

Cryptococcus and *Cryptococcosis* in Human and Animal Model: An Overview

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How to cite this paper: da Silva, E.G., Leite-Jr., D.P., da Silva Maia, M.L., de Souza Silva, S.M., de Assis Baroni, F., Auler, M.E., Dias, A.L.T., Moreira, D., Gandra, R.F., Ramos, R.T.B., Gasparetto, A., de Oliveira Dantas, E.S., Costa, V.T. and Paula, C.R. (2020) *Cryptococcus* and *Cryptococcosis* in Human and Animal Model: An Overview. *Advances in Microbiology*, 10, 180-193.

<https://doi.org/10.4236/aim.2020.104014>

Received: March 19, 2020

Accepted: April 21, 2020

Published: April 24, 2020

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Abstract

Background: Yeasts from the genus *Cryptococcus* (species *C. neoformans* and *C. gattii*) are opportunistic pathogens that afflict humans with low immunity caused by diseases such as AIDS or by the usage of immunosuppressive drugs. It is a fatal disease that has been studied around the whole world and its most fatal form is the brain *cryptococcosis*. **Purpose:** The present review describes the disease from pathogen isolation, the various clinical presentations of the disease, the most important virulence factors of yeast in human and animal model and their clinical issues. **Methods:** On this review, several published studies about the disease are presented. **Results:** Numerous researches have been done worldwide in order to find a kind of therapy that is more effective against the disease. Amphotericin B, in all forms is still the drug of choice in the treatment of the *cryptococcosis*. Fluconazole, as well as voriconazole in combination with amphotericin B, is recommended in the cases of treatment failure. **Conclusion:** This study presented has elucidated a little more about the disease. Further studies should be conducted to find more diagnoses that are accurate as well as more effective treatments for eradicating the disease. In this study, the bibliographic survey makes reference to

the world literature; with regard to ecology, taxonomy, main factors related to virulence, the clinical manifestations, the action of antifungal drugs and histopathological analysis used in an animal model, were the objectives deleterious aspects of this study, thus informing, in a simple way, the importance of this microorganism for research and researchers working with this global disease, called *Cryptococcosis*.

Keywords

Cryptococcus, *Cryptococcosis*, Animal Model, *Cryptococcus neoformans*, *Cryptococcus gattii*, Virulence Related Factors

1. Introduction

1.1. Isolation of Yeast into the Environment

There has been significant progress in the ecological distribution, as well as in the population genetics, in molecular epidemiology and in the clinical characteristics of *Cryptococcus* genus [1]. Church towers in metropolitan areas are large habitats of pigeons, thus facilitating the spread of microorganism propagules [2] [3].

Cryptococcus neoformans are usually found in places of a high concentration of people such as, urban areas [2] [4]. Trees of the genus *Eucalyptus spp.*, as well as pigeons present in some specific places, such as nursing homes and hospitals, should be considered main risk factors for the immunocompromised population [5].

1.2. Genus *Cryptococcus*

There are now hundreds of emerging pathogenic fungi. *Cryptococcus neoformans* and *Cryptococcus gattii*, are currently two of the main opportunistic/pathogenic species for humans [6] [7]. *C. neoformans* and *C. gattii* cause infections in humans and animals, and the yeast is acquired through infective particles of the environment. Hollow decaying trees and soil are taken as the ecological niche of yeast [8]; as well as other possible sources and microhabitats of infection [9].

1.3. *Cryptococcus neoformans* and *Cryptococcus gattii*

Various researches reveal that *C. neoformans* in immunocompromised individuals causes meningoencephalitis, which can lead to death. *C. gattii* affects immunocompromised and immunocompetent patients [10]. Several molecular biology techniques have been used for the genetic identification of *C. neoformans* and *C. gattii* with the aim of comparing the groups.

