

Cryptococcosis in Atlántico, Colombia: an approximation of the prevalence of this mycosis and the distribution of the etiological agent in the environment

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ABSTRACT

Introduction: Cryptococcosis is an invasive disease acquired by inhalation of infectious propagules from the environment. Currently, compulsory notification of the spread of this disease is not required in Colombia. However, reporting of human immunodeficiency virus (HIV)/acquired immune deficiency syndrome cases to the National Surveillance System has suggested that there is a growing population at risk of contracting cryptococcosis. Few studies have described the occurrence of cryptococcosis in Colombia. Therefore, in this study, we examined the pathology of this disease in Atlántico, Colombia and determined the distributions of *Cryptococcus neoformans* and *Cryptococcus gattii* in the environment. **Methods:** Clinical samples/isolates were gathered from cases of cryptococcosis previously diagnosed at health institutions in Atlántico, and surveys were completed by clinicians. The environmental study considered 32 sampling points and three tree species, i.e., Quickstick (*Gliricidia sepium*), Almond (*Terminalia catappa*), and Pink trumpet (*Tabebuia rosea*). Environmental and clinical samples/isolates were analyzed for phenotypic and genotypic confirmation. **Results:** From 1997-2014, 41 cases of cryptococcosis were reported. The mean patient age was 40.5 years (range: 18-63 years); 76% were men, and 78% were HIV positive. Isolation was possible in 38 cases (*C. neoformans*, molecular type VNI in 37 cases and *C. gattii*, molecular type VGI in one case). In 2012-2014, 2,068 environmental samples were analyzed with a positivity of 0.4% (*C. neoformans*, molecular type VNI) in Almond and Pink trumpet trees. **Conclusions:** *Cryptococcus neoformans*, molecular type VNI had a higher prevalence than *C. gattii* and was associated with human exposure and the pathogenesis of cryptococcosis in this geographical region.

Keywords: *Cryptococcus neoformans*. *Cryptococcus gattii*. Cryptococcosis. Colombia. Environment.

INTRODUCTION

Cryptococcosis is a life threatening fungal disease that affects man and other vertebrates. Nearly one million individuals will be diagnosed with cryptococcosis annually, with an increased incidence among patients with human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS). The highest incidence of cryptococcosis has been reported in Sub-Saharan Africa, where the mortality rate associated with this disease is estimated to range from 50% to 70%; however, other countries, such as the United States of America, Brazil, and Australia have also reported a significant number of cases. Therefore, this mycosis may be considered an AIDS-defining condition^{(1) (2) (3)}.

The main etiological agents of cryptococcosis are two basidiomycetous yeasts, *Cryptococcus neoformans* and *Cryptococcus gattii*. *Cryptococcus neoformans* can be further classified in two varieties, *Cryptococcus neoformans* var. *grubii* and *Cryptococcus neoformans* var. *neoformans*; both of these species can be differentiated into four serotypes (A and D or B and C, respectively), several hybrids (AD, AB, and BD)⁽⁴⁾, and eight molecular types (molecular types VNI-IV, and molecular types VGI-IV)^{(1) (5)}. In 2003, Meyer et al.⁽⁵⁾ performed a molecular typing study that included 340 Ibero-American clinical, veterinary, and environmental isolates from Argentina, Brazil, Chile, Colombia, Mexico, Peru, Venezuela, Guatemala, and Spain; for each participating country, these data facilitated the identification of the eight major molecular types of these two pathogens through polymerase chain reaction (PCR) fingerprinting⁽⁵⁾. Additionally, according to current studies, the natural habitat for both species has been expanding and includes environments in rural as well as urban settings in native and non-native trees in decaying wood, hollows, bird droppings, and water^{(1) (5) (6) (7) (8) (9) (10)}.

