

## Frequency of *Cryptococcus* Species and Varieties in México and their Comparison with some Latin American Countries

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**ABSTRACT.** Cryptococcosis frequency is increasing in Mexico. Few epidemiologic and clinical surveys exist even though 10-15% of patients with AIDS develop this mycosis. To know the situation about the prevalence and etiological agents of cryptococcosis in our country, a study including isolated *Cryptococcus* spp. strains took place from 1989 to August 1998. A total of 211 *Cryptococcus* spp. strains obtained from patients of diverse hospital centers in Mexico City were studied. This study describes cryptococcosis epidemiological data, in our country. *C. neoformans* was the dominant isolation (97.15%), followed by two *C. albidus* isolations and one *C. uniguttulatus* isolation. 92.5% of the strains was isolated from cerebral spinal fluid and the remaining were from bone marrow, blood, ganglion, sputum, bronchial lavage and liver biopsy. AIDS was the principal opportunistic factor (85%). Strains were found prevails in males (87.3%). It was found that the age groups where the disease appears most frequently are in the fourth (37.5%) and third (33.8%) decade of life. Finally, the isolation frequency of the *C. neoformans* var. *neoformans* (vn) and var. *gattii* (vg) obtained in this study (vn 86.7% and vg 10.4%) was compared with the ones registered in Argentina (vn 97.6%, vg 2.4%); Brazil (vn 83.3%, vg 15.8%); Cuba (vn 97.6%, vg 2.4%); Paraguay (vn 0%, vg 100.0%); Puerto Rico (vn 100.0%, vg 0%) and Venezuela (vn 66.7%, vg 29.6%).

**Key words:** *Cryptococcus*, Epidemiology.

**RESUMEN.** La criptococosis está incrementando su frecuencia en México. Pocos estudios epidemiológicos y clínicos existen aún cuando entre el 10-15% de los pacientes con SIDA, desarrollan esta micosis. Para conocer la prevalencia y los agentes etiológicos de la criptococosis en nuestro país, se efectuó un estudio que incluyó cepas de *Cryptococcus* spp. aisladas desde 1989 hasta agosto de 1998. Un total de 211 cepas de *Cryptococcus* spp. obtenidas de pacientes atendidos en diversos centros hospitalarios ubicados en la Ciudad de México fueron estudiadas. *C. neoformans* fue el agente dominante (97.15%), seguido de *C. albidus* y *C. uniguttulatus*. El 92.5% de las cepas se aislaron a partir de líquido cefalorraquídeo y las restantes provinieron de médula ósea, sangre, ganglio, esputo, lavado broncoalveolar y biopsia de hígado. El SIDA se describió como el principal factor de oportunismo. Se encontró que la enfermedad predomina en el sexo masculino (87.3%) y en personas entre la tercera (33.8%) y cuarta (37.5%) décadas de la vida. Finalmente, la frecuencia de aislamiento de *C. neoformans* var. *neoformans* (vn) y var. *gattii* (vg) obtenida (vn 86.7% y vg 10.4%), fue comparada con la reportada en: Argentina (vn 97.6%, vg 2.4%); Brasil (vn 83.3%, vg 15.8%); Cuba (vn 97.6%, vg 2.4%); Paraguay (vn 0%, vg 100.0%); Puerto Rico (vn 100.0%, vg 0%) y Venezuela (vn 66.7%, vg 29.6%).

**Palabras clave:** *Cryptococcus*, Epidemiología

### INTRODUCCIÓN

Cryptococcosis is a systemic disease caused by *Cryptococcus neoformans* encapsulated yeast; other *Cryptococcus* species like *C. albidus*<sup>17,31</sup> y *C. laurentii*<sup>17</sup> are oddly pathogenic in humans. The increasing incidence of cryptococcosis is caused by the immune-commitment of T cells and AIDS.<sup>1</sup> There are several works that have published data referring to the prevalence of the cryptococcosis mainly in

U.S.A, Australia, Japan and diverse European countries,<sup>18</sup> nevertheless, with the exception of Brazil, few reports have presented the disease condition in Latin America. The present study was carried out retrospectively to determine the identification, and the prevalence of the species and varieties of *Cryptococcus* in isolates of patients from different hospital centers of Mexico City.

Also, the data registered in this one and other Mexican surveys,<sup>7,14,20</sup> were compared with the statistics published

Table 1. Hospitals from which *Cryptococcus* spp. Strains were obtained.

