Note

Environmental sampling of *Ceratonia siliqua* (carob) trees in Spain reveals the presence of the rare *Cryptococcus gattii* genotype AFLP7/VGIV

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**ABSTRACT**

**Background:** *Cryptococcus gattii* is a pathogenic basidiomycetous yeast that is emerging in temperate climate zones worldwide. *C. gattii* has repetitively been isolated from numerous tree species. Ongoing environmental sampling and molecular characterization is essential to understand the presence of this primary pathogenic microorganism in the Mediterranean environment.

**Aims:** To report the first isolation of the rare *C. gattii* genotype AFLP7/VGIV from the environment in Europe.

**Methods:** Samples were collected from woody debris of carob trees (*Ceratonia siliqua*) and olive trees (*Olea europea*) in El Perelló, Tarragona, Spain. Cryptococcus species were further characterized by using URAS-RFLP, MALDI-TOF, AFLP and MLST. The antifungal susceptibility profile to amphotericin B, 5-fluorocytosine, fluconazole, itraconazole, posaconazole and voriconazole was determined using Sensititre Yeast One and E-test.

**Results:** Cultures from one carob tree revealed the presence of ten *Cryptococcus*-like colonies. One colony was identified as *C. gattii*, and subsequent molecular characterization showed that it was an α mating-type that belonged to the rare genotype AFLP7/VGIV. Antifungal susceptibility testing showed values within the range of sensitivity described for other isolates of the same genotype and within the epidemiological cutoff values for this species.

**Conclusions:** The isolation of the rare *C. gattii* genotype AFLP7/VGIV in Spain is the first report in the European environment, implying the possible presence in other regions of the Mediterranean area, and underlines that clinicians must be aware for *C. gattii* infections in healthy individuals.

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**MUESTREOS MEDIOAMBIENTALES DE ALGARROBOS (CERATONIA SILIQUA) ESPAÑOLES REVELAN LA PRESENCIA DEL RARO GENOTIPO AFLP7/VGIV DE CRYPTOCOCCUS GATTII**

**RESUMEN**

**Antecedentes:** *Cryptococcus gattii* es una levadura perteneciente a los basidiomictos y considerada un patógeno emergente en climas templados. *C. gattii* se ha aislado en un gran número de especies de árboles en todo el mundo. El muestreo medioambiental y la caracterización molecular de *C. gattii* es esencial para entender la presencia de este patógeno primario en el entorno de la cuenca mediterránea.

**Objetivos:** Comunicar la presencia del raro genotipo AFLP7/VGIV de *C. gattii* en el medio ambiente en Europa.

**Métodos:** Se tomaron muestras de detritos de algarrobos (*Ceratonia siliqua*) y olivos (*Olea europea*) en las afueras de El Perelló (Tarragona, España). La colonia de *C. gattii* aislada se sometió a análisis mediante URAS-RFLP, MALDI-TOF, AFLP y MLST. Se llevó a cabo un estudio de sensibilidad in vitro a la anfotericina B,
Cryptococcus gattii is a pathogenic basidiomyceteous yeast that mainly causes infection by the inhalation of the desiccated yeast cells or basidiospores, in which small size allows them to pass the lung alveoli. \(^{25}\) During the past two decades the number of C. gattii infections has increased in regions that are within temperate climate zones, mainly due to several large ongoing outbreaks in North America. \(^{11,12,24}\) Since the onset of the HIV/AIDS-pandemic during the early 1980s, cryptococcal infections among immunocompromised individuals have raised dramatically. It has been estimated that annually approximately one million HIV-infected patients develop cryptococcal meningitis, and that approximately 625,000 people die due to this fungal infection. \(^{23}\)

Cryptococcus neoformans and C. gattii differ in their host predilection, ecology and physiology. \(^{2,12,24}\) The former has a global distribution while C. gattii was restricted to tropical and subtropical climate zones. \(^{1,11,12,24}\) But C. gattii is nowadays emerging in temperate climate zones similar to that of Mediterranean Europe. \(^{11}\) Until recently it was believed that certain Eucalyptus species were the exclusive ecological niche for C. gattii, but this assumption was refuted by large-scale environmental screening initiated after several C. gattii outbreaks. \(^{5,7,15,24}\)