In Colombia, cryptococcosis does not require compulsory notification. However, various reports have confirmed the importance of cryptococcosis in this country. Since 1997, a national survey focusing on cryptococcosis has been carried

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out voluntarily at medical institutions in order to report and describe cases of cryptococcosis, allowing researchers to study the demographic and clinical data associated with each case. According to data amassed by the Colombian Cryptococcosis Study Group, the mean annual incidence rates of cryptococcosis between 1997 and 2005 and between 2006 and 2010 in the general population were both 2.4×10^6 individuals; in AIDS patients, the rate rose from 3.0×10^3 individuals during the first period to 3.3×10^3 individuals during the second period. *C. neoformans* var. *grubii* was found to be prevalent during both periods (95.9% and 96.7%, respectively) in comparison with *C. gattii* (3.3% and 2.2%, respectively)⁽¹¹⁾⁽¹²⁾.

Studies have suggested that the eight molecular types of *Cryptococcus* are distributed within South America and at least seven of these types are actually circulating in Colombia⁽¹⁾⁽¹³⁾⁽¹⁴⁾⁽¹⁵⁾⁽¹⁶⁾⁽¹⁷⁾. Determination of the ecological niches of genotypes of both species is important. To this end, previous reports have demonstrated the presence of these species associated with eucalyptus, ficus, and almond trees and with pigeon droppings in several regions throughout Colombia⁽¹⁵⁾⁽¹⁶⁾⁽¹⁷⁾. Therefore, studies of the specific distributions of these etiological agents within Atlántico, Colombia are necessary, particularly considering the variety of trees that belong to the local flora, including Pink trumpet and Quickstick trees. Studies focusing on specific areas in Colombia where *C. neoformans*/*C. gattii* are established are necessary to predict possible endemic areas for these two pathogens in the future through ecological niche modeling.

Therefore, the aim of this study was to determine the epidemiology of cryptococcosis in Atlántico, Colombia by analyzing data collected through the National Surveillance Program and to investigate the geographical distribution of the molecular types/serotypes found in this part of Colombia in order to obtain important information for future surveillance purposes.

METHODS

Study area

The Department of Atlántico is one of the political divisions of Colombia, South America and is geographically located in the northern coast of the country. Barranquilla is the capital city of Atlántico⁽¹⁸⁾. Topographically, the Department of Atlántico is predominantly flat, and the climate is hot and dry, with an average annual temperature of 28°C. The windy season usually covers part of the last quarter of one year and the first quarter of the next. Additionally, the rainy season during the year is bimodal (May to June and September to November), with an average annual precipitation between 500 and 1,500mm; dry periods occur from December to March and June to July⁽¹⁹⁾.

Study design

This was a descriptive observational study in which clinical and epidemiological data were gathered from surveys collected across a 17-year period (1997-2014) and from corresponding samples/isolates from patients with cryptococcosis diagnosed

in the Department of Atlántico, Colombia. The survey also explored whether cryptococcosis was an AIDS-defining illness, inquiring about diagnosis date, clinical findings, type of treatment, outcomes, types of diagnostic assays employed (clinical laboratory/imagenology), and treatment.

Clinical and environmental studies

Clinical study: a survey was completed for every case of cryptococcosis diagnosed at a public or private institution throughout the Department of Atlántico. Whenever possible, the survey was submitted with a corresponding isolate or sample to the Departmental Public Health Laboratory in Barranquilla. Data and isolates/samples were finally submitted to the Instituto Nacional de Salud (INS). At this national study center, a database was created for surveillance purposes using Biolumics software, and all national data were collected. Analysis of the information was performed using the statistical program Epi Info 6.1.

Each clinical isolate given to the INS was confirmed using conventional laboratory techniques (i.e., presence of a capsule through the Indian ink exclusion method, melanin production in *Guizotia abyssinica* media, urease production, or use of nitrates) or detection using cryptococcal antigen latex agglutination system (CALAS) (Meridian Bioscience, Inc., OH, USA) for cases in which cerebrospinal fluid (CSF) or serum samples were provided. Species determination was performed using canavanine-glycine-bromothymol blue agar (CGB). The isolates were maintained in sterile distilled water and as glycerol stocks at -70°C.

