Epidemiology and Host- and Variety-Dependent Characteristics of Infection Due to *Cryptococcus neoformans* in Australia and New Zealand

Sharon Chen,[1] Tania Sorrell,[1] Graeme Nimmo,[3] Bryan Speed,[4] Bart Currie,[5] David Ellis,[6] Deborah Marriott,[7] Tania Pfeiffer,[6] Dinah Parr,[8] Karen Byth,[2] and the Australasian Cryptococcal Study Group[a]

[1]Centre for Infectious Diseases and Microbiology, Westmead Hospital, Sydney, New South Wales, and [2]Westmead Institute of Health Research, Westmead, New South Wales, [3]Department of Microbiology, Princess Alexandra Hospital, Brisbane, Queensland, [4]Department of Infectious Diseases, Austin, and Repatriation Medical Centre, Melbourne, Victoria, [5]Menzies School of Health Research, Darwin, Northern Territory, [6]Mycology Unit, Women's and Children's Hospital, Adelaide, South Australia, [7]Department of Microbiology, St. Vincent's Hospital, Sydney, New South Wales, Australia; and [8]Auckland Hospital, Auckland, New Zealand

A prospective population-based study was conducted in Australia and New Zealand during 1994–1997 to elucidate the epidemiology of cryptococcosis due to *Cryptococcus neoformans* var. *neoformans* (CNVN) and *C. neoformans* var. *gattii* (CNVG) and to relate clinical manifestations to host immune status and cryptococcal variety. The mean annual incidence per 10^6 population was 6.6 in Australia and 2.2 in New Zealand. Of 312 episodes, CNVN caused 265 (85%; 98% of the episodes in immunocompromised hosts) and CNVG caused 47 (15%; 44% of the episodes in immunocompetent hosts). The incidence of AIDS-associated cases in Australia declined annually ($P < .001$). Aborigines in rural or semirural locations ($P < .001$) and immunocompetent males ($P < .001$) were at increased risk of CNVG infection. Cryptococcomas in lung or brain were more common in immunocompetent hosts ($P \leq .03$) in whom there was an association only between lung cryptococcomas and CNVG. An AIDS-associated genetic profile of CNVN serotype A was confirmed by random amplification of polymorphic DNA analysis. Resistance to antifungal drugs was uncommon. The epidemiology of CNVN infection has changed substantially. Clinical manifestations of disease are influenced more strongly by host immune status than by cryptococcal variety.

Cryptococcus neoformans is the commonest cause of fungal meningitis worldwide and the fourth most common life-threatening opportunistic infection in individuals with AIDS [1]. Cryptococcosis is not a notifiable disease, and there are few precise estimates of incidence [2]. Most cases are caused by *C. neoformans* var. *neoformans* (CNVN) serotype A [1]. In certain tropical and subtropical countries, including Australia and Papua New Guinea, however, *C. neoformans* var. *gattii* (CNVG) serotype B is also an important pathogen and causes major neurological morbidity [1–5]. The 2 varieties have different environmental reservoirs [6]. Epidemiological and clinical studies in Europe and North America have necessarily focused on CNVN, especially in patients with AIDS [7–9]. Retrospective Australian studies have associated CNVN with immunocompromised hosts and CNVG with previously healthy hosts, concurrent focal lesions in the lung and CNS, and severe neurological sequelae [2, 3].

Substantial changes in the epidemiology of cryptococcosis have occurred with evolution of the AIDS epidemic. As the epidemic developed, there was a marked increase but subsequent fall in the frequency of AIDS-associated cryptococcosis as a result of widespread use of the antifungal agent fluconazole as prophylaxis for oral candidiasis in HIV-infected patients [10]. Furthermore, the incidence of AIDS-defining illnesses in general has declined in western countries since the introduction of highly active antiretroviral therapy (HAART) [11, 12]. One recent study reported an annual AIDS-specific incidence of cryptococcosis of 17–66 per 10^3 persons living with AIDS during 1992–1994 in 4 areas of the United States [8]. In Australia, HAART was introduced in early to mid-1996 [13], but it is not known to what extent more effective treatment strategies have influenced the incidence of AIDS-associated cryptococcosis.

Genetic differences among strains of cryptococci have been definitively demonstrated by a number of molecular typing methods, such as Southern blot hybridization with DNA probes based on repetitive DNA sequences from *C. neoformans* [14] and multilocus enzyme electrophoresis [15]. Using the technique

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[a] Group members are listed at the end of the text.

Reprints or correspondence: Dr. Sharon Chen, Centre for Infectious Diseases and Microbiology, Westmead Hospital, Westmead, New South Wales, Australia (sharonc@icpmr.wsahs.nsw.gov.au).

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of random amplification of polymorphic DNA (RAPD), we established that there is a high level of genetic concordance between clinical and environmental isolates of CNVG [16]. We also noted a predominant genetic profile among serotype A strains from patients with AIDS and other immunocompromised patients [17].

Because there have been no national comparative population-based studies delineating the relative influence of host immune status and cryptococcal variety on the epidemiology and clinical features of human disease, we established the Australasian Cryptococcal Study Group (ACSG) in 1994 to collect comprehensive data on the demography, epidemiology, and clinical features of cryptococcosis caused by the 2 varieties of *C. neoformans* in Australia and New Zealand and to monitor trends over a 3-year period. We also determined the in vitro antifungal susceptibilities and RAPD profiles of cryptococcal isolates.

Methods

Study design. Epidemiological and clinical data on all possible cases of cryptococcal infection in Australia and New Zealand were recorded from 1 March 1994 to 1 March 1997. Participants from microbiology/mycology laboratories and infectious diseases services of all university hospitals, physicians in private practice or at public health facilities, and microbiologists at private pathology laboratories recorded the following data on a standard questionnaire for each case: coded name and hospital record number; sex and date of birth; postal code of residence; date of presentation or diagnosis; ethnic background by continent (i.e., Australasia [white, Australian aborigine, or Pacific Islander], Africa, Asia, or North or South America); underlying disease (AIDS, other causes of immunodeficiency, pregnancy, alcoholism, liver disease, or diabetes mellitus); site of infection; source(s) of culture-positive specimens; results of cryptococcal antigen tests and histology; variety of *C. neoformans* cultured; chest radiography (CXR) results; and findings of thoracic and cerebral CT scans and MRIs.

