

## First Case of Human Cryptococcosis Due to *Cryptococcus neoformans* var. *gattii* in Spain

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**We report the first case of human cryptococcosis due to *Cryptococcus neoformans* var. *gattii* described in our country, which was presented as brain cryptococcoma in an immunocompetent patient. An extensive sampling of the patient's environment was carried out to find the source of infection.**

### CASE REPORT

A 60-year-old heterosexual Spanish farmer came to the Hospital General de Alicante in July 2003, having suffered for several days from cephalalgia and somnolence. He had never traveled abroad. A diabetes mellitus type 2 identified 2 years previously was the only clinical antecedent of interest. Human immunodeficiency virus serology was investigated with repeated negative results. General and neurological exploration included computerized tomography scanning, which disclosed a brain mass lesion in basal ganglions. Capsulated yeasts were seen in a stereotaxic brain puncture sample, and *Cryptococcus neoformans* was suspected to be the causative agent. *C. neoformans* capsular antigen was detected in blood and cerebrospinal fluid (CSF) several times during the process (maximum values detected, 1/256 and 1/32, respectively). The yeast was cultured from a surgical drainage sample of the brain abscess. Species identification was carried out on the basis of microscopic morphology, growth at 37°C, a urease test, phenoloxylase production, and the carbohydrate assimilation pattern (Auxacolor; Bio-Rad). Further testing such as canavanina glycine bromothimol blue agar growth, serotype determination (Cryptocheck test; Iatron), and genotype analysis revealed that the strain was *C. neoformans* var. *gattii* serotype B. The strain identification and serotype were confirmed in another mycology laboratory (IMIM, Barcelona, Spain). Two antifungal drug sensitivity tests were performed (Sensititre and Etest). Both tests showed low amphotericin B (AMB) and ketoconazole MICs but different results with fluconazole (MIC<sub>Sensititre</sub>, 8 µg/ml; MIC<sub>Etest</sub>, 64 µg/ml) and 5-flucytosine (MIC<sub>Sensititre</sub>, 2 µg/ml; MIC<sub>Etest</sub>, 32 µg/ml). Voriconazole was only tested with the Sensititre test (MIC<sub>Sensititre</sub>, 12 µg/ml). Genotype analysis

consisted of the study of five molecular DNA targets: internal transcribed spacer-5.8S rRNA gene sequence analysis (36), 5S rRNA gene and URA5 gene restriction fragment length polymorphism (RFLP) analysis (24), and amplification patterns of two highly repeated minisatellite sequences (M13 and GACA<sub>4</sub>) (14, 24). Nucleotide sequence analysis of the internal transcribed spacer-5.8S rRNA gene confirmed the identification at a species level by comparing it to ribosomal sequence databases (EMBL and GenBank). RFLP of the URA5 gene showed a VGI molecular type (Fig. 1) which is characteristic of *C. neoformans* var. *gattii*. RFLP 5S rRNA gene and minisatellite amplification of M13 and GACA<sub>4</sub> also displayed molecular patterns attributable to *C. neoformans* var. *gattii* strains (14, 24).

Some different antifungal therapies were followed, depending on the clinical evolution and the antigen levels (serum and CSF). Intravenous AMB at 200 mg/day (Ambisome) was the first choice, but fluconazole (400 mg/12 h) and AMB at 400 mg/day combined with 5 flucytosine (2.5g/6 h) were also prescribed. Three months after diagnosis, neurological symptoms and serum and CSF positive antigen detection persisted. Therefore, intra-abscess amphotericin B deoxicolate was administered by a surgical procedure, and a symptomatic improvement was rapidly detected. Although during the next 3 months serum antigen detection remained positive at low levels (1/16), 6 months after diagnosis, the serum level increased again to 1/64. Therefore, oral voriconazole was prescribed as a maintenance therapy. At present the patient is asymptomatic, and serum remained negative for *C. neoformans* antigen for more than 6 months.

An extensive sampling of the patient's area of work was carried out, including different soil samples, bird droppings, and vegetal tissues from all the tree species around; no eucalyptus was seen in the area. Some *Cryptococcus* and other yeast species were found, but, unfortunately, none of them corresponded to *C. neoformans*. Moreover, the patient's family reported the presence of a species of parrot in the house next door. Our interest in visiting the neighbors and taking samples

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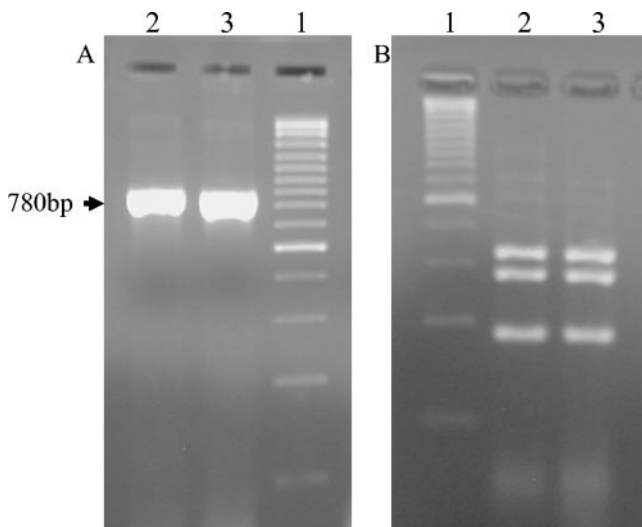