Environmental study: for the environmental study, a census of trees was performed in 32 sampling points (local parks and areas with a high density of trees) in Barranquilla for three common trees species, i.e., Almond, Pink trumpet, and Quickstick trees. At every sampling point, each tree was identified in alphanumeric order with a letter: M for Quickstick, A for Almond, and R for Pink trumpet, with a consecutive number for each case. The census was introduced into Google Maps; this layer allowed the georeferenciation of all sampling points in terms of geographical coordinates. Representative samples included leaves, flowers, soil, bark, and hollows. The samples (approximately 10g each) were collected between September 2012 and November 2014 within different quarters each year to sample variable climatic conditions. Environmental data from Barranquilla were obtained from the Instituto de Hidrología, Meteorología y Estudios Ambientales de Colombia (IDEAM)⁽¹⁹⁾.

Environmental samples were processed using conventional techniques⁽¹⁴⁾⁽¹⁵⁾⁽¹⁶⁾ and plating of the processed samples in *G. abyssinica* selective media. Typical brownish colonies, indicating melanin production, were subcultured in Sabouraud dextrose agar media to determine the purity of isolate growth and confirmed using conventional laboratory techniques, as described for the clinical study. When possible, more than one colony was recovered from positive samples.

Molecular typing

DNA extraction and molecular typing: high-molecular-weight deoxyribonucleic acid (DNA) was extracted as previously described⁽²⁰⁾. Primers of the microsatellite-specific sequence

(GTG)₅ were used for the genotyping of the isolates by PCR fingerprinting⁽²¹⁾. Molecular types were assigned by comparison with the reference strains of the eight major molecular types loaded on each gel. Isolates were also typed using restriction fragment length polymorphism (RFLP) analysis of the URA5 gene, which was amplified with the two flanking primers URA5 (5'-ATGTCCTCCCAAGCCCTCGACTCCG-3') and SJ01 (5'-TTAAGACCTCTGAACACCGTACTC-3')⁽⁵⁾. Restriction fragment length polymorphism patterns were assigned visually by comparison with the patterns obtained from standard strains (VNI-VNIV and VGI-VGIV).

Determination of mating type by PCR: two PCR primer pairs for *C. neoformans* specific for mating types α and a were used. The MAT α -specific mating type primers were MF α U (5'-TTCAGTCCATCTTCACCACC-3') and MF α L (5'-TCTAGGCGATGACACAAAGGG-3'). The MAT a -specific mating type primers were MF a 2U (5'-ACACCGCCTGTTACAATGGAC-3') and MF a 2L (5'-CAGCGTTTGAAGATGGACTTT-3'). Amplification reactions were performed as described previously⁽²²⁾; for mating type a , the primer concentration was 50ng/mL. The PCR products were separated on 2% agarose gels in 1× Tris Borate EDTA buffer containing 0.5mg/mL ethidium bromide for 1h at 100V.

Ethical considerations

The survey was designed according to the guidelines established by the European Confederation of Medical Mycology and was approved by the Ethics Committee of the Instituto Nacional de Salud (INS). Health professionals in public, private, and educational institutions completed the forms. Demographic data, including gender, age, date and place of birth, and place of residence, were collected. Additionally, the presence of any risk factors, such as HIV infection/AIDS and others, were considered.

RESULTS

Clinical isolates and surveys

Forty-one cases of cryptococcosis were reported in the Department of Atlántico, Colombia between 1997 and 2014. Thirty-one (76%) cases were adult men (median age: 40.5 years; range: 18-63 years). Thirty-two (78%) cases were positive for HIV; of these, 25% denied having received antiretroviral therapy (ART). In three (7.3%) cases, cryptococcosis diagnosis defined the AIDS condition. Other risk factors included solid tumors in three (7.4%) cases, autoimmune disease in one (2.4%) case, and kidney transplant in one (2.4%) case.

