Mycopathologia (2019) 184:45–51
https://doi.org/10.1007/s11046-018-0313-2

SHORT COMMUNICATION

Genetic Diversity of the *Cryptococcus gattii* Species Complex in Mato Grosso State, Brazil

Fernanda Harumi Maruyama · Daphine Ariadne Jesus de Paula · Isabela de Godoy Menezes · Olívia Cometti Favalessa · Rosane Christine Hahn · Arleana do Bom Parto Ferreira de Almeida · Valéria Régia Franco Sousa · Luciano Nakazato · Valéria Dutra

Received: 11 September 2018 / Accepted: 7 December 2018 / Published online: 9 January 2019

Abstract Cryptococcosis is caused by fungi of the genus *Cryptococcus*. Owing to its importance, this study aimed to analyze the genetic diversity of *C. gattii* isolates from animals, humans, and the environment in Mato Grosso State (MT), Brazil, during November 2010–December 2017. All isolates of the *C. gattii* species complex were subjected to molecular genotyping via Restriction Fragment Length Polymorphism (PCR–RFLP) and Multi-locus Sequence Typing (MLST). PCR–RFLP analysis revealed that 21 isolates presented the genotype VGII, which is considered the most common and virulent genotype globally among. MLST analysis revealed the presence of 14 sequence types (STs), of which 5 are considered new genotypes. Clonal Complex (CC) CC182 (n = 5; 23,80%) and CC309 (n = 3; 14,28%) were the most frequent. CC distribution in relation to origin revealed that three CCs were found in animals with a predominance of CC182 (66,66%), while nine were found in humans, and two CCs were found in the environment. Extensive genetic variability was observed among the isolates in the State of Mato Grosso. STs belonging to the already described clonal complexes (CC) indicate the global expansion and adaptation of isolates in several other countries. Therefore, detection of clonal complexes and STs already described in other regions and the occurrence of new STs in the present study help further the current understanding of the geographic dispersion and genetic origin of the *C. gattii* species complex.

Keywords *Cryptococcus gattii* · Multi-locus sequence typing · Genotyping · Cryptococcosis

Handling Editor: Ferry Hagen.

F. H. Maruyama (✉) · D. A. J. de Paula · I. d. G. Menezes · A. d. B. P. F. de Almeida · V. R. F. Sousa · L. Nakazato · V. Dutra
Postgraduate Course in Veterinary Sciences, Department of Veterinary Sciences, Federal University of Mato Grosso, Cuiabá, Brazil
e-mail: fer_19_nanda@hotmail.com

O. C. Favalessa · R. C. Hahn
Postgraduate Course in Health Sciences, Department of Molecular Biology, Federal University of Mato Grosso, Cuiabá, Brazil

Introduction

Cryptococcosis is a disease caused by fungi of the genus *Cryptococcus*, affecting immunocompetent and immunocompromised humans and animals. The estimated of the global incidence, in 2014, of cryptococcal meningitis to be substantial at 223,100 cases annually, resulting in 181,100 annual deaths [1–3]. Owing to nomenclatural controversy, the species of the genus *Cryptococcus* can be classified in accordance with Kwon-Chung et al. [4] and are divided in two complexes of greater relevance: the "*Cryptococcus neoformans* species complex" and "*C. gattii* species

complex." Accurate taxonomy was proposed by Hagen et al. [3], that recognized seven species, excluding diploid/aneuploid hybrids, in the *C. neoformans* species complex (*C. neoformans* and *C. deneoformans*) and *C. gattii* species complex (*C. gattii; C. deuterogattii*, *C. bacillisporus, C. tetragattii* and *C. decagattii*).

The *C. gattii* species complex has been extensively studied because studies have indicated their expansion to temperate regions, wherein they were previously associated with tropical and subtropical climates [5, 6]. Considered rare, this *C.gattii* species complex gained importance in an outbreak in humans and animals on Vancouver Island (Canada) in 1999 [7].

