

Cryptococcal meningitis epidemiology: 17 years of experience in a State of the Brazilian Pantanal

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Abstract

Introduction: This study aimed to describe cryptococcal meningitis (CM) cases and the associated demographic, clinical, and microbiological data obtained from cities in the State of Mato Grosso do Sul in the Midwestern region of Brazil. **Methods:** The data from 129 patients with laboratory-confirmed CM admitted from 1997 to 2014 were retrospectively reviewed. The molecular types of *Cryptococcus neoformans* and *Cryptococcus gattii* isolated from cerebrospinal fluid were analyzed to determine their geographic distribution. **Results:** The patients had a mean age of 37 years and consisted mostly of men (76.7%). Most of the *Cryptococcus* isolates were obtained from patients infected with human immunodeficiency virus (HIV) and included 105 (87.5%) and 5 (55.6%) isolates of *C. neoformans* and *C. gattii* complexes, respectively. A restriction fragment length polymorphism (RFLP) analysis of *URA5* revealed that most of the isolates were *C. neoformans* molecular type VNI (89.1%), whereas the molecular types VGII (7%) and VNII (3.9%) were observed less frequently. Notably, 65% of the cases with a time from symptom onset to laboratory diagnosis of more than 60 days resulted in fatalities, and sequelae were observed among the patients who survived. **Conclusions:** The present study documents the occurrence of neurocryptococcosis, which is mainly caused by *C. neoformans* VNI, in Mato Grosso do Sul, Brazil, with probable autochthonous cases in the Brazilian Pantanal, the world's largest tropical wetland, and a biome where cryptococcosis has not yet been explored.

Keywords: Cryptococcosis. Aids. *Cryptococcus*. Molecular typing. *Cryptococcus neoformans*. *Cryptococcus gattii*.

INTRODUCTION

Cryptococcosis is a systemic mycosis caused primarily by *Cryptococcus neoformans* species complex, which includes the molecular types VNI, VNII, VNB, VNIII, and VNIV, and the *Cryptococcus gattii* species complex, which includes the molecular types VGI, VGII, VGIII, and VGIV¹⁻³. Differences in geographical distribution, ecological niches, epidemiology, clinical presentation, and molecular characteristics have been observed among various *Cryptococcus* spp⁴.

The Pantanal is the world's largest tropical wetland area and home to approximately 3,500 plants species and more than 730 species of birds, many of which undergo intercontinental

and intracontinental migration^{5,6}, which may influence the geographic distribution of *Cryptococcus* spp.

Yeasts are distributed in the environment and can infect the human body primarily through the respiratory tract, causing lung disease in some cases. Cryptococcal meningitis is the most common clinical manifestation due to the remarkable tropism of *Cryptococcus* spp. toward the central nervous system^{7,8}.

Worldwide, the epidemiology of cryptococcosis has been influenced by acquired immunodeficiency syndrome (AIDS)^{8,9}. In Latin American countries, 54.4 cases of cryptococcal meningitis are estimated to occur annually in every set of 1,000 cases of AIDS¹⁰. In Brazil, the disease has been identified as one of the most life-threatening fungal infections in this group of patients¹¹. The mortality rates associated with cryptococcal meningitis vary regionally. Despite improvements in medical care and antifungal treatment, a significant decrease in mortality, which is estimated to be greater than 50% in less developed countries, has not been observed to date^{10,12,13}.

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Currently, there are limited data on the molecular epidemiology of cryptococcal meningitis in the Midwestern region of Brazil, and this study aimed to provide new information to the scientific community.

METHODS

We retrospectively analyzed 129 cases of cryptococcal meningitis diagnosed at the University Hospital, Federal University of Mato Grosso do Sul, Campo Grande, the capital of Mato Grosso do Sul, from May 1997 to May 2014.

