exists as yeast and reproduces by budding. These haploid, unicellular yeasts are the only forms of C. neoformans that have been recovered from human infections.

The sexual (perfect) state of C. neoformans has only been observed in the laboratory. It is unknown whether sexual reproduction occurs in nature. The yeast forms can exist in one of two mating types designated a and alpha. Coculture of yeast of each mating type on certain agars can result in conjugation which produces the sexual state. Conjugation between the a and b types results in the formation of the teleomorph which consists of dikaryotic hyphae that contain clamp connections. Some of the hyphae develop into specialized structures called basidia.

Meiosis occurs at the terminal portion of the basidia resulting in formation of uninucleate basidiospores. These spores are initially unencapsulated, but can quickly

develop capsules when released from the basidia. Budding may begin after encapsulation, thereby completing the life cycle.

Growth and identification — C. neoformans produces white, mucoid colonies on a variety of agars that usually become visible to the naked eye within 48 hours (show picture 1). The identification of C. neoformans in the clinical laboratory is supported by the presence of an urease-positive, encapsulated yeast. Further confirmation can be achieved with biochemical tests contained in commercial kits and by detection of the enzyme phenol oxidase which is solely produced by C. neoformans. However, most clinical microbiology laboratories do not stock the expensive agars required for detecting this enzyme. Most laboratories use a variety of sugar fermentations contained in commercial kits to identify the organism.

Histologic identification — The yeast form of cryptococci can be identified in histopathologic specimens using methenamine silver stain (show histology 1A-1B). Mucicarmine stain shows both the yeast form and the capsule, and Fontana-Masson stain reveals melanin contained in the yeast.

Capsule — The polysaccharide capsule surrounding C. neoformans can be visualized in a suspension of India ink when examined under the microscope. The capsule appears as a clear area amidst the ink particles (show picture 2). The thickness of the capsule can vary, but can comprise more than 50 percent of the diameter of the yeast cell in some isolates.

The capsule has antiphagocytic properties and is an important virulence determinant [4] . Mutant cryptococci that are either hypocapsular or acapsular are less virulent in animal models than encapsulated strains, and are associated with increased phagocytosis by white blood cells in vitro [5,6] .

Cryptococcus neoformans strains can undergo phenotypic switching with prolonged in vitro passage, and one group has demonstrated this phenomenon in vivo in mice that resulted in increased virulence and death [7] . In this model, phenotypic switching led to changes in the polysaccharide capsule, which further reduced alveolar macrophage phagocytosis and promoted a more vigorous inflammatory reaction that was destructive to lung tissue.

Melanin production — The presence of the enzyme phenol oxidase in C. neoformans is unique among members of the genus. This enzyme catalyzes one step in the conversion of phenolic compounds to melanin. A wide variety of phenolic

substrates can be utilized by the cryptococcal phenol oxidase including catecholamines such as dopamine and epinephrine.

The phenol oxidase enzyme may be an important virulence factor for cryptococcal infection. On the other hand, cryptococcal mutants lacking phenol oxidase activity are avirulent in animal models and are more susceptible to antibody-mediated phagocytosis [8] .

Phenol oxidase may promote virulence via one of several possible mechanisms:

- The high level of dopamine in the central nervous system may serve as a substrate for melanin production by the organism [9] . In addition, the ability to degrade catecholamines may protect the yeast from toxic effects of catecholamines in the central nervous system [10] .
- The melanin that is produced accumulates in the cell wall where it may protect against attack by immune effector cells [11] .

ECOLOGY — Significant differences in the ecology of the two varieties of C. neoformans account for the distribution of infections due to these organisms.

C. neoformans var grubii and neoformans — C. neoformans var grubii and neoformans has been found in soil samples from around the world in areas frequented by birds, especially pigeons and chickens [12] . This fungus has also been isolated from roosting sites of pigeons and in association with rotting vegetation [13] .

The basis for the association of pigeons with C. neoformans var neoformans is uncertain. Pigeons do not become infected with C. neoformans in nature. The organism is probably inhibited within pigeons by their elevated body temperature (over 40ºC). Pigeons can, however, harbor the yeast as saprophytes in their gastrointestinal tract.