At the molecular level, the *C. gattii* species complex can be classified into five main genetic groups) with varying ecology and epidemiology in accordance with the geographic region [3]. The differentiation and molecular typing of these species, using methods including Restriction Fragment Length Polymorphism (PCR–RFLP), Amplified Fragment Length Polymorphism Analysis (AFLP), and Multi-Locus Sequence Typing (MLST) [8], are necessary to verify the distributions of their molecular patterns and, consequently, to provide knowledge regarding possible virulent genes and drug resistance [3].

This study aimed to investigate the molecular diversity of *C. gattii* species complex in Mato Grosso (MT) state, Brazil. This geographic location, with different biomes in the region, which favors high genetic variability among isolates of the *C. gattii* species complex, can further the current understanding of the dynamic distribution of this yeast and to the prevention and effective control of this disease.

Materials and Methods

Isolation and Culture of Genus *Cryptococcus*

Isolates were obtained from samples sent to the routine of the Veterinary Hospital of the Federal University of Mato Grosso (UFMT) as well as the University Hospital of Julio Muller. It was carried out from November 2010 to December 2017. The environmental isolates were incubated by the Mycology Laboratory (UFMT). Routinely, the isolation of cultures suspects of genus *Cryptococcus* was cultured on Sabouraud dextrose agar 2%, Sabouraud dextrose agar plus chloramphenicol (0.05 g/L), and niger seed agar (*Guizotia abysinica*) [9]. The material was incubated at 30 °C and 37 °C and evaluated daily for 7 d, followed by morphological evaluation using India ink for microscopic morphological analysis and biochemical test on CGB agar (L-Canavanine–Glycine–Bromothymol blue).

DNA Isolation and PCR

For molecular characterization, the isolates of genus *Cryptococcus* were incubated on Sabouraud dextrose agar at 30 °C for 48 h. After sample growth, DNA was extracted from each isolate and transferred to a 2-mL microtube containing 500 µl of extraction buffer, followed by glass pearls and phenol/chloroform, as described by Del Poeta et al. [10]. The material was stored at − 20 °C for use in molecular testing.

Subsequently, PCR was performed to identify the isolates in accordance with the protocol of Aoki et al. [11]. CNA 70A and CNA 70S oligonucleotide pairs specific for *C. neoformans* species complex, and CNB 49A and CNB 49S specific for the *C. gattii* species complex, were used to determine the species of the isolates, by amplifying products of 695 bp and 448 bp, respectively. For the identification of the *SOD1* (superoxide dismutase) gene and confirmation of the *C. gattii* species complex, PCR was performed as described by D'Souza et al. [12]. Amplification products, stained with Gel Red (Biotium), were subjected to 1.5% agarose gel electrophoresis at 100 V for 90 min and visualized on ChemiDocTM XRS using ImageLabTM® software.

PCR–RFLP Technique

PCR–RFLP analysis for genotyping was performed in accordance with the method of Meyer et al. [13]. The amplification products of the *URA5* gene were double digested with Sau96I (10 U/L) and HhaI (20 U/µl) for 3 h and separated by electrophoresis on agarose gel (3%). PCR–RFLP patterns were attributed by comparison with band-profile strain references (VNI-VNIV and VGI-VGIV) [13].

MLST Technique

In the MLST for isolates of the *C. gattii* species complex, seven genetic loci (*CAP59 GPD1*, *LAC1*, *PLB1*, *SOD1*, *URA5*, and *IGS1*) were used to

distinguish the closely related strains as described by Meyer et al. [14]. Each amplified locus was purified using the GFX^{TM} PCR kit DNA and Gel Band Purification (GE Healthcare). Subsequently, the samples were sequenced on the automatic ABI-PRISM 3500 Genetic Analyzer.

Software Analysis

The sequences were trimmed and compared to sequences from each locus published in the MLST database of the *C. gattii* species complex (http://mlst.mycologylab.org) [15]. Subsequently, STs were analyzed in goeBurst software to better comprehend the distribution and possible identification of a common ancestor of the isolates.