The University Hospital is a 255-bed university teaching reference center for the diagnosis and treatment of infectious diseases in the entire State of Mato Grosso do Sul. This state ranked sixth in terms of tertiary extension, with an area of 357,145,534 km², which corresponds to 4.19% of the total area of Brazil (8,5515,767,049 km²) and 22.2% of the area of the Midwest region. This state was estimated to have 2,651,235 inhabitants in 2015, giving the state a ranking of 21 in terms of population in Brazil^{14,15}.

The cases were selected from a database and included 72 cases diagnosed between 1997 and 2005 (participants of the study conducted by Lindemberg et al.) and 57 cases diagnosed from 2006 to 2014¹⁶.

Cryptococcal meningitis is identified based on the clinical features of meningeal infection and the isolation of *Cryptococcus* spp. from cerebrospinal fluid (CSF) culture.

Patient characteristics were obtained from medical records and the Microbiological Research Laboratory database. Demographic (gender, age, occupation, and area of residence) and clinical characteristics [human immunodeficiency virus (HIV) coinfection, previous use of antiretroviral therapy, time elapsed between symptom onset of cryptococcosis to diagnosis, length of hospital stay, and clinical outcomes during the hospitalization period], and the species and molecular type of *Cryptococcus* spp. were investigated.

Ethical considerations

The study was approved by the institutional ethics committee.

In this study, polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) was used to distinguish *C. neoformans* and *C. gattii* species. The *URA5* gene was amplified by PCR using the primers *URA5* (5' ATGCCTCCCAAGCCCTCGACTCCG 3') and *SJ01* (5' TTAAGACCTCTGAACACCGTACTC 3'). The amplified PCR product was double digested using the *HhaI* and *Sau96I* enzymes, as previously described³. The RFLP profiles were visually analyzed by comparison with reference strains.

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 23.0 (IBM Corporation, Armonk, NY, USA). Normality was assessed using the Shapiro–Wilk test. The groups were compared using Student's *t*-test or the Mann-Whitney U test for continuous variables and Pearson's chi-square test or Fisher's exact test for categorical variables. A significance level of 0.05 was adopted.

RESULTS

A total of 129 CSF isolates were recovered during the 17-year study period. One hundred eleven (86%) of the samples were obtained from AIDS patients, and 63 (56.8%) of these had cluster of differentiation 4 (CD4) counts less than 200 cells/mm³.

Ninety-nine (76.7%) patients were men, and the mean [standard deviation] age of the patients was 37 [11] years (range: 10-74 years). With regard to occupation, 15.5% (20/129) of the patients were general service providers, 13.2% (17/129) were construction workers, 10.8% (14/129) were household workers, 7.8% (10/129) were rural workers, and 16.3% (21/129) had other occupations; however, the profession of 36.4% (47/129) of the cases was not recorded. Other patient characteristics are summarized in **Table 1**.

No significant differences were observed in the sex ratio, time from symptom onset to diagnosis, and patient outcomes between patients with HIV infection and those without HIV infection. However, patient age, area of residence, and cryptococcal species responsible for causing the infection showed significant differences between patients with AIDS and HIV-negative patients (*P*-value < 0.05).

The mean age of the AIDS patients was 35.7 [9.5] years, which is lower than the mean age of the HIV-negative patients, which was 44.6 [14.9] years (Student's *t*-test; *P*-value < 0.01).

Thirty-five (41.6%) patients with a previous diagnosis of AIDS reported the use of highly active antiretroviral therapy (HAART) (**Figure 1**), but only 20.2% (17/84) regularly received this therapy.

The average time from symptom onset to the diagnosis of cryptococcal meningitis was 41 [60.9] days (range: 1-297 days). A total of 69 (53.5%) patients survived, and 60 (46.5%) died. Although not statistically significant, 65% (13/20) of the patients with a time from symptom onset to the laboratory diagnosis of cryptococcosis of more than 60 days died, and 42.8% (39/91) of the patients who received a laboratory diagnosis less than 60 days after the onset of disease symptoms died. The period from the onset of disease symptoms to laboratory diagnosis could not be identified in 18 cases. Patients who were discharged from the hospital (n=70) developed various complications, including visual sequelae (n=5; 7.1%), cognitive impairment (n=4; 5.7%), and hearing loss (n=1; 1.4%).

