

Case Report

First case report of cryptococcosis due to *Cryptococcus decagattii* in a pediatric patient in Argentina

Ana Berejnoi^[1], Constanza Giselle Taverna^[2], Mariana Mazza^[2], Matías Vivot^[2],
Guillermina Isla^[2], Susana Córdoba^[2] and Graciela Davel^[2]

[1]. Hospital Público Materno Infantil, Salta, Argentina.

[2]. Departamento Micología, INEI (Instituto Nacional de Enfermedades Infecciosas) “Dr. Carlos G. Malbrán”, ANLIS (Administración Nacional de Laboratorios e Institutos de Salud), Ciudad Autónoma de Buenos Aires, Argentina.

Abstract

We report the first case of cryptococcosis due to *Cryptococcus decagattii* in an immunocompetent pediatric patient from an indigenous community in Argentina with a successful outcome. Two isolates (blood, cerebrospinal fluid) were genotyped by restriction fragment length polymorphism of the orotidine monophosphate pyrophosphorylase (*URA5*) gene as VGIV and identified by multi-locus sequence typing as *C. decagattii*. Matrix-assisted laser desorption/ionization time of flight mass spectrometry identification indicated genotype VGIII. The minimum inhibitory concentration of amphotericin B, fluconazole, itraconazole, and voriconazole was determined (cerebrospinal fluid: 0.25, 16, 0.12, and 0.12, blood: 0.25, 4, 0.12, and 0.06, respectively, all in mg/L).

Keywords: Cryptococcosis. *Cryptococcus decagattii*. Pediatric patient.

INTRODUCTION

Cryptococcus gattii sensu lato (s.l.) is a primary human pathogen that causes respiratory and neurological diseases, particularly meningoencephalitis, in immunocompetent patients¹. This pathogen is categorized into 5 genotypes, VGI/AFLP4, VGII/AFLP6, VGIII/AFLP5, VGIV/AFLP7, and VGIIIc/VGIV/AFLP10¹⁻³, but it was recently proposed that these genotypes represent in fact 5 different species, namely *C. gattii sensu stricto*, *C. deuterogattii*, *C. bacillisporus*, *C. tetragattii*, and *C. decagattii*, respectively³. We report here the first case of cryptococcosis caused by *C. decagattii* in Argentina. Isolates from the patient were genotyped VGIV based on restriction fragment length polymorphism (RFLP) of the orotidine monophosphate pyrophosphorylase gene (*URA5*), but analysis of the samples by matrix-assisted laser desorption/ionization

time of flight mass spectrometry (MALDI-TOF MS) and multi-locus sequence typing (MLST) indicated genotype VGIII.

CASE REPORT

A seven-year-old female of the indigenous community Wichi (Salta Province, Argentina) was admitted to a local medical institution on July 17, 2017 with postprandial vomiting, abdominal pain, and constipation. On July 19, 2017 (day 0) she began to show clinical signs of meningoencephalitis and was referred to a public hospital. The girl presented with a depressed level of consciousness, lethargy, psychomotor excitation, stiff neck, a cardiac frequency of 115 bpm, a respiratory frequency of 16 bpm, and a Glasgow scale of 10/15 (motor response: 4, eye response: 3, verbal response: 3). Ophthalmological assessment revealed pupils with slight anisocoria, strabismus and left-sided nystagmus, and left unilateral papilledema grade II. Signs of cerebral edema were observed in a computed tomography scan.

Blood (BS) and cerebrospinal fluid (CSF) samples were obtained and empirical antimicrobial treatment with ceftriaxone (1 g/12 h), amphotericin B lipid complex (2.5 mg/kg/d), and acyclovir (60 mg/kg/d) was initiated on day 0. Her body weight was 21.5 kg and her blood glucose level 90 mg/dL.