Results and Discussion

Isolation and Culture of Genus *Cryptococcus*

Of the 21 isolates, 13 (61.90%) were from humans, six (28.57%) were from animals, and two (9.52%) were from environmental samples. Indian ink was used to observe the capsule of the genus *Cryptococcus*. All the samples on CGB agar were positive and confirmed via PCR analysis. In humans, 11 (84.61%) samples were from neurologic cases, 1 (7.69%) was from a pulmonary case, and 1 (7.69%) was from a case showing both clinical forms. The animal isolates had (dog, cat, and guinea pig) lesions on the nose or skin and, to a lesser extent, the neurologic form (Table 1).

PCR–RFLP Analysis

PCR–RFLP analysis revealed that all the isolates belonged to genotype VGII. In previous reports, genotype VGI was considered the most frequent; however, currently, VGII has been the most commonly found genotype in global isolates [16]. This genotype is distributed worldwide and has been described as the most virulent strain responsible for infections in both immunocompetent and immunocompromised humans and animals [17, 18]. In animals, the importance of isolating this genotype can contribute to the identified the sentinel markers of the disease and verify that this genotype can adapt to several hosts, thus increasing its dispersion.

Associated with the outbreak on Vancouver Island, Canada, it is believed that the evolutionary origin of this molecular type may be traced to South America, adapting to the temperate zones [19–21]. Hagen et al. [18] reported that phylogenetic and recombination analysis (AFLP and SCAR-MLST) suggested that the oldest lineage originated in Brazil, where the Amazon region is the most plausible for the origin of the VGII genotype, causing outbreaks in British Columbia (Canada) and the Pacific Northwest (USA) and cases in Australia. The first VGII isolate (LMM 293) identified in Brazil was in the state of Rio de Janeiro in 1988, from a patient in the Northern region of the country [22]. The genotype was also described in the Northeast, South, Southeast, and Midwest Brazil [22–26].

Analysis of MLST Technique

Genotyping analysis of 21 isolates via MLST revealed a considerable genetic diversity in comparison with PCR–RFLP analysis. The presence of 14 STs was observed, of which 5 are considered new (ST 485, ST 486, ST 487, ST 488, and ST 489). These STs are distributed in 12 Clonal Complexes and four were "Singletons" (Fig. 1). Several genotypes were verified in Brazil and these new STs probably resulted from clonal propagation or genetic recombination [25, 27]. Furthermore, Lockart et al. [28] affirmed the existence of a large genetic diversity that according to Souto et al. [25], results from the ability to emerge from the original habitat, adaptation, and colonization of new environments and hosts.

The clonal complexes CC182 (n = 5; 23,80%) and CC309 (n = 3; 14.28%) were the most frequent. The distribution of CC, in relation to the sample origin, shows that three CCs with a predominance of CC182 (66,66%) in animals. In humans, nine CCs with a predominance of CC309 (15,38%), CC40 (15,38%), CC20(15,38%), and CC306 (15,38%) were observed. In the environment, only two CCs were observed (Fig. 2). CC182 and CC309 have been reported previously in Brazil; however, the CC associated with ST40 was the most frequent, as reported by Souto et al. [25]. In addition, these same authors affirmed that the Brazilian isolates do not show a population structure established in accordance with the geographic region, indicating that Brazilian regions are dominated by different genotypes.

Table 1 Characteristics of *C. gattii* species complex isolates during the years 2010–2017 from humans, animals, and the environment in the State of Mato Grosso, Brazil