Some of these techniques highlight polymerase chain reaction (PCR) fingerprinting, amplified fragment length polymorphisms (AFLP), microsatellite typ-

ing [11]; study of the length of restriction fragment polymorphism (RFLP) [12] and more recently, whole genome sequencing (WGS) [13]. Based on capsule structural and differences techniques these genus yeasts can be classified into *Cryptococcus neoformans* species complex (serotype A, VN I) and *Cryptococcus gattii* species complex: *C. gattii* sensu stricto (VGI), *Cryptococcus deuterogattii* (VGII), *Cryptococcus bacillisporus* (VGIII), *Cryptococcus tetragattii* (VGIV) and *Cryptococcus decagattii* (VGIII/VGIV) [14] [15] [16].

1.4. Virulence

The ability to cause *cryptococcosis*, comes from the capacity of these yeasts to reproduce itself and grow inside the host, which offers conditions for the microorganism to survive in its interior [17]. Some issues as polysaccharide capsule, melanin production, urease production, and phospholipases are the main virulence factors of *C. neoformans* and *C. gattii*, although the yeast is considered an accidental pathogen since it does not complete its life cycle in the host [18]. One of the important facilitating attributes for the colonization of pathogenic fungi is the body temperature responsible for infection in humans [17]. The main difference among *C. neoformans* and *C. gattii* species and other fungal pathogens is the capsule that surrounds the cell's surface.

This capsule is composed of complex polysaccharide polymers (mannan, xylose, glucuronic acid) as well as the cell wall that is associated with the capsule, which is a matrix composed of alpha and beta glucans, chitin, chitosan and mannoproteins [19]. While the molecular structure of melanin is still controversial, some significant studies have been carried out to understand the particular aspects related to macro and microstructure in the last decades [20]. According to some authors, urease is an important role in ensuring the use of urea produced by the host and used by the yeast as a nutrient [21] [22].

Urease is responsible for the dissolution of the microvessels of the central nervous system (CNS) [23]. Phospholipase is highly likely to be another enzyme responsible for the pathogenicity of yeast [24], being one more crucial factors in the virulence of the microorganism [25].

1.5. Diagnosis of *Cryptococcosis*

Cryptococcosis is related to death in immunocompromised patients. In recent years many researches and clinical trials have led to improvement, such as disease prevention. An accurate diagnosis, and correct therapy have collaborated in the treatment of this disease [26]. One of the main components of the *C. neoformans* and *C. gattii* capsule is glucuronoxylomannan (GXM), detected by the antigen agglutination test (CALAT) [27].

Particularly useful in the detection of *Cryptococcus* species the Film Array multiplex PCR panel, is a promising platform for a rapid diagnosis of the disease, predicting the sterility of the culture, as well as differentiating IRIS in positive recurrences among people with recurrent symptoms. Otherwise, a less costly

method as well as higher velocity and greater variety of species is the polymorphism size analysis (FSP) that analyzes the size of the ITS1 and ITS2 regions [28] (Table 1).

1.6. Disease

Cryptococcosis is an opportunistic mycosis that affects immunosuppressed humans and animals; this disease is acquired by inhalation of encapsulated yeast as well as by basidiospores found in the environment [29] [30].

1.7. Humans

The most common manifestation is the involvement of the central nervous system, but pulmonary disease also occurs [31], in immunosuppressed patients, especially with the progression of acquired immunodeficiency (AIDS) and is related to deaths [28].

1.8. Clinical Presentation of *Cryptococcosis*

Cryptococcus spp., is a major cause of meningitis around the world. *Cryptococcosis* meningitis is more common in adult patients infected with the HIV virus [32]. Pulmonary *cryptococcosis* can manifest itself in several ways: subclinical, granulomatous, infection of the respiratory tract with isolated pulmonary nodule or even acute respiratory insufficiency that can lead to death of the patient [33]. Some reports indicate that *Cryptococcosis* of the central nervous system and

Table 1. Molecular characterization, virulence and diagnosis.