Corresponding author: MSc. Constanza Giselle Taverna.

e-mail: ctaverna@anlis.gov.ar

Orcid: 0000-0001-6985-2699

Received 5 October 2018

Accepted 17 December 2018

The abdominal ultrasound and chest radiograph were normal, as were blood cell counts and liver and kidney function tests. Electrolyte levels in the blood were also relatively normal with a slight hyponatremia and elevated C-reactive protein (23.1 mg/dL). The erythrocyte sedimentation rate was determined as 7 mm/h, white blood cell (WBC) count as 7,300 cells/ μ L (68 % neutrophils, 21 % lymphocytes), the hematocrit as 37 %, and hemoglobin as 121 g/L. Microscopic observation of the CSF specimen after staining with India ink showed the presence of rounded encapsulated yeasts consistent with *Cryptococcus*. Further analysis of the sample revealed an elevated WBC count (250 cells/ μ L), a glucose concentration of 0.44 g/L, and a protein level of 0.47 g/L. Yeasts compatible with *Cryptococcus* were also observed in the BS following 3 days incubation in the Bact/ALERT[®] PF culture medium (BioMérieux Hazelwood, MO, USA). Both CSF and BS cultures yielded colonies on blood agar within 48 h of incubation at 35°C. Ziehl-Neelsen staining and an acid-fast bacteria culture of the CSF sample were both negative. Yeasts were identified as *C. neoformans* by the automated Vitek[®] 2 Compact system (BioMérieux, Hazelwood, MO, USA). The girl was not infected with HIV or hepatitis B or C and did not take any medications. Her main risk factors were malnutrition and low socio-economic status. On July 25, 2017 acyclovir and ceftriaxone were discontinued, amphotericin B lipid complex replaced by amphotericin B deoxycholate (20 mg/24 h), and fluconazole (200 mg/12 h) was added. On July 26, 2017 she started to experience intracranial hypertension and therefore therapeutic CSF drainage was performed every 72 h. The India ink test was still positive at this point although the cultures were negative. After six additional weeks of treatment with fluconazole (10 mg/kg/d) she showed improvement of her symptoms and the treatment was changed to oral fluconazole (375 mg/d). On September 25, 2017 (day 68) she presented with frontal headache and vomiting. Lumbar puncture revealed an elevated opening pressure (70 cm H₂O) and colorless CSF containing 161 WBC/ μ L, 0.41 g/L glucose, and 0.5 g/L protein. The India ink test was positive again and cultures still negative. Therapeutic CSF drainage was performed 7 times every 72 h. A new treatment regimen with amphotericin B deoxycholate (1 mg/kg/d) and fluconazole (10 mg/kg/d) was initiated. On October 9, 2017 (day 82) meprednisone (1 mg/kg/d) was added because the high intracranial pressure persisted (101 cm H₂O). On October 23, 2017 (day 96), the CSF was colorless and contained 8 WBC/ μ L, 0.49 g/L glucose, and 0.3 g/L protein. India ink staining remained positive and the cultures negative. On October 30, 2017 (day 103) the girl was in a good clinical condition and was discharged with the following treatment instructions: oral fluconazole (200 mg/d) for 3 months, diphenylhydantoin (2.5 mL/12 h) and oral meprednisone (40 mg/d) for 7 days, and medical check-ups every 2 weeks in the local medical institution.

Isolates obtained from the initial CSF and BS cultures were sent to the National Reference Laboratory for further studies. They showed mucoid colonies in yeast medium agar plates and rounded budding yeasts in 5% malt extract, growing well at 37°C. Urease, phenoloxidase, and canavanine-glycine-bromothymol blue (CGB) tests were positive. Identification

by MALDI-TOF MS was performed as described previously⁴. This technique identified both isolates as *C. gattii s.l.* Best match results were obtained with the main spectra (MSP) of genotype VGIII strains. Molecular characterization by RFLP of the *URA5* gene was performed as described previously² and both isolates showed a genotype VGIV profile. MLST was performed according to the International Society for Human and Animal Mycology (ISHAM) consensus scheme⁵. MLST clustering analysis showed that our isolates represent a novel sequence type related to the genotype VGIII that clustered with the strains WM1802 (ST100) and WM1804 (ST101) from Mexico (**Figure 1**). These strains are related to the previously named “VGIIIc” strain 7685027 from Southern California⁶ and to the AFLP10 strain CBS11687 isolated in Spain from a Mexican patient that is the type strain of *C. decagattii*³. The alleles and sequence types were: *ST*=545, *CAP59*=95, *GPD1*=61, *IGS1*=110, *LAC1*=41, *PLB1*=45, *SOD1*=131, and *URA5*=57. All alleles except for *LAC1* represent a new allele type and result in a new sequence type entry in the MLST database. The *URA5* sequence showed a single nucleotide polymorphism (SNP) at position 528, which is the restriction site of the *SAU96I* enzyme, leading to a VGIV genotype profile.

