Domestic Birds as Source of Cryptococcus deuterogattii (AFLP6/VGII): Potential Risk for Cryptococcosis

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Abstract Cryptococcosis is an infection caused by encapsulated basidiomycetous yeasts belonging to the Cryptococcus neoformans/Cryptococcus gattii species complexes. It is acquired through inhalation of infectious propagules, often resulting in meningitis and meningoencephalitis. The ecological niche of these agents is a wide variety of trees species, as well as pigeon, parrot and passerine excreta. The objective of this study was to isolate Cryptococcus yeasts from excreta of commercially traded parrots and passerines. The 237 samples were collected between October 2018 and April 2019 and processed using conventional methodologies. Nineteen colonies with a dark brown phenotype, caused by phenol oxidase activity, were isolated, suggesting the presence of pathogenic Cryptococcus yeasts. All isolates tested positive for urease activity. URA5-RFLP fingerprinting identified 14 isolates (68.4%) as C. neoformans (genotype AFLP1/VNI) and 5 (26.3%) as C. deuterogattii (genotype AFLP6/VGII). Multi-locus sequence typing was applied to investigate the relatedness of the C. deuterogattii isolates with those collected globally, showing that those originating from bird-excreta were genetically indistinguishable from some clinical isolates collected during the past two decades.

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Introduction

Cryptococcosis is a fungal infection in animals and humans caused by encapsulated basidiomycetous yeasts belonging to the Cryptococcus neoformans and Cryptococcus gattii species complexes [1, 2]. By inhalation of infectious propagules, the spores, or desiccated yeast cells, reach the pulmonary alveoli and evolve into the pulmonary form of the disease and ultimately spread to the central nervous system causing meningitis or meningoencephalitis [2, 3].

The C. neoformans species complex comprises the two species C. neoformans (serotype A; genotypes AFLP1/VNI, AFLP1A/VNB/VNII, and AFLP1B/VNII), C. deneoformans (serotype D, genotype AFLP2/VNIV) and their hybrids (serotype AD, genotype AFLP3/VNIII) [1]. The C. gattii species complex includes five pathogenic species: C. gattii sensu stricto (serotype B, genotype AFLP4/VGI), C. bacillisporus (serotype B and C, genotype AFLP5/VGIII), C. deuterogattii (serotype B, genotype AFLP6/VGII), C. tetragattii (serotype C, genotype AFLP7/VGIV) and C. decagattii (serotype B, genotype AFLP10/VGVI) [1, 2].

Cryptococcus species have been isolated from several ecological niches, such as soil, pigeon droppings and debris in tree holes, and new reservoirs are reported. Environmental sampling attributes to a better understanding of the epidemiology of the disease. The C. neoformans species complex has a cosmopolitan distribution and is primarily causing disease in immunocompromised individuals, such as HIV-infected subjects [1, 2]. The C. neoformans species complex is associated with organic components in the excreta of pigeons, captive birds, dust and decaying trees of various species [4]. The members of the C. gattii species complex are emerging pathogens and were initially considered as ‘tropical and subtropical pathogens’ [5]. However, studies from the past two decades showed that infections and environmental occurrence has expanded to temperate regions, including North America and the north-western part of Europe [6, 7]. In addition, many ecological niches have been investigated worldwide in an attempt to elucidate the environmental reservoirs [7, 8]. In Brazil C. gattii sensu lato has been identified from hollow trees [9, 10] and even dust in houses and libraries [11, 12].

Epidemics caused by members of C. gattii species complex have been described and ranging from local small outbreaks affecting goats (Spain) [13], sheep (Australia) [14], and parrots in an aviary in São Paulo, Brazil [15]. However, the largest C. deuterogattii outbreak so far was first reported early 2000’s from Vancouver Island (B.C. Canada) and has expanded since then to the Pacific Northwest of the U.S.A. [16].

Environmental studies conducted in the state of Mato Grosso, Brazil, have identified C. neoformans and C. gattii species complexes from different ecological niches [9, 12, 17] as well as clinical isolates from humans and animals [18, 19] contributing to elucidate the epidemiology of cryptococcosis. The aim of this study was to investigate the presence of Cryptococcus yeasts in the excreta of captive birds such as parrots and passerines.