Data were forwarded to the ACSG coordinating center and entered into the Clinical Report System (CRS) version 3.04/4 database (CRS Private Ltd., Sydney, Australia). Survey information was disseminated at selected national meetings during the study period, and 3 of the authors (S.C., G.N., and D.P.) maintained regular contact (once a month) with participants. Audits every 6–12 months ensured completeness of reporting and identified missing cases. Isolates of *C. neoformans* were forwarded to a mycology reference laboratory (see below).

Case definitions. Cases were included if there were clinical features consistent with cryptococcosis and ⩾1 of the following occurred: isolation of *C. neoformans* from a normally sterile site or bronchoalveolar lavage; a positive india ink–stained preparation of CSF; significant cryptococcal antigen titers (⩾8 by latex agglutination) in CSF and/or serum; and/or histologic identification of encapsulated yeastlike forms in biopsy specimens. Cerebral cryptococcosis included cases in which cryptococcomas (lesions of ⩾1 cm in diameter) were demonstrated on CT scan and/or *C. neoformans* was recovered from or visualized in brain tissue. Abnormalities on CXR and/or thoracic CT scan were documented independently by a radiologist and classified as alveolar/interstitial infiltrates, nodules, or circumscribed lesions of ⩾1 cm in diameter (cryptococcomas) [18]. When a case was reported as a recurrence of cryptococcosis, it was not taken into account in the analysis unless it occurred >6 months after the previous episode.

Population and pregnancy data from the 1996 census were obtained from the Australian Bureau of Statistics and population data from Statistics New Zealand [19–23]. Rates of notified cases of AIDS were available from the Australian National HIV data sets and from the AIDS Epidemiology Group, New Zealand Health Information Service [13, 24, 25]. The incidence of AIDS-associated cryptococcosis was calculated with use of the number of patients with cryptococcosis as the numerator and the number living with AIDS during the calendar year as the denominator [13, 25] (and N. Dickson, personal communication).

Statistical analysis. Categorical data were analyzed by χ^2 or Fisher's exact test (2-tailed) as appropriate. ORs were obtained by univariate logistic regression analysis to quantify the degree of association between variables. Independent predictors of outcome were identified by multivariate logistic regression analysis with the backward-elimination procedure. Data were analyzed with SPSSPC+ software (version 7.0; SPSS, Chicago). $P < .05$ was considered statistically significant.

Laboratory studies. The identity and variety of isolates were confirmed by standard methods [26]. Serotyping was performed by the Crypto Check agglutination test (Iatron Laboratories, Tokyo). Isolates were stored at 4°C in sterile water and at −70°C in nutrient broth containing 10% horse serum (CSL, Parkville, Victoria, Australia) for subsequent susceptibility testing and genetic analysis by the RAPD method. Before testing, organisms were subcultured onto Sabouraud's dextrose agar (Difco, Detroit) at 30°C for 72 h to ensure purity.

Antifungal susceptibility testing. Isolates were selected from the ACSG culture collection at random for susceptibility testing, but approximately equal numbers from each of the 3 years of the study were included. Isolates were tested for susceptibility to amphotericin B (AmB), flucytosine, fluconazole, and itraconazole by the National Committee for Clinical Laboratory Standards (NCCLS) broth microdilution technique, according to the protocol outlined in document M27-A [27], but with yeast nitrogen broth (Difco) used in place of RPMI 1640 medium [28]. The reference strains *C. neoformans* ATCC 90112 and *C. neoformans* ATCC 90113 were included in each test batch to ensure quality control.

Testing was performed in 96-well microtiter plates [27], which were agitated mechanically before measurements were made. MICs were determined by measurement of the optical density at 530 nm with a Vmax microplate reader (Bioclone Australia, Marrickville, New South Wales). The MIC of AmB was defined as the lowest concentration of drug at which there was 100% inhibition of growth, whereas the MICs of flucytosine and the azoles were defined as the lowest concentration at which there was 80% inhibition of growth in comparison with that in drug-free controls.

RAPD analysis. Thirty-four isolates (22 CNVN, 12 CNVG) recovered from patients in this study were subjected to RAPD analysis with use of the method of Chen et al. [17]. The CNVG isolates were selected for testing at random; 10 CNVN isolates were obtained from HIV-infected patients, 2 from otherwise im-

Table 1. Sex ratios for patients with cryptococcosis in Australasia, 1994-1997.

Characteristic	No. (%) of patients	Male:female ratio	P[a]
All patients	350 (100)	2.9:1	<.001
Aboriginal	21 (6.0)	2.1:1	.1
Underlying disease	349 (99.7)		
AIDS[b]	149 (42.6)	15.4:1	<.001
NAI	91 (26.0)	1.2:1	.3
Immunocompetent	109 (31.1)	1.9:1	.001
Unknown	5 (1.4)	—	—

NOTE. NAI, non-AIDS, immunocompromised.

[a] Sex ratio in comparison with that in total population of 10.5×10^6 (49%) males, 10.9×10^6 (51%) females [19, 23].

[b] Comparable male:female ratio (1991 males, 108 females) for all patients with AIDS [13, 24, 25].

munocompromised patients, and 10 from immunocompetent individuals. Isolates whose RAPD profiles had been previously established to be reproducible over time [16, 17] were included in the analysis as known "standards." These comprised isolates W10 (RAPD profile Ia), W16 (profile Ib), FMC1 (profile Ic), and W16 (profile II), all of which were CNVN. The CNVG isolate "standards" were strains W8 (profile VGI), E698 (profile VGIII), and ATCC strain 32609 (profile VGII; see also results).