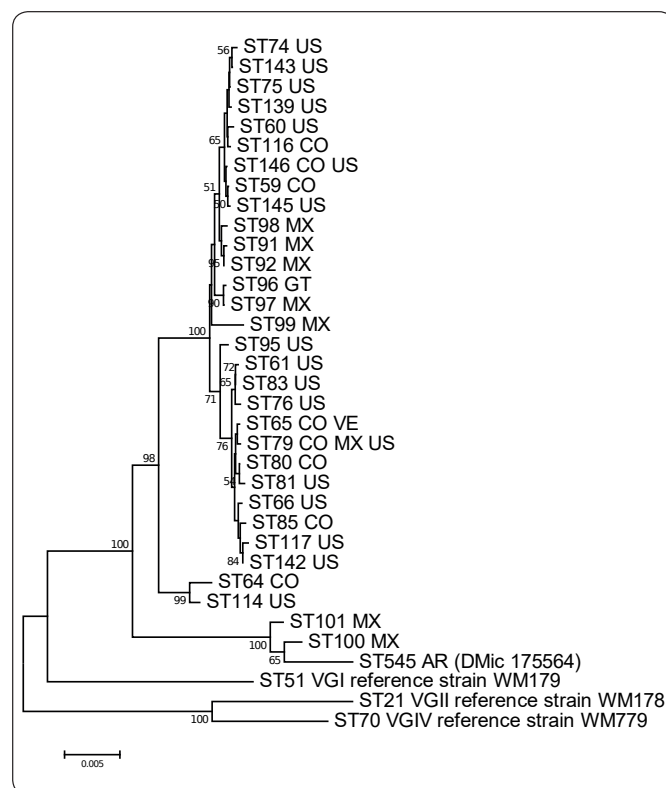


FIGURE 1: Genetic relationship between the Argentinian VGIII genotype and VGIII isolates reported in other countries inferred by the Neighbor-Joining method using the concatenated data set of the seven multi-locus sequence typing (MLST) loci. The number at each branch indicates bootstrap values >50 % based on 1,000 replicates. The taxa nomenclature includes the sequence type (ST) numbers and country of isolation. **AR:** Argentina, **CO:** Colombia, **ES:** Spain, **GT:** Guatemala, **MX:** Mexico, **US:** United States of America, **VE:** Venezuela. The tree is drawn to scale, with branch lengths measuring the number of substitutions per site.

Antifungal susceptibility tests were carried out by determining the minimum inhibitory concentration (MIC) according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) Definitive Document (E. Def) 7.3.1 reference method⁷. Isolates were tested against amphotericin B, fluconazole, itraconazole, and voriconazole (Merck, Buenos Aires, Argentina). The MIC values for the CSF isolate were 0.25 mg/L amphotericin B, 16 mg/L fluconazole, 0.12 mg/L each itraconazole and voriconazole, and for the BS isolate were 0.25 mg/L amphotericin B, 4 mg/L fluconazole, 0.12 mg/L itraconazole, and 0.06 mg/L voriconazole. Current methodologies to determine the MIC do not include clinical breakpoints for *C. gattii s.l.*, and hence no categorical interpretation of results was possible. We used the epidemiological cutoff value (ECV) proposed by Espinel Ingroff et al. to interpret our results⁸⁻⁹. Both isolates were categorized as wild-type (WT) for all antifungals tested, except for non-WT categorization of the CSF isolate for fluconazole (MIC=16 mg/L).

DISCUSSION

Previous studies have reported the presence of *C. gattii* genotypes VGI, VGII, and VGIII in clinical and environmental sources in Argentina and other South American countries, but genotype VGIV has only been isolated in Colombia¹⁰⁻¹¹. *C. decagattii* has been rarely isolated and with only 5 reports worldwide³. We present here the first case report of cryptococcosis caused by *C. decagattii* in Argentina and also in South America in a patient belonging to the indigenous community Wichí whose people live in huts made of branches in close physical contact with nature and domestic animals. This extensive exposure to nature may be a potential source of infection¹. On the other hand, the patient presented with signs of malnutrition that may also have been a predisposing factor.

