

## *Cryptococcus gattii* Infection: Characteristics and Epidemiology of Cases Identified in a South African Province with High HIV Seroprevalence, 2002–2004

Juliette Morgan,<sup>1</sup> Kerrigan M. McCarthy,<sup>3</sup> Susan Gould,<sup>3</sup> Ke Fan,<sup>2</sup> Beth Arthington-Skaggs,<sup>1</sup> Naureen Iqbal,<sup>1</sup> Karen Stamey,<sup>1</sup> Rana A. Hajjeh,<sup>2</sup> and Mary E. Brandt,<sup>1</sup> for the Gauteng Cryptococcal Surveillance Initiative Group

<sup>1</sup>Mycotic Diseases Branch and <sup>2</sup>Coordinating Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; and <sup>3</sup>Mycology Reference Unit, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa

**We describe 46 *Cryptococcus gattii*-infected persons identified by population-based surveillance conducted in South Africa. Most patients with *C. gattii* infection presented with meningitis. The mortality rate during hospitalization was 36%. We found no significant differences between persons with and persons without *C. gattii* infection with regard to clinical presentation, acquired immunodeficiency syndrome diagnosis, concomitant conditions, or prior opportunistic infections. *C. gattii* isolates had low MICs to the tested antifungal drugs.**

Meningitis caused by *Cryptococcus neoformans* is one of the most life-threatening opportunistic diseases in HIV-infected individuals. In sub-Saharan Africa, an area with a high prevalence of HIV infection, the incidence and mortality of cryptococcosis are largely unknown. In Gauteng Province, South Africa, a population-based surveillance for cryptococcosis initiated in 2002 documented an overall incidence rate of 15.6 cases per 100,000 population, with a 27% mortality rate [1].

The major species involved in AIDS-associated cryptococcosis is *Cryptococcus grubii* (serotype A; also known as *Cryptococcus neoformans* var. *grubii*). Infections caused by *C. neoformans* (serotype D; also known as *C. neoformans* var. *neoformans*) and *C. neoformans* serotype AD occur in lesser proportions. These species have a worldwide distribution. A

smaller proportion of disease is caused by *Cryptococcus gattii* (serotypes B and C; also known as *C. neoformans* var. *gattii*), which occupies a more restricted habitat [2]. AIDS-associated cryptococcosis is caused primarily by *C. grubii* or *C. neoformans*, even in areas where *C. gattii* is endemic. The proportion of non-AIDS-associated infections caused by *C. gattii* did not change after the advent of the AIDS pandemic [3–6], whereas the number of AIDS-associated *C. grubii* and *C. neoformans* infections increased significantly. Among persons not infected with HIV, *C. gattii* infection frequently presents as a lesion localized in the lung (as pulmonary nodules) or CNS (as localized CNS lesions) [7–9], whereas in HIV-infected individuals, infection presents largely as meningitis, with or without fungemia. We report the clinical characteristics, epidemiology, and antifungal susceptibility data from a surveillance case series of *C. gattii* performed in South Africa.

**Methods.** Population-based surveillance for cryptococcosis was conducted in Gauteng Province (population, 9 million), South Africa, from March 2002 through February 2004 [1]. For this analysis, a case of *C. gattii* infection was defined as the incident isolation of *C. gattii* from a Gauteng Province resident during the study period. A patient was considered to be HIV infected when a positive HIV test result or a history of a positive test result was documented during the incident hospitalization. Patients with a documented negative HIV test result obtained during the incident hospitalization were considered to be HIV uninfected. Patients were considered to have AIDS if they met the Centers for Disease Control and Prevention's (CDC's) revised 1993 AIDS classification [10]. Surveillance officers collected supporting medical data weekly from laboratories and medical records.