Chest X-rays were performed in 14 (34.1%) of the 41 patients, six of whom showed positive findings characterized by pleural effusion with pulmonary nodules, alveolar infiltrates, acute bronchopathy, and unspecified abnormalities. Brain computed tomography revealed recurrent hydrocephalus in two patients and cysticercosis in one patient.

Isolation of the fungus was possible in 38 (92.7%) of the 41 cases. Most isolates were obtained from the CSF, followed by blood culture (25 and 10 cases, respectively). *C. neoformans* var. *grubii*, VNI was identified in 37 (97.4%) cases, whereas *C. gattii*, VGI was identified in one (2.6%) case (**Table 1**). This single *C. gattii* isolate was recovered from the blood culture of a male patient with AIDS hospitalized with pulmonary symptoms who was not receiving ART and eventually died at a tertiary care institution during intravenous treatment with amphotericin B plus fluconazole.

In two cases (both patients with AIDS who had not received ART, one of whom also had cysticercosis), the diagnosis of cryptococcosis was made through cryptococcal antigen latex agglutination tests; one patient was positive using a 1:32 dilution

TABLE 1 - Laboratory findings in 41 patients with cryptococcosis in Atlántico Colombia, 1997-2014.

Diagnostic test	Number	Percentage	Results
Culture			
cerebrospinal fluid	25	61.0	<i>Cryptococcus neoformans</i> var. <i>grubii</i> , molecular type VNI
lung biopsy	2	5.0	<i>Cryptococcus neoformans</i> var. <i>grubii</i> , molecular type VNI
skin lesion	1	2.4	<i>Cryptococcus neoformans</i> var. <i>grubii</i> , molecular type VNI
blood culture	9	22.0	<i>Cryptococcus neoformans</i> var. <i>grubii</i> , molecular type VNI
blood culture	1	2.4	<i>Cryptococcus gattii</i> , molecular type VGI
Total cultures	38	92.8	
Latex agglutination			
serum	1	2.4	positive 1:32
cerebrospinal fluid	1	2.4	positive 1:64
Direct exam	1	2.4	positive Indian ink test
Total	41	100.0	

from a serum sample, and the other was positive using a 1:64 dilution from CSF. Culturing of CSF in this case was also performed with a negative isolation report, and no information about antifungal treatment was provided. This patient also showed meningeal signs.

One case was diagnosed through Indian ink exclusion staining of CSF by identification of typical encapsulated yeasts. In this case, the patient was HIV positive, showed meningeal signs, received treatment with amphotericin B (50mg/day) plus fluconazole (400mg/day), and was discharged after hospitalization.

Two (4.9%) of the 41 patients experienced relapse of mycosis; one had a kidney transplant, and the other had an AIDS-related condition but did not receive ART. This latter patient had a CD4⁺ cell count of 40 cells/mm³ during initial hospitalization. For these cases, isolation of *C. neoformans* var. *grubii*, VNI was possible from pulmonary biopsy and CSF, respectively. The patient with the kidney transplant also had skin lesions (cellulitis of lower limbs) and died during hospitalization while receiving amphotericin B.

For treatment of cryptococcosis, two (4.9%) patients received amphotericin B plus fluconazole, 12 (29.3%) received monotherapy with amphotericin B, and seven (17.1%) received only fluconazole.

Environmental study

A total of 2,068 environmental samples were collected. Of these samples, 13% were collected in 2012, 58% were collected during 2013, and 29% were collected during 2014 (Table 2). *C. neoformans* var. *grubii* was isolated from nine samples (positivity: 0.4%) from Almond and Pink trumpet trees, yielding

40 colonies [25 colony-forming units (CFU) from Almond trees and 15 CFU from Pink trumpet trees]. The positive samples were collected and processed during the last quarter of 2013, accounting for 32.1% of the total (Table 2). There were no significant differences in terms of sample type, and no *C. gattii* isolates were detected in any of the samples analyzed during this period, as shown in Table 3. There were no environmental isolates from samples associated with Quickstick trees.