Preparation of DNA from the isolates and subsequent amplification of DNA by PCR were done with use of the same primer combinations and under experimental conditions identical to those described elsewhere [16, 17]. Three 20- to 22-mer primers, designated CN1, 5SOR, and MYC1, were used in 3 combinations: CN1/MYC1, MYC1/5SOR, and 5SOR/CN1. The 12-mer primers FPK1-01, FPK1-05, and FPK 1-07 were used in 2 combinations: FPK1-01/FPK1-05 and FPK1-05/FPK1-07. The sequences of these primers have been published elsewhere [17]. Products of amplification were electrophoresed in a 7% polyacrylamide gel and visualized by silver staining (BioRad, Ryde, New South Wales, Australia). Band sizes were estimated from comigrating 100-bp DNA molecular size standards (GIBCO BRL, Five Dock, New South Wales, Australia). RAPD profiles of CNVN and CNVG were scored and defined as described elsewhere [16, 17]. Subtypes within a major profile were designated with a lowercase letter (a, b, etc.) if a consistent difference was noted with 1 primer pair. The reproducibility of the method used has been confirmed [17].

Results

Three hundred fifty-five evaluable episodes of cryptococcal infection were identified in 350 patients; permission to include information on 4 additional patients with AIDS in Australia was refused. Of those 355 episodes, 152 (42.8%) occurred in year 1 the survey, 92 (25.9%) in year 2, and 111 (31.3%) in year 3. The mean incidence of cryptococcosis was 6.6 per 10^6 population per year in Australia (8.0 in year 1, 4.8 in year 2, and 5.7 in year 3) and 2.2 per 10^6 population per year in New Zealand (2.2 in year 1, 1.9 in year 2, and 2.5 in year 3). Of 312 isolates biotyped, 265 (84.9%) were CNVN and 47 (15.1%) were CNVG. Of 73 CNVN isolates serotyped, 71 were serotype A, 1 was serotype AD, and 1 was untypeable. All 47 CNVG isolates were serotype B.

Underlying host disease. Potential risk factors for cryptococcosis were known in 349 (98.3%) of 355 episodes. Two hundred and forty (68.8%) occurred in immunocompromised hosts: 149 (42.9% overall) with AIDS (all with $CD4^+$ cell counts of $<100 \times 10^6$/L), 32 with malignant disease (19 had a lymphoproliferative disorder, 10 had leukemia, and 3 had breast cancer), 12 organ transplant recipients (all renal transplantation patients), 5 with sarcoidosis, 14 with collagen vascular disorders, and 28 receiving long-term immunosuppressive therapy for other disorders. There were 109 episodes of infection (31.3%) in immunocompetent hosts.

Four of 42 women of child-bearing age with cryptococcosis were pregnant, but this was no more than the expected rate for the general population (an estimated 250,000 viable pregnancies annually among women aged 15–49 years [21]; OR, 2.0; 95% CI, 0.7–5.7; $P = .2$). Eight patients (2.3%) had diabetes mellitus (4 HIV-negative immunocompromised hosts and 4 [3.7% of 109] immunocompetent hosts), compared with an estimated prevalence in the Australian population of 3.9% [29]. Nine patients had chronic alcoholism with or without cirrhosis.

The annual incidence of cryptococcosis per 10^3 persons living with AIDS in Australia decreased from 39.1 in year 1 to 20.3 in year 2 ($P < .001$) and to 13.5 in year 3 ($P = .1$); the incidences in HIV-negative cases were 4.0 per 10^6 general population in year 1, 2.6 per 10^6 in year 2, and 4.2 per 10^6 in year 3. In New Zealand, the annual incidence of cryptococcosis was 38.4 per 10^3 persons living with AIDS in year 1, 49.4 per 10^3 in year 2, and 49.0 per 10^3 in year 3; among HIV-negative individuals, the incidence remained constant at a mean of 1.1 per 10^6 general population (range, 1.07–1.12) [24] (and N. Dickson, personal communication).

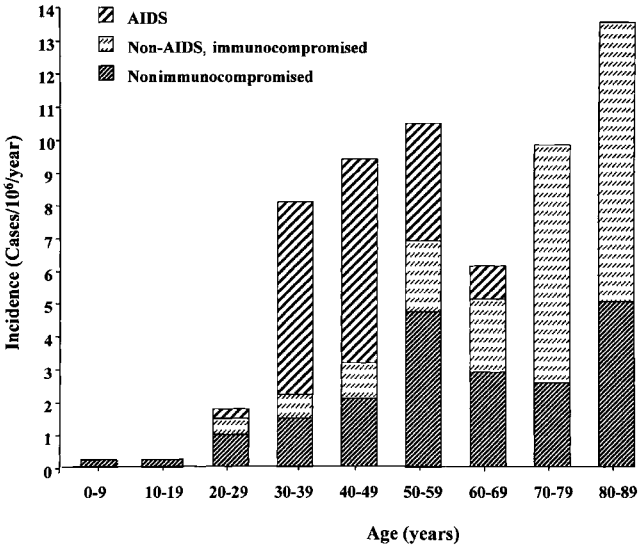

Figure 1. Annual incidence of cryptococcosis per 1 million population in Australasia, by patient age and host immunologic status, from 1 March 1994 to 1 March 1997.

Table 2. Distribution of incidence of cryptococcosis in Australia and New Zealand in 1994-1997, as related to underlying HIV infection, according to cryptococcal variety.

	CNVN		CNVG	
Jurisdiction	AIDS patients[a]	Non-AIDS patients	AIDS patients	Non-AIDS patients
New South Wales	3.2	2.0	0.06	0.6
Queensland	1.4	4.9	—	0.9
Victoria	1.7	1.3	—	0.8
Western Australia	2.9	2.1	—	1.2
Northern Territory	—	8.5	—	8.5
South Australia	0.7	1.2	—	0.5
Australian Capital Territory	3.3	1.1	—	1.1
Tasmania	—	1.5	—	—
New Zealand	1.1	1.0	—	0.09

NOTE. Data are expressed as incidences of infection per million population per year in each of the jurisdictions, which was calculated by dividing the average number of cases recorded per year for the particular patient group by the population of each jurisdiction estimated in the census in 1996 [19]. CNVG, *C. neoformans* var. *gattii;* CNVN, *Cryptococcus neoformans* var. *neoformans*.