Materials and Methods

Study Site

Brazil’s third largest state of Mato Grosso (latitude -13° 0’0” S and longitude -56° 0’0” O) covers an area of 903,206,997 km² in the midwestern region with Cuiabá being its capital city. Mato Grosso, with approximately 3.5 million inhabitants, has three of the Brazilian main ecological systems: The Amazon Forest, Cerrado and Pantanal. The climate is tropical, with rainfall during summer, low humidity during winter, and temperatures ranging from 24 °C to 40 °C [20].

Sample Collection and Isolation

Two-hundred thirty seven excreta samples were taken from freestanding cages containing a single species of parrots or passerines, collection was performed between October 2018 and April 2019 in Campo Verde, Várzea Grande and Cuiabá. Samples were sent to the Laboratory of Medical Mycology/Research at the Federal University of Mato Grosso (UFMT), where processing was performed according to the
protocols described previously by Filiu and co-workers [21] and Lazéra and colleagues [22] with few modifications. Briefly, two grams of the sample was suspended in 8 mL of distilled water and 0.4 g of chloramphenicol was added. The samples were thereafter homogenized and allowed to settle for 1 h. From each sample, 100 μL of the supernatant was seeded onto ten Niger Seed Agar (NSA) plates that were supplemented with chloramphenicol (0.4 g/L) and amikacin (120 μL/L). Media were incubated at 35 °C for 72 h. Thereafter, media were examined for brown colonies suggestive of being members of the *C. neoformans/C. gattii* species complexes. All isolate with characteristics suggestive for being *Cryptococcus* were inoculated onto urea medium and incubated at 35 °C for 72 h and examined [23].

Molecular Characterization

DNA extraction was performed according to the protocol described by Del Poeta and colleagues [24]. DNA was stored at –20 °C until further use. Cryptococcal isolates were genotyped using URA5-RFLP according to the methodology described by Meyer and co-workers [25]. The URA5 amplicons were overnight digested at 37 °C with Sau96I (10 μL) and Hhal (20 μL) endonucleases (New England Biolabs), fingerprints were visualized onto a 3% agarose gel that included the reference strains for each of the molecular types [25]. Isolates that were identified as *C. deuterogattii* were further molecularly investigated by multi-locus sequence typing (MLST) as previously described [26]. Obtained sequence-data was added to a reference set of *C. deuterogattii* MLST data representing all known sequence types for this species, subsequently phylogenetic analysis was performed in MEGA v7 using the maximum likelihood method as previously described [26–28].

Results

Among the 237 collected excreta samples, 142 (59.9%) originated from the order of parrots and 95 (40.1%) from passerines, comprising 16 genera and 17 species. The majority of samples came from cockatiels (*Nymphicus hollandicus*) (n = 71/237), followed by the Atlantic canaries (*Serinus canarius*) (n = 50/237) and budgerigars (*Melopsittacus undulatus*) (n = 33/237). Samples were collected in the capital Cuiabá (175/237; 73.8%), 39/237 (16.5%) in Campo Verde and 23/237 (9.7%) in Várzea Grande (Table 1).

Out of the 237 samples collected and plated onto NSA, 19 (8.0%) isolates showed morphological characteristics suggestive for members of the *C. neoformans/C. gattii* species complexes, as they had dark brown coloration of the colonies due to phenol oxidase production. These isolates were all urease positive. The molecular types of the isolates were determined by URA5-RFLP. Fourteen out of nineteen isolates (73.2%) were *C. neoformans* sensu stricto molecular type VNI (= genotype AFLP1/VNI) and five (26.3%) were *C. deuterogattii* molecular type VGII (= genotype AFLP6/VGII). For *C. neoformans* sensu stricto, cockatiels (*N. hollandicus*) showed a greater number of positive results (n = 12, 85.6%), followed by Red rumped parrots (*P. haematotus*) and the Atlantic canary (*S. canarius*), both represented by one isolate (each 7.2%). On the other hand, *C. deuterogattii* was obtained from cockatiels (*N. hollandicus*) excreta (n = 4; 80%) and Bourke’s parrot (*N. bourkii*) excreta (n = 1; 20%). The maximum likelihood phylogenetic analysis of the MLST data showed that all five *C. deuterogattii* isolates were genetically indistinguishable from each other and from clinical isolates collected up to 2 decades ago from Brazil, Caribbean Islands, France, French Guiana and China (Fig. 1). Sequences were deposited in NCBI GenBank under accession numbers MZ393809-MZ393843 (Table 2).

