

Short Communication

Prevalence of cryptococcosis in Atlántico, department of Colombia assessed with an active epidemiological search

María Clara Noguera^[1], Patricia Escandón^[2], Merle Arévalo^[1],
Yina García^[1], Luz Estela Suárez^[3] and Elizabeth Castañeda^[2]

[1]. Grupo Caribe de Enfermedades Infecciosas, Universidad Metropolitana, Barranquilla, Atlántico, Colombia.

[2]. Grupo Microbiología, Instituto Nacional de Salud, Bogotá, Colombia.

[3]. Programa Maestría en Microbiología, Universidad Metropolitana, Barranquilla, Atlántico, Colombia.

Abstract

Introduction: Cryptococcosis is the second most frequent cause of opportunistic infections in human immunodeficiency virus (HIV) patients in Colombia. We aimed to determine the prevalence of cryptococcosis in the Colombian department of Atlántico. **Methods:** An active search for cryptococcosis cases was conducted between 2015 and 2017 in health institutions by distributing surveys to clinicians and characterizing samples phenotypically and genotypically. **Results:** Thirty-eight cryptococcosis cases were identified (81.6% men, 76.3% HIV patients). The calculated annual prevalence was 5.08/1 million inhabitants. *Cryptococcus neoformans* var. *grubii* VNI was isolated in 34 cases. **Conclusions:** These results provide the basis for passive surveillance of cryptococcosis.

Keywords: Cryptococcosis. *Cryptococcus neoformans*. *Cryptococcus gattii*. Colombia. Active epidemiological search. Annual prevalence.

Cryptococcosis is a life-threatening fungal disease that affects humans and other vertebrates. Cases are reported worldwide every year, particularly among patients with human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS). Nevertheless, the greatest burden of this mycosis is identified in Sub-Saharan Africa with an estimated mortality of 50%-70%. The main etiological agents are 2 basidiomycetous yeasts, *Cryptococcus neoformans* and *Cryptococcus gattii*. *C. neoformans* has the highest global prevalence and is further classified into the varieties *C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans*. *C. gattii* is clinically more virulent and considered a deadly pathogen¹. Both species can be differentiated into serotypes (A and D for *C. neoformans*, B and C for *C. gattii*), hybrids (AD, AB, and BD), and molecular types (VNI, VNII, VNIII, VNIV,

VNB, VGI, VGII, VGIII, and VGIV)¹. A new taxonomy of the *C. neoformans* and *C. gattii* species complexes has recently been proposed, but the use of “*C. neoformans* species complex” and “*C. gattii* species complex” seems to be the most appropriate and least confusing reference until a consensus is achieved^{2,3}.

Although notification of the national public health surveillance system in Colombia is not mandatory for cryptococcosis, a national survey designed in accordance with the guidelines of the European Confederation of Mycology has been carried out voluntarily within medical institutions since 1997⁴. According to a report by Escandón et al., the average annual incidence rate of cryptococcosis in the general Colombian population was 0.23 per 100,000 inhabitants (1997-2016), while in AIDS patients, the rate rose to 1.1 per 1000 inhabitants. *C. neoformans* var. *grubii* was more prevalent (87.5%) than *C. gattii* (3.1%); VNI was the most prevalent (96.1%) molecular type, while VGII predominated in *C. gattii* isolates (54.3%)⁴. Previous data based on voluntary notifications indicated 41 cases of cryptococcosis reported between 1997 and 2014 in the Atlántico department of Colombia, with an annual average prevalence of 1.04 per 1 million inhabitants⁵.

Corresponding author: Dra. María Clara Noguera.
e-mail: bacteriologos@unimetro.edu.co / maclarnog@gmail.com
Orcid: 0000-0002-6761-9110
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This study aimed to develop an active epidemiological search for cryptococcosis cases in Atlántico, department of Colombia with participation of health professionals to obtain accurate prevalence data of this mycosis.