[a] The incidences of AIDS per million population per year were 56.4 (New South Wales), 21.9 (Queensland), 28.9 (Victoria), 21.5 (Western Australia), 5.5 (Northern Territory), 21.0 (South Australia), 29.2 (Australian Capital Territory), 14.8 (Tasmania), and 16.0 (New Zealand), as estimated for the calendar year 1996 [13, 25].

Sex and age. Males were affected more commonly than females, but male sex was a significant risk factor only for immunocompetent patients (table 1), among whom it was associated with CNVG (male:female ratio, 3.3:1; OR, 3.4; 95% CI, 1.7–6.7; $P < .001$). For immunocompetent, CNVN-infected patients, the ratio was 1.3:1 ($P = .2$). The average age was 36.8 years for males and 47.7 years for females. Age distribution by host category is shown in figure 1. Of 2 children aged <9 years, one was an Australian aborigine and the other was from Samoa.

Geographic distribution, epidemiology, and variety of C. neoformans. The incidence of cryptococcosis and distribution by variety of *C. neoformans* are shown for all jurisdictions of Australia in figure 2; the distribution of incidence of disease by underlying HIV infection is summarized in table 2. The incidence of CNVN infection in New Zealand was 2.1 cases per 10^6 population per year, and the incidence of and CNVG was 0.09 cases per 10^6. Within Australia, the overall incidence of disease ranged from 2.1 (in Tasmania) to 18.8 (in the Northern Territory) cases per 10^6 population per year. The highest number of cases occurred along the eastern seaboard of the Australian mainland, where 5%–7% of individuals with AIDS were affected (table 2). Among immunocompetent hosts, the incidence of infection was highest in the Northern Territory and Queensland (18.8 and 3.2 cases per 10^6 population per year, respectively). A disproportionate number of CNVN infections (77% of cases) in Queensland affected HIV-negative individuals. The ratio of CNVN to CNVG infection was highest in New Zealand (23:1) and lowest in the Northern Territory of Australia (1:1). When AIDS patients were excluded, this ratio was reduced substantially in all jurisdictions except Queensland, where it fell only to 5.4:1 (table 2).

Epidemiological characteristics of CNVN and CNVG infection are summarized in table 3. Immunocompromised patients were typically infected with CNVN (80% of patients), whereas 89% of CNVG infections occurred in immunocompetent hosts. Cases due to CNVN (79.3%) were concentrated in major cities, whereas 47% of CNVG infections occurred in individuals living in rural or semirural locations. Data on ethnic background were available for all cases. Australian aborigines (21 cases; annual incidence, 19.8 per 10^6 population) and New Zealand Maoris (11 cases; annual incidence, 11.4 per 10^6 population) were disproportionately affected. Two patients were Tongan, 5 Melanesian, 11 Asian, and 1 black. The incidence of CNVG infection among aborigines was 10.4 cases per 10^6 population per year, compared to 0.7 cases per 10^6 population per year among nonaborigines (OR, 11.7; 95% CI, 6.0–22.4; $P < .001$). Indigenous Australians were also at greater risk of CNVN infection than were nonaborigines (incidence, 8.5 vs. 4.4 cases per 10^6 population per year; OR, 1.8; 95% CI, 0.9–3.5; $P = .08$); this was statistically significant for HIV-negative patients (incidence, 6.6 vs. 1.9 cases per 10^6 population per year; OR, 3.6; 95% CI, 1.6–7.6; $P = .001$).

Univariate analysis revealed that CNVG infection was associated with aboriginal race, a rural or semirural domicile, and absence of host immunosuppression (table 3). Multivariate analysis indicated that host immune status was the most significant determinant of infection due to each particular variety and that a rural or semirural domicile remained an independent risk factor for CNVG (table 3).

Population-based analysis revealed a strong interaction between aboriginal race and rural or semirural domicile. Seventy percent of indigenous Australians lived in rural or semirural

Table 3. Comparison of epidemiological characteristics of infection due to *Cryptococcus neoformans* var. *neoformans* (CNVN) and *C. neoformans* var. *gattii* (CNVG) by univariate and multivariate analysis.

	No. (%) of patients with			
Characteristic	CNVG	CNVN	OR (95% CI)	*P*
Univariate analysis				
All patients	47 (100)	265 (100)	—	—
Male	36 (77)	193 (73)	1.2 (0.6–2.6)	.42
Aboriginal	11 (23)	9 (3.4)	8.2 (3.2–21)	<.001
Rural or semirural domicile	22 (47)	55 (21)	3.3 (1.7–6.2)	<.001
Underlying host disease				
Immunocompetent	41 (87)	52 (20)	—[b]	
AIDS	1 (2.1)	133 (50)	0.01 (0–0.07)	<.001
NAI	4 (8.5)	76 (29)	0.07 (0.02–0.2)	<.001
Unknown	1 (2.1)	4 (1.5)	—	—
Multivariate analysis[a]				
Rural or semirural domicile			3.4 (1.5–7.7)	.004
Underlying host disease				
Immunocompetent			—[b]	—
AIDS			0.01 (0–0.08)	<.001
NAI			0.07 (0.03–0.2)	<.001

NOTE. NAI, non-AIDS, immunocompromised.

[a] ORs for each of these independent predictors have been adjusted for the other variables.

[b] Reference category.