FIG. 1. Agarose gel electrophoresis of DNA obtained by PCR amplification of the URA5 gene (A) and subsequent RFLP fingerprinting after DNA enzymatic digestion (B). Lanes 1, molecular ruler; lanes 2, DNA obtained from *C. neoformans*-cultured cells; lanes 3, DNA obtained from the clinical sample (cryptococcoma drainage).

of the parrot's feces was misunderstood, and they therefore got rid of the parrot, together with the possibility of studying a putative source of the infection (30, 32, 35).

Cryptococcosis is an infectious disease caused by the yeast *C. neoformans*. It presents different clinical manifestations and a wide range of severity, depending not only on the patient's risk factors but also on the yeast variety involved. This microorganism was traditionally described as a unique species, *C. neoformans*, that included two pathogenic varieties, *C. neoformans* var. *neoformans* and *C. neoformans* var. *gattii*. Important differences between the two varieties have recently raised *C. neoformans* var. *gattii* to species status as *Cryptococcus gattii* (20).

Cryptococcosis from *C. neoformans* var. *neoformans* infection is cosmopolite. Patients are commonly immunocompromised, mostly with a cellular immunity alteration, as in human immunodeficiency virus-infected individuals. *C. neoformans* var. *gattii* is believed to behave more aggressively than *C. neoformans* var. *neoformans* and to cause infections in immunocompetent patients more frequently (8). Until now, this yeast was considered to be restricted to warm areas (tropical and subtropical climates), but this statement is under discussion after the recent outbreak of cryptococcosis infection by *C. gattii* in the temperate climate of Vancouver Island (British Columbia, Canada) (18). Both pathogenic varieties also show different natural habitats. *C. neoformans* var. *neoformans* is widely associated with bird feces, with a strong presence in pigeon excreta (19). The association of *C. neoformans* var. *gattii* with eucalyptus trees has been demonstrated (13), and some strains have also been isolated from a wide range of different natural sources, including some tropical birds (2, 12, 16, 23, 26, 27). Cryptococcoma is not the usual clinical presentation of the disease, although a number of cases were described in coun-

tries all over the world, including Spain (28). They usually appear in the literature as case reports because of their low incidence. They are more common in immunocompetent hosts and usually have a better prognosis than disseminated cryptococcosis (17, 22).

In Spain, *Cryptococcus* and cryptococcosis have already been studied by some authors with various points of view (3, 25). The presence of the yeast in relation to birds has been demonstrated in different studies and in various locations (11, 15, 29). Some reports on human and animal cryptococcosis have also been reported since 1971 (1). Among these studies, the first report of the presence of *C. neoformans* var. *gattii* in our country is remarkable (4). In 1998, 13 strains of *C. neoformans* var. *gattii* were isolated from the tissues of different dead goats. All of them suffered from invasive disease. At that time, a national epidemiological study for human cryptococcosis had just started in Spain (10). During a 6-year period (1998 to 2003) all strains found in the human study (a total of 64) belonged to *C. neoformans* var. *neoformans* until July 2003, when the present case came to our laboratory.

Finding the source of the infection is very important to clarify whether it could be an autochthonous infection or an imported case. Some published cases of *C. neoformans* var. *gattii* cryptococcosis outside the area of endemicity (5, 6, 31, 33, 34) had a clear source of infection. Most of them occurred in patients who had traveled to Australia or South America. For some others, without a probable contact with eucalyptus trees and without any prior travel, the source remains uncertain. An exceptional situation has recently been described in Vancouver Island (Canada). An extended cryptococcosis outbreak of *C. gattii* involved some locations on the east coast of the island, and a large number of humans and animals were affected. The exhaustive environmental sampling carried out in the area allowed the detection of the possible natural reservoirs of the disease. The yeast was present in some tree hollows, soils, and other materials (18). This fact marks an important change in the understanding of the geographical distribution and natural life cycle of *C. gattii* and highlights the importance of finding the source of the infection. In order to demonstrate the presence of the yeast in our patient's environment, a total of 43 samples were taken all around his working area. Samples included vegetal material from different tree species (not including eucalyptus), different kinds of soils, and some pigeon droppings. As with other works focused on the isolation of *C. neoformans* var. *gattii* from eucalyptus and other trees outside the zones of endemicity (7, 9, 21), until now the presence of the yeast in the natural environment has not been proved. All these findings encouraged the search for a *C. gattii* environmental niche in Spain.

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