Molecular Characterization			
Species	Serotype	Genotypes	Authors
<i>C. neoformans</i> var. <i>grubii</i>	A	VNI, VNIII, VNB	
<i>C. gattii</i> , <i>C. bacillisporus</i> , <i>C. deuterogattii</i> ,			Hurst <i>et al.</i> , 2019 [15]
<i>C. tetragattii</i> and <i>C. decagattii</i>	B/C	VGI-IV	Hagen <i>et al.</i> , 2015 [14]
Virulence		Authors	
Survival at mammalian body temperature			
Production of polysaccharide capsule		Rone-Egstron <i>et al.</i> , 2001 [21]; Ruane <i>et al.</i> , 1988 [22]	
Production of melanin		Pini <i>et al.</i> , 2017 [24]; Lev <i>et al.</i> , 2013 [25];	
Positive urease		Casadevall and Pirofski, 2012 [18]	
Phospholipase			
Diagnosis		Authors	
Detection of glucuronoxylomannan (GXM)			
<i>Cryptococcus</i> antigen latex test (CALAT)		Khodati <i>et al.</i> , 2017 [28];	
Multiplex PCR		Tone <i>et al.</i> , 2016 [27];	
Analysis of the ITS1 and ITS2 regions			

pulmonary infection; are common in patients who express the disseminated disease [34]. The fungus can lead the patient to extensive fibrosis in the subarachnoid space to find small veins, inducing venous congestion, causing cerebral infarction, and thus may be fatal [35].

1.9. Animal Model

The infection of central nervous system in the murine model is responsible for the death of the animals [36]. The choice of the ideal model depends on the research objectives. In animals, studies have evaluated *C. neoformans* infection in mammals [37]. These yeasts are found in silkworm, fruit fly, nematode and larvae [38] [39] [40] [41].

Invertebrate models such as silkworm, pneumatoid, fruit fly, and larvae have been used in experiments in the study of this disease [41]. The use of an animal model is very convenient in the study of the pathogenicity of microorganism, as well as understanding the evolution of the disease [42]. C57BL/6 mouse is an excellent model used to study the inflammatory response caused by *C. gattii* as it is more susceptible to disease due to its lower inflammatory response [43]. In severe SCID (Combined Immunodeficiency Disorder) animal models, combined immunodeficiency is very useful for the therapeutic and immunological study that simulates immunodeficient patients [36] [44] [45] [46].

2. Histopathologic Analysis

2.1. Brain Tissue—Mouse Model

Histological study of brain tissue in immunodeficient mice showed on the first day right after inoculation, the presence of edemas and, congested vessels. On the second day, liquefaction area and focal necrosis appeared and intercalated with fragments of necrotic cells after the 5 days. The yeast are visualized in the meninges on the seventh day and this condition which persisted for up to 15 days from the initial of inoculation, *C. neoformans* was also observed in the brain parenchyma (Figures 1(A)-(J)).

2.2. Lung Tissue—Mouse Model

In pulmonary tissue, focal areas of bleeding and mild neutrophilic inflammatory infiltrate into the alveolar wall are observed one day after inoculation, progressing to pulmonary congestion. On the 11th day of infection, edema, congestion, hemorrhage, diffuse and focal neutrophilic infiltrate, as well as intense amount of *C. neoformans* interspersed with necrotic cell fragments (Figures 2(A)-(D)).

2.3. Immunology

Evidence shows that *C. neoformans* can damage host cells and tissues, although this yeast is not considered a cytotoxic pathogen [47]. Studies have shown that the male mouse strain type is useful for studying brain infection after fungemia, also arguing that the main immune cells are dendritic cells and resident alveolar