Discussion

The natural habitat of members of the *C. neoformans* and *C. gattii* species complexes has been extensively studied, especially in areas where the incidence of cryptococcosis is relatively high [5, 28]. The primary ecological niche of *C. neoformans* sensu stricto was repetitively found to be bird excreta, especially pigeon excreta. Three decades ago, *C. gattii* sensu lato was isolated from plant debris under a *Eucalyptus camaldulensis* tree in Australia [29]. The distribution pattern of *E. camaldulensis* was associated with the relatively high proportion of cryptococcal infections among rural aboriginals, compared to other areas [30].
Since then, the investigation of *C. neoformans/C. gattii* species complex members has been carried out in a large variety of niches, which repetitively showed that *C. neoformans* sensu stricto was mostly isolated from bird excreta while *C. gattii* sensu lato has been associated with tree/plant debris [9–13].

In Brazil, isolation of *Cryptococcus* yeast species from captive birds’ excreta has been reported from the states of Paraná, Rio de Janeiro, Pará and Mato Grosso do Sul [17, 21, 31–35]. While analyzing the number of isolates obtained in the current study, a greater number of positive samples were observed from cockatiel excreta (*N. hollandicus*). This species is popular, and more expensive, among the commercialized birds. In contrast, Pereira and colleagues [35] reported a low isolation rate for this species while more isolates were obtained from the excreta of the budgerigar (*Melopsittacus undulatus*). On the other hand, Lugarini and co-workers [32] obtained a higher number of isolates from Saffron finches (*Sicalis flaveola*) and it was observed that the yeasts may be distributed via excreta of Psittaciformes and Passeriformes regardless of the bird species.

In the current study, 13 out of 19 (72.2%) isolates were *C. neoformans* sensu stricto and were isolated from Psittaciformes and Passeriformes excreta. This corroborates results from the studies by Lugarini and colleagues [32] performed in the state of Paraná where 25.5% (*n = 36/141*) were *C. neoformans* sensu stricto, and Passoni and co-workers [33] performed in the city of Rio de Janeiro where 4.3% (*n = 54/1,268*) of the samples were *C. neoformans* sensu lato positive. Other studies have shown higher numbers of *C. neoformans* sensu stricto isolates from pigeon excreta in the city of Belém [31], and Campo Grande [21]. In Cuiabá, *C. neoformans* sensu stricto was also identified, mostly from niches such as pigeon excreta at various locations in the city as reported by Takahara and co-workers [17].

Cryptococcosis, an opportunistic fungal infection, is often diagnosed among HIV/AIDS patients, and is also a major cause of morbidity and mortality, with *C. neoformans* sensu stricto molecular type VNI being the most prevalent worldwide among clinical and environmental strains [1, 35]. This holds true for Brazil, where *C. neoformans* sensu stricto VNI predominates in clinical isolates among HIV-infected patients, mainly in the Southern, Southeastern and Midwestern regions [4, 36, 37].

It was believed that the River red gum tree (*Eucalyptus camaldulensis*) was the niche of *C. gattii* sensu lato, consequently others investigated these