During the study period, the mean temperature and relative humidity remained within the averages of 28°C and 78.93%, respectively. In 2012 and 2013, the annual days of rain were similar (95 and 91 days, respectively); however, this number decreased in 2014 (75 days). Annual rainfall remained similar for all 3 years (51.4 in 2012, 49.2 in 2013, and 56.3 in 2014), with the highest periods of rain occurring during the third quarters of 2013 and 2014 (Table 4).

Molecular typing

Thirty-seven (97.4%) of the 38 clinical isolates were molecular type VNI; the remaining clinical isolate (2.6%) was identified as VGI. All of the environmental isolates (40 colonies) were identified as VNI (Figure 1). All isolates were mating type α .

DISCUSSION

Pathogenic species of *Cryptococcus* have been isolated from clinical and environmental samples in Colombia⁽³⁾⁽¹¹⁾⁽¹²⁾⁽¹⁵⁾⁽¹⁶⁾⁽¹⁷⁾. In the Department of Atlántico in Colombia, only 41 cases of cryptococcosis have been reported in the last 17 years. However, because notification of cryptococcosis is not compulsory

TABLE 2 - Environmental sampling for *Cryptococcus neoformans*/*Cryptococcus gattii* in Barranquilla, Colombia, 2012-2014

Year	Quarter	Samples collected		Positive findings
		n	%	
2012	third	36	1.7	-
	fourth	226	10.9	-
2013	first	139	6.7	-
	second	190	9.2	-
	third	202	9.8	-
	fourth	665	32.1	+
2014	first	156	7.6	-
	second	0	0.0	-
	third	330	16.0	-
	fourth	124	6.0	-
Total		2,068	100.0	

TABLE 3 - Distribution of 40 isolates from environmental sampling according to place, type, and number of trees sampled in Barranquilla, Colombia during October 2013 (fourth quarter).

Place of sampling	Type of tree	Positive trees/ number of trees sampled	Number of colonies recovered
Venezuela Park	Almond	3/17	10
Venezuela Park	Pink trumpet	2/11	10
José Martí Park	Pink trumpet	1/11	5
Villa Santos Boulevard	Almond	3/17	15
Total		9/56	40

TABLE 4 - Weather variables detected during environmental sampling for *Cryptococcus neoformans*/*Cryptococcus gattii* in Barranquilla, Colombia, 2012-2014*.

Weather variables	Years												Average
	2012				2013				2014				
	quarters				quarters				quarters				
	1	2	3	4	1	2	3	4	1	2	3	4	
Mean temperature (°C)	26.66	28.30	28.67	28.30	27.53	28.46	28.13	27.80	27.00	28.63	28.63	27.90	28.00
Max temperature (°C)	31.03	33.60	34.07	33.13	32.53	34.43	34.10	33.10	32.37	34.40	34.60	33.40	33.40
Min, temperature (°C)	23.80	25.00	24.87	25.07	24.47	25.13	24.23	24.43	24.07	25.40	25.30	24.77	24.71
Rainfall (mm)	0.00	63.34	52.49	38.35	0.00	60.45	103.63	32.68	0.00	30.28	102.87	92.03	48.01
Relative humidity (%)	77.30	75.57	82.53	80.93	75.97	78.50	80.87	83.96	79.30	63.74	82.13	86.33	78.93
Days of rain	0	32	36	27	1	29	34	27	0	11	34	30	21.75

*Adapted from IDEAM data (www.ideam.gov.co). Highlighted numbers represent the quarter in which we observed positive findings.

in Colombia, the possibility of under registration of clinical cases cannot be ruled out. Accordingly, we propose that surveillance of cryptococcosis within Colombia should be improved through the application of a national survey and studies of clinical *Cryptococcus* isolates.