Molecular typing showed that most of the isolates obtained from the studied region belonged to *C. neoformans* molecular type VNI (n=115; 89.1%); however, VNII (n=5; 3.9%) and *C. gattii* VGII (n=9; 7%) types were also found (**Figure 2**).

Three out of five *C. neoformans* VNII strains were isolated from patients originating from two other states: São Paulo (n=1) and Paraná (n=2). The molecular types of *Cryptococcus* spp. according to HIV infection status and clinical outcomes are described in **Table 2**.

DISCUSSION

This study is relevant because it describes the epidemiological aspects of cryptococcal meningitis cases during a period of almost two decades from cities of Mato Grosso do Sul state.

TABLE 1: Demographic and clinical characteristics of patients with cryptococcal meningitis who either had AIDS or were HIV negative.

Variables	Total (n=129)		AIDS (n=111)		HIV negative (n=18)		P-value
	n	%	n	%	n	%	
Gender							0.62*
male	99	76.7	86	77.5	13	72.2	
female	30	23.3	25	22.5	5	27.8	
Age group (years)							<0.01*
10–29	24	18.6	22	20.0	2	10.5	
30–49	90	69.8	80	72.6	10	52.6	
≥50	15	11.6	8	7.3	7	36.8	
Geographic distribution							0.36*
Campo Grande City	86	67.4	75	67.6	12	66.7	
Countryside cities	40	31.0	34	30.6	6	33.3	
other states	2	1.6	2	1.8	-	-	
Area							< 0.01*
urban	116	89.9	103	93.6	13	68.4	
rural	13	10.1	7	6.4	6	31.6	
Time between symptom onset and diagnosis (days)							0.09*
<30	64	49.6	58	52.7	6	31.6	
≥30	65	50.4	52	47.3	13	68.4	
Species							0.03**
<i>Cryptococcus neoformans</i>	120	93.0	115	95.5	5	78.8	
<i>Cryptococcus gattii</i>	9	7.0	5	4.5	4	21.1	
Length of stay (days)							0.23*
<30	72	55.8	59	53.6	13	68.4	
≥30	57	42.2	51	46.4	6	31.6	
Clinical outcome							0.40*
discharge	69	53.5	61	55.0	8	44.4	
death	60	46.5	50	45.0	10	55.6	

AIDS: acquired immunodeficiency syndrome; **HIV:** human immunodeficiency virus. *Fisher's exact test. **Chi-squared test.

Although numerous studies have investigated cryptococcal meningitis throughout the world, few studies have been conducted in this large Brazilian territory.

The results of this study showed that approximately 7.6 cases of cryptococcal meningitis were diagnosed annually at the MAPUH, and the diagnosis rate was six-fold higher in AIDS patients than in HIV-negative patients. In Goiás, another state of the Brazilian Midwest, Souza et al.¹⁷ described a similar annual incidence rate (5.9) of cryptococcal meningitis.

Corroborating the findings of previous studies, a greater involvement of men (76.7%) than women was observed in

this study. Approximately 61%-82% of the population has been found to be affected with cryptococcal meningitis, as reported in several studies, indicating that men might be more susceptible to infection by *Cryptococcus* spp¹⁷⁻¹⁹. The underlying causes for this increased susceptibility may be related to the following factors: occupational exposure; behavioral aspects, including alcoholism and smoking; and HIV infection^{12,20}. Hormonal and immunological factors that predispose men to infection by *Cryptococcus* spp have also been investigated, and testosterone seems to induce greater capsule production and lower macrophage efficiency²¹.

