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RESEARCH ARTICLE

Cryptococcosis in HIV/AIDS patients in northern Brazil: Clinical aspects, molecular types and isolation of agents from environmental samples associated with patients

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Abstract

Objectives: In the state of Amazonas, northern Brazil, cryptococcosis is endemic, with a predominance of *Cryptococcus neoformans* in individuals with HIV/AIDS, and *Cryptococcus gattii* VGII in non-HIV individuals. This study analysed the clinical isolates and clinical–epidemiological characteristics of HIV/AIDS patients diagnosed with cryptococcosis in a tertiary healthcare facility in Manaus, Amazonas and investigated the presence of agents of cryptococcosis in environmental samples.

Methods: A survey was made of data from HIV/AIDS patients diagnosed with cryptococcosis between January 2017 and December 2019, and environmental samples were collected at the patients' and their neighbours' homes. The isolates were submitted to morphophysiological analysis and PCR-RFLP typing to determine the molecular types.

Results: Clinical–epidemiological characteristics of 55 patients and 75 clinical isolates were analysed. Neurocriptococcosis was the clinical form observed in 98.2% (n = 54/55) of patients. A total of 38.1% (n = 21/55) of patients died within 100 weeks, of which 21.8% (n = 12/55) died less than a month after the diagnosis of cryptococcosis. *C. neoformans* VNI (n = 68/75), *C. neoformans* VNII (n = 1/75), *C. gattii* VGI (n = 3/75) and *C. gattii* VGII (n = 3/75) were identified. Mixed infection was observed in two patients, one by *C. neoformans* VNI and VNII and the other by *C. neoformans* VNI and *C. gattii* VGI. *Cryptococcus* VNI was detected in three (n = 3/51) households, one of a patient (n = 1/17) and two households that neighbour patients' houses (n = 2/34).

Conclusions: This study demonstrated the prevalence of *C. neoformans* VNI, which is a cause of cryptococcosis in patients with HIV/AIDS in the state of Amazonas, and revealed a greater diversity of molecular types affecting these patients in the region than in previous studies. In the studied group, a high mortality rate was observed, which reflects the importance of early diagnosis, and evidences cryptococcosis as an AIDS-defining disease and an important public health problem in the region. The home environment proved to be a potential source of infection/reinfection by *C. neoformans* VNI.

Sustainable Development Goal: Good Health and Wellbeing.

KEYWORDS
Amazonas, cryptococcosis, *Cryptococcus*, HIV/AIDS, PCR-RFLP

INTRODUCTION

Cryptococcosis is a potentially fatal respiratory and neurological disease that affects humans and animals worldwide. This systemic mycosis is acquired through the inhalation of infectious propagules from the environment and is caused by pathogenic members of the genus *Cryptococcus*, including the *Cryptococcus neoformans* species' complex molecular types VNI, VNII, VNIII, VNIV and the *Cryptococcus gattii* species' complex molecular types VGI, VGII, VGIII, VGIV) [1] as well as the newly identified VGV and VGVI [2]. Recently, seven species of agents of cryptococcosis have been proposed by Hagen et al. [3]; however, this nomenclature is still incipient and we treat these agents as two complexes and their respective molecular types or genotypes, according to Kwon-Chung et al. [1]

Cryptococcus neoformans is primarily responsible for infection in immunosuppressed patients, particularly patients with AIDS [4]. Rajasingham et al. [5] estimated an incidence of 223,100 cases of cryptococcal meningitis and approximately 181,100 deaths annually, cryptococcosis being the second most common cause of AIDS-related death. In Brazil, cryptococcosis is the most recurrent systemic mycosis in immunocompromised patients, mainly in the form of meningitis [6]. When associated with AIDS, it corresponds to 4.4% of diagnosed cases, with an estimated 1,000,000 cases and 250,000 related deaths annually [7]. By contrast, *C. gattii* predominantly cause a primary infection in immunocompetent individuals. It is worth noting that the endemic cryptococcosis caused by *C. gattii* shows a regional pattern in Brazil, and is mostly reported in the north and northeast of the country, where it occurs usually in immunocompetent hosts, including children and young adults [8]. In the state of Amazonas, cryptococcosis is prevalent in HIV patients and its main agent is *C. neoformans* VNI, while in non-HIV patients, *C. gattii* VGII predominates as an agent of cryptococcosis [9–11].