Hospital	No. of strain
Centro Médico Nacional La Raza, IMSS	114
Instituto Nacional de Neurología y Neurocirugía, SS	65
Centro Médico Nacional Siglo XXI, IMSS	14
Hospital Juárez, SS	4
Hospital Ignacio Zaragoza, ISSSTE	6
Instituto Nacional de Enfermedades Respiratorias, SS	3
Instituto Nacional de Pediatría, SS	2
Other Hospitals	3
<b>Total</b>	<b>211</b>

in Argentina,<sup>2-5</sup> Brazil,<sup>6, 9, 19, 21, 23, 24, 26, 27</sup> Cuba,<sup>9-11</sup> Paraguay,<sup>18</sup> Puerto Rico<sup>13</sup> and Venezuela<sup>30</sup> with the purpose of appraising the incidence of the cryptococcosis etiological agents in these Latin American countries.

## MATERIALS AND METHODS

*Cryptococcus* sp. strains were provided by diverse hospital centers located in Mexico City (Table 1). Each one of the obtained strains were identified by means of a routine's etiological diagnosis examinations: examination in fresh with India ink, a culture media of agar dextrose Sabouraud at 30 and 37 °C, urease test, phenoloxidase test, carbon auxanogram by VITEK system and a culture media of glycine-canavanine-bromothymol blue.

Only some strains were identified by the serotype to which they belonged and this was confirmed by the Pasteur Institute of Paris, France.

Only some patients from whom the isolated were obtained, the following epidemiological data were registered: name, gender, age, occupation, place of residence, origin place, pathological product obtained and underlying disease.

## RESULTS

A total of 211 *Cryptococcus* strains were studied. It was found that *C. neoformans* was the dominant isolation (97.15%), followed by two *C. albidus* and, one *C. uniguttulatus*; three *Cryptococcus* species could not be determined (Table 2). In spite of its low frequency, the finding

Table 2. Frequency of *Cryptococcus* species and varieties isolated from mexican patients with cryptococcosis.

Etiological Agent	No. of Strains (%)
<i>Cryptococcus neoformans</i> var. <i>neoformans</i>	183 (86.73)
<i>Cryptococcus neoformans</i> var. <i>gattii</i>	22 (10.42)
<i>Cryptococcus albidus</i>	2 (0.94)
<i>Cryptococcus uniguttulatus</i>	1 (0.50)
<i>Cryptococcus</i> sp.	3 (0.42)
<b>Total</b>	<b>211 (100.00)</b>

of species different from *C. neoformans*, epidemiologically calls the attention, since diverse existing works<sup>17,31</sup> report the isolation of *C. albidus* or *C. laurentii* as agents who, in an relevant way, attempt against the life of patients.

Regarding to strains pertaining to var. *neoformans*, only three serotypes could be identified (two serotype A and one serotype D); concerning to var. *gattii*, only one strain was serotype C, the others turned out serotype B. The presence of serotypes C and D is surprising, since a great majority of serotype C cases come from the south of California (U.S.A.) and the serotype D is predominant in the European continent.<sup>18</sup>

Regarding to pathological products from which strains were isolated: 186 were isolated from cerebral spinal fluid (CSF), seven from bone marrow, four from blood, one from ganglion, one from sputum, one from bronchial lavage and one from a liver biopsy (Table 3). In three patients, *C. neoformans* was isolated from two different sites: C.S.F. and bone marrow (in two) and C.S.F. and blood (in one). In the three cases, identification test were made to discard that, in each patient, the strains were different; when confirming (until serotype) that it was the same strain, only the corresponding to C.S.F. were entered in this study.

As far as the investigated predisposition factors: 110 of them suffered from AIDS, 18 patients apparently with no factors and seven with some immunosuppression factors different from AIDS (Table 4). These results confirm that *C. neoformans*, is an opportunistic pathogen frequently found in AIDS patients, an association that becomes narrower taking into account that the systemic infection is combined with a production of capsular polisaccharides specific of *C. neoformans*, inhibitors of phagocytosis and supposedly responsible for the acute course of cryptococcosis in AIDS.<sup>1</sup> However, regardless of AIDS patients, 17 out of 25 (68%) belonged to var. *gattii*, and only the remaining 32% were var. *neoformans*. These data, possibly it would reflect the Mexico situation before the AIDS era.

In relation to the distribution by gender, and based on

Table 3. Pathological products from which 201 *Cryptococcus* spp. Strains were obtained.