This observational descriptive study was conducted for 30 months between January 2015 and June 2017 in the department of Atlántico, a geographical region in the Northern part of Colombia. Epidemiological and clinical data were recorded by clinicians at public and private institutions as part of the voluntary national survey approved by the Ethics Committee of the Instituto Nacional de Salud (INS, Act No. 2/2012).

An epidemiological search for new cryptococcosis cases was carried out by previous sensitization and periodic communication with clinicians in different health facilities. A survey was completed for every case of cryptococcosis diagnosed at participating public or private institutions. Each survey was submitted with a corresponding isolate/sample to the Universidad Metropolitana in Barranquilla for confirmation using conventional techniques. Species differentiation was performed by culturing samples/isolates in canavanine glycine bromothymol blue (CGB) agar^{4,5}. Surveys and samples/isolates were finally submitted to the INS for confirmation and molecular typing. The cryptococcal antigen latex agglutination system (CALAS, Meridian Bioscience, Ohio, USA) was used in cases where cerebrospinal fluid (CSF) or serum samples were provided.

Molecular typing was performed according to the protocol described by Meyer et al. in 2003⁶. The isolates were maintained in sterile distilled water at room temperature and in glycerol at -70°C.

A database was created at the national study center using BioloMICS® Bio-Aware (Hannut, Belgium) for the collection and surveillance of all national data. Analysis of the information was performed using the statistical program Epi Info™ version 6.1 Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA).

Thirty-eight cases of cryptococcosis were reported for an annual average prevalence of 5.08 cases in 1 million inhabitants. Thirty-one (81.6%) cases were adult men (median age: 35.4 years, range: 10-58 years). Twenty-nine (76.3%) cases were HIV-positive patients and the mycosis was the AIDS-defining condition in 5 of these patients (17.2%). One case each (11.1%) was identified among the remaining cases with the following conditions: solid tumor, autoimmune disease, diabetes, hematologic malignancy, and immune disorder. In 3 cases (33.3%) the underlying risk factor remained unknown, and for 1 case (11.1%) this part of the survey was not completed. There were 9 fatalities (23.7%).

Isolation of fungi was successful in 37 cases (97.4%), with most of the isolates obtained from CSF (31 cases) and some by blood culture (6 cases). *C. neoformans* var. *grubii* molecular type VNI was identified in 34 cases (91.9%), whereas *C. gattii* molecular types VGI and VGII were confirmed in 1 HIV-positive male patient (2.7%) who died and in two HIV-negative patients (5.4%), respectively. Of the latter cases, one was a boy who died at the hospital without further immunological studies performed; the other was a man with a laboratory-confirmed low cluster of differentiation 4 (CD4) count (284 cells/μL) who received

treatment with amphotericin B (50 mg/day) and fluconazole (400 mg/day) and was discharged from the hospital with visual and motor sequelae. Only 1 (2.6%) case of cryptococcosis was identified by qualitative latex agglutination of the CSF sample because the culture was unsuccessful.

Headaches were the most prevalent symptom reported in 26 cases (68.4%), followed by fever in 23 (61.0%), confusion in 19 (50.0%), coughing in 11 (29.0%), seizures in 7 (18.4%), and meningeal signs in 5 cases (13.1%). Only 1 patient (2.6%), who was HIV-positive, had a relapse during the study period 6 months after his initial diagnosis and *C. neoformans* var. *grubii* molecular type VNI was identified again. He received inpatient treatment with amphotericin B (50 mg/day) and fluconazole (400 mg/day) and was discharged.

Of the 10 patients requiring inpatient treatment, 4 received monotherapy with amphotericin B and 3 were treated with amphotericin B and 5-fluorocytosine. These 7 patients were discharged. One patient who received amphotericin B and fluconazole died. The remaining 2 inpatients received monotherapy with fluconazole; one of these patients died, the other was discharged.