Id isolates	Year	Source	Clinical signs	City	URA5-RFLP	MLST	CC
53	2012	Human	Neurocryptococcosis	Várzea Grande	VGII	20	20
MASC	2010	Human	Neurocryptococcosis	Cuiabá	VGII	20	20
3174	2012	Human	Neurocryptococcosis	Cuiabá	VGII	40	40
2416	2011	Human	Neurocryptococcosis	Cuiabá	VGII	46	*(46)
M953-16	2016	Human	Neurocryptococcosis	Cuiabá	VGII	182	182
3330	2013	Human	Pulmonary cryptococcosis	Cuiabá	VGII	306	Sg(306)
741-05	2010	Human	Neurocryptococcosis	Cuiabá	VGII	306	Sg(306)
987-08	2010	Human	Neurocryptococcosis	Cuiabá	VGII	309	*(309)
2285	2011	Human	Neurocryptococcosis	Cuiabá	VGII	309	*(309)
638-08	2010	Human	Pulmonary and Neurocryptococcosis	Cuiabá	VGII	316	*(316)
96-06	2010	Human	Cryptococcal meningitis	Cuiabá	VGII	485[b]	*(485)
623-06	2010	Human	Cryptococcal meningitis	Sinop	VGII	487[b]	40
741-06	2010	Human	Neurocryptococcosis	Cuiabá	VGII	488[ab]	*(488)
662	2011	Canine	Apathy and increased abdominal volume	Sinop	VGII	182	182
142	2012	Feline	Pulmonary cryptococcosis	Cuiabá	VGII	182	182
M638-17	2017	Guinea pig	Nasal injury	Cuiabá	VGII	182	182
865-11	2011	Canine	Skin injury	Cuiabá	VGII	489[b]	182
147	2012	Feline	Nasal injury	Cuiabá	VGII	309	*(309)
M226-16	2016	Feline	Skin injury and neurocryptococcosis	Cuiabá	VGII	486[ab]	Sg(486)
148C	2014	Public Library	Nd*	Cuiabá	VGII	264	Sg(264)
12	2010	Tree	Nd*	Cuiabá	VGII	310	Sg(310)

*Do not have founder defined

Nd* Nothing to declare

Sg Singletons

[a]New allele

[b]New ST

CC182 was isolated in four distinct species (human, feline, canine, and guinea pig) and is related to ST181 and the new ST489. One study reported that guinea pigs were naturally infected by genus *Cryptococcus* [29]. However, the molecular identification of genus *Cryptococcus* and its genotyping has not been described yet to complete the characterization process.

The occurrence of CC182 in tropical and temperate countries has been described by several authors in China, Caribbean Islands, and Guyana [15]. Moreover, according to Souto et al. [25], this worldwide distribution proves the proximity between many Brazilian STs and the STs present globally, demonstrating the capacity for expansion, recombination, and adaptation of these strains.

CC309 was isolated from two humans and one cat (ST309) and, had already been described in São Paulo [30]. This ST belongs to the same lineage as that of the new ST488, which was isolated from humans and is associated with a new allele for the *SOD1* gene.

The clonal complex formed by the founder ST20 is considered hypervirulent and has already been described in North America (related to the outbreak at Vancouver Island), Europe and South America, the Amazon region, and the Southeast region of Brazil (São Paulo state) [2, 25, 30–32]. In addition, it has been modified and expanded to several other regions, based on the appearance of new strains. Adaptation and/or microevolution in the environment may be associated with the high frequency of this complex in