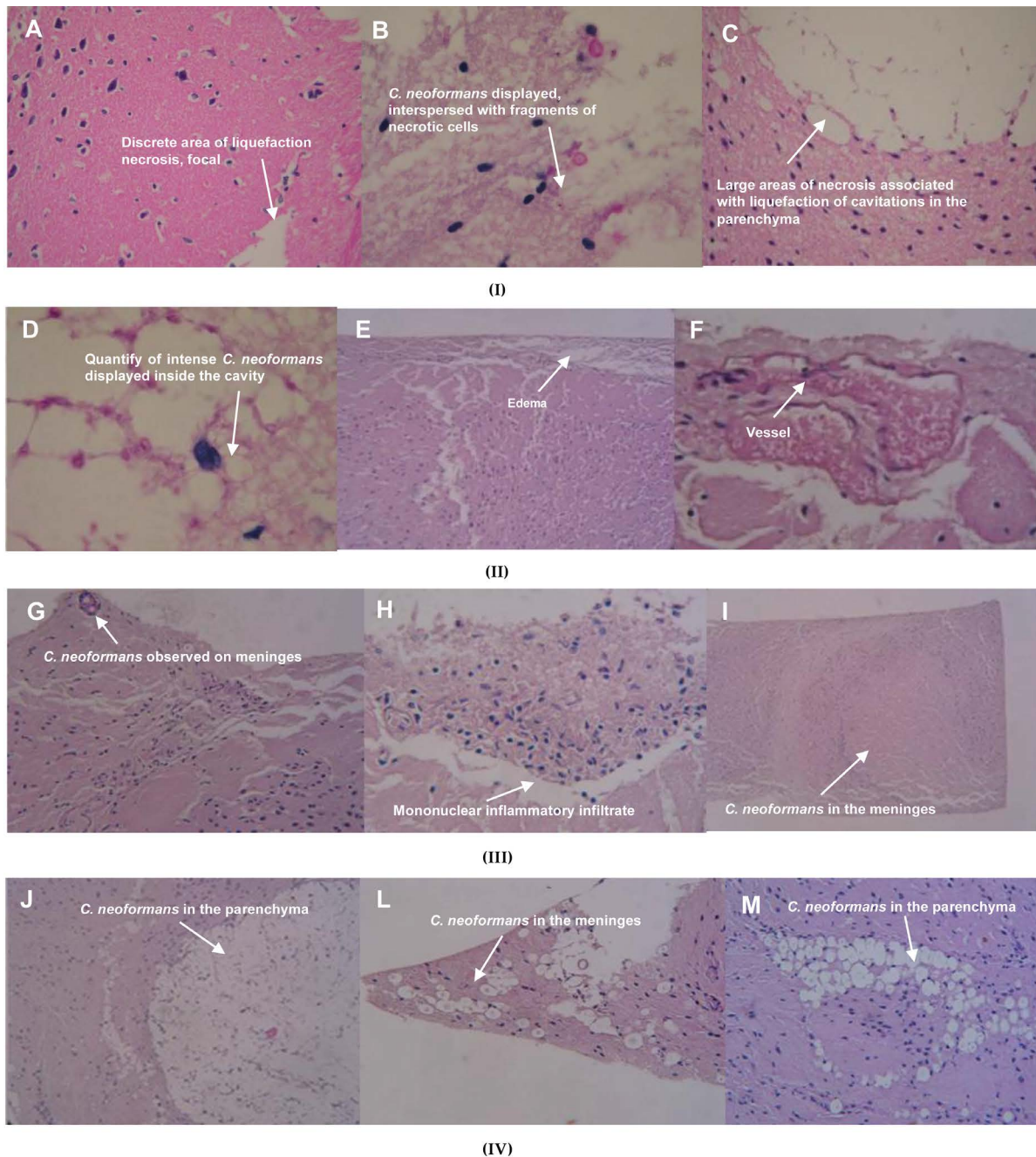


Figure 1. Histological analysis of brain tissue, of immunocompetent mice (image (A)-(J)). (I) Histopathology of brain tissue in immunodeficient murine model, 5 days after inoculation, (A) presenting a discrete area of liquefaction necrosis, focal HE, 400 \times , (B) *C. neoformans* displayed, interspersed with fragments of necrotic cells, 7 days after inoculation, PAS, 400 \times . (II) Histopathology of brain tissue in immunodeficient murine model at 11 days after the inoculation; (C) presenting large areas of necrosis associated with liquefaction of cavitation's in the parenchyma, HE, 400 \times ; (D) quantify of intense *C. neoformans* displayed inside the cavities, PAS, 400 \times . (III) Histopathology of brain tissue in immunocompetent murine model. (E) 24 hours after inoculation presenting edema, HE, 200 \times , (F) 48 hours after inoculation vessel congested, HE, 200 \times . (IV) Histopathology of brain tissue in immunocompetent murine model. (G) *C. neoformans* observed on meninges, 7 days after inoculation, HE, 200 \times , (H) mononuclear inflammatory infiltrate in the 9th day after inoculation, HE, 500 \times . (V) Histopathology of brain tissue in immunocompetent murine model, 15 days after inoculation. (I) *C. neoformans* in the meninges, HE, 200 \times , (J) *C. neoformans* in the parenchyma, HE, 500 \times . (VI) Histopathology of brain tissue in immunocompetent murine model, 15 days after inoculation. (L) *C. neoformans* in the meninges, HE, 200 \times , (M) *C. neoformans* in the parenchyma, HE, 500 \times . Author: Prates, R.A.

macrophages [48].