| Bird Order | Species name | Total | Percentage |
|------------|--------------|-------|------------|
| Cockatiel  | Psittaciformes | *Nymphicus hollandicus* | 71 | 30 |
| Atlantic canary | Passeriformes | *Serinus canarius* | 50 | 21 |
| Budgerigar | Psittaciformes | *Melopsittacus undulatus* | 33 | 11 |
| Lovebird | Psittaciformes | *Agapornis* species | 23 | 10 |
| Ring-necked parakeet | Psittaciformes | *Psittacula krameri* | 13 | 6 |
| Eastern rosella | Psittaciformes | *Platycercus eximius* | 7 | 4 |
| Grey parrot | Psittaciformes | *Psittacus erithacus* | 6 | 2.6 |
| Gouldian finch | Passeriformes | *Erythrura gouldiae* | 6 | 2.6 |
| Zebra finch | Passeriformes | *Taeniopygia guttata* | 6 | 2.6 |
| Java sparrow | Passeriformes | *Padda oryzivora* | 4 | 1.8 |
| Society finch | Passeriformes | *Lonchura striata domestica* | 4 | 1.7 |
| Bourke’s parrot | Psittaciformes | *Neopsephotus bourkii* | 4 | 1.7 |
| Red-rumped parrot | Psittaciformes | *Psophotus haematontotus* | 3 | 1.5 |
| American kestrel | Falconiformes | *Falco sparverius* | 2 | 1 |
| Alexandrine parakeet | Psittaciformes | *Psittacula eupatria* | 2 | 1 |
| Rock dove | Columbiformes | *Columba livia* | 1 | 0.5 |
| Plum-headed parakeet | Psittaciformes | *Psittacula cyanocephala* | 1 | 0.5 |
| Barred parakeet | Psittaciformes | *Bolborhynchus lineola* | 1 | 0.5 |
| Total | – | – | 237 | 100 |
Fig. 1 Multi-locus sequence typing-based phylogenetic analysis of Cryptococcus deuterogattii isolates. Maximum likelihood phylogenetic analysis was performed in MEGA v7 using settings as previously described [26–28]. The tree with the highest log likelihood (−12,291.85) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.1000)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 43.83% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 270 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 3997 positions in the final dataset. The five C. deuterogattii isolates from the current study are indicated with a green circle, other Brazilian C. deuterogattii isolates reported previously are demarcated with a dark blue triangle, while bright blue triangles indicate isolates that originated from other Latin American countries. Isolates WM161, IHEM14941S, WM179 and WM779 served as outgroup, representing C. bacillisporus, C. decagattii, C. gattii sensu stricto and C. tetragattii reference strains, respectively.
trees in other countries such as India, United States, Mexico [30, 38, 39]. That only E. camaldulensis was the single niche for C. gattii sensu lato has been disputed successfully by others. For example, Laze´ra and colleagues reported the isolation of two cryptococcal species, C. deneoformans (cited as C. neoformans var. neoformans) and C. gattii sensu lato (cited as C. neoformans var. gattii) from a single Cassia javanica tree in the city of Teresina (Brazil). And large-scale environmental screening for C. deuterogattii in British Columbia (Canada) showed that (decaying) trees in general are the primary niche [8, 40].

Cryptococcus gattii sensu lato is rarely isolated from excreta of captive birds, however, the present study observed 26.3% (n = 5/19) positive samples harbouring C. deuterogattii. Which differs from the study by Abegg and colleagues who isolated C. gattii sensu stricto (genotype AFLP4/VGI) from bird excreta [41]. Cryptococcus deuterogattii in this study came from samples collected from cockatiel excreta at two locations, namely a veterinary clinic and from a small farm. From the latter location samples were collected from six tree holes near the bird cages, but these samples yielded no cryptococcal growth. A case cluster caused by C. gattii sensu lato yeasts has been reported to have affected Psittaciformes species in an aviary zoo in the state of S˜ao Paulo [15]. Yeasts from the nasal region, excreta and liver were isolated from one of the birds and identified as serotype B [15] and subsequently confirmed by molecular tools as being C. deuterogattii [42].

Studies conducted in the city of Cuiabá showed the presence of C. deuterogattii, the agent causing the ongoing and expanding outbreak on Vancouver Island [41], in library dust [12], in tree holes (Plathymenia reticulata) located in the central urban area of the city [9], in clinical isolates mostly from immunocompetent patients and also from HIV/AIDS patients [4]. The

Table 2  Distribution of Cryptococcus neoformans/Cryptococcus gattii species complexes isolated from bird excreta in commercial establishments