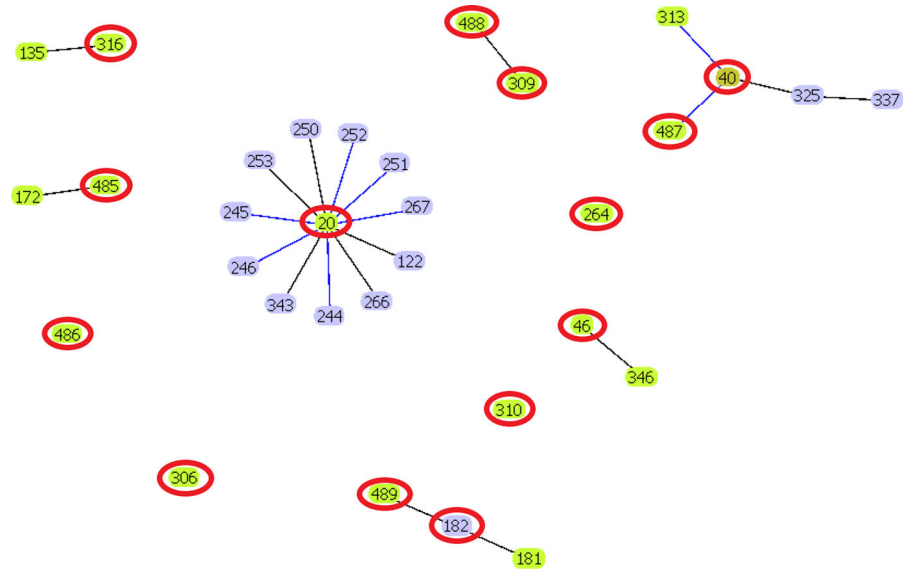

Fig. 1 Analysis of isolates from the State of Mato Grosso, using the goeBurst software showing the sequence typing, formation of the clonal complex, and respective lineages. Central STs: Light Green = founding group, Brown = founding subgroup; Light blue: common central ST. The samples, circled in red, are those included in this study

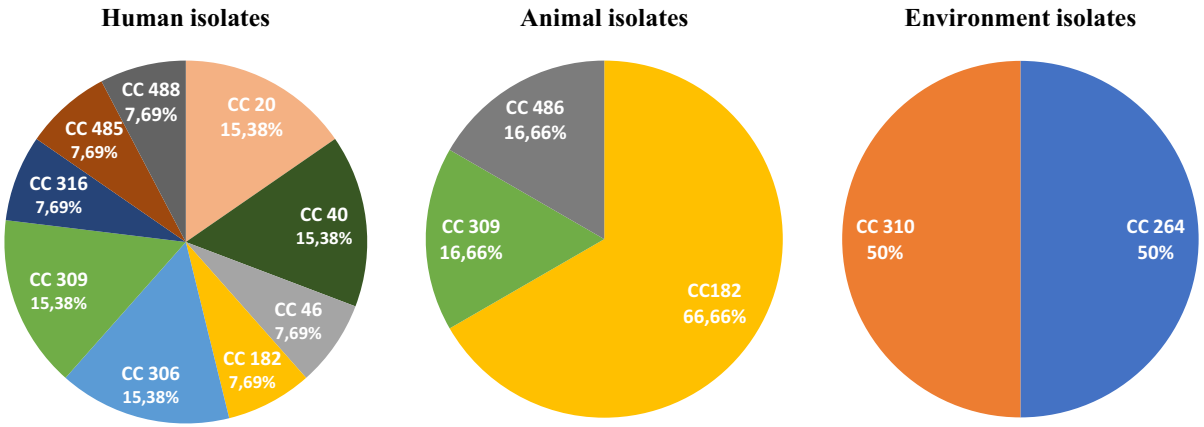

Fig. 2 Percentage distribution of the isolates of *C. gattii* VGII in relation to their origin and Clonal Complex (CC) in the State of Mato Grosso during the period from 2010 to 2017

the north of Brazil [25] and, the isolation of ST20 may be related to human migration or the proximity of the center west to the northern region (Amazonica).

In relation to the CC formed by the founder ST40, this has already been described in Bahia, São Paulo, Rio de Janeiro, and Mato Grosso do Sul [25, 30, 33], demonstrating the capacity for dispersion. Owing to potential genetic recombination, this complex has also been shown to be associated with the new lineage, ST487.

There was no defined founder in the group formed by ST46 and ST346; however, ST46 was already described in Norte de Santander (Colombia) [34] and in Amazonas (Brazil) [35]. ST316, described in humans [30] is related to ST135, which was isolated in the state of Mato Grosso do Sul (Brazil) [25]. Further, the new ST485 is in the same lineage as ST172, which was isolated from humans in Brasília (Brazil) [36].

The singleton isolates ST264, ST306, ST310, and ST486 were not correlated with any other genotype in the MLST database. However, in São Paulo, Brazil, ST264, ST306, and ST310 had already been reported in humans [30]. In addition, ST264 was also isolated

from environmental samples in Amazonas [32], as reported herein. A new allelic profile and singleton were discovered in this study, represented by ST486.