The role of pigeon guano in the pathogenesis of human infections is obscure. A history of intense pigeon exposure is only rarely elicited from patients with cryptococcosis. In addition, outbreaks of the disease have never been traced to pigeon roosting areas. It is possible that pigeons come into contact with C. neoformans var grubii and var neoformans by eating contaminated vegetation. Human infection may result from a similar exposure to contaminated vegetation.

C. gattii — As opposed to var neoformans and var grubii, C. gattii has never been cultured from bird guano. It has been cultured from river red gum trees (Eucalyptus camaldulensis) and forest red gum trees (Eucalyptus tereticornis) in Australia [14,15] . These trees were exported from Australia to various parts of the world. Strains of Cryptococcus gattii have been recovered from river red gum trees in San Francisco [16] . It has also been recovered from some trees native to Vancouver Island, including Douglas fir, alder, and Garry oak [17] .

EPIDEMIOLOGY — Cryptococcosis is a world-wide infection that only rarely causes disseminated infection in healthy individuals. The vast majority of patients with cryptococcosis are immunocompromised due to one of the following conditions (listed in order of decreasing frequency):

- Acquired immunodeficiency syndrome (AIDS)
- Prolonged treatment with corticosteroids
- Organ transplantation
- Malignancy
- Sarcoidosis

An incidence of 4.9 cases of cryptococcosis per 100,000 population was found in both San Francisco and Atlanta in 1992 [18] . Underlying AIDS and African-American race were independently associated with cryptococcal infection in these cities.

Cryptococcal infection is the fourth most common opportunistic infection in patients with AIDS [19,20] . Approximately 6 to 10 percent of patients with AIDS in the pre-HAART era in the United States and Europe were diagnosed with cryptococcosis. The number of cryptococcosis cases have declined since the availability of HAART [21] , but this infection is still a relatively common AIDS-presenting illness. The rate of cryptococcal meningitis in patients with AIDS in sub-Saharan Africa is 15 to 30 percent [20] .

As noted above, despite the finding of this fungus in pigeon guano, direct transmission from pigeon to human has not been reported. However, one case has been documented of transmission of C. neoformans from a pet cockatoo to a patient taking immunosuppressive medications following renal transplantation who developed cryptococcal meningitis [22] . Spread of infection from person to person has not been documented. Interestingly, cryptococcosis is very uncommon in children, even children with AIDS. Why this occurs is not known.

An outbreak of C. gattii infection has been identified in Vancouver Island, British Columbia [17,23,24] . Starting in 1999, there was a significant increase in the incidence of cryptococcosis in Vancouver Island, and between 1999 and 2002, at least 59 patients were found to have infections [17] . Most of the patients were immunocompetent, and had pulmonary disease [17,24] .

Serotypes — C. neoformans is the principal pathogenic member of the genus. The serotypes of C. neoformans in cases of cryptococcosis vary according to geographic location and whether the patient has HIV infection as a predisposing condition.

An extensive review of 725 clinical isolates of C. neoformans obtained from around the world prior to the AIDS epidemic found that var grubii and var neoformans accounted for 80 percent of isolates [25] . Almost all cryptococcal isolates from patients with AIDS have been var grubii, regardless of geographic location.

The isolates of C. gattii have been almost exclusively found in tropical and subtropical areas such as Hawaii, Brazil, Australia, Southeast Asia, and Central and sub-Saharan Africa [25,26] . The most common site of C. gattii infection in the United States was in Southern California. The C. gattii cryptococci found in patients in temperate areas may have been acquired in a tropical area, except for the outbreak of infection that has been described in Vancouver Island [17] .

A series of 133 cases from Australia occurring over a 10 year period suggests some possible differences in the clinical illness caused by the two varieties [3] .

- All C. gattii infections occurred in healthy hosts while 90 percent of C. neoformans var neoformans infections occurred in immunosuppressed hosts.
- C gattii causes focal CNS and pulmonary disease primarily in immunocompetent patients; however, in a study of 176 cryptococcal isolates from AIDS patients with meningoencephalitis in sub-Saharan African, C. gattii was isolated in 13 percent of patients from Malawi and Botswana [27] .
- Isolation of C. neoformans from blood and urine was associated with immunosuppression and var neoformans infection.
- The mortality among patients with var grubii and neoformans infection was high, but this was most likely due to the underlying diseases found in the infected patients.