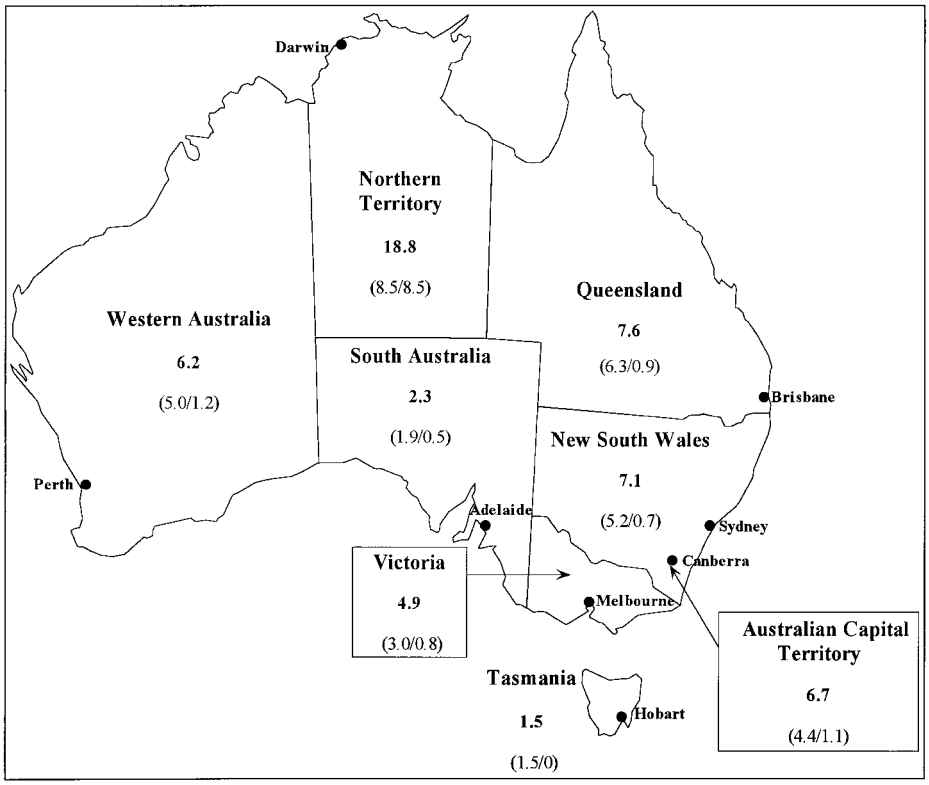

Figure 2. Map of Australia showing the jurisdictions in which the diagnosis of cryptococcosis was made during 1994–1997. The bold figures show the incidence of infection per 10^6 population in each of these regions. The figures in parentheses are the corresponding incidences of CNVN infection/CNVG infection per 10^6 population. All incidences were calculated by dividing the average number of cases recorded per year by the population of each region that was estimated in the census in 1996 [19]. Note that the sum of the incidences of CNVN and CNVG infection are not equivalent in all cases to the total incidence of infection, as biotyping was not possible in all instances.

areas, compared with 37.3% of the general population [22]. Seventy-two percent of the Maori and 80% of the general population of New Zealand lived in major cities [23]. Aborigines in rural or semirural areas were at significantly increased risk of CNVG infection, compared with the reference group of nonaborigines in major cities (OR, 12.7; 95% CI, 6.3–25.5; $P <$ .001). Neither city-dwelling aborigines nor nonaborigines in rural or semirural areas were at significantly increased risk of CNVG infection.

Clinical characteristics. Cryptococcosis was diagnosed by isolation of *C. neoformans* in 317 (89.3%) episodes, detection of cryptococcal antigen in serum and/or CSF in 28 cases (18 patients had AIDS), and histologic examination in 10 cases (9 pulmonary and 1 cerebral infection). Cerebral CT scans revealed cryptococcomas in 30 of 257 cases (2 also with hydrocephalus), hydrocephalus alone (7 cases), or nonspecific meningeal enhancement (7 cases).

Clinical presentation was closely related to host status (table 4). Pulmonary disease was more common in immunocompetent hosts (60%), and meningitis was most common in those with AIDS (86%). Brain cryptococcomas, with or without meningitis, were more frequent in immunocompetent hosts (15 of 84 vs. 15 of 173; OR, 2.3; 95% CI, 1.1–4.9; $P = .03$). Multivariate analysis of the relationship between sex, race, domicile, and host immune status and the presence of cerebral cryptococcomas indicated that the only independent predictor of cryptococcomas was the absence of host immunosuppression (OR, 3.3; 95% CI, 1.4–10; $P = 007$). Brain cryptococcomas were associated with CNVG by univariate analysis (10 of 30 vs. 16 of 171 with CNVN; $P = .001$).

As host immune status and cryptococcal variety were interdependent variables, we tested the association between variety and brain cryptococcomas in immunocompetent hosts with CNS infection. This was not statistically significant (9 of 25 CNVG infections vs. 4 of 17 CNVN infections; OR, 2.0; 95% CI, 0.5–7.3; $P = .4$). Cryptococcomas were multiple in 26 patients for whom the cryptococcal variety was known (3 of 10 with CNVG and 9 of 16 with CNVN infections). Cryptococcomas were located in the basal ganglia ($n = 10$), parietal lobe ($n = 8$), and/or cerebellum ($n = 5$).

CXR abnormalities were attributed to cryptococcosis in 120 cases (table 4). Alveolar and/or interstitial infiltrates were present in 70 (58.3%) of the 120 episodes and were most common in HIV-negative immunocompromised hosts (92% of episodes).

Cryptococcomas were evident in 46 (38%) of 120 cases and most frequently affected immunocompetent patients (48.4%). The absence of immunosuppression was independently associated with lung cryptococcomas by multivariate analysis (OR, 2.5; 95% CI, 1.3–5; $P = .01$). In immunocompetent patients with lung disease, pulmonary cryptococcomas were also associated with CNVG (20 of 24 CNVG infections vs. 9 of 31 CNVN infections; OR, 12.2; 95% CI, 3.3–46; $P < .001$). Forty-one patients (24 immunocompromised, 17 immunocompetent; 26 infected with CNVN, 15 with CNVG) presented with concurrent lung and CNS disease that was associated with CNVG (OR, 4.3; 95% CI, 2–10; $P < .001$) but not with host immune status. Serum cryptococcal antigen was detected in 54% (65) of 120 patients with pulmonary infection, 66% (27) of 41 with lung plus CNS infection, and 74.4% (125) of 169 with CNS disease only.

