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CASE REPORTS

First Reported Isolation of *Cryptococcus neoformans* var. *gattii* from a Patient in Singapore

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Cryptococcal meningitis has long been known to afflict immunocompetent patients in Singapore. We report the first identification of an isolate of *Cryptococcus neoformans* var. *gattii* from a Singaporean resident; this variety can cause invasive disease in the immunocompetent. Meningitis in a traveler returning from this area may be cryptococcal.

CASE REPORT

A previously healthy 47-year-old Singaporean Chinese beverage seller presented in August 2000 with a several-week-long history of confusion, productive cough, fever, and loss of weight. He had visited Bangkok, Thailand, in April 2000 and Kuala Lumpur, Malaysia, in June 2000; he also made regular day trips to Johor Bahru, Malaysia, which is just on the other side of the causeway from Singapore. Upon admission, general and neurological examinations of the patient were normal other than disorientation in time and person; however, a chest X ray revealed consolidation in the right upper lobe and an enlarged right hilum. A magnetic resonance head scan showed an enhancing lesion in the right frontal lobe with surrounding edema and midline shift. Capsulated yeasts were seen in a bronchial biopsy, and *Cryptococcus neoformans* was subsequently cultured from a stereotaxic brain biopsy; further testing revealed that this isolate was *Cryptococcus neoformans* var. *gattii*. His flat was close to a railway station inhabited by large numbers of pigeons; his family were unable to give any information regarding eucalyptus trees in the vicinity of his home or workplace. Bronchoscopic specimens failed to reveal any malignant cells. Human immunodeficiency virus serology tests were negative, and all other laboratory indices of immune function, including $CD4^+$-cell count, were unremarkable. He was treated for 3 weeks with intravenous amphotericin B (0.4 mg/kg of body weight/day) and flucytosine (150 mg/kg/day), which he tolerated well, and this was then changed to fluconazole (400 mg per day orally) for maintenance therapy. His chest radiographic signs resolved; however, although his confusional state improved slightly, there was only minimal resolution of the frontal abscess on a repeat magnetic resonance scan, despite an increase in the dose of fluconazole to 800 mg daily for 3 months.

C. neoformans is an encapsulated yeast which causes infections, usually of the central nervous system, typically in immunocompromised patients. *Cryptococcus neoformans* var. *neoformans* is the variety most commonly isolated worldwide. This variety is commonly isolated from bird (especially pigeon) feces throughout the world. It is uncertain whether avian droppings are the ultimate source of most human infections or in what form the agent is transmitted (yeast or basidiospore) (1). *C. neoformans* var. *gattii* is particularly associated with eucalyptus trees, with few reports from other sources (1); most clinical isolates of this variety are from patients from the tropics and subtropics. The two may be distinguished by capsule serotype (*C. neoformans* var. *neoformans*, serotype A, D, or AD; *C. neoformans* var. *gattii*, serotype B or C) (1). They may also be distinguished by growth pattern on canavanine-glycine-bromothymol blue agar: *C. neoformans* var. *gattii* produces a color change (green to blue) while *C. neoformans* var. *neoformans* does not (4). Only serotype B has been isolated from eucalyptus trees (1). It has been suggested that all serotype A isolates be reclassified as a new variety, *Cryptococcus neoformans* var. *grubii* (3).

C. neoformans var. *gattii* is believed to behave more aggressively than *C. neoformans* var. *neoformans*, more often causing infections in immunocompetent patients (1). In a 10-year Australian study of patients with cryptococcal infections (5), the varietal status of 71 isolates was determined. Forty-six of 51 patients infected with *C. neoformans* var. *neoformans* were in some way immunocompromised while all 20 patients with *C. neoformans* var. *gattii* were judged to be free of underlying immune defects. Additionally, pulmonary involvement was more frequent in the group infected with *C. neoformans* var. *gattii*. In a study published in 1972, 30 Singaporean patients with cryptococcal meningitis were reported (6). Only one-third

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of the patients had some variety of preexisting chronic disease (four had systemic lupus erythematosus and were on long-term steroid immunosuppressive treatment, and the remaining six had a disparate set of diagnoses: two of pulmonary tuberculosis, and one each of myasthenia gravis, schizophrenia, chronic lung disease, and hypertension), and none of them had a malignancy or reticulosis. Ten of these patients had pulmonary lesions attributed to cryptococcal infection. Seven patients required some form of ventricular decompression (shunting to the peritoneum or atrium). It remains the perception among physicians in Singapore that cryptococcal infections behave aggressively here and frequently affect patients with no underlying immune defect.

We examined a series of 24 isolates of *C. neoformans* isolated from 22 patients admitted to Tan Tock Seng Hospital over an 18-month period. One grew on canavanine-glycine-bromothymol blue agar with the production of a blue color change; this organism was a yeast which yielded a positive reaction after incubation on Christensen's urea agar and which was determined by microscopy to be capsulated from an India ink preparation. Subculture to bird seed agar (BBL) yielded brown mucoid colonies. It was reconfirmed as *C. neoformans* using the API C auxotyping system, yielding the profile 2547133. The isolate was serotyped by using the antisera provided in the Crypto Check Iatron kit (Iatron Laboratories, Tokyo, Japan) and determined to be type B. It was isolated from the patient described above.

We are unable to find any previous report of *C. neoformans* var. *gattii* isolated from a patient in Singapore in the literature. Clinicians should be aware that this variety of *C. neoformans* may cause disease in previously fit individuals living on the island. In addition, Singapore receives several million tourists every year; returning travelers who develop meningitis may be infected with this fungus. There are many eucalyptus trees in Singapore, including specimens of the species most frequently identified as a source of *C. neoformans* var. *gattii*, *Eucalyptus camaldulensis* (2), and we continue to seek this yeast from these and other local environmental sources.

REFERENCES

1. **Casadevall, A., and J. R. Perfect.** 1998. *Cryptococcus neoformans.* ASM Press, Washington, D.C.
2. **Ellis, D. H., and T. J. Pfeiffer.** 1990. Natural habitat of *Cryptococcus neoformans* var. *gattii*. J. Clin. Microbiol. **28:**1642–1644.
3. **Franzpot, S. P., I. F. Salkin, and A. Casadevall.** 1999. *Cryptococcus neoformans* var. *grubii*: separate varietal status for *Cryptococcus neoformans* serotype A isolates. J. Clin. Microbiol. **37:**838–840.
4. **Kwon-Chung, K. J., I. Polacheck, and J. E. Bennett.** 1982. Improved diagnostic medium for separation of *Cryptococcus neoformans* var. *neoformans* (serotypes A and D) and *Cryptococcus neoformans* var. *gattii* (serotypes B and C). J. Clin. Microbiol. **15:** 535–537
5. **Speed, B., and D. Dunt.** 1995. Clinical and host differences between infections with the two varieties of *Cryptococcus neoformans*. Clin. Infect. Dis. **21:**28–34.
6. **Tay, C. H., W. L. S. Chew, and L. C. Y. Lim.** 1972. Cryptococcal meningitis: its apparent increased incidence in the Far East. Brain **95:**825–832.