Isolates were identified as *C. neoformans* at the originating institutions and were then sent to the National Health Laboratory Service Mycology Reference Unit of the National Institute for Communicable Diseases (Johannesburg, South Africa) for species confirmation and serogrouping using canavanine-glycine–bromthymol blue agar [11]. Most *C. gattii* isolates and a subset of non-*gattii* *Cryptococcus* isolates were sent to the CDC's Fungus Reference Unit (Atlanta, GA), where the species was confirmed by serotyping using the Crypto-Chek kit (Iatron). Two methods for MIC testing were compared: the M-27A2 broth microdilution method [12] and the microdilution method of Ghannoum et al. [13], which were both performed in accordance with the published protocols.

Categorical data were compared using Fisher's exact test with

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Reprints or correspondence: Dr. Mary Brandt, Centers for Disease Control and Prevention, 1600 Clifton Rd., Mailstop G-11, Atlanta, GA 30333 (MBrandt@cdc.gov).

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**Table 1. Viable *Cryptococcus* isolates recovered, by HIV status of the patient, during cryptococcosis surveillance in Gauteng Province, South Africa, 2002–2004.**

HIV status	No. (%) of isolates				Total (n = 1912)
	Non- <i>gattii</i> species of <i>Cryptococcus</i> <sup>a</sup> (n = 1866)	<i>C. gattii</i>			
		Serotype B (n = 19)	Serotype C (n = 22)	Not serotyped (n = 5)	
HIV infected	1203 (64)	13 (68)	11 (50)	4 (80)	1231
HIV uninfected	3 (0.16)	1 (5.3)	0	0	4
Unknown	660 (35)	5 (26.3)	11 (50)	1 (20)	677

<sup>a</sup> A total of 143 isolates were serotype A; the rest were not serotyped.

SAS software, version 9.1 (SAS Institute). A *P* value of  $\leq .05$  was considered to be statistically significant.

**Results.** Incident cryptococcosis was reported in 2753 Gauteng residents during the 2-year surveillance period. In 2434 cases (88%), *C. neoformans* was recovered at the submitting laboratory. The National Institute for Communicable Diseases laboratory received 1912 viable isolates (78% of isolates) and sent 219 isolates from 186 patients to the CDC for confirmation and MIC testing. All isolates tested at the CDC were either serotype A, B, or C; no serotype D or AD isolates were identified (table 1). A total of 46 isolates (2.4%) were confirmed to be *C. gattii*, and 41 were serotyped (serotype B, 19 isolates; serotype C, 22 isolates). In addition, 5 isolates were identified as *C. gattii* in South Africa but were not serotyped.

The median age of the 46 *C. gattii*-infected patients was 35 years (range, 10–54 years). Twenty-four patients (53%) were female, and all (100%) were of black race. Most of the *C. gattii*-infected patients presented with symptoms of meningitis: 35 (76%) complained of headache, 31 (67%) had a stiff neck, and 25 (54%) had fever (figure 1). Less specific symptoms included nausea and/or vomiting, cough, shortness of breath, and night sweats. Twenty-nine patients (63%) had a concomitant AIDS-associated infection or illness present at the time of hospitalization: 39% had oral candidiasis, 24% had pulmonary tuberculosis, 4% had *Pneumocystis jiroveci* pneumonia or another type of pneumonia, 22% had wasting, and 11% had lymphadenopathy.