Antifungal susceptibility testing. Seventy-seven isolates (59 CNVN, 18 CNVG) were tested by broth microdilution. None was resistant to itraconazole (MIC, ≤0.25 mg/L), and 1 was resistant to flucytosine (MIC, ≥64 mg/L). One isolate, from a patient with AIDS who relapsed with cryptococcal meningitis following an initial response to AmB, was resistant to AmB; testing of sequential isolates revealed a stepwise increase in MIC, from 1 mg/L to 16 mg/L [30]. The MIC of fluconazole was ≤8 mg/L ("susceptible") for 68 isolates, 16–32 mg/L ("intermediately susceptible") for 6 isolates, and ≥64 mg/L ("resistant") for 3 isolates (figure 3). There were no significant differences between CNVN and CNVG in median MICs (both 4 mg/L) or the proportion of isolates for which MICs were ≥8 mg/L (20 of 59 CNVN vs. 9 of 18 CNVG). There was also no

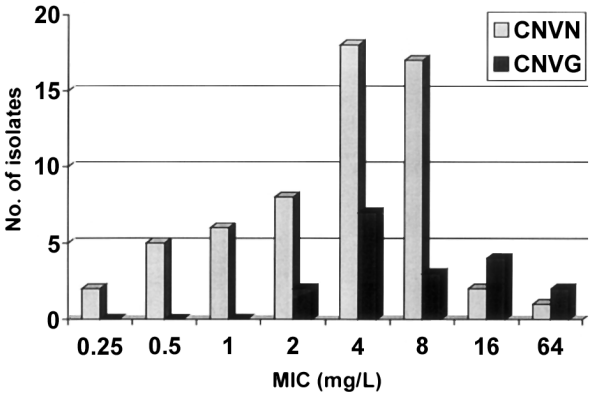

Figure 3. In vitro susceptibilities of CNVN isolates ($n = 59$) and CNVG isolates ($n = 18$) to fluconazole, as determined by broth microdilution.

Table 4. Site of infection, abnormal laboratory parameters, and radiological features in cases of cryptococcosis in Australasia, 1994-1997.

Site of infection, laboratory finding	No. of patients	Underlying disease				Crytococcus biotype (if known)		
		AIDS ($n = 149$)	NAI ($n = 91$)	None ($n = 105$)	P[a]	CNVN ($n = 265$)	CNVG ($n = 47$)	P[b]
CNS	209/295	128	35	46		171	30	
Meningitis	200/295	128/139	31/66	41/90	<.001	165/221	27/42	.2
Brain	30/257	6/128	9/45	15/84	.03	16/190	10/38	.003
CSF india ink positive	209/295	93/128	23/31	36/41	.05	—	—	
CSF antigen positive	209/295	112/128	28/31	40/41	.1	—	—	
CSF culture positive	209/295	111/128	28/31	39/41	.2	147/165	22/27	.3
Cerebral CT	44/257[c]							
Cryptococcoma		6/128	9/35	15/46	<.001	16/171	10/30	.001
Hydrocephalus		2/128	1/35	6/46	.004	4/171	5/30	.003
Lung	120/308	17/127	39/76	64/105	<.001	81/230	30/45	<.001
Chest radiograph	120/308[d]							
Cryptococcoma		5/17	10/39	31/64	.02	18/81	24/30	<.001
IAI		14/17	36/39	20/64	<.001	57/81	5/30	<.001
Adenopathy		—	5/39	—	—	2/81	2/30	.3
Pleural effusion		3/17	7/39	13/64	.7			
Blood	50	36	11	3		50	—	
Urine	9	5	4	0		9	—	
Skin	19	0	11	8		16	3	
Bone marrow	3	3	—	—		3	—	
Larynx	1	—	1	—		1	—	
Lymph node	5	3	2	—		5	—	
Synovial fluid	1	—	1	—		1	—	

NOTE. Data are no. of patients or no. of patients with indicated characteristic/total no. tested. CNVG, *C. neoformans* var. *gattii;* CNVN, *Cryptococcus neoformans* var. *neoformans;* IAI, interstitial or alveolar infiltrate; NAI, non-AIDS, immunocompromised.

[a] Comparison of variables affecting all immunocompromised patients versus immunocompetent hosts.

[b] Comparison of variables in CNVN- versus CNVG-infected individuals.

[c] Number of episodes in which abnormalities were demonstrated on cerebral CT scans of patients with CNS cryptococcosis.

[d] Number of episodes in which radiological abnormalities were demonstrated on the chest radiographs of patients with lung infection.

significant change in median MICs over the period of study. Median fluconazole MICs for CNVN isolates from AIDS and non-AIDS patients were identical (4 mg/L for both).

RAPD analysis. Two major RAPD profiles were identified amongst the 12 isolates of CNVG studied; 9 isolates were assigned to profile VGI and 3 to profile VGII, as defined elsewhere [16] (data not shown). Two of the latter were recovered from aborigines living in the Arnhemland region ("Top End") of the Northern Territory and the other from a resident of a semirural area of New South Wales.

We have previously established that CNVN isolates from Australia can be assigned to 3 major RAPD profiles [17]. Representative profiles (profiles Ia, Ib, Ic, and II) of known "standard" strains with use of the primer pair 5SOR/CN1, which have been defined elsewhere [17], are shown in figure 4. The 22 isolates tested in this study were all serotype A. Isolates recovered from patients with AIDS ($n = 10$) were genetically indistinguishable and assigned to profile Ia. One each of 2 isolates obtained from patients who were immunocompromised for reasons other than AIDS was assigned to profile Ia and profile II. Of 10 isolates recovered from immunocompetent hosts, 7 were assigned to profile II, 2 to profile Ia, and 1 to profile Ic.