The main urban reservoir of *C. neoformans* is pigeon droppings, but it occurs in several substrates, including decaying wood [12]. *C. gattii* has also been related to tree species and decaying wood [13, 14]. *Cryptococcus neoformans* has been isolated from dust in households in Rio de Janeiro, suggesting regular exposure in daily life [15]. Alves et al. [16] conducted a study in public spaces, such as schools, squares as well as residences and buildings in Manaus, Amazonas, and showed the presence of *C. neoformans* type VNI in pigeon droppings and also in the excrement of captive birds, and *C. gattii* molecular type VGII in a hollow tree, evidencing the same molecular types of *Cryptococcus* that are responsible for infections in Manaus. Brito-Santos et al. [17] identified *C. gattii* of molecular type VGII in household dust samples in wooden houses in a municipality located in the upper Negro River region, in the Amazonas state. Environmental studies show the importance of several microenvironments as a potential source of human infection.

In Brazil, cryptococcosis is not a disease that requires compulsory notification and data on the distribution of cases of the disease and its causative agents are scarce. Between 2017 and 2019, 1,372 cases of AIDS were reported at the Dr. Heitor Vieira Dourado Tropical Medicine Foundation (FMT-HVD) [18], which is a reference centre for HIV/AIDS cases in the state of Amazonas, Brazil, of whom 83 had cryptococcosis associated, representing 6% of all AIDS cases. In this work, we analysed the cases of cryptococcosis in patients with HIV/AIDS diagnosed during a three-year period (2017–2019) at FMT-HVD to characterise the clinical–epidemiological data of these patients, the molecular types causing cryptococcosis and we also analysed the environmental sources in order to determine the presence of agents of cryptococcosis in the homes of these patients and their neighbours' homes.

METHODS

Patient data collection and ethical approval

The sociodemographic, clinical and laboratory information of the patients were obtained via the electronic medical record IDoctor and subsequently tabulated using Microsoft® Excel. The following clinical data were extracted: diagnosis of HIV/AIDS and cryptococcosis, symptoms, clinical form of cryptococcosis, CD4T cell count, patient evolution; sociodemographic data: age, sex and city of residence; and epidemiological data related to exposure to the fungus.

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Tropical Medicine Foundation Doctor Vieira Dourado (protocol code CAAE No. 82715917.4.0000.0005).

Sampling of clinical isolates

Cryptococcus isolates were obtained from individuals with HIV/AIDS diagnosed with cryptococcosis in a tertiary health unit in Manaus, Amazonas, Brazil, from January 2017 to December 2019. All isolates were maintained in Sabouraud dextrose agar tubes and stored at 4°C at the Medical Mycology Laboratory at FMT-HVD who kindly provided the isolates for the experiments that were carried out in the Mycology Laboratory of the Instituto Leônidas e Maria Deane/FIOCRUZ.

Isolation of fungi from environmental samples

We collected 77 environmental samples in 51 households (17 from patients homes and 34 neighbours' homes), comprising

51 samples of household dust, 8 soil samples, one bird excrement sample and 17 samples of air. Household dust was collected in all the homes visited, air collection was carried out only in the patients' homes and soil only in patients' homes where there was a yard with exposed soil. The air was collected for 15 min in each household (850 litres analysed in each household) using a 6-layer impactor (9–0.65 μm) (Bioaero 6, Energética containing plates with Niger seed agar (NSA) medium. The plates with air samples were incubated at 28°C and observed daily for up to 5 days. The remaining samples were processed according to Lazera et al. [19], 10 plates were used for each sample. Plates with NSA were incubated for up to 5 days at 28 °C. Isolates were also subjected to growth at 37°C.