Cryptococcosis is a life-threatening fungal disease and thousands of individuals are diagnosed every year. The prevalence among HIV/AIDS patients is particularly high, and this mycosis is responsible for 15% of AIDS-related deaths globally^{7,8}. *C. neoformans* var. *grubii* is the predominant causative variant with the highest incidence reported in Sub-Saharan Africa. *C. gattii* is less prevalent worldwide but endemic in the province of British Columbia, Canada and in the Northern and Western territories of Australia, where repeated outbreaks have led to obligatory reporting of new cases to the national surveillance systems. In many regions of the world, cryptococcosis remains a neglected disease, which probably leads to underreporting of cases^{1,9}.

According to a study by Rajasingham et al. that assessed global HIV cases reported until December 2013, 31.8 million adults were living with HIV, with 21.7 million of these cases in Sub-Saharan Africa⁸. The authors estimated that there are 223,100 annual cases of cryptococcal meningitis globally, with most of these cases occurring in Sub-Saharan Africa (73%, 162,500), followed by Asia and the Pacific (19%, 43,200 cases), and Latin America (2.4%, 5,300 cases)⁸.

The national public health surveillance system in Colombia (Sistema de Vigilancia en Salud Pública/SIVIGILA) indicates that the incidence of HIV/AIDS has been increasing in the country since 2007. Data obtained by SIVIGILA in 2012 showed that the district of Barranquilla (capital city of the department of Atlántico) had a high HIV/AIDS prevalence of 33.7 cases per 100,000 inhabitants, which was more than twice the national average (16.4 per 100,000 inhabitants). Similarly, the incidence of HIV/AIDS reported for the entire department of Atlántico (19.4 per 100,000 inhabitants) was also higher than the national average¹⁰. According to these epidemiological reports, the number of cases of cryptococcosis in Barranquilla and Atlántico should be higher than the observed in our study and many cases may not have been reported. Data from the World Health

Organization (WHO) indicate that only 34% of patients with HIV in Colombia were receiving anti-retroviral therapy (ART) in December 2010¹¹. Thus, the untreated seropositive population may be at a higher risk of developing an opportunistic disease such as cryptococcosis.

Notification of cryptococcosis cases is not compulsory in many countries of the world. Thus, underreporting of cases leads to misinformation and distortion of the real magnitude of this pathology. Different reports have confirmed the importance of this mycosis in Colombia^{4,5,12-14}. A strategy of improving the surveillance for cryptococcosis through an active search was implemented in this study with the participation of trained clinical staff in hospitals and health institutions. Improved surveillance led to the identification of 38 cases in 30 months, which represents a higher than average annual prevalence. Most cases reported herein were adult males with predominant compromise of the CNS and with compromise of the blood in some cases. The prevalence of *C. neoformans* and *C. gattii* detected in this study is in accord with previous studies. Predominant symptoms were headache, fever, and confusion, followed by coughing, seizures, and meningeal signs, which were also reported in previous studies^{4,5,7,12,13}.

One male patient from whom *C. gattii* VGII was isolated survived with neurological sequelae and low CD4 counts. It is known that cryptococcal meningitis poses a significant problem for patients with defects in cell-mediated immunity because it causes high rates of mortality despite therapy in this population. Idiopathic CD4 lymphopenia (a disease with unknown cause) is detected in 27% of reported cryptococcosis cases and considered a predominant predisposing genetic factor for this mycosis in apparently "normal hosts"⁷.

In conclusion, we identified 38 cases of cryptococcosis in 30 months in the department of Atlántico. Compared with the previous study reporting 41 cases in 18 years, these results indicate that the average annual prevalence was almost 5-fold higher than previous estimates and this active search significantly improved the registry of cases in this region. These results form the basis for a functional strategy for the national surveillance program to implement passive surveillance for cryptococcosis. Of 202 national surveys forwarded voluntarily to the national surveillance program during the study period, 19% were from the department of Atlántico, indicating an increasing commitment of this department to cryptococcosis surveillance compared to a proportion of only 2.2% of all notifications during the previous 18 years. Moreover, given the global recognition of cryptococcosis as a sentinel disease of AIDS, these new findings indicate a need to modify the surveillance in Colombia from a passive to an active system, particularly regarding factors that still contribute to high mortality rates.

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