After being recognized and phagocytosed by effector cells, such as neutrophils, alveolar macrophages and dendritic cells, alveolo and distal airways provide conditions for the yeast to reproduce outside of these associated cells or after recognition by the respiratory epithelium [49].

The main routes for entry of *C. neoformans* into the host are the lungs, where the organism escapes from the organ intracellularly within of the macrophage or through other phagocytes. This yeast migrates out of the lungs with a mechanism called a “Trojan horse”, or via transcytosis. These cells go directly to the affected organs through respiratory etial cells [50]. The blood-brain barrier (BBB) is responsible for invasion in the central nervous system by yeast through two mechanisms: transcytosis (yeast carried in endothelial cells) and a mechanism known as a trojan horse (yeast transported within of the macrophage) [51].

Cryptococcus is an intracellular pathogen that survives by hosting cells and reproducing inside infected macrophages [52]. The immune response in *cryptococcosis* is mediated by macrophages and helper T cells (Th1 and Th2) [53] [54]. IL-4 is responsible for the regulation of B-cells for the secretion of IgG1 and interferon- γ antibodies through the stimulation of IgG2a antibodies, making

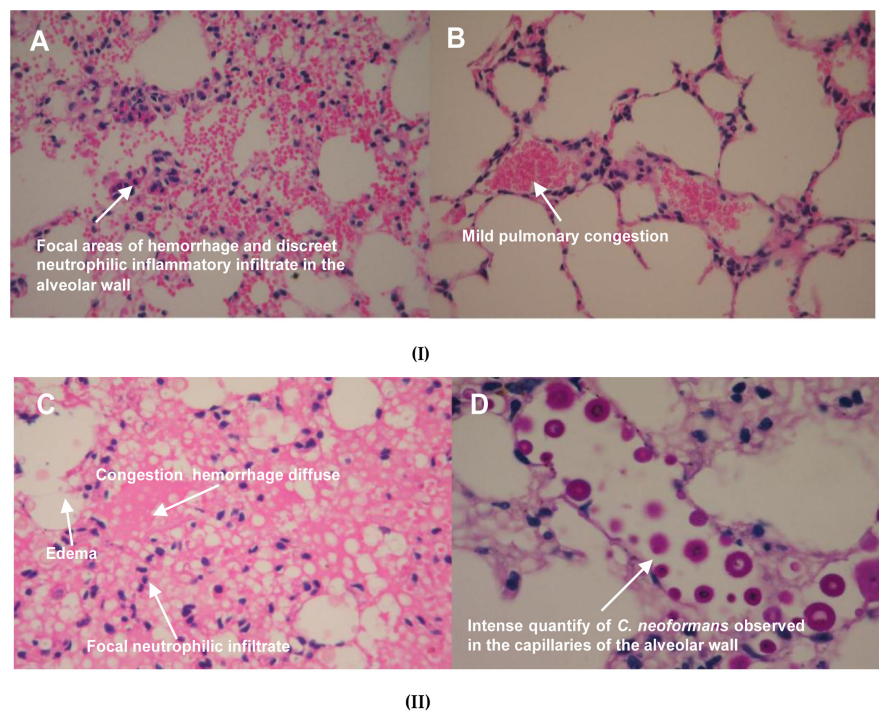


Figure 2. Histological analysis of lung tissue, of immunocompetent mice (image (A)-(D)). (I) Histopathology of lung tissue in immunodeficient murine model. (A) 1 day after inoculation showing focal areas of hemorrhage and discrete neutrophilic inflammatory infiltrate in the alveolar wall, (B) 5 days after inoculation with mild pulmonary congestion, HE, 400 \times . (II) Histopathology of lung tissue in immunodeficient murine model 11 days after inoculation. (C) Presenting edema, congestion hemorrhage diffuse and focal neutrophilic infiltrate, HE, 400 \times , (D) Intense quantify of *C. neoformans* observed in the capillaries of the alveolar wall, PAS, 1000. Author: Prates, R.A.

one of the Th1 or Th2 isotopes an indicator of the response [55] [56].