Morphophysiological identification of *Cryptococcus*

Isolates that were positive for phenoloxidase (brown colonies) were observed under a microscope using slides with 4% NaOH, Indian ink (nanquim) and cotton blue lactophenol. Colonies with capsulated yeasts, without hyphae, and producing melanin were subcultivated in NSA and subsequently identified in canavanine-glycine-bromothymol blue medium (CGB).

PCR-RFLP typing

The DNA of the clinical and environmental isolates was extracted with the use of the QIAamp Tissue and Blood extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and included a pre-phase of mechanical maceration using glass beads. Amplification of the *URA5* gene was performed followed by double digestion with *HhaI* and *Sau961* (ThermoScientific), according to Meyer et al. [20]. To identify the molecular types, the following reference strains (conceded by Fiocruz/CFP from their collection of pathogenic fungi) were used: CFP 55 (serotype A, VNI), CFP 56 (serotype A, VNII), CFP 57 (serotype AD, VNIII), CFP 58 (serotype D, VNIV), CFP 59 (serotype B, VGI), CFP 60 (serotype B, VGII), CFP 61 (serotype B, VGIII) and CFP 62 (serotype C, VGIV).

RESULTS

Clinical and epidemiological data

Eighty-three individuals with HIV/AIDS were diagnosed with cryptococcosis between January 2017 and December 2019, with an average of 28 cases per year. We included 55 patients whose clinical isolates were viable for the study and obtained 75 isolates. Of these, 81.8% (*n* = 45/55) either resided or have resided in the city of Manaus, capital of Amazonas State, with a greater frequency in the eastern and northern areas of the city (Figure 1); 20% (*n* = 11/55) came from other municipalities of the state (Table 1). Manaus is located in the middle of the Amazon Rainforest, at the confluence of the Negro and Solimões Rivers, with a territory of 11,401 km^2 and 2.2 million inhabitants and has ecological reserves such as the Adolpho Ducke Forest Reserve (Figure 1).

Cryptococcus neoformans VNI was the aetiological agent in 94.5% (*n* = 52/55) of the cases, with one case of mixed infection by *C. neoformans* VNI and VNII, and one case of mixed infection by *C. neoformans* VNI and *C. gattii* VGI. VGII was identified in 5.4% (*n* = 3/55) of patients (Table 1). 74.5% (*n* = 41/55) of the patients are male and patient age ranged from 18 to 53 years, with a mean age of 35.3 years. The most frequent clinical manifestations were severe headache 63.6% (*n* = 35/55), fever 40% (*n* = 22/55), vomiting 36.3% (*n* = 20/55), myalgia 12.7% (*n* = 7/55), mental confusion 29.1% (*n* = 16/55), dyspnoea 29.1% (*n* = 16/55), dementia 25.4% (*n* = 14/55), weight loss 25.4% (*n* = 14/55), seizure 18.1% (*n* = 10/55) and psychomotor agitation 12.7% (*n* = 7/55). Neurological sequelae, such as visual impairment 29.1% (*n* = 16/55), motor deficit 10.9% (*n* = 6/55) and hearing deficit 7.3% (*n* = 4/55) were also observed. Neurocryptococcosis was observed in 98.2% (*n* = 54/55), and only 1.8% (*n* = 1/55) patient had pulmonary cryptococcosis. Cerebrospinal fluid (CSF) was the type of clinical sample with the most *Cryptococcus* isolates recovered, that is in 85.4% (*n* = 47/55) of the patients.

The CD4 + T cells count performed at the closest date to the diagnosis of cryptococcosis was observed. The mean CD4+ T cells were 87.1 cells/mm^3: 49% (*n* = 27/55) < 50 cells/mm^3, 16.3% (*n* = 9/55) 50–100 cells/mm^3, 3.6% (*n* = 2/55) 100–150 cells/mm^3, 20% (*n* = 11/55) >150 cells/mm^3, for 10.9% (*n* = 6/55) there were no data. 21.8% (*n* = 12/55) were diagnosed with cryptococcosis at the same time as they received the diagnosis of HIV/AIDS, with a difference of 1–3 days between the results. Fluconazole and amphotericin B were the medications used in the treatment of patients. Hospital death was observed in 38.1% (*n* = 21/55) patients, and 21.8% (*n* = 12/55) died less than one month after diagnosis.