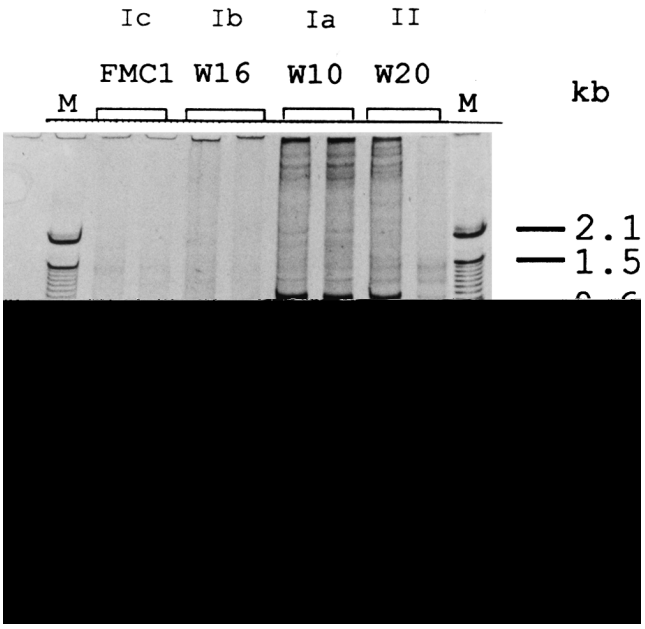

Figure 4. Profiles obtained by random amplification of polymorphic DNA (RAPD) of known "standards" of CNVN isolates, amplified with the primer pair 5SOR/CN1, illustrating the major types I and II and subtypes Ia, Ib, and Ic. *M* is the molecular-size reference marker (100-bp) ladder; other lanes are amplified DNA fragments from duplicate DNA preparations of individual strains as named. Bands were included in analysis regardless of intensity. RAPD profiles or subprofiles (types) are shown at top.

Discussion

This survey provides the first prospective, nationwide analysis of cases of cryptococcosis in a region in which *both* varieties of *C. neoformans* are endemic. Consistent with previous observations, the overwhelming majority of CNVN and all CNVG infections were due to serotype A and serotype B, respectively; serotype D accounts for ~1% of CNVN isolates in Australia [31]. The study differs from a number of others [7–9] in that >50% of cases occurred in HIV-negative subjects, and characteristics of infection in immunocompromised and immunocompetent hosts could therefore be compared. In addition, the large number of immunocompetent individuals with documented CNVG and CNVN infection allowed us to determine the relative influence of host immune status and biotype on disease manifestations.

A rapid increase in cryptococcosis worldwide has followed the AIDS pandemic. AIDS, which accounted for a doubling in the incidence of cryptococcosis in the late 1980s in 1 Australian state [2] and for 43% of cases in the present study, has had an even greater impact on the prevalence of cryptococcosis elsewhere: 86% of cases in France in 1985–1993 were associated with AIDS, as were 86% in 4 United States locations in 1992–1994, and 68% in Durban, South Africa, in 1991–1994 [7, 8, 32]. The overall incidence of cryptococcosis in Australia fell by 29% during the study period. This was due to a reduction in AIDS-associated cases and is consistent with National AIDS Registry data on the declining incidence of initial AIDS-defining illnesses [13].

HAART has been linked to a reduction in AIDS-associated opportunistic infections and was introduced in Australia late in the second year of our study [13]. Use of the azole drug fluconazole may also have been a contributory factor, as has been reported elsewhere [8], although this drug was marketed in Australia in late 1991 and used as treatment or prophylaxis for AIDS-associated candidiasis from 1992. The observed decline in annual incidence of AIDS-specific cryptococcosis per 10^3 persons living with AIDS in Australia during 1994–1997 (39.1–13.5) was similar in magnitude to that reported from Atlanta (66–30) and San Francisco (22–17) [8].

Our study found that the infection rate among HIV-negative subjects remained stable. Estimates of the incidence of cryptococcosis in several countries have been published, although are not directly comparable with our results because they were usually retrospective, they made extrapolations from individual hospitals or cities, and/or they examined small numbers of patients. These estimates have ranged from 0.1 case per 10^6 population per year in one region of France (all CNVN), to 48.8 cases per 10^6 population in selected districts of Papua New Guinea (mostly CNVG), to 140 cases per 10^6 population in Arnhemland in the Northern Territory of Australia (all CNVG) [4, 7, 33].

The present study reports substantial regional variation in the geographic distribution of CNVN within Australia that has not

been previously recognized. Most episodes of infection due to CNVN affected immunocompromised patients living in major cities, with the proportion of AIDS patients who developed cryptococcosis being similar in the eastern states, where the incidence of AIDS is greatest. Unexpectedly, the highest incidences of CNVN infection were reported from the Northern Territory and Queensland. Most of these cases involved HIV-negative individuals, raising the possibility of increased exposure to an unknown environmental source of CNVN in these regions.

Infections due to CNVG were significantly more common in rural or semirural areas on the Australian mainland, broadly corresponding to the distribution of the environmental reservoir of this variety, *Eucalyptus camaldulensis* and *Eucalyptus tereticornis* [6]. CNVG infection also occurs in southern California, Italy, and India, where recent reports have identified CNVG in samples from local (introduced) eucalyptus [6, 34, 35]. The incidence of CNVG infection was highest in the Northern Territory, consistent with a similarly high incidence reported amongst Australian aborigines in this jurisdiction [33]. Aborigines have been overrepresented in previous reports of cryptococcosis from centers in Northern Australia [33, 36]. Although disease due to CNVG was disproportionately high in aborigines living in rural or semirural areas, suggesting that environmental exposure is the dominant risk factor for CNVG infection, the incidence of infection due to CNVN was also increased in this group. This new observation indicates that factors other than environmental exposure need to be explored; for example, variables such as genetic susceptibility, socioeconomic status, and nutritional status.

Immune deficiency was the major risk factor for cryptococcosis due to CNVN, whereas CNVG was associated with immunocompetent hosts, as reported elsewhere [2, 3]. Diabetes mellitus and pregnancy have been considered to be risk factors for cryptococcosis, but our data did not show an association with either. Diabetes was not identified as an independent risk factor in a case-control study of AIDS-associated cryptococcosis in the United States [8].

Age-related differences in incidence have been reported. As confirmed in our study, cryptococcosis is uncommon in children, even those with AIDS, probably because they are exposed less frequently to environmental reservoirs [37]. Cryptococcosis was more common in elderly patients, a finding that suggests age-associated immunodeficiency may be a risk factor. In a recent study of aged mice, mortality associated with cryptococcosis was increased, although this was not correlated with reduced T cell function [38]. Most studies of patients with cryptococcosis have shown a male predominance, which has been attributed to increased environmental exposure, hormonal influences, and/or genetic predisposition. In the present study, only immunocompetent males infected with CNVG were disproportionately affected.