- Infections due to C. gattii were more likely to present in an insidious fashion and to be associated with neurologic complications such as hydrocephalus and cranial nerve deficits.

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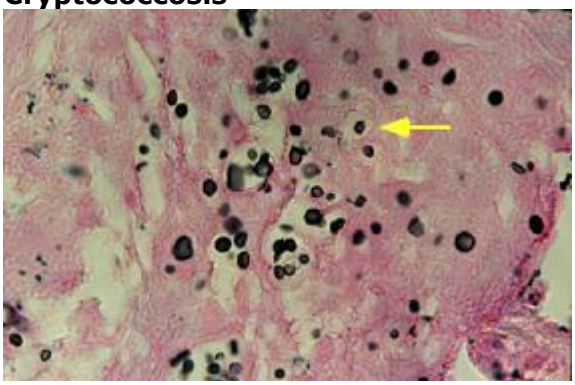
GRAPHICS

Cryptococcus neoformans on Sabouraud's agar

Culture of Cryptococcus neoformans on Sabouraud's agar. *Courtesy of Harriet Provine.*

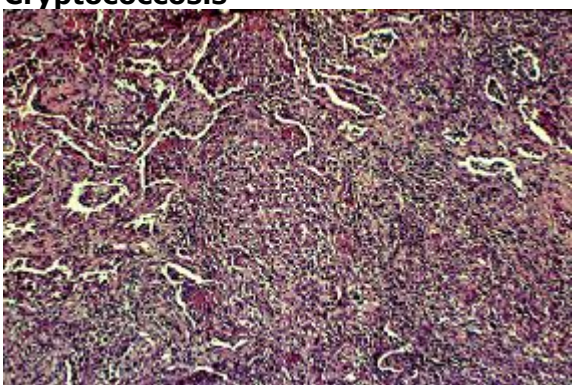
Cryptococcus silver stain lung

Cryptococcosis



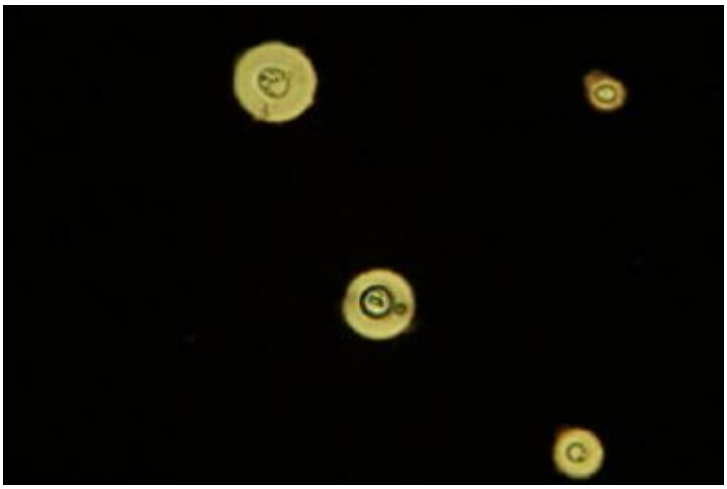
Photomicrograph of a silver stained slide shows multiple organisms of Cryptococcus neoformans. Many of the organisms are surrounded by a pale halo reflecting the presence of a polysaccharide capsule (arrow). The yeast forms themselves tend to be variable in size and shape and reproduce by a process of narrow neck budding, in contrast to broad-based budding in blastomycosis. *Courtesy of Jeffrey L Myers, MD.*

Cryptococcosis



Low power photomicrograph shows a nonspecific pattern of organizing pneumonia in a patient with cryptococcosis. Well-formed granulomas can occur but are lacking in this example. *Courtesy of Jeffrey L Myers, MD.*

Cryptococcus neoformans in an india ink preparation



India ink preparation of cerebrospinal fluid (x400) shows a prominent clear zone around individual yeasts, consistent with the capsule of Cryptococcus neoformans. The yeast in the center of the slide is budding. *Courtesy of Harriet Provine.*

Source from UPTODATE 2008.