Similar to the study by Souto et al. [25], wide genetic diversity can generate highly virulent strains, either by factors such as changes in species composition, stress, climate change, or habitat, adaptation to regions of dry and humid climate, and variations in temperature. Thus, the State of Mato Grosso, comprising three biomes, Amazon, Cerrado, and Pantanal [37], may influence the clonal dispersion and/or recombination among the genotypes. Thus, the pathogenicity of the *C. gattii* species complex is probably related to its genetic diversity, global dispersion of isolates, and adaptation to different hosts [36].

Conclusion

Considerable genetic diversity of the *C. gattii* species complex was observed along with the appearance of news STs in MT. The higher frequency of CC309 and CC182 isolates, affecting both humans and animals, differed in the state of Mato Grosso from that in other regions studies, probably owing to the presence of different biomes in the region, which favor high genetic variability among isolates of the *C. gattii* species complex. This characteristic of the pathogen is a challenge for public health owing to the effect of this dispersion, virulence, and resistance on treatment. In addition, higher detection of neurologic cases in humans than in animals leads us to question the route of infection and the mechanism of action of this pathogen in the organisms of each species, thus indicating the need for further studies on the virulence of this complex.

Acknowledgements Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval This study was certificated of the Animal and Human Ethics Committee No. 888/CEP-HUJM/2012.