Households of patients with cryptococcosis and assessment of the presence of *Cryptococcus*

It was possible to make a home visit for environmental collection in 17 patients' homes, since 12 resided in locations that are distant from Manaus and 26 could not be contacted. For every patient's home that was visited, the two neighbouring houses were also visited, thus totalling 51 houses.

Cryptococcus neoformans VNI was detected in 5.9% (*n* = 3/51) households and was viable for growth at 37 °C. Household dust was positive in 3.9% (*n* = 2/51) of samples, while soil was positive in 12.5% (*n* = 1/8) of the households where this substrate was collected. The isolates were obtained from households with some type of yard, while houses without a yard or apartments were not positive (Table 2). In 55% (*n* = 28/51) of the households, there was a report of the presence of pigeons outside the home; however, there

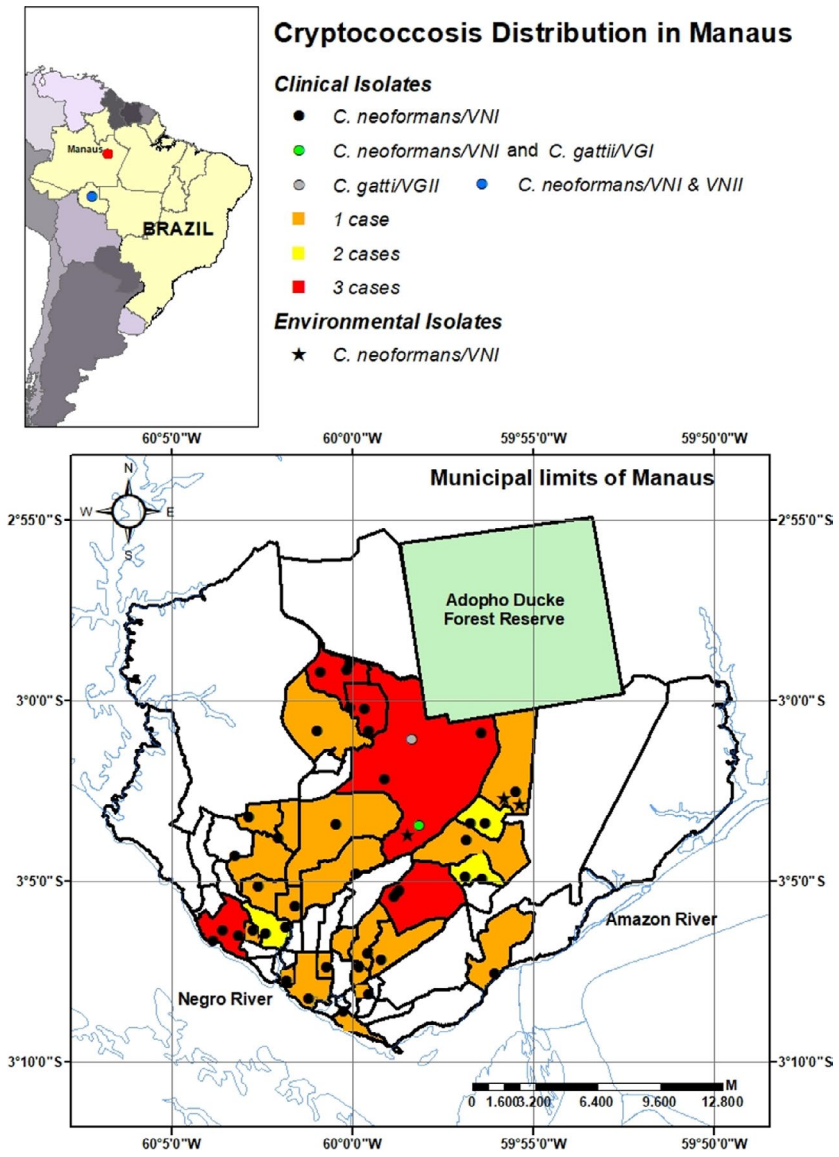

FIGURE 1 Distribution of cryptococcosis cases and environmental isolates in the municipality of Manaus, Brazil according to districts and molecular types; • Patient (P5) resides in another state but was diagnosed with cryptococcosis in Manaus

was accumulation of pigeon excreta only in one household, whose sample was negative. *Cryptococcus* was not detected in any of the air samples.

Cryptococcus neoformans VNI was isolated from the soil of one patient's home, as well as dust obtained from his neighbour's home. The patient has lived in the house for about 17 years, but this was undergoing renovation that began shortly after the diagnosis of cryptococcosis. The soil sample was collected from soil that had been turned over (Figure 2). No new re-infection has been reported in this patient so far.

Dust sample 2, which was positive for *C. neoformans* VNI, was obtained from a neighbouring home of another patient (P13); however, there was no detection of the fungus in the home of the patient who had lived there for about 20 years. Several clinical isolates were obtained from this patient at different times, with mixed infection by *C. neoformans* VNI and *C. gattii* VGI. The patient reported regular visits to a friend's smallholding, which is located in an area with recent deforestation, road formation and urbanisation. In addition, there were pigeons in a school in front of his residence. The location of these cases is shown in Figure 1.

Molecular type of Cryptococcus isolates obtained from patients and the environment

The restriction fragment length polymorphism (RFLP) of the *URA5* gene showed that 90.6% (*n*= 68/75) of the clinical isolates were *C. neoformans* VNI, though other molecular types were also observed, such as *C. neoformans* VNII (*n* = 1/75), *C. gattii* VGI (*n* = 3/75) and *C. gattii* VGII (*n* = 3/75). The three environmental isolates were *C. neoformans* VNI. Patient P5 presented two different molecular types of *C. neoformans* (VNI

TABLE 1 Description of the 55 HIV/AIDS patients diagnosed with cryptococcosis from January 2017 to December 2019, according to place of residence, age, type of sample, CD4T cell count, aetiological agent, treatment and outcome