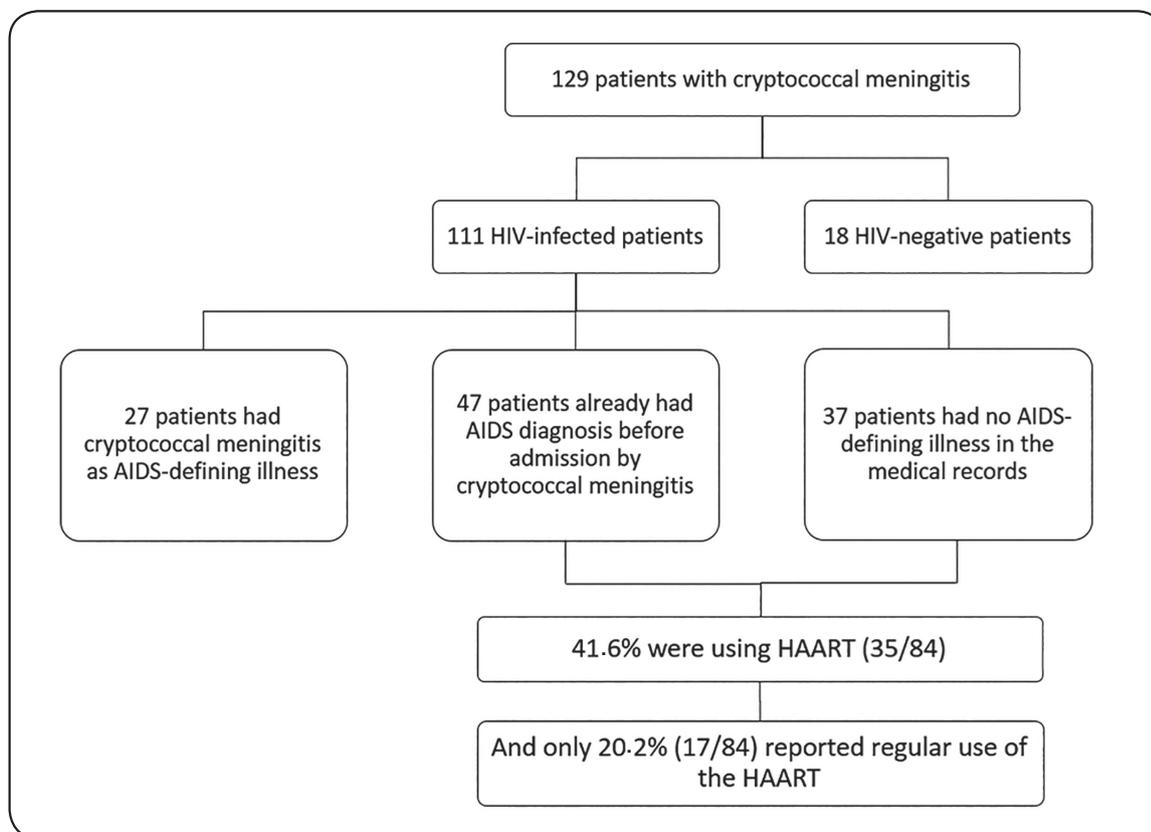


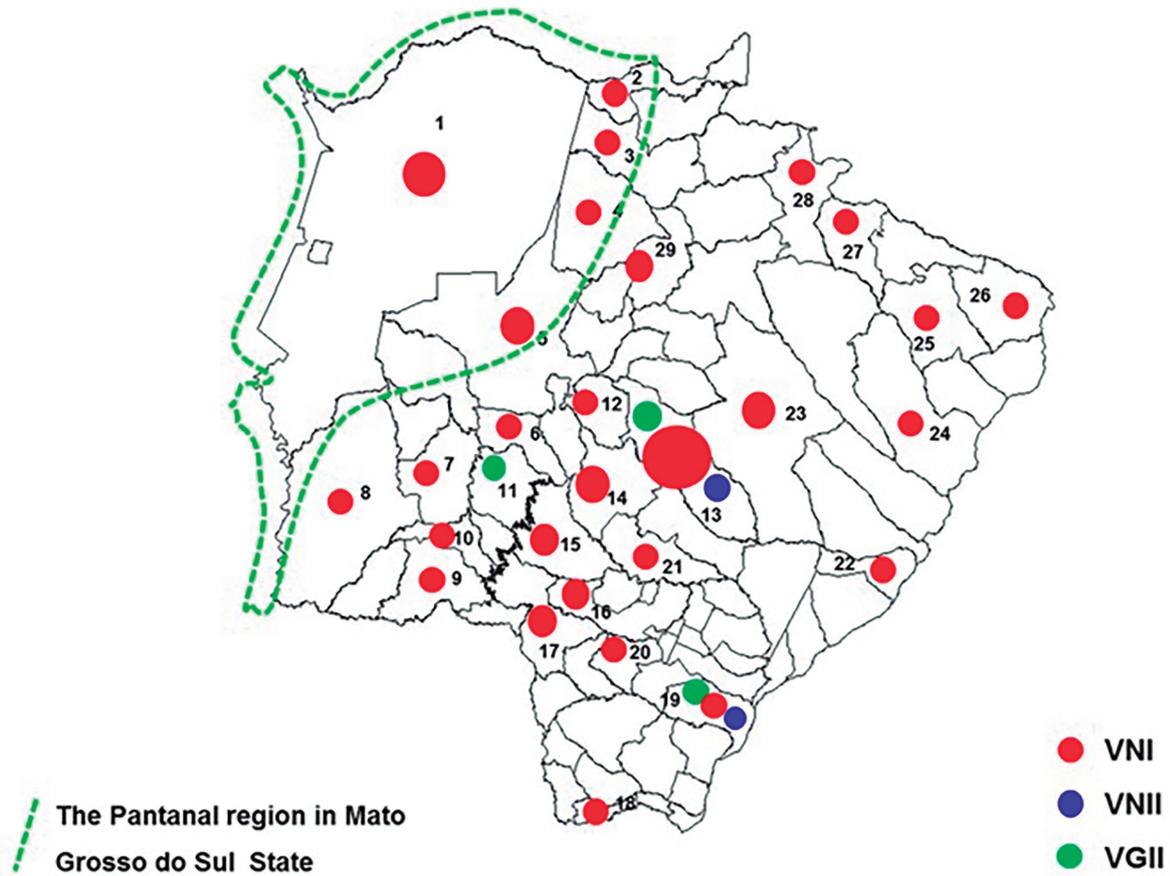
FIGURE 1: Distribution of patients with cryptococcal meningitis according to HIV infection status and antiretroviral therapy use. **HIV:** human immunodeficiency virus; **AIDS:** acquired immunodeficiency syndrome; **HAART:** highly active antiretroviral therapy.

TABLE 2: Patients with cryptococcal meningitis according molecular type, HIV infection status, and clinical outcomes.

Variables	<i>Cryptococcus neoformans</i>		<i>Cryptococcus gattii</i>		P-value*
	VNI and VNII (N=120)		VGII (N=9)		
	n	%	n	%	
AIDS					0.03
yes	105	87.5	5	55.6	
HIV negative	15	12.5	4	44.4	
Clinical outcomes					0.04
discharged	62	51.7	8	88.9	
death	58	48.3	1	11.1	

HIV: human immunodeficiency virus; **AIDS:** acquired immunodeficiency syndrome. *Fisher's exact test.