Data from the *Sistema Nacional de Vigilancia en Salud Pública* (SIVIGILA) indicate that the incidence of HIV/AIDS has been increasing in Colombia since 2007. In 2012, data from this reporting system showed that the district of Barranquilla had a high incidence of HIV/AIDS (33.7×10^5 individuals), which was more than twice the national indicator (16.4×10^5 individuals). Similarly, the incidence of HIV/AIDS reported in Atlántico (19.4×10^5 individuals) was also higher than the national average. Therefore, based on these epidemiologic reports, the number of cases of cryptococcosis in these regions should be higher, and many cases may not have been reported. These data also suggest that there is a population at risk that could be suffering from an opportunistic disease, such as cryptococcosis, or be more prone to acquiring this disease. Data from the World Health Organization showed that only 34% of patients with HIV in Colombia were receiving ART as of December 2010⁽²³⁾ ⁽²⁴⁾. Thus, the untreated seropositive population may be at higher risk of developing an opportunist infection, such as cryptococcosis. These numbers are consistent

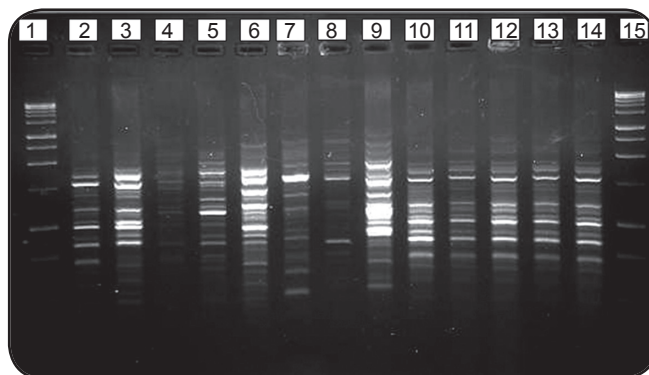


FIGURE 1 - PCR fingerprinting using (GTG)₅ primer of environmental isolates of *Cryptococcus neoformans* var. *grubii* recovered in Atlántico, Colombia. 1. Molecular Weight Marker 1kb; 2. VNI: control strain; 3. VNII: control strain; 4. VNIII: control strain; 5. VNIV: control strain; 6. VGI: control strain; 7. VGII: control strain; 8. VGIII: control strain; 9. VGIV: control strain; 10-14: environmental isolates, molecular type VNI; 15. Molecular Weight Marker 1kb. PCR: polymerase chain reaction.

with those found in a study in Colombia between 1996 and 2010, where only 23.8% of patients with AIDS were receiving ART⁽³⁾.

In our study, all infected patients were adults. This is consistent with previous reports in which the majority of cases

of cryptococcosis have been reported to occur in adults, with the exception of cases in the Northern part of Brazil, where mycosis is frequently observed in younger individuals⁽²⁾⁽³⁾⁽¹¹⁾⁽¹²⁾⁽²⁵⁾⁽²⁶⁾. The high prevalence of cryptococcosis in patients with HIV in the current study is also consistent with previous reports in which HIV/AIDS, transplantation, and other immunodeficiencies have been shown to be the main risk factors for cryptococcosis⁽¹⁾⁽²⁾⁽³⁾⁽⁴⁾. Finally, our findings that most infected individuals were men are also consistent with other studies, including some studies of populations in Colombia⁽³⁾⁽¹¹⁾⁽¹²⁾.

In this study, the diagnosis of cryptococcosis was possible from CSF in the majority of cases, and most isolates were *C. neoformans* molecular type VNI. These significant findings provide insights into the epidemiology of this disease in the Colombian population and are consistent with the predominant fungal tropism for the central nervous system, similar to results reported by other investigators⁽²⁾⁽³⁾⁽¹¹⁾⁽¹²⁾. However, one of the isolates obtained from blood culture was identified as *C. gattii*, VGI. This result is consistent with other findings showing the reduced clinical frequency of this species worldwide and the low prevalence of the molecular type VGI in South America⁽¹⁾. Our findings are also similar to the results of the National Surveillance Program of Cryptococcosis in Colombia from 1997 to 2010⁽¹¹⁾⁽¹²⁾. Furthermore, in a study by Meyer et al.⁽⁵⁾ in 2003, the frequency of this molecular type was found to be low in Colombian isolates⁽⁵⁾. However, the observation that cryptococcosis caused by *C. gattii* is associated predominantly with hosts having normal immunity may no longer be true, as supported by the identification of underlying conditions such as HIV infection and cancer of solid organs as risk factors for cryptococcosis⁽²⁷⁾. For the patient in our study with *C. gattii* infection, we did not have information available concerning the patient's travel history to geographical areas in which *C. gattii* has been identified; this information may be useful for identification of the possible source of infection.