Patient	Place of residence	Age	Sample	CD4+ T cells/mm^3	Molecular type	Treatment	Outcome
P1	Manaus	29	CSF	>150	VNI	AmB + FCZ	Death
P2	Manaus	45	CSF	13	VNI /VNI	AmB + FCZ	Death
P3	Manaus	39	CSF	>150	VNI	AmB + FCZ	OC
P4	Rio Preto da Eva	37	CSF	>150	VNI	AmB + FCZ	OC
P5[a]	RONDONIA	28	CSF	4	VNI/VNII	AmB + FCZ	Death
P6	Manacapuru	29	CSF	18	VNI	No data	Death
P7	Careiro Castanho	28	CSF	55	VNI/VNI	AmB + FCZ	OC
P8	Manaus	28	CSF	9	VNI/VNI	AmB + FCZ	OC
P9	Parintins	27	CSF	No data	VNI/VNI	AmB + FCZ	Death
P10	Manacapuru	30	CSF	12	VGII	AmB + FCZ	Death
P11	Manaus	22	CSF	12	VNI/VNI	AmB	OC
P12	Manaus	44	CSF	80	VNI	AmB + FCZ	OC
P13[a]	Manaus	34	CSF	7	VGI/VGI/VNI/VGI	AmB + FCZ	HD
P14	Itacoatiara	35	CSF	18	VNI	AmB + FCZ	OC
P15	Manaus	23	CSF	7	VNI	AmB + FCZ	Death
P16	Manaus	43	CSF	15	VNI	AmB + FCZ	Hospitalised
P17	Manaus	53	CSF	10	VNI	AmB	OC
P18	Manaus	40	Blood	14	VNI	AmB	Death
P19	Manaus	30	CSF	47	VNI	No data	OC
P20	Manaus	28	CSF	>150	VNI	No data	Death
P21	Beruri	26	CSF	No data	VNI/VNI	No data	Death
P22	Manaus	43	CSF	120	VGII	AmB + FCZ	OC
P23	Manaus	53	CSF	74	VNI/VNI	FCZ	Death
P24	Manaus	30	CSF	75	VNI	AmB	OC
P25	Manaus	34	CSF	23	VNI/VNI/VNI/ VNI/VNI	AmB + FCZ	Death
P26	Autazes	51	CSF	>150	VGII	AmB + FCZ	Death
P27	Manaus	40	CSF	>151	VNI/VNI	FCZ	OC
P28	Manaus	48	TA	No data	VNI	No data	Death
P29	Manaus	43	Blood	6	VNI	AmB + FCZ	OC
P30	Manaus	18	CSF	62	VNI	FCZ	Death
P31	Manaus	22	CSF	24	VNI	AmB + FCZ	OC
P32	Manaus	40	Blood	23	VNI	FCZ	Death
P33	Manaus	46	Blood	4	VNI/VNI	AmB	Death
P34	Manaus	36	CFS	93	VNI	FCZ	OC
P35	Manaus	25	CFS	13	VNI	AmB + FCZ	OC
P36	Manaus	37	CFS	>150	VNI	AmB + FCZ	OC
P37	Manaus	44	CFS	No data	VNI	AmB + FCZ	Death
P38	Manaus	51	CFS	No data	VNI	AmB + FCZ	Death
P39	Manaus	49	CFS	49	VNI/VNI	AmB + FCZ	OC
P40	Manaus	29	Blood	>150	VNI	FCZ	OC
P41	Manaus	18	CFS	50	VNI	AmB + FCZ	Death
P42	Rio Preto da Eva	36	CFS	43	VNI	AmB + FCZ	OC
P43	Manaus	36	CFS	63	VNI	AmB + FCZ	OC
P44	Careiro da Varzea	32	CFS	103	VNI	AmB + FCZ	OC

(Continues)

TABLE 1 (Continued)

Patient	Place of residence	Age	Sample	CD4+ T cells/mm^3	Molecular type	Treatment	Outcome
P45	Manaus	37	CFS	>150	VNI/VNI	AmB + FCZ	OC
P46	Manaus	36	Blood	20	VNI	AmB + FCZ	OC
P47	Manaus	34	CFS	>150	VNI	AmB + FCZ	OC
P48	Manaus	35	CFS	16	VNI	AmB + FCZ	OC
P49	Manaus	53	CFS	25	VNI	AmB + FCZ	HD
P50	Manaus	50	CFS	No data	VNI	AmB + FCZ	Death
P51	Manaus	29	BAL	21	VNI	AmB + FCZ	OC
P52	Iranduba	27	CFS	30	VNI	AmB + FCZ	HD
P53	Manaus	26	CFS	>150	VNI	AmB + FCZ	OC
P54	Manaus	31	CFS	7	VNI/VNI	AmB + FCZ	OC
P55	Manaus	28	CFS	66	VNI	AmB + FCZ	OC

Abbreviations: CFS, Cerebrospinal fluid; TA, Tracheal aspirate; BAL, Bronchoalveolar lavage fluid; AmB, Amphotericin B; FCZ, Fluconazole; OC, Outpatient care; HD, Hospital discharge.

[a]Mixed infection case.

TABLE 2 Types of housing visited and data related to the collections carried out at the home of patients and neighbours

Type of housing	Nº	Sample	Nº	Positive sample	Nº
House without a backyard	13	House dust	13	No	0
		Air	3	No	0
House with a concreted backyard	13	House dust	13	Yes	1
		Air	3	No	0
House with a dirt backyard	15	House dust	15	Yes	1
		Soil	8	Yes	1
		Pigeon Droppings	1	No	0
		Air	8	No	0
Apartment	10	House dust	10	No	0
		Air	3	No	0
Total	51		77		3

and VNII), while patient P13 presented one isolate of *C. neoformans* (VNI) and three isolates of *C. gattii* (VGI).