2.4. Treatments

Amphotericin B associated with flucytosine has been used to treat *cryptococcosis*. According to studies, fluconazole has a fungistatic effect, although it has been used as an alternative for the treatment of amphotericin and flucytosine. Regarding the authors, the drug does not effectively eliminate the fungus and has been associated with clinical relapse, as well as increasing the risk of drug resistance [57] [58]. When resources are limited, Amphotericin B associated with flucytosine over a period of two weeks, has been effective in treating this disease [59].

The treatment of choice in disseminated *cryptococcosis* is Amphotericin B associated with intravenous flucytosine. Amphotericin B deoxycholate associated with flucytosine and administered intravenously for a period of at least two weeks, may be a second therapeutic option, however, in HIV-positive patients as well as, transplanted, it is recommended to administer the combination of these drugs for a longer period (four to six weeks) [60]. The most effective therapy, which decreases the toxicity of these associated drugs, such as the encapsulation of Amphotericin B and fluconazole in fibrin microspheres, has been used successfully by bringing these associated drugs into the correct target [59]. Voriconazole associated with Amphotericin B in murine model, with fluconazole resistant strains is effective in the treatment of experimental *cryptococcosis*, significantly reducing the isolation of yeast in the lungs and brains of these animals, as well as increasing their survival [44]. The production of pro-inflammatory cytokines mediated by amphotericin B and voriconazole is dependent on the concentration administered [46].

3. Conclusion

Cryptococcosis is a fatal disease; many studies have been conducted with the aim of discovering a more accurate diagnosis as well as a more effective treatment for this disease. The review presented aims to elucidate and contribute a little more about it. Further studies should be performed to find more accurate diagnoses as well as more effective treatments for the eradication of this disease. This survey seeks to bring to light some factors related to virulence, taxonomy, ecology and action to antifungal drugs and the diverse clinical and histopathological manifestations of this fungal entity called *Cryptococcus*, where they were elaborated in an animal model, aiming to bring information in a simple way to researchers who develop research with this microorganism.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Research Involving Human Participants and/or Animals

N/A.

Informed Consent

N/A.

Authors' Contributions

EGS prepared the study, carried out and was involved in writing the manuscript. CRP participated in the review of the manuscript and was the chief researcher. DPLJ collaborated in the final revision of the manuscript. All other authors were involved in the examination of biological materials, data analysis and the writing of the manuscript. All authors read and approved the final manuscript.