Basidiomycetous yeasts of the genera *Cryptococcus* have already been isolated from decaying wood, bark, and tree hollows of more than 36 arboreal species⁽⁸⁾⁽⁹⁾⁽¹³⁾⁽¹⁴⁾⁽¹⁵⁾⁽¹⁶⁾⁽²⁸⁾⁽²⁹⁾⁽³⁰⁾⁽³¹⁾⁽³²⁾. The isolation of a significant number of colonies both from Almond and Pink trumpet trees is consistent with these findings. Further studies are needed to better establish the association between the presence of *Cryptococcus* in tree species and infection in humans in Colombia.

VNI isolates were identified in both clinical and environmental isolates, suggesting an association between the environmental exposure to the fungus and the acquisition of the disease. This association is being studied by our group using more discriminative molecular assays, such as multilocus sequence typing (MLST) of seven consensus genes, allowing us to determine the specific associations between clinical and environmental isolates in our country. Previous studies have identified the molecular association between clinical and environmental isolates in Colombian strains, supporting previous studies showing that cryptococcosis can be acquired by exposure

to environmental sources⁽¹³⁾. Our findings confirmed that VNI was the major molecular type circulating among patients in Colombia and that this molecular type was found with mating type α , which is considered more virulent than mating type a and is not very frequently reported for isolates of *C. neoformans* var. *grubii*.

In this study, we analyzed the data from samples collected under different climate conditions. Notably, the mean temperature and relative humidity were similar for all years during the environmental sampling period. In the geographical area examined in this study, the rainy season is usually characterized by periods of intense precipitation. Moreover, rainfall during the fourth quarter (the rainy season in Barranquilla) in both 2012 and 2013 (38.35 and 32.68mm, respectively) was lower than that during the same quarter in 2014 (92.03mm). Despite this, the number of samples collected during the fourth quarter of 2014 was low (6%), which may have affected our results (**Table 2**). Furthermore, the occurrence of the El Niño weather pattern in 2012 may have had a unique impact on rainfall and spread of *Cryptococcus* species in the Colombian Caribbean Region. During this period, the IDEAM reported only 52.5mm of rainfall for the third quarter of 2012 for Barranquilla, which was only half of that reported during the same period in 2013 and 2014 (103.6 and 102.9mm, respectively)⁽¹⁹⁾; these weather patterns may have affected the results of this study, particularly considering that 10.9% of all samples were collected and processed during this last quarter of 2012. Precipitation may have favored the recovery of *C. neoformans* in the last quarters of 2013, consistent with reports by Granados et al., who showed higher recovery of the fungus during periods of increased rainfall⁽³³⁾. Additionally, the increased number of samples (32.1%) collected during the fourth quarter of 2013 support the possibility that the weather patterns may have increased the opportunity to isolate the fungus.

To the best of our knowledge, this paper is the first attempt to determine the epidemiology of cryptococcosis in this part of Colombia, where no data were previously available. Our results showed that the prevalence of *C. neoformans* was higher than that of *C. gattii* from both clinical and environmental sources. The identification of this fungus in this region in Colombia in Almond trees, and particularly in Pink Trumpet trees, suggests the need for further studies focused on the ability of both causative species to colonize different niches considering variations in meteorological conditions in order to provide stronger tools for the development of an ecological niche model in Colombia.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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