DISCUSSION

Cryptococcosis is an important public health problem that contributes significantly to morbidity and early mortality in individuals with HIV/AIDS [21]. Worldwide, *C. neoformans* VNI is the most common molecular type [7, 8, 22], while *C. neoformans* VNII appears in 1–16% of cases in South America, Africa and Oceania [22]. In the Amazonas State, *C. neoformans* VNI remains the aetiological agent that is most present in cases of cryptococcosis associated with AIDS [8–10] and, about 10 years after the first report in the state of Amazonas [9], a case of *C. neoformans* VNII was again identified.

Cryptococcus gattii VGII and *C. gattii* VGI were identified in this study. *C. gattii* is usually associated with immunocompetent individuals in areas with either a tropical or subtropical climate [11, 22–24], but they also infect immunocompromised individuals [25], and VGI has started to emerge in Europe, Australia and Asia [22, 26, 27]. VGI was observed as an agent of cryptococcosis for the first time in northern Brazil in the state of Pará and the case involved an immunocompetent child [28], though it was evidenced for the first time in the Amazonas state in the present study in an individual with HIV/AIDS.

A total of 98.2% (n = 54/55) patients presented neurocryptococcosis, while 1.8% (n = 1/55) (P51) presented the isolated pulmonary form, which is poorly diagnosed, since the spread of infection to the central nervous system normally occurs [28]. Meningitis is the most common form and has a higher mortality rate in individuals with HIV/AIDS [29, 30]. The clinical manifestations and neurological sequelae observed in our study corroborate the work of Rocha et al. [10]. Mental alterations are contributing factors for the progression of these patients to death [31], and seizures were observed in 18.1% of patients, indicating and advanced stage of the disease. Pastick et al. [32] associated seizures with reduced neurocognitive function and increased mortality at 10 weeks.

FIGURE 2 *Cryptococcus* isolation process from environmental samples collected at the patient's home: (a) Typical dirt backyard where soil was collected; (b) Sample processed in Niger seed agar (NSA) for isolation of *Cryptococcus*; (c) phenoloxidase positive colony subcultivated in NSA

Although access to highly active antiretroviral therapy (HAART) has improved globally, there is still a substantial number of individuals with HIV with CD4T cell counts <100 cells/mm^3 [33]. Cryptococcosis, in many cases is the first indication that HIV infection has evolved into AIDS, it is considered a defining disease of AIDS since it is associated with low counts of CD4T lymphocytes in the blood (below 100 cells) [34, 35]. In our study, 21.8% (n = 12/55) of patients discovered that they had HIV when they were hospitalised for cryptococcosis.

Mortality was observed mainly when the CD4T cell count was <50 cells/mm^3 and in cases of diagnosis of cryptococcosis and HIV/AIDS. Early death was observed in 21.8% (n = 12/55). Of these, 7.2% (n = 4/55) did not use antifungal agents, since they died within 1–3 days of hospitalisation. In another study conducted in Manaus, Souza et al. [36] also observed early death of patients with a mean survival of 15 days.

Irregular adherence to the use of HAART, the unavailability of rapid methods for early diagnosis and the lack of antifungal treatment lead to a high frequency of deaths from cryptococcosis; in addition, the appearance of severe clinical presentations also indicate late diagnosis [5, 7, 28]. Vidal et al. [37] suggest the adoption of routine cryptococcal antigen (CrAg) screening in patients infected with HIV with CD4T <200 cells/mm^3, regardless of HAART status.