| Sample ID* (CFP accession nr.)* | Popular name | Scientific name | Cryptococcus species (molecular type) |
|-------------------------------|--------------|----------------|-------------------------------------|
| E5G2 (CFP00952)               | Atlantic canary | S. canarius | C. neoformans (VNI)                 |
| E9G4A (CFP00984)              | Cockatiel | N. hollandicus | C. deuterogattii (VGI)               |
| E9G4B (CFP00985)              | Cockatiel | N. hollandicus | C. deuterogattii (VGI)               |
| E9G5 (CFP00986)               | Bourke’s parrot | N. bourkii | C. deuterogattii (VGI)               |
| E9G6 (CFP00953)               | Cockatiel | N. hollandicus | C. deuterogattii (VGI)               |
| E9G7 (CFP00954)               | Cockatiel | N. hollandicus | C. deuterogattii (VGI)               |
| E9G8 (CFP00955)               | Cockatiel | N. hollandicus | C. neoformans (VNI)                 |
| E9G9 (CFP00956)               | Cockatiel | N. hollandicus | C. neoformans (VNI)                 |
| E9G11 (CFP00957)              | Cockatiel | N. hollandicus | C. neoformans (VNI)                 |
| E9G14 (CFP00958)              | Cockatiel | N. hollandicus | C. neoformans (VNI)                 |
| E9G15 (CFP00959)              | Cockatiel | N. hollandicus | C. neoformans (VNI)                 |
| E9G16 (CFP00960)              | Red rumped parrot | P. haematonotus | C. neoformans (VNI)                 |
| E9G17 (CFP00961)              | Cockatiel | N. hollandicus | C. neoformans (VNI)                 |
| E9G19 (CFP00962)              | Cockatiel | N. hollandicus | C. neoformans (VNI)                 |
| E9G20 (CFP00963)              | Cockatiel | N. hollandicus | C. neoformans (VNI)                 |
| E9G21 (CFP00987)              | Cockatiel | N. hollandicus | C. neoformans (VNI)                 |
| E9G24 (CFP00988)              | Cockatiel | N. hollandicus | C. neoformans (VNI)                 |
| E9G34 (CFP00989)              | Cockatiel | N. hollandicus | C. neoformans (VNI)                 |
| E9G35 (CFP00990)              | Cockatiel | N. hollandicus | C. neoformans (VNI)                 |

*E = Establishment/store; G = collection cage
# = FIOCRUZ culture collection accession number

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current study records the first isolation of *C. deutero-gattii* from Psittaciiformes and Passeriformes excreta.

Other environmental studies that reported isolation of bird-associated *C. gattii* sensu lato isolates [15, 21, 31, 32, 41], obtained cryptococcal isolates from excreta but it could not be excluded that the excreta was from other bird species. The isolation of members of the *C. neoformans/C. gattii* species complexes from bird excreta does not mean that a particular bird species has a specific role as a reservoir. Nevertheless, it indicates that bird excreta contributes to the aerial dispersion of infectious *Cryptococcus* propagules, allowing its transmission to humans and other mammals [43]. Captive bird breeders’ staff, who frequently perform daily cage cleaning and bird grooming, may be exposed to high concentrations of infectious propagules that enables the acquisition of a cryptococcal infection.

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**Conflict of interest** None declared.

**Ethical Approval** Not applicable.

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**References**

1. Hagen F, Khayhan K, Theelen B, Kolecka A, Polacheck I, Sionov E, Falk R, Parmen S, Lumbsch HT, Boekhout T. 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.

2. Francisco EC, de Jong AW, Hagen F. Cryptococcosis and *Cryptococcus*. Mycopathologia. 2021;186(5):729–731. https://doi.org/10.1007/s11046-021-00577-7.

3. Velagapudi R, Hseuh YP, Geunes-Boyer S, Wright JR, Heitman J. Spores as infectious propagules of *Cryptococcus neoformans*. Infect Immun. 2009;77(10):4345–55. https://doi.org/10.1128/IAI.00542-09.

4. Favalessa OC, de Paula DA, Dutra V, Nakazato L, Tadano T, LazeraMdos S, Wanke B, Trilles L, Walderez SM, Silva D, Hahn RC. Molecular typing and in vitro antifungal susceptibility of *Cryptococcus* spp from patients in Midwest Brazil. J Infect Dev Ctries. 2014;8:1037–43. https://doi.org/10.3855/jidc.4446.

5. Springer DJ, Chaturvedi V. Projecting global occurrence of *Cryptococcus gattii*. Emerg Infect Dis. 2010;16(1):14–20. https://doi.org/10.3201/eid1601.090369.

6. Acheson ES, Galanis E, Bartlett K, Klinkenberg B. Climate classification system-based determination of temperate climate detection of *Cryptococcus gattii* sensu lato. Emerg Infect Dis. 2019;25(9):1723–6. https://doi.org/10.3201/eid2509.181884.