References

- [1] Fang, W., Fa, Z. and Liao, W. (2015) Epidemiology of *Cryptococcus* and *Cryptococcosis* in China. *Fungal Genetics and Biology*, **78**, 7-15. <https://doi.org/10.1016/j.fgb.2014.10.017>
- [2] Takahara, D.T., Lázera, M.S., Wanke, B., Trilles, L., Dutra, V., Paula, D.A.J., Nakazato, L., Anzai, M.C., Leite-Jr., D.P., Paula, C.R. and Hahn, R.C. (2013) First Report on *Cryptococcus neoformans* in Pigeon Excreta from Public and Residential Locations in the Metropolitan Area of Cuiabá, State of Mato Grosso, Brazil. *The Revista do Instituto de Medicina Tropical de São Paulo*, **55**, 371-376. <https://doi.org/10.1590/S0036-46652013000600001>
- [3] Baroni, F.A., Paula, C.R., Silva, E.G., Viani, F.C., Rivera, I.N., Oliveira, M.T. and Gambale, W. (2006) *Cryptococcus neoformans* Strains Isolated from Church Towers in Rio de Janeiro City, RJ, Brazil. *The Revista do Instituto de Medicina Tropical de São Paulo*, **48**, 71-75. <https://doi.org/10.1590/S0036-46652006000200003>
- [4] Montenegro, H. and Paula, C.R. (2000) Environmental Isolation of *Cryptococcus neoformans* var. *gattii* and *C. neoformans* var. *neoformans* in the City of São Paulo, Brazil. *Medical Mycology*, **38**, 385-390. <https://doi.org/10.1080/mmy.38.5.385.390>
- [5] Kamari, A., Sepahvand, A. and Mohammadi, R. (2017) Isolation and Molecular Characterization of *Cryptococcus* Species Isolated from Pigeon Nests and Eucalyptus Trees. *Current Medical Mycology*, **3**, 20-25. <https://doi.org/10.29252/cmm.3.2.20>
- [6] Kwon-Chung, K.J. and Bennett, J.E. (1984) Epidemiologic Differences between the Two Varieties of *Cryptococcus neoformans*. *American Journal of Epidemiology*, **120**, 123-130. <https://doi.org/10.1093/oxfordjournals.aje.a113861>
- [7] Freij, J.B., Fu, M.S., De Leon Rodriguez, C.M., Dziejczak, A., Jedlicka, A.E., Dragotakes, Q., Rossi, D.C.P., Jung, E.H., Coelho, C. and Casadevall, A. (2018) Conservation of Intracellular Pathogenic Strategy among Distantly Related Cryptococcal Species. *Infection and Immunity*, **86**, 1-15. <https://doi.org/10.1128/IAI.00946-17>
- [8] Cogliati, M., D'Amicis, R., Zani, A., Montagna, M.T., Caggiano, G., De Giglio, O., Balbino, S., De Donno, A., Serio, F., Susever, S., *et al.* (2016) Environmental Distribution of *Cryptococcus neoformans* and *C. gattii* around the Mediterranean Basin. *FEMS Yeast Research*, **16**, fow086.
- [9] Leite-Jr., D.P., Amadio, J.V.R.S., Martins, E.R., Simões, S.A.A., Yamamoto, A.C.A., Leal-Santos, F.A., Takahara, D.T. and Hahn, R.C. (2012) *Cryptococcus* spp Isolated from Dust Microhabitat in Brazilian Libraries. *Journal of Occupational Medicine and Toxicology*, **7**, 1-7. <https://doi.org/10.1186/1745-6673-7-11>

- [10] Kwon-Chung, K.J., Fraser, J.A., Doering, T.L., Wang, Z., Janbon, G., Idnurm, A. and Bahn, Y.S. (2014) *Cryptococcus neoformans* and *Cryptococcus gattii*, the Etiologic Agents of Cryptococcosis. *Cold Spring Harbor Perspectives in Medicine*, **4**, a019760. <https://doi.org/10.1101/cshperspect.a019760>
- [11] Meyer, W. and Mitchell, T.G. (1995) Polymerase Chain Reaction Fingerprinting in Fungi Using Single Primers Specific to Minisatellites and Simple Repetitive DNA Sequences: Strain Variation in *Cryptococcus neoformans*. *Electrophoresis*, **16**, 1648-1656. <https://doi.org/10.1002/elps.11501601273>
- [12] Meyer, W., Castañeda, A., Jackson, S., Huynh, M., Castañeda, E. and Group ICS (2003) Molecular Typing of IberoAmerican *Cryptococcus neoformans* Isolates. *Emerging Infectious Diseases*, **9**, 189-195. <https://doi.org/10.3201/eid0902.020246>
- [13] D'Souza, C.A., Kronstad, J.W., Taylor, G., Warren, R., Yuen, M., Hu, G., Jung, W.H., Sham, A., Kidd, S.E., Tangen, K., *et al.* (2011) Genome Variation in *Cryptococcus gattii*, an Emerging Pathogen of Immunocompetent Hosts. *mBio*, **2**, e00342. <https://doi.org/10.1128/mBio.00342-10>
- [14] Hagen, F., Khayhan, K., Theelen, B., Kolecka, A., Polacheck, I., Sionov, E., Falk, R., Parnmen, S., Lumbsch, H.T. and Boekhout, T. (2015) Recognition of Seven Species in the *Cryptococcus Gattii/Cryptococcus Neoformans* Species Complex. *Fungal Genetics and Biology*, **78**, 16-