



# Varieties and serotypes of *Cryptococcus neoformans* clinical isolates in Colombia

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**Summary** The aim of this study was to contribute to the knowledge of the geographic distribution of *Cryptococcus neoformans* varieties, in Colombian patients, and to determine the mating types of such varieties. A total of 370 clinical isolates were studied. *C. neoformans* var. *neoformans* was identified in 95.2% of them. *C. neoformans* var. *gattii* in 4.8%, 4.5% being serotype B and 0.3%, serotype C. Fifty-five of the 74 (74%) isolates studied were mating type "α", all of them *C. neoformans* var. *neoformans*. Serotype C had been previously reported only once in South American countries.

**Key words** *Cryptococcus neoformans* var. *neoformans*, *C. neoformans* var. *gattii*, Cryptococcosis

## Variedades y serotipos de aislamientos clínicos de *Cryptococcus neoformans* en Colombia

**Resumen** El objetivo de éste trabajo fue el de contribuir al conocimiento de la distribución geográfica de las variedades de *Cryptococcus neoformans* en pacientes colombianos y determinar el tipo de pareja sexual que presentan estas variedades. Se estudiaron 370 aislamientos clínicos. *C. neoformans* var. *neoformans* se determinó en 95,2% de ellos, *C. neoformans* var. *gattii* en 4,8%, el serotipo B con una frecuencia de 4,5% y el C con 0,3%. Cincuenta y cinco de los 74 (74%) aislamientos estudiados fueron pareja "α", todos ellos *C. neoformans* var. *neoformans*. El serotipo C había sido previamente informado solamente una vez en América del Sur.

**Palabras clave** *Cryptococcus neoformans* var. *neoformans*, *C. neoformans* var. *gattii*, Criptococosis

Cryptococcosis is a worldwide opportunistic mycosis whose etiologic agent, *Cryptococcus neoformans*, is an encapsulated basidiomycetous yeast that has been classified into two varieties and five serotypes: *C. neoformans* var. *neoformans* (serotypes A, D and AD) and *C. neoformans* var. *gattii* (serotypes B and C) [1,2]. In 1999, Franzot *et al.*, based on molecular studies, proposed a separate variety for serotype A as *C. neoformans* var. *grubii* [3]; however the new variety is not completely accepted. The two or three varieties of *C. neoformans* isolated from patients differ in certain aspects, among which, geographic distribution is mentioned frequently [4].

Kwon-Chung showed that *C. neoformans* has two basidiomycetous teleomorphs, *Filobasidiella neoformans* var. *neoformans* and *F. neoformans* var. *bacillispora*; the mating type of each one can be "α" or "a" [5,6]. Alpha isolates are the most frequent mating types reported among clinical isolates [6].

The aim of this study was to determine the geographic distribution of the two *C. neoformans* varieties and serotypes in Colombian clinical cases and, additionally, to determine the mating types of such varieties.

Three hundred and seventy *C. neoformans* clinical isolates were recovered from cryptococcosis cases diagnosed or confirmed between 1972 and 1996 in the Microbiology Laboratory at the Instituto Nacional de Salud. For each isolate, information was obtained about collection date, demographic data, serological test results for the human immunodeficiency virus (HIV) infection and geographic origin.

Control strains were supplied by Dr. KJ Kwon-Chung from the National Institute of Allergy and Infectious Diseases, NIH, Bethesda. There were *C. neoformans* NIH 68, NIH 52, NIH 112 serotype B and NIH 18 serotype C, *F. neoformans* D3501 (α), D3502 (a) and *F. bacillispora* NIH 444 (α), NIH 191 (a).

Each isolate was identified by standardized methods [7-10]. The varieties by culture were done in canavanine-glycine-bromothymol blue agar (CGB) [11]

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and the serotype by slide agglutination test with specific rabbit antisera obtained from intravenous injection with formalin-killed yeast cells of each serotype, according to Kwon-Chung's protocol (personal communication). The antisera were first adsorbed with heterologous serotype cells and titrated with control strains [12]. Additionally, all *C. neoformans* var. *gattii* serotypes, and the isolate serotype D, were confirmed using the Cryptocheck kit (Iatron, Lab, Tokio, Japan). The mating test was determined in sucrose biotin agar medium (SBA) [6].