Etiological agent	No. of strains	Sputum	Ganglion	Liver	Bronchial Lavage	Cerebral Spinal Fluid	Bone Marrow	Blood
<i>C. albidus</i>	1	-	-	1	-	-	-	-
<i>C. neoformans</i> var. <i>gattii</i>	22	-	-	-	1	21	-	-
<i>C. neoformans</i> var. <i>neoformans</i>	175	1	1	-	-	162	7	4
<i>C. uniguttulatus</i>	1	-	-	-	-	1	-	-
<i>Cryptococcus</i> sp	2	-	-	-	-	2	-	-
Total	201	1	1	1	1	186	7	4

Table 4. Predisposed factors found in 135 patients with cryptococcosis.\*

Etiological agent	No. of strains	Opportunistic factors		
		None	AIDS	Non AIDS
<i>C. albidus</i>	1	-	1	-
<i>C. neoformans</i> var. <i>gattii</i>	18	15	1	2
<i>C. neoformans</i> var. <i>neoformans</i>	114	3	106	5
<i>C. uniguttulatus</i>	1	-	1	-
<i>Cryptococcus</i> sp.	1	-	1	-
Total	135	18	110	7

\* Data obtained by authors

Table 5. Distribution by gender in 166 patients with cryptococcosis.

Etiological agent	No. of strains	Gender	
		Female	Male
<i>C. albidus</i>	2	1	1
<i>C. neoformans</i> var. <i>gattii</i>	20	9	11
<i>C. neoformans</i> var. <i>neoformans</i>	140	11	129
<i>C. uniguttulatus</i>	1	-	1
<i>Cryptococcus</i> sp.	3	-	3
Total	166	21	145

\* Data obtained by authors

the studied patients, it was found that disease prevails in males in an approximated ratio of 7:1 (Table 5). In relation to the registered age, it was found that the age groups where the disease appears most frequently are in the third, fourth and fifth decade of life (Table 6). Of two isolated

strains from children, it attracts the attention that one of them belonged to a new born, an outstanding epidemiologist data because, since the passage of *C. neoformans* has not been transplacentally registered up to now, in this case is very difficult to determine the infection source, taking



Table 6. Distribution by age groups from 136 patients with cryptococcosis.\*

Etiological agent	No. of strains	Age groups							
		0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80
<i>C. albidus</i>	1	-	-	-	1	-	-	-	-
<i>C. neoformans</i> var. <i>gattii</i>	19	2	5	3	5	3	1	-	-
<i>C. neoformans</i> var. <i>neoformans</i>	114	-	5	43	43	17	3	2	1
<i>C. uniguttulatus</i>	1	-	-	-	1	-	-	-	-
<i>Cryptococcus</i> sp	1	-	-	-	1	-	-	-	-
Total	136	2	10	46	51	20	4	2	1

\* Data obtained by authors

Table 7. Frequency of *Cryptococcus neoformans* varieties in some Latin American countries.

Country <sup>Reference</sup>	<i>Cryptococcus neoformans</i> var. <i>neoformans</i> a / b (%)	<i>Cryptococcus neoformans</i> var. <i>gattii</i> a / b (%)	Not identified a / b (%)
Argentina <sup>2-5, 18</sup>	279 / 286 (97.6)	7 / 286 (2.4)	0
Brazil <sup>6, 9, 18, 19, 21, 23, 24, 26, 27</sup>	495 / 594 (83.3)	94 / 594 (15.8)	5 / 594 (0.9)
Cuba <sup>10-12</sup>	80 / 82 (97.6)	2 / 82 (2.4)	0
Mexico <sup>7, 14, 20, §</sup>	234 / 266 (88.0)	32 / 266 (12.0)	0
Paraguay <sup>18</sup>	0 / 1 (0)	1 / 1 (100.0)	0
Puerto Rico <sup>13</sup>	12 / 12 (100.0)	0 / 12 (0)	0
Venezuela <sup>30</sup>	18 / 27 (66.7)	8 / 27 (29.6)	1 / 27 (3.7)

A, No. of strains; b, Total No. of studied strains; §, present study.

into account that, apparently, the baby could not have had contact with pigeon droppings or similar, at the hospital.

Since the prevailing species was *C. neoformans*, finally the isolation frequency of the two species varieties obtained in this study was compared with the ones registered in other countries of Latin America. It can be noted in Table 7 that *C. neoformans* var. *neoformans* counts on a greater distribution within the human cases of cryptococcosis in the general population of the countries registered.