7. Cogliati M, D’Amicis R, Zani A, Montagna MT, Caggiano G, De Giglio O, Balbino S, De Donno A, Serio F, Susever S, Ergin C, Velegraiki A, Ellabib MS, Nardoni S, Macci C, Oliveri S, Trovato L, Dipinetto L, Rickerts V, McCormick-Smith I, Akcaglar S, Tore O, Mlinaric-Missoni E, Bertout S, Malliê M, Martins MD, Vencà AC, Vieira ML, Sampaio AC, Pereira C, Criseo G, Romeo O, Ranque S, Al-Yasiri MH, Kaya M, Cerckicgulo N, Marchese A, Vezzulli L, Ilkît M, Desnos-Olliver M, Pasquale V, Korem M, Polacheck I, Scopa A, Meyer W, Ferreira-Paim K, Hagen F, Theelen B, Boekhout T, Lockhart SR, Tintelnot K, Tortorano AM, Drometer F, Varmà A, Kwon-Chung KJ, Inàcio J, Alonso B, Colomb MF. Environmental distribution of *Cryptococcus neoformans* and *C. gattii* around the Mediterranean basin. FEMS Yeast Res. 2016. https://doi.org/10.1093/lemsy/fow045.

8. Kidd SE, Chow Y, Mak S, Bach PJ, Chen H, Hingston AO, Kronstad JW, Bartlett KH. Characterization of environmental sources of the human and animal pathogen *Cryptococcus gattii* in British Columbia, Canada, and the Pacific Northwest of the United States. Appl Environ Microbiol. 2007;73(5):1433–43.

9. Anzai MC, Lazera Mdos S, Wanke B, Trilles L, Dutra V, de Paula DA, Nakazato L, Takahara DT, Simi WB, Hahn RC. *Cryptococcus gattii* VGII in a *Platymenia reticulata* hollow in Cuiabá, Mato Grosso, Brazil. Mycoses. 2014;57:414–8. https://doi.org/10.1111/myc.12177.

10. Fortes ST, Lazéra MS, Nishikawa MM, Macedo RC, Wanke B. First isolation of *Cryptococcus neoformans* var. *gattii* from a native jungle tree in the Brazilian Amazon rainforest. Mycoses. 2001;44(5):137–40.

11. Brito-Santos F, Barbosa GG, Trilles L, Nishikawa MM, Wanke B, Meyer W, Carvalho-Costa FA, Lazera MS. 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(2):e0115866. https://doi.org/10.1371/journal.pone.0115866.

12. Leite DP Jr, Amadio JV, Martins ER, Simões SA, Yama-moto AC, Leal-Santos FA, Takahara DT, Hahn RC. *Cryptococcus* spp isolated from dust microhabitat in Brazilian
libraries. J Occup Med Toxicol. 2012;7:11. https://doi.org/10.1186/1745-6673-7-11.

13. Colom MF, Hagen F, Gonzalez A, Mellado A, Morera N, Linares C, Garcia DF, Peñatko JS, Boekhout T, Sánchez M. Ceratonia siligua (carob) trees as natural habitat and source of infection by Cryptococcus gattii in the Mediterranean environment. Med Mycol. 2012;50(1):67–73. https://doi.org/10.3109/13693786.2011.574239.

14. McGill S, Malik R, Saul N, Beets S, Secombe C, Robertson I, Irwin P. Cryptococcus in domestic animals in Western Australia: a retrospective study from 1995–2006. Med Mycol. 2009;47(6):625–39. https://doi.org/10.1080/1369378080215219.

15. Raso TF, Werther K, Miranda ET, Mendes-Giannini MJ. Cryptococcosis outbreak in psittacine birds in Brazil. Med Mycol. 2004;42(4):355–62.

16. Byrnes EJ 3rd, Bartlett KH, Perfect JR, Heitman J. Cryptococcus gattii: an emerging fungal pathogen infecting humans and animals. Microbes Infect. 2011;13(11):895–907. https://doi.org/10.1016/j.micinf.2011.05.009.