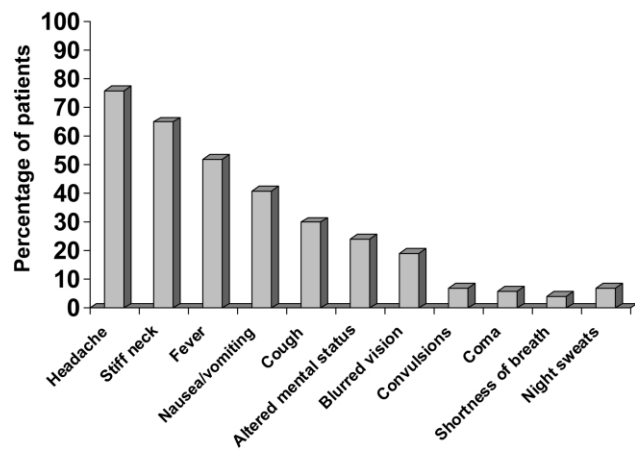
*C. gattii*-infected patients included 28 patients (61%) who were known to be HIV infected; the HIV infection status was unknown for 17 patients (37%), and 1 patient was not HIV infected (2.2%) (table 1). Among patients who were known to be HIV infected, 18 (64%) had experienced an AIDS-defining condition before hospitalization for cryptococcosis; pulmonary tuberculosis was the most common (39%), followed by *P. jiroveci* pneumonia, extrapulmonary tuberculosis, and other pneumonias (7% for all). CD4 cell counts were determined for 8 patients, and the median CD4 cell count was 54 cells/ $\mu$ L (range, 8–176 cells/ $\mu$ L). Similar percentages of HIV-infected patients were infected with each of the cryptococcal serotypes (table 1).

Lumbar puncture was performed for all 46 *C. gattii*-infected

patients. India ink stain test results were positive for 44 patients (96%), cryptococci were recovered from 43 (97%), and cryptococcal latex agglutination test results were positive for 20 (87%). Similar CSF laboratory results were seen for the overall surveillance [1]. Blood samples were obtained for culture from 5 (11%) of the 46 *C. gattii*-infected patients; 3 (60%) of these cultures yielded positive results. The 2 MIC testing methods generated essentially identical results. Isolates generally demonstrated low MICs for the tested antifungal drugs (table 2).

Most *C. gattii*-infected patients (84%) received antifungal treatment (fluconazole was administered to 69%, amphotericin B was administered to 30%, and 11% received both drugs). Sixteen patients (35.6%) died during the incident hospitalization. The median time from hospitalization to death was 9 days (range, 0–100 days). Of the patients who were discharged from the hospital, 72% were given outpatient fluconazole treatment.

Table 3 shows the results of univariate analysis of demographic and clinical characteristics. No significant differences were found with regard to clinical signs or symptoms, such as headache, neck stiffness, fever, cough, and shortness of breath (data not shown). Given the paucity of CD4 cell count data, the diagnosis of AIDS and prior opportunistic infections were



**Figure 1.** Clinical manifestations of *Cryptococcus gattii*-infected patients determined from cryptococcosis surveillance in Gauteng Province, South Africa, 2002–2004.

**Table 2. MICs for 41 *Cryptococcus gattii* isolates collected during cryptococcosis surveillance in Gauteng Province, South Africa, 2002–2004.**

Serotype, antifungal agent	MIC range, $\mu\text{g}/\text{mL}$	MIC <sub>50</sub> , $\mu\text{g}/\text{mL}$	MIC <sub>90</sub> , $\mu\text{g}/\text{mL}$
Serotype B (n = 19)			
Fluconazole	0.5–8	1	4
Itraconazole	0.03–0.5	0.125	0.25
Voriconazole	0.015–0.125	0.03	0.06
Serotype C (n = 22)			
Fluconazole	0.5–8	2	4
Itraconazole	0.03–0.5	0.125	0.25
Voriconazole	0.015–0.125	0.03	0.06

used as surrogate markers of immunosuppression when we assessed the correlation between serogroup and degree of immunosuppression; no significant differences were found for these variables between *C. gattii*-infected patients and patients without *C. gattii* infection or between patients infected with *C. gattii* serotype B and those infected with serotype C (table 3). A significantly lower proportion of *C. gattii*-infected patients received treatment, compared with patients who were not infected with *C. gattii* ( $P = .02$ ). The mortality associated with cryptococcosis was also not influenced by the serogroup and/or serotype.