State of Mato Grosso do Sul: municipalities



Legend

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| 1: Corumbá (VNI); n=3 | 15: Maracaju (VNI); n=2 |
| 2: Sonora (VNI); n=1 | 16: Dourados (VNI); n=2 |
| 3: Coxim (VNI); n=1 | 17: Ponta Porã (VNI); n=2 |
| 4: Rio Verde de Mato Grosso (VNI); n=1 | 18: Sete Quedas (VNI); n=1 |
| 5: Aquidauana (VNI); n=2 | 19: Naviraí (VNI, VNII, VGII); n=1, 1, 1 |
| 6: Anastácio (VNI); n=1 | 20: Caarapó (VNI); n=1 |
| 7: Bonito (VNI); n=1 | 21: Rio Brilhante (VNI); n=1 |
| 8: Porto Murtinho (VNI); n=1 | 22: Bataguassu (VNI); n=1 |
| 9: Bela Vista (VNI); n=1 | 23: Ribas do Rio Pardo (VNI); n=2 |
| 10: Jardim (VNI); n=1 | 24: Três Lagoas (VNI); n=1 |
| 11: Nioaque (VGII); n=1 | 25: Inocência (VNI); n=2 |
| 12: Terenos (VNI); n=1 | 26: Paranaíba (VNI); n=1 |
| 13: Campo Grande (VNI, VNII, VGII); n=75, 4, 6 | 27: Chapadão do Sul (VNI); n=1 |
| 14: Sidrolândia (VNI); n=2 | 28: Costa Rica (VNI); n=1 |
| | 29: São Gabriel do Oeste (VNI); n=2 |

FIGURE 2: Distribution of *Cryptococcus neoformans* and *Cryptococcus gattii* molecular types in the cities of Mato Grosso do Sul according to the place of residence of the patients with cryptococcal meningitis.

Previous studies have shown that cryptococcosis often affects adults, especially those with altered cellular immunity^{17,18,21}. Worldwide, men aged 30-45 years are at greater risk of developing cryptococcal meningitis^{12,22,23}. In accordance with the global trends, cryptococcal meningitis in the Mato Grosso do Sul State is closely related to HIV infection^{16,24}.

The number of patients aged older than 50 years was proportionally higher in the HIV-negative group. Aging increases an individual's susceptibility to infectious diseases, even those without HIV, due to the comorbidities associated with anatomical and physiological changes and poor immune responses²⁵.

Only three (2.3%) cases of cryptococcal meningitis in children were recorded during the 17-year study period. A similar prevalence was found in the Rio Grande do Sul and Rio de Janeiro states^{22,26}.

The reason why children are less affected by cryptococcosis remains unknown²⁷. In the Northern and Northeastern regions of Brazil, primarily the States of Bahia (32%), Pará (24%), and Piauí (9.5%), a high prevalence of cryptococcosis in children was reported, and a majority of these cases are children living in rural areas^{18,28,29}.

This study showed an association between living in a rural area and an HIV-negative status. Cryptococcosis should be investigated in patients living in endemic areas who have these characteristics.

Confirming the cosmopolitan nature of this disease, most patients lived in urban areas, primarily Campo Grande City. This finding may have been obtained because the urban population in Mato Grosso do Sul is almost six-fold higher than the rural population according to the census conducted in 2010 by the Brazilian Institute of Geography and Statistics^{14,15}.

Another reason for this finding is that urban centers are often important ecological niches of domestic pigeons (*Columba livia*), which are natural reservoirs of *Cryptococcus* sp. In fact, this yeast was observed in the excreta of captive birds and in public areas that are commonly used by humans³⁰⁻³².

Vidal et al.¹¹ stated that in Latin American countries, those who live in poverty and are affected by opportunistic diseases have limited access to medical care. Although the present study did not aim to study the socioeconomic status of patients, in Brazil, the average amount of time from symptom onset to the diagnosis of cryptococcosis ranges from several days to several months, as observed in the studied population^{18,28}. Delays in diagnosis may reflect the difficulties that patients face accessing health services as well as the limited diagnostic resources (serological tests, microscopic findings, and culture) available in Mato Grosso do Sul cities. From this perspective, the number of cryptococcal meningitis cases in the South Pantanal region may be underestimated. Focused efforts to improve the current diagnostic capacity and identify the agents of cryptococcal disease are urgently warranted.