The different molecular types exhibit different virulence and susceptibility patterns to antifungal agents, and it should be noted that mixed infections may be of particular importance for patient treatment [38]. Mixed infections have been observed in other studies [38–41], including patients with up to three distinct molecular types of *Cryptococcus*. We identified two cases of mixed infection (Table 1); one case (P5) from the state of Rondônia, in which *C. neoformans* VNI and *C. neoformans* VNII that were obtained from different collections during the same hospitalisation, with the patient presenting a CD4T of 4 cells/mm^3, and another patient (P13) with CD4T of 7 cells/mm^3, a resident of Manaus that presented one isolate of *C. neoformans* VNI and three isolates of *C. gattii* VGI (Table 1). It is noteworthy that the isolates of VGI, VGI and VNI, in this sequence, were obtained with intervals of one month from CSF samples and again, after five months, in CSF a third isolate of VGI was identified. It is possible that a re-infection has occurred, but the most likely cause in this case is a relapse caused by the same strain, since the fungal agent can persist for a long time in lesions in the host [38].

Infection by *C. neoformans*/*C. gattii* is caused by inhalation of infective fungal propagules present in nature [42, 43] and depends on the exposure of individuals to certain environments where there is the presence of the pathogen.

The size of the inhaled fungus, the time of exposure to this agent, the virulence of the strain and the immune response of the host are very important in this context [44]. In our work, we identified three environmental isolates of *C. neoformans* VNI, and in other studies, VNI was also isolated from household dust [14, 45] and soil from the homes of AIDS patients [45], decaying wood and woodlice [14], as well as pigeon excreta [46]. These studies show household dust as a potential source for human infection [18], in addition to the importance of this environment, and the follow-up of patients that have already been treated due to the possibility of reinfection after return to the home [46].

In our study, most cases are concentrated in the city of Manaus, where there are recurrent anthropogenic actions such as areas of the forest being cleared for expansion of the city (Figure 1). Although *C. gattii* was not isolated in any household, the molecular type VGII has already been isolated in the urban environment of Manaus [17, 46], which was in a sample of decomposing wood collected in the Adolpho Ducke Forest Reserve and also in water samples of the Negro River and Tarumã River, and these are important areas in Manaus that suffer from anthropic actions [47].

In most of the homes visited, the presence of pigeons in the extradomicile was reported. Pigeons have become increasingly present in urban ecosystems, and have become an environmental and public health problem [48]; and *C. neoformans* VNI has been isolated in this environment [16, 48]. In addition, activities, such as soil disturbance, fallen trees and deforestation, are also associated with *Cryptococcus* infection [12]. An interesting fact is that the three households in which isolates were found were houses with a yard, and one of the isolates was obtained from soil that had been turned over. Household dust and soil may have been the sources of infection by *C. neoformans* VNI.

Air impaction collectors are used indoors to control air quality since it is possible to control the amount of samples and homogeneous distribution of particles [49]. The method used for air collection only allowed us to evaluate the air at the specific time of collection; however, there was no detection of the fungus. Nonetheless, it is possible that the fact that suspended airborne particles depend on factors such as wind direction, intensity of atmospheric current, air humidity and circulation of people [50] and this could been considered a limitation of our study.

CONCLUSIONS

Cryptococcus neoformans VNI is the most common molecular type that causes neurocryptococcosis in individuals, with cryptococcosis being associated with HIV/AIDS in Manaus, Amazonas and an important indicator of AIDS. A greater diversity of molecular types was observed affecting these patients and *C. gattii* VGI was identified as an agent of cryptococcosis for the first time in the Amazonas state, and two cases of mixed infection were observed. Although the study was carried out with a small number of individuals, the high lethality demonstrates a late diagnosis of cryptococcosis, in addition to the diagnosis of HIV/AIDS itself and, consequently, late treatment. Our study shows the presence of *C. neoformans* VNI in the home environment, which constitutes a potential source of infection or reinfection for patients. These data suggest a need for continuous monitoring of cases of cryptococcosis associated with HIV/AIDS in the state, and both clinical and epidemiological data and the investigation of environmental sources for better case management, which should facilitate early diagnosis, adequate therapy and the reduction of severe clinical forms and the mortality rate.

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