Given the high incidence of CNVG infection in rural and semirural areas of Australia, increased environmental exposure is the most likely explanation. A similar conclusion was reached following a seroepidemiological study in Papua New Guinea [37]. Although the male predominance amongst aborigines with cryptococcosis failed to achieve statistical significance, relatively few aborigines were studied, and a larger sample size is required to rigorously test this association. We did not find an increased risk of cryptococcal disease in men with AIDS, consistent with findings of a case-control study in the United States [8].

Clinical features of cryptococcosis were clearly dependent on host immune status. Lung disease was uncommon in patients with AIDS (11%) and frequent in immunocompetent hosts (60%). Conversely, 90% of AIDS patients presented with meningitis, but only 30%–40% of the HIV-negative groups. In immunocompetent patients, cryptococcomas in the lung were 10-fold more common and cryptococcomas in the brain were 3-fold more common than in patients with AIDS, which is consistent with local containment of infection by a granulomatous host response [1]. Cryptococcomas visible by CT were concentrated in the region of the basal ganglia; cryptococci were similarly distributed in an autopsy study [39]. This may reflect high local concentrations of L-3-(3,4-dihydroxyphenyl)-1-alanine (L-DOPA), a catecholamine substrate metabolized by *C. neoformans* phenol oxidase enzyme to form melanin and other products of catecholamine oxidation; the ability to form such products is considered to be integral to cryptococcal virulence [40]. Skin lesions, which have been associated with *C. neoformans* serotype D [7], were noted in 5% of our patients; 84% were caused by serotype A. Dissemination to other sites was characteristic of CNVN infection in immunocompromised patients.

Cryptococcomas in lung or brain occurred more frequently in patients infected with CNVG, as has been reported elsewhere [2, 3]. However, removal of the interdependent variable, host immune status, showed that only lung cryptococcomas were significantly associated with CNVG; these patients were also more likely to present with combined lung and CNS disease. The observation that brain cryptococcomas were not more often associated with CNVG differs from the findings of a retrospective study by Mitchell et al. [3].

MICs of antifungal drugs were comparable for both cryptococcal varieties. The sole AmB-resistant isolate was recovered during relapse of infection after initial treatment with AmB. Only 1 other case linking the development of in vitro resistance to clinical relapse has been reported [41]. Notably, median MICs of fluconazole were not increased in isolates from patients with AIDS, despite widespread use of fluconazole as prophylaxis for candidiasis in this group. Likewise, significant changes in fluconazole MICs over time were not observed.

The results of RAPD analysis of CNVN isolates lend support to our previous observation of a common molecular profile of CNVN serotype A in patients with AIDS in Australia [17]. Possible explanations for this finding include a point source of infection, exposure to a predominant environmental strain, and the selection of a particular molecular type of *C. neoformans* in the

presence of immunodeficiency. The first possibility cannot readily explain the occurrence of the same genetic type at several sites across Australia; the second is unlikely, as we have previously demonstrated a broad spectrum of RAPD patterns in isolates from immunocompetent patients from similar locations [17].

Limited numbers of isolates were tested in the present study, and the possible effect of linkage disequilibrium, or the nonrandom association between genetic markers, on the finding of a predominant RAPD pattern cannot be completely excluded. However, we consistently observed a predominant genetic profile among isolates from AIDS patients with use of several primer combinations and after repeated independent PCR amplifications; in our previous study, the same predominant RAPD profile(s) had been observed in 28 isolates recovered from AIDS patients [17].

Evidence of linkage disequilibrium in *C. neoformans* has been demonstrated by Brandt et al. [15], who reported a disproportionate predominance of genotypes with use of multilocus enzyme electrophoresis and RAPD analysis of isolates in the United States. In their study, no differences were seen in the genotype distribution of isolates from AIDS and non-AIDS patient populations [15]. The hypothesis that amplicons obtained by RAPD may represent sequences linked to cryptococcal virulence requires further study. As expected, the majority of CNVG isolates tested were of RAPD profile VGI, consistent with the hypothesis that human disease is derived from exposure to host eucalyptus trees, since all Australian eucalyptus isolates to date have been of profile VGI [16]. The occurrence of a different genetic profile, VGII, amongst isolates recovered from patients residing in Arnhemland, where the known host eucalyptus trees do not occur naturally, remains unexplained.

In conclusion, our study is the first to directly compare the clinical manifestations of cryptococcosis in immunocompetent hosts, HIV-negative immunocompromised individuals, and patients with AIDS. Clinical manifestations are strongly dependent on host immune status. There is a significant association between lung (but not brain) cryptococcomas and CNVG in immunocompetent patients. The striking changes in incidence of cryptococcosis from the tropical north to the south of Australasia are noteworthy. Although Australian aborigines in rural or semirural locations have the greatest risk for CNVG infection, the observation that the incidence of infection due to both CNVG and CNVN is increased indicates that factors other than environmental exposure should be explored. Pregnancy was not found to be a risk factor for cryptococcosis. The fact that disease due to CNVG is virtually confined to immunocompetent hosts remains unexplained and invites further study of factors that determine susceptibility to cryptococcosis.

Australasian Cryptococcal Study Group

Members of the study group are: A. Allworth, M. Beaman, R. Benn, S. Chen, P. Collignon, B. Currie, D. Ellis, R. Ellis-Pegler, J. Ferguson, D. Fisher, J. Faoagali, R. Forgan-Smith, A. Fuller, C. Gollege, D. Gordon, I. Gosbell, T. Gottlieb, C. Heath, J. Hoy, S. Hume, P. Jones, R. Kelly, K. Kocuiba, G. Kotsiou, S. Lang, J. LeBrooy, G. Lum, D. Marriott, R. McAleer, J. McCormack, H. Merrell, A. Mijch, D. Muir, R. Munro, P. Newton, G. Nimmo, K. Ott, K. Papanoum, R. Pritchard, D. Parr, J. Pearman, T. Pfeiffer, E. Reiss-Levy, J. Robson, J. Schooneveldt, D. Shaw, V. Sinickas, M. Slavin, B. Speed, T. Sorrell, M. Tilse, F. Tosolini, M. Whitby, and G. Wood.

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