*C. neoformans* clinical isolates were obtained from cerebrospinal fluid (CSF) (94.0%) urine (1.9%), blood (1.4%), pus (1.4%), bronchoalveolar lavage (0.8%), bone marrow (0.3%) and sputum (0.3%). Meningitis was the most common clinical manifestation of cryptococcosis in Colombia similar to the cases reported from around the world, especially for the HIV positive group [13-17].

The most frequent variety diagnosed was *C. neoformans* var. *neoformans* with 352 (95.2%) cases (351 serotype A and 1 serotype D), followed by 18 (4.8%) *C. neoformans* var. *gattii* (17 serotype B, and 1 C). The proportion of varieties was similar to those reported from Argentina, Brazil [16,18,19] and North America, excluding South California [4], except for the *C. neoformans* var. *gattii* serotype C, which had been previously reported only once in the area [20]. In contrast, these results were different from figures published in Venezuela and in Mexico [21,22].

*C. neoformans* var. *neoformans* was recovered in all years studied, but *C. neoformans* var. *gattii* was not isolated in some years. The reason for this behavior is not clear at present. However, these results could reflect changes in factors which may affect the fungus habitat [23].

Two hundred and eleven (57%) patients were HIV(+), 52 (14%) HIV(-) and in 107 (29%) no data was available (Table 1). *C. neoformans* var. *neoformans* isolates increased four-fold between 1980 and 1990, mostly due to the HIV infected patients with cryptococcosis who were diagnosed during the same period. Similar observations have been published around the world [13,24].

Demographic characteristics of the 370 patients established that 291 (78.6%) were male and 72 (19.5%) female, in 7 (1.9%) no data was recorded. The distribution by age group in 239 patients showed 21 (8.8%) between 1 and 19 years, 144 (60.3%) between 20 and 39 years, 53 (22.2%) between 40 and 59, and 21 (8.8%) between 60 and 79 years. *C. neoformans* var. *neoformans* was diagnosed more frequently in adult males (79.5%), 20-39 years old (40%), 59.4% being AIDS patients, out of whom 21 (10%) were females. *C. neoformans* var. *gattii* was diagnosed mostly in males (61%), aged 40-59 (41%), in whom no immunodeficiency was reported.

**Table 1.** Distribution of *Cryptococcus neoformans* by HIV results and varieties.

Years	HIV (+) / Total	<i>C. neoformans</i>	
		var. <i>neoformans</i>	var. <i>gattii</i>
1972 - 1979	0 / 3	3	0
1980 - 1985	0 / 18	18	0
1986	0 / 8	6	2
1987	0 / 7	7	0
1988	5 / 17	16	1
1989	10 / 22	18	4
1990	8 / 25	22	3
1991	19 / 34	32	2
1992	16 / 29	29	0
1993	21 / 31	28	3
1994	41 / 58	56	2
1995	41 / 55	54	1
1996	50 / 63	63	0
Total	211 / 370	352	18

Regarding geographical distribution, *C. neoformans* var. *neoformans*, serotype A was diagnosed in 20 of the 33 (61%) departments in the country, mainly in the central Andean region, while serotype D in one patient from Cundinamarca, a central department. In contrast, *C. neoformans* var. *gattii*, serotype B, was isolated in only seven departments. Furthermore, most (61.1%) of the *C. neoformans* var. *gattii* isolates came from a single northeastern department, to be more precise from Cúcuta city, where our laboratory has been searching for *C. neoformans* var. *gattii* habitat for various years. Serotype C was recovered from one patient from Arauca, an Eastern region department. Recent findings of our group proved almond trees in Cúcuta city were positive for *C. neoformans* var. *gattii*, serotype C [25].

Fifty-five of the 74 (74%) *C. neoformans* var. *neoformans* isolates under study were mating type "α", whereas sexual mating was unsuccessful in the other 19 and also in the seven *C. neoformans* var. *gattii* isolates studied. Similar to other countries, the mating type "α" was the most prevalent among clinical isolates [6,26]. Sexual mating was unsuccessful for all the *C. neoformans* var. *gattii* isolates studied. The reason for these results is not clear; however, the number of isolates studied was not significant as to show definite results.

More studies about the natural habitat of *C. neoformans* are required and some are currently in progress for a better understanding of the geographical distribution of varieties and serotypes of this pathogen in clinical Colombian isolates.

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