References

1. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, et al. Global burden of disease of HIV-associated cryptococcal meningitis: na updated analysis. Lancet Infect Dis. 2017;17:873–81. https://doi.org/10.1016/S1473-3099.
2. Singer LM, Meyer W, Firacative C, Thompson GR, Samitz E, Sykes JE. Antifungal drug susceptibility and phylogenetic diversity among Cryptococcus isolates from dogs and cats in North America. J Clin Microbiol. 2014;52:2061–70. https://doi.org/10.1128/JCM.03392-13.
3. 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. https://doi.org/10.1016/j.fgb.2015.02.009.
4. Kwon-Chung KJ, Bennett JE, Wickes BL, Meyer W, Cuomo CA, Wollenburg KR, Bicanic TA, et al. The case for adopting the "species complex" nomenclature for the etiologic agents of cryptococcosis. mSphere. 2017;2:e00357-16. https://doi.org/10.1128/msphere.00357-16.
5. Harris J, Lockhart S, Chiller T. *Cryptococcus gattii*: Where do we go from here? Med Mycol. 2012;50:113–29. https://doi.org/10.3109/13693786.2011.607854.
6. Kwon-Chung KJ, Fraser JA, Doering TL, Wang ZA, Janbon G, Idnurm A, et al. *Cryptococcus neoformans* and *Cryptococcus gattii*, the etiologic agents of cryptococcosis. Cold Spring Harb Perspect Med. 2014;4:1–27. https://doi.org/10.1101/cshperspect.a019760.
7. Stephen C, Lester S, Black W, Fyfe M, Raverty S. Multispecies outbreak of cryptococcosis on southern Vancouver Island, British Columbia. Can Vet J. 2002;43:792–4.
8. Espinel-Ingroff A, Kidd SE. Current trends in the prevalence of *Cryptococcus gattii* in the United States and Canada. Infect Drug Resist. 2015;8:89–97. https://doi.org/10.2147/IDR.S57686.
9. Shields AB, Ajello L. Medium for selective isolation of Cryptococcus neoformans. Science. 1966;151:208–9.
10. Del Poeta M, Toffaletti DN, Rude TH, Dykstra CC, Heitamn J, Perfec JR. Topoisomerase I is essential in Cryptococcus neoformans: role in pathobiology and as an antifungal target. Genet Soc Am. 1999;152:167–78.
11. Aoki FH, Imai T, Tanaka R, Mikami Y, Taguchi H, Nishimura NF, et al. New PCR primer pairs specific for *Cryptococcus neoformans s*erotype A or B prepared on the basis of random amplified polymorphic DNA fingerprint pattern analyses. J Clin Microbiol. 1999;37:315–20.
12. D'Souza C, Hagen F, Boekhout T, Cox GM, Heitman J. Investigation of the basis of virulence in serotype A strains of *Cryptococcus neoformans* from apparently immunocompetent individuals. Curr Genet. 2004;46:92–102. https://doi.org/10.1007/s00294-004-0511-y.
13. Meyer W, Castañeda A, Jackso S, Huynh M, Castañeda E, I.C.S. Group. Molecular typing of IberoAmerican *Cryptococcus neoformans* isolates. Emerg Infect Dis. 2003;9:189–95. https://doi.org/10.3201/eid0902.020246.
14. 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;47:561–70. https://doi.org/10.1080/13693780902953886.
15. Dou HT, Xu YC, Wang HZ, Li TS. Molecular epidemiology of *Cryptococcus neoformans* and *Cryptococcus gattii* in China between 2007 and 2013. Eur J Clin Microbiol Infect Dis. 2015;34:753–63. https://doi.org/10.1007/s10096-014-2289-2.
16. Chen SC, Meyer W, Sorrell TC. *Cryptococcus gattii* infections. Clin Microbiol Rev. 2014;27:980–1024. https://doi.org/10.1128/CMR.00126-13.
17. Springer DJ, Chatuverdi V. Projecting global occurrence of *Cryptococcus gattii*. Emerg Infect Dis. 2010;16:14–20. https://doi.org/10.3201/eid1601.090369.
18. Hagen F, Ceresini PC, Polacheck I, Ma H, Nieuwerburgh FV, Gabaldón T, et al. Ancient dispersal of the human fungal pathogen *Cryptococcus gattii* from the Amazon rainforest. PLoS ONE. 2013;8:e71148. https://doi.org/10.1371/journal.pone.0071148.
19. Kidd SE, Hagen F, Tscharke RL, Huynh M, Bartlett KH, Fyfe M, et al. A rare genotype of *Cryptococcus gattii* caused the cryptococcosis outbreak on Vancouver Island (British Columbia, Canada). Proc Natl Acad Sci USA. 2004;101:17258–63. https://doi.org/10.1073/pnas.0402981101.
20. Feng X, Yao Z, Ren D, Liao W, Wu J. Genotype and mating type analysis of *Cryptococcus neoformans* and *Cryptococcus gattii* isolates from China that mainly originated from non-HIV-infected patients. FEMS Yeast Res. 2008;8:930–8. https://doi.org/10.1111/j.1567-1364.2008.00422.x.