In summary, within the interesting results, it can be found: a) one strain of *C. neoformans* var. *neoformans* serotype D, b) one strain of *C. neoformans* serotype C, c) one strain of *C. neoformans* var. *gattii* associated to AIDS, c) one strain of *C. albidus* isolated from liver, d) one strain of *C. uniguttulatus* isolated from C.S.F. and e) *C. neoformans* var. *neoformans* is the main etiological agent of the human Cryptococcosis in Latin America, even though Brazil, Cuba, Mexico and Venezuela count on climatic conditions

that could characterize them like of tropical-subtropical climate in where, apparently, var. *gattii* would prevail.<sup>18</sup>

## DISCUSSION

In spite of the low frequency with which *C. uniguttulatus* and *C. albidus* were isolated, the frequency of reports being added about the isolation of these leavenings is greater day to day. The appearance and settlement of these fungal agents, who during long time have been considered as innocuous for man, are very important because these findings reflect the great deterioration of the immune response of individuals from whom the isolated ones are obtained.

According to data obtained in Mexico, the most common clinical presentation of cryptococcosis is meningoencephalitis. In the present survey, none of the isolated ones

were obtained from urine, sperm or prostate secretion, specimens whose study is of interest because the prostate can be an asymptomatic niche, colonized by *C. neoformans* and also because the persistence of the yeast in the genitourinary tract is due to the inefficiency the antimycotic treatment has in that corporal area.<sup>1</sup>

Most of the cryptococcosis cases, mainly caused by *C. neoformans* var. *neoformans*, are AIDS associated, as well as to other opportunist sufferings; nevertheless it is necessary to take into account the observations conducted by Arastéh *et al.*,<sup>1</sup> who have reported that in order to a patient with AIDS present systemic infection by *C. neoformans*, the CD4 lymphocytes count must be smaller than 150/ $\mu$ l.

On the other hand, var. *gattii* is mainly related to patients with non opportunist factors (primary cryptococcosis); in fact, this variety is exceptionally isolated from patients with AIDS, since as far as 1996 only eight cases were known at world-wide level, being the ninth one our Mexican case.<sup>7</sup>

Data related to gender and age surely is influenced by the amount of patients presenting AIDS, since, when having this disease as an opportunist factor, the demographic data is in terms of this factor, rather than to the cryptococcosis itself. Due to that is a retrospective study, unfortunately in this survey important epidemiological data like as foreign travels, type and response to treatment, severity or development of the disease, hospitalization time, etc., we can not recorded.

Although the cryptococcosis is a disease of a world-wide distribution, the registered data of this disease in Latin America is very poor. In addition to the countries reported in this paper, some like Aruba,<sup>25</sup> Chile,<sup>29</sup> Colombia,<sup>15</sup> Costa Rica,<sup>22</sup> Granada,<sup>16</sup> Jamaica,<sup>25</sup> Panama,<sup>28</sup> Peru<sup>32</sup> and Uruguay<sup>8</sup>, have data published at world-wide level. However, this countries were not included in our study because they do not comment on varieties of *C. neoformans* found.

The obtained Mexican data, compared with the reported ones in other Latin countries, although to few isolates from Paraguay and Puerto Rico were studied, reflect a great similarity. Most interesting of these surveys is the high prevalence of var. *neoformans*, a variety that was not considered as a dominant one in these countries, which are considered of semitropical or tropical climate.<sup>18</sup>

From the study performed by Kwon-Chung,<sup>18</sup> before AIDS the regions having a high prevalence of *C. neoformans* var. *gattii*, had warm temperatures ( $\geq 10^{\circ}\text{C}$  in the cold season) that means, only in tropical and subtropical climates; the countries list in table 7 have this condition, nevertheless the obtained data show the high frequency of var. *neoformans*. It is clear, all data included in this study were obtained after 1980 (AIDS era), why the AIDS patients are more susceptible to var. *neoformans*, remains to be studied.

It is a fact that world-wide registers a constant increase of opportunist factors, such as: chronic diseases, immuno-

deficiencias, cortico, antibiotic and immunotherapies and organs transplanting. cryptococcosis is one of the mycosis with a high risk of being acquired by patients who have some or several of the opportunist factors above mentioned; reason why this infection should have a study priority, from a clinical viewpoint, of laboratory diagnosis and therapeutic handling.

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