17. Takahara DT, Lázera Mdos S, Wanke B, Trilles L, Dutra V, Paula DA, Nakazato L, Anzai MC, Leite Júnior DP, Paula CR, Hahn RC. First report on Cryptococcus neoformans in pigeon excreta from public and residential locations in the metropolitan area of Cuiabá, state of Mato Grosso, Brazil. Rev Inst Med Trop S Paulo. 2013;55:371–6. https://doi.org/10.1590/S0036-46552013000600001.

18. Favalessa OC, de Paula DA, Dutra V, Nakazato L, Tadano T, Lázera Mdos S, Wanke B, Trilles L, Walderez Szeszs M, Silva D, Hahn RC. Molecular typing and in vitro antifungal susceptibility of Cryptococcus spp from patients in Midwest Brazil. J Infect Dev Ctries. 2014;8(8):1037–43. https://doi.org/10.3855/jidc.4446.

19. Favalessa OC, Ribeiro LC, Tadano T, Fontes CJF, Dias FB, Coelho BPA, Hahn RC. Primeira descrição da caracterização fenotípica e susceptibilidade in vitro a drogas de leveduras do gênero Cryptococcus spp isoladas de pacientes HIV positivos e negativos, Estado de Mato Grosso. Rev Soc Bras Med Trop. 2009;42:661–5.

20. IBGE - Instituto Brasileiro de Geografia e Estatística, Cidade e Estados. https://www.ibge.gov.br/cidades-e-estados/mt/html/ Last accessed on December 21st, 2019.

21. Filut WFDO, Wanke B, Aguiña SM, Vilela VO, Macedo RCL, Lázera M. Avian habitats as sources of Cryptococcus neoformans in the city of Campo Grande, Mato Grosso do Sul, Brazil. Rev Socied Bras Med Trop. 2002;35:591–5.

22. Lázera M, Cavalcanti M, Trilles L, Nishikawa M, Wanke B. Cryptococcus neoformans var. gattii—evidence for a natural habitat related to decaying wood in a pottery tree hollow. Med Mycol. 1998;36:119–22.

23. Christensen WB. Urea decomposition as a means of differentiating Proteus and paracolon cultures from each other and from Salmonella and Shigella types. J Bacteriol. 1946;52:461.

24. Del Poeta M, Toffaletti DL, Rude TH, Dykstra CC, Heitman J, Perfect JR. Topoisomerase I is essential in Cryptococcus neoformans: role in pathobiology and as an antifungal target. Genetics. 1999;152:167–78.

25. Meyer W, Castañeda A, Jackson S, Huynh M, Castañeda E, IberoAmerican Cryptococcal Study Group. Molecular typing of IberoAmerican Cryptococcus neoformans isolates. Emerg Infect Dis. 2003;9:189–95.

26. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7 for bigger datasets. Mol Biol Evol. 2016;33:1870–4. https://doi.org/10.1093/molbev/msw054.

27. Hagen F, Colom MF, Swinne D, Tintelnot K, Iatta R, Montagna MT, Torres-Rodríguez JM, Cogliati M, Velegraki A, Burgraff A, Kamermans A, Sweere J, Meis JF, Klaassen CH, Boekhout T. Autochthonous and dormant Cryptococcus gattii infections in Europe. Emerg Infect Dis. 2012;18:1618–24. https://doi.org/10.3201/eid1810.120068.

28. Filiu WFDO, Wanke B, Aguêna SM, Vilela VO, Macedo MP, Rodriguez Garcia E, Garcia-Benayas E, Rojo-Amigo R, Rodrigue-Galleco JC, Hagen F, Colom MF. Successful isavuconazole salvage therapy for a Cryptococcus deuterogattii (AFLP6/VGII) disseminated infection in a European immunocompetent patient. Mycopathologia. 2021;186:507–18. https://doi.org/10.1007/s11046-021-00566-w.

29. Ellis DH, Pfeiffer TJ. Natural habitat of Cryptococcus neoformans var. gattii. J Clin Microbiol. 1990;28:1642–4.

30. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender N, Robertson I, Irwin P. Cryptococcosis in domestic animals in South Africa. J Clin Microbiol. 2004;42(4):355–62.