Notably, in the present study, 65% of the cases involving patients who received their laboratory diagnosis more than 60 days after symptom onset resulted in fatalities, and sequelae

were observed among those who survived. Because the disease has a high mortality rate if not diagnosed and treated appropriately, we reaffirm the importance of early diagnosis for survival and better prognosis.

In the United States, the mortality associated with cryptococcosis seems to be decreasing²⁷. However, in less developed countries, the estimated mortality rate is 55%, and this rate is even higher in Sub-Saharan Africa (70%), where the occurrence of AIDS is high¹⁰.

Overall, approximately 957,900 cases (range: 371,700-1,544,000) of cryptococcal meningitis occur every year, and 624,700 deaths (range: 125,000-1,124,900) occur within 3 months after the onset of infection. In Latin America, studies have investigated the factors responsible for the high mortality of AIDS-related cryptococcal meningitis¹¹. In Brazil, the mortality rate ranged from 32% to 60%^{18,33,34}; however, there was limited information on the fatality rate of HIV-infected patients with cryptococcal meningitis in the Midwestern region of Brazil^{16,23}.

The World Health Organization recommended the early diagnosis and treatment of HIV (HAART) as one of the most important and cost-effective preventive strategies for reducing the incidence and mortality associated with cryptococcal meningitis¹¹. When available, screening tests, including investigations of the presence of cryptococcal capsular antigen in asymptomatic and symptomatic patients, also contribute to the optimization of the diagnosis and treatment of cryptococcosis³⁵.

Various studies have shown that the incidence of cryptococcal meningitis can be minimized by the regular use of HAART^{8,11}. Late and incomplete access to antiretroviral therapy may have resulted in the high rate of cryptococcal infection observed in our study.

Previous studies have shown that the VNI molecular pattern is the most common pattern worldwide, whereas VNII was observed in 1%-16% of cases reported in southern continents, such as South America, Africa, and Oceania^{2,3}. *C. gattii* VGII was also observed in South America, North America^{3,27,36}, and Oceania². Interestingly, in Brazil, several regions exhibited a high prevalence of the VNI molecular type and sporadic cases of the VNII and VGII molecular types^{3,16,29,37,38}. The results obtained in this study add new information to the sparsely available data on the molecular epidemiology of cryptococcal meningitis in the Midwestern region of Brazil. The predominance of the VNI molecular type and the identification of the VGII molecular type have been described in the States of Goiás and Mato Grosso as well as other Brazilian states^{16,37} where VGII is endemic³⁸, except for the states in the North and Northeast region.

This is the first report to describe the molecular types of *Cryptococcus* causing cryptococcal meningitis in Campo Grande, Mato Grosso do Sul. Similar to the findings of a previous study²⁴, VNI was observed throughout the state. We highlight the isolation of VNII in patients from Campo Grande and Naviraí, cities in close proximity to São Paulo State, where this molecular type was previously described³⁸⁻⁴⁰.

In addition to Campo Grande and Nioaque City, the VGII molecular type was also found in Naviraí, which borders

Paraná State, where VGII was previously reported³⁸. Naviraí and Campo Grande are the only cities where three molecular types (VNI, VNII, and VGII) were detected. Further studies should be conducted to investigate this relation.

One of the primary limitations of our study is that it employed a cross-sectional design, and this type of design precludes the analysis of causal relations and only suggests associations between various variables. Another primary limitation is that a convenient sample was used, which makes it difficult to extrapolate the results to a larger population.

In conclusion, the present study documents the occurrence of neurocryptococcosis, which is mainly caused by *C. neoformans* VNI, in Mato Grosso do Sul, Brazil, with probable autochthonous cases in the Pantanal, a biome where cryptococcosis has not yet been explored. The time from symptom onset to clinical diagnosis is crucial for patient recovery but is largely dependent on the patient's access to healthcare and on the available diagnostic resources available.

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Conflict of interest

The authors declare that there is no conflict of interest.

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