Meningoencephalitis caused by *C. gattii s.l.* usually requires aggressive management of the elevated intracranial pressure and extensive antifungal treatment¹. In the present case, several therapeutic CSF drains were required to alleviate the intracranial pressure and the antifungal treatment regimen included administration of amphotericin B and fluconazole twice for an extensive period of time. Rapid differentiation between *C. gattii s.l.* and *C. neoformans s.l.* was possible with the CGB test and MALDI-TOF MS. This differentiation is important because these complexes differ in their clinical presentation and management^{1,3}. Molecular characterization of *C. gattii* species is important for ecological and epidemiological purposes. In the present case, RFLP of the *URA5* gene misidentified the isolates as genotype VGIV, but the *URA5* sequence contained the same SNP described previously for two isolates of the VGIII serotype C from Mexico¹². MALDI-TOF MS identification of the isolates showed the best match results with the MSP of the genotype VGIII strains. This is in agreement with previous studies demonstrating a high potential of this technique to correctly differentiate between the major genotypes⁴.

Acknowledgments: We thank Luciana Trilles for her help with the MLST analysis.

Conflict of Interest: The authors have no conflicts of interest to declare.

REFERENCES

- Chen SC-A, Meyer W, Sorrell TC. *Cryptococcus gattii* infections. Clin Microbiol Rev. 2014;27(4):980-1024.
- Meyer W, Castañeda A, Jackson S, Huynh M, Castañeda E, IberoAmerican Cryptococcal Study Group. Molecular typing of IberoAmerican *Cryptococcus neoformans* isolates. Emerging Infect Dis. 2003;9(2):189-95.
- Hagen F, Khayhan K, Theelen B, Kolecka A, Polacheck I, Sionov E, et al. Recognition of seven species in the *Cryptococcus gattii*/*Cryptococcus neoformans* species complex. Fungal Genet Biol. 2015;78:16-48.
- Taverna CG, Mazza M, Bueno NS, Alvarez C, Amigot S, Andreani M, et al. Development and validation of an extended database for yeast identification by MALDI-TOF MS in Argentina. Med Mycol. 2018. doi: 10.1093/mmy/myy021.
- Meyer W, Aanensen DM, Boekhout T, Cogliati M, Diaz MR, Esposto MC, et al. Consensus multi-locus sequence typing scheme for *Cryptococcus neoformans* and *Cryptococcus gattii*. Med Mycol. 2009;47(6):561-70.
- Springer DJ, Billmyre RB, Filler EE, Voelz K, Pursall R, Mieczkowski PA, et al. *Cryptococcus gattii* VGIII isolates causing infections in HIV/AIDS patients in Southern California: identification of the local environmental source as arboreal. PLoS Pathog. 2014;10(8):e1004285.
- Arendrup MC, Meletiadis J, Mouton JW, Lagrou K, Petr Hamal, Guinea J, et al. Eucast Definitive Document E.DEF 7.3.1 E.DEF 7.3.1. Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts. 2017.
- Espinel-Ingroff A, Chowdhary A, Cuenca-Estrella M, Fothergill A, Fuller J, Hagen F, et al. *Cryptococcus neoformans*-*Cryptococcus gattii* species complex: an international study of wild-type susceptibility endpoint distributions and epidemiological cutoff values for amphotericin B and flucytosine. Antimicrob Agents Chemother. 2012;56(6):3107-13.
- Espinel-Ingroff A, Aller AI, Canton E, Castañón-Olivares LR, Chowdhary A, Córdoba S, et al. *Cryptococcus neoformans*-*Cryptococcus gattii* species complex: an international study of wild-type susceptibility endpoint distributions and epidemiological cutoff values for fluconazole, itraconazole, posaconazole, and voriconazole. Antimicrob Agents Chemother. 2012;56(11):5898-906.
- Mazza M, Davel G, Taverna C, Refojo N, Canteros CE, Bueno N, et al. Contribution of the National Mycology Laboratory Network to Surveillance of Cryptococcosis in Argentina. Curr Fungal Infect Rep. 2017;11(4):209-19.
- Firacative C, Lizarazo J, Illnait-Zaragozí MT, Castañeda E, Latin American Cryptococcal Study Group. The status of cryptococcosis in Latin America. Mem Inst Oswaldo Cruz. 2018;113(7):e170554.
- Firacative C, Roe CC, Malik R, Ferreira-Paim K, Escandón P, Sykes JE, et al. MLST and Whole-Genome-Based Population Analysis of *Cryptococcus gattii* VGIII Links Clinical, Veterinary and Environmental Strains, and Reveals Divergent Serotype Specific Sub-populations and Distant Ancestors. PLoS Negl Trop Dis. 2016;10(8):e0004861.