**Discussion.** We identified 2.4% of incident cryptococcal isolates as *C. gattii* serotypes B or C during our 2-year-long, population-based cryptococcal surveillance in Gauteng Province, South Africa. The true incidence may be underrepresented, because viable isolates are required for serotyping, and the capacity to perform diagnostic assessments for cryptococcal disease in this resource-poor area of the world is limited. Presently, cryptococcosis diagnosis in Gauteng hospitals is based on culture results and the findings of examinations of India ink-stained CSF spec-

imens obtained from patients with meningitis [1]; thus, examiners miss patients who have systemic symptoms (fever and headache without meningeal signs) or who have pulmonary nodules or localized CNS lesions. In a study from France, a more wealthy country, 29% of HIV-infected persons received diagnoses without the use of CSF culture [14].

The lack of surveillance in Africa and the predominance of *C. grubii* since the advent of the AIDS pandemic suggested that infection due to *C. gattii* serotype C was essentially nonexistent in this continent [15]. In this surveillance, we found that 54% of the *C. gattii* isolates belonged to serotype C. In a recent study from Malawi and Botswana, all of the *C. gattii* isolates examined were serotype C [16].

We found no clinical differences or any difference in the level of immunosuppression between *C. gattii*-infected and *C. gattii*-uninfected patients who presented with cryptococcal meningitis. We cannot generalize these data to populations that may have access to more sophisticated means of diagnosis, because diagnostic limitations in South Africa prevent detection of cerebral mass lesions or pulmonary nodules. For such a study, case-finding that includes more extensive diagnostic practices for a wider range of clinical presentations beyond meningitis would be necessary.

Previous studies suggest that *C. gattii* isolates have higher MICs than do non-*C. gattii* isolates [17–21], raising concerns that *C. gattii*-infected patients might demonstrate slower responses to antifungal therapy [7, 22, 23]. We tested the largest series, to our knowledge, of *C. gattii* isolates from human cryptococcal disease, and we found that the MICs for serotype B and C isolates were low for all 3 azole drugs tested. Although there are no established interpretive breakpoints for cryptococci with any antifungal drugs, these results are comparable to those obtained for cryptococcal isolates in the United States [24] and

**Table 3. Univariate analysis of demographic and clinical characteristics of patients with and without *Cryptococcus gattii* infection from cryptococcosis surveillance in Gauteng province, South Africa, 2002–2004**

Characteristic	No. of patients with characteristic/no. of patients with data (%)		P
	<i>C. gattii</i> -infected patients (n = 46)	Non- <i>C. gattii</i> -infected patients (n = 1866)	
Female sex	21/45 (46.7)	885/1860 (47.6)	NS
Adult (age, >16 years)	44/46 (95.7)	1805/1866 (96.7)	NS
HIV infected	28/29 (96.6)	1203/1206 (99.8)	NS
AIDS	18/28 (64.3)	741/1203 (61.6)	NS
Concomitant condition	29/46 (63)	1089/1866 (58.4)	NS
Lumber puncture performed	46/46 (100)	1827/1866 (98)	NS
Received treatment	38/45 (84.4)	1695/1800 (94.2)	.02
Died	16/45 (35.6)	531/1810 (29.3)	NS
Discharged from the hospital while receiving fluconazole	27/29 (93.1)	1200/1277 (94)	NS

**NOTE.** NS, not significant.

Africa [25]. Methods for MIC testing of cryptococci in the laboratory remain nonstandardized, and correlation with clinical outcome is still unknown.

Although the mortality associated with *C. gattii* infection in this study was high (35.6%), it was not significantly different from that for non-*C. gattii* infections. We did find that *C. gattii*-infected patients were less likely than *C. gattii*-uninfected patients to receive antifungal treatment, but the significance of this finding is not clear.

In summary, AIDS-associated *C. gattii* infection is likely to be more common in Africa than was previously thought, but we found no differences in clinical presentation, outcome illness, or antifungal susceptibility between *Cryptococcus* species. Identification of *Cryptococcus* species does not appear to be necessary for routine management in this HIV-infected population, because *C. gattii* infection does not appear to impact patient management or outcome more severely than do infections with other species. However, in this era of widely available antiretroviral treatment, ongoing surveillance for cryptococcal disease will be important to monitor epidemiologic trends.

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