21. Ngamskulrungroj P, Gilgado F, Faganello J, Litvintseva AP, Leal AL, Tsui KM. Genetic diversity of the *Cryptococcus* species complex suggests that *Cryptococcus gattii* deserves to have varieties. PLoS ONE. 2009;4:e5862. https://doi.org/10.1371/journal.pone.0005862.
22. Trilles L, Lázera MS, Wanke B, Oliveira RV, Barbosa GG, Nishikawa MM, et al. Regional pattern of the molecular types of *C. neoformans* and *C. gattii* in Brazil. Memoria Instituto Oswaldo Cruz. 2008;103:455–62.
23. Casali AK, Goulart L, Silva LKR, Ribeiro AM, Amaral AA, Alves SH, et al. Molecular typing of clinical and environmental *Cryptococcus neoformans* isolates in the Brazilian state Rio Grande do Sul. FEMS Yeast Res. 2003;3:405–15. https://doi.org/10.1016/S1567-1356(03)00038-2.
24. Paula DAJ, Almeida ABPF, Cruz FS, Furlan FH, Colodel EM, Sousa VRF, et al. Occurrence and molecular characterization of cryptococcosis in dogs and cats in Mato Grosso, Brazil. Pesquisa Veterinária Brasileira. 2014;34:167–72.
25. Souto ACP, Bonfietti LX, Ferreira-Paim K, Trilles L, Martins M, Ribeiro-Alves M, et al. Population genetic analysis reveals a high genetic diversity in the brazilian *Cryptococcus gattii* VGII population and shifts the global origin from the amazon rainforest to the semi-arid desert in the northeast of Brazil. PLOS Negl Trop Dis. 2016;10:1–19. https://doi.org/10.1371/journal.pntd.0004885.
26. Silva EC, Guerra JM, Torres LN, Lacerda AMD, Gomes RG, Rodrigues DM, et al. *Cryptococcus gattii* molecular type VGII infection associated with lung disease in a goat. BMC Vet Res. 2017;13:1–6. https://doi.org/10.1186/s12917-017-0950-6.
27. Barcellos VA, Martins LMS, Fontes ACL, Rewsaat JCV, Squizani WD, Araújo GRS, et al. Genotypic and phenotypic diversity of *Cryptococcus gattii* VGII clinical isolates and its impact on virulence. Front Microbiol. 2018;9:132. https://doi.org/10.3389/fmicb.2018.00132.
28. Lockhart SR, Igbal N, Harris JR, Grossman NT, Debess E, Wohrle R, et al. *Cryptococcus gattii* in the United States: genotypic diversity of human and veterinary isolates. PLoS ONE. 2013;8:e74737. https://doi.org/10.1371/journal.pone.0074737.
29. Herck HV, Ingh TSGAMVD, Hage MHVD, Zwart P. Dermal cryptococcosis in a guineapig. Lab Anim. 1988;22:88–91.
30. Bonfietti LX, Pham CD, Szeszs MW, Silva DC, Martins MA, Lockhart SR, et al. Diversidade de isolados clínicos de *Cryptococcus gattii* por *multi-locus sequence typing* no Estado de São Paulo. Boletim do Instituto Adolfo Lutz. 2014;24:36–9.
31. Carriconde F, Gilgado F, Arthur I, Ellis D, Malik R, Wiele NV, et al. Clonality and a—a recombination in the Australian *Cryptococcus gattii* VGII population—an emerging outbreak in Australia. PLoS ONE. 2011;6:e16936. https://doi.org/10.1371/journal.pone.0016936.
32. Brito-Santos F, Barbosa GG, Trilles L, Nishikawa MM, Wanke B, Meyer W, et al. Environmental isolation of *Cryptococcus gattii* VGII from indoor dust from typical wooden houses in the deep Amazonas of the Rio Negro basin. PLoS ONE. 2015;10:e0115866. https://doi.org/10.1371/journal.pone.0115866.
33. Engelthaler DM, Hicks ND, Gillece JD, Roe CC, Schupp JM, Driebe EM, et al. *Cryptococcus gattii* in North American Pacific Northwest: whole-population genome analysis provides insights into species evolution and dispersal. MBio. 2014;5:e0146414. https://doi.org/10.1128/mBio.01464-14.
34. Lizazaro J, Escandón P, Agudelo CI, Firacative C, Meyer W, Castañeda E. Retrospective study of the epidemiology and clinical manifestations of *Cryptococcus gattii* infections in Colombia from 1997 to 2011. PLoS Negl Trop Dis. 2014;8:e3272. https://doi.org/10.1371/journal.pntd.0003272.
35. Alves GSB, Freire AKL, Bentes AS, Pinheiro JFS, Souza JVB, Wanke B. Original article molecular typing of environmental *Cryptococcus neoformans/C. gattii* species complex isolates from Manaus, Amazonas, Brazil. Mycoses. 2016;59:509–15. https://doi.org/10.1111/myc.12499.
36. Hagen F, Colom MF, Swinne D, Tintelnot K, Iatta R, Montagna MT, et al. Autochthonous and dormant *Cryptococcus gattii* infections in Europe. Emerg Infect Dis. 2012;18:1618–24. https://doi.org/10.3201/eid1810.120068.
37. IBGE. Mapas de biomas do Brasil: escala 1:5.000.000. 2017. Disponível em. http://mapas.ibge.gov.br/biomas2/viewer.htm. Acesso em 02 maio 2017.