31. Cuetara MS, Jusdado Ruiz-Capillas JJ, Nunez-Valentin MP, Rodriguez Garcia E, Garcia-Benayas E, Rojo-Amigo R, Rodriguez-Galleco JC, Hagen F, Colom MF. Successful isavuconazole salvage therapy for a Cryptococcus deuterogattii (AFLP6/VGII) disseminated infection in a European immunocompetent patient. Mycopathologia. 2021;186:507–18. https://doi.org/10.1007/s11046-021-00566-w.

32. Passoni LFC, Wanke B, Nishikawa MM, Laze´ra MS. Cryptococcus neoformans molecular type VGII and Cryptococcus neoformans molecular type VNI from environmental sources in the city of Belém, Pará, Brazil. Mem Inst Oswaldo Cruz. 2009;104:662–4. https://doi.org/10.1590/S0074-02681219880000481.

33. Pereira JR, Campos FL, de Abreu DPB, de Assis BF, Ferreira FM, Vainstein MH. Cryptococcus neoformans isolated from Passerine and Psittacine bird excreta in the state of Paraná, Brazil. Mycopathol. 2008;166:61–9. https://doi.org/10.1007/s11046-008-9122-3.

34. Lugarini C, Goebel CS, Condas LAZ, Muro MD, de Farias MR, Ferreira FM, Vainstein MH. Cryptococcus neoformans isolated from Passerine and Psittacine birds in the city of Belém, Pará, Brazil. Mem Inst Oswaldo Cruz. 2019;114:1. https://doi.org/10.1590/S0074-026820090000400023.

35. Rodrı´guez Garcia E, Garcia-Benayas E, Rojo-Amigo R, Rodriguez-Galleco JC, Hagen F, Colom MF. Successful isavuconazole salvage therapy for a Cryptococcus deuterogattii (AFLP6/VGII) disseminated infection in a European immunocompetent patient. Mycopathologia. 2021;186:507–18. https://doi.org/10.1007/s11046-021-00566-w.

36. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7 for bigger datasets. Mol Biol Evol. 2016;33:1870–4. https://doi.org/10.1093/molbev/msw054.
37. Rozenbaum R, Gonçalves AJ. Clinical epidemiological study of 171 cases of cryptococcosis. Clin Infect Dis. 1994;18(3):369–80.
38. Chakrabarti A, Jatana M, Kumar P, Chatha L, Kaushal A, Padhye AA. Isolation of Cryptococcus neoformans var. gattii from Eucalyptus camaldulensis in India. J Clin Microbiol. 1997;35:3340–2.
39. ArgüeroLicea B, Garza Garza D, Flores Urbieta V, Cervantes Olivares RA. Isolation and characterization of Cryptococcus neoformans var. gattii from samples of Eucalyptus camaldulensis in Mexico city. Rev Iberoam Micol. 1999;16:40–2.
40. Lazera MS, Cavalcanti MAS, Londero AT, Trilles L, Nishikawa MM, Wanke B. Possible primary ecological niche of Cryptococcus neoformans. Med Mycol. 2000;38:379–83. https://doi.org/10.1080/mmy.38.5.379.383.
41. Abegg MA, Cella FL, Faganello J, Valente P, Schrank A, Vainstein MH. Cryptococcus neoformans and Cryptococcus gattii isolated from the excreta of Psittaciformes in a southern Brazilian zoological garden. Mycopathologia. 2006;161:83–91. https://doi.org/10.1007/s11046-005-0186-z.
42. Hagen F, Ceresini PC, Polacheck I, Ma H, van Nieuwburgh F, Gabaldón T, Kagan S, Pursall ER, Hoogveld HL, van Iersel LJ, Klau GW, Kelk SM, Stougie L, Bartlett KH, Voelz K, Pryszzcz LP, Castañeda E, Lazera M, Meyer W, Deforce D, Meis JF, May RC, Klaassen CH, Boekhout T. Ancient dispersal of the human fungal pathogen Cryptococcus gattii from the Amazon rainforest. PLoS ONE. 2013;8(8): e71148. https://doi.org/10.1371/journal.pone.0071148.
43. Marietto-Gonçalves GA, Grandi F. Are all psittacine birds carriers of Cryptococcus neoformans? Mem Inst Oswaldo Cruz. 2011;106:781–2. https://doi.org/10.1590/S0074-02762011000600023.

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