Within the C. neoformans/C. gattii species complex 13 genotypes can be discerned by using molecular techniques such as PCR fingerprinting, restriction fragment length polymorphism (RFLP) fingerprinting (using the PLB1 and UR5 loci), amplified fragment length polymorphism (AFLP) fingerprinting, multi-locus microsatellite (MLMT) and multi-locus sequence typing (MLST). \(^{2,12,13,18}\) C. neoformans can be classified into five genotypes: AFLP1/VNI, AFLP1A/VNI/VNB and AFLP1B/VNI for C. neoformans variety grubii (serotype A), AFLP2/VNI for C. neoformans variety neoformans (serotype D) and AFLP3/VNI for the hybrid form (serotype AD). \(^{2,18}\) C. gattii can be split into five genotypes known as AFLP4/VGI, AFLP6/VGII and AFLP10/VGIV for serotype B isolates, and AFLP5/VGII and AFLP7/VGIV for serotype C isolates. \(^{12,13,18}\) Isolates with genotypes AFLP5/VGII, AFLP7/VGIV and AFLP10/VGIV have frequently been isolated from immunocompromised individuals; on the contrary, the other C. gattii genotypes are often isolated from individuals that apparently do not have any underlying disease. \(^{2,12,13,16,24}\) Although rarely found, interspecies hybrids between haploid C. gattii and C. neoformans genotypes exist, and so far three interspecies hybrid genotypes (all originated from clinical sources) have been described. \(^{14}\)

In Europe, infections with C. gattii are being increasingly reported, with the Mediterranean area being the hotspot for autochthonous acquired infections, while among northern European citizens infections were found to be acquired elsewhere. \(^{7,12}\) Animals have been reported as being sentinels for the environmental presence of C. gattii, \(^{1,7,20,21}\) and several outbreaks of C. gattii infections among animals have been reported from the Iberian Peninsula.

5-fluorocytosina, fluconazol, itraconazol, posaconazol and voriconazol mediante las pruebas comerciales Sensititre Yeast One \(^{8}\) y E-test \(^{8}\).

**Resultados:** De una de las muestras de un algarrobo se aislaron 10 colonias susceptibles de ser Cryptococcus. Una de ellas fue estudiada e identificada como C. gattii, y su subsecuente caracterización molecular mostró que se trataba de un tipo sexual α y que pertenecía al raro genotipo AFLP7/VGIV. El estudio de la sensibilidad a los antifúngicos mostró valores similares a los de otras cepas del mismo genotipo y dentro del rango de valores de corte epidemiológicos para la especie.

**Conclusiones:** El aislamiento en España de C. gattii con el genotipo AFLP7/VGIV es el primero descrito en el medioambiente en Europa; podría encontrarse también en otros países de la cuenca mediterránea, donde debería tenerse un especial cuidado por la posibilidad de infección en individuos no inmunodeprimidos.

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AFLP7/VGIV cases have been reported from Africa, India, and a travel-related case from Sweden. Based on PCR fingerprinting, this genotype has been observed as cause of cryptococcal disease in Latin America. Subsequent molecular characterization revealed that these clinical Latin American isolates formed a polyphyletic cluster and support recent data that the PCR fingerprint molecular type VGIV can be separated into two distinct clusters by AFLP and MLST genotyping, namely AFLP7/VGIV for serotype C isolates and AFLP10/VGIV for serotype B isolates.

From the current report, as well as from others, it is clear that genotype AFLP7/VGIV and its sibling AFLP10/VGIV are the least reported and least studied genotypes within the *C. gattii/C. neoformans* species complex. The occurrence of these rare genotypes remains enigmatic, but ongoing local environmental sampling initiatives, such as the current study and the recently initiated European Environmental *C. gattii* Sampling Initiative, will reveal more insights into the life-style of these rarely reported *C. gattii* genotypes.

Conflict of interest

The authors declare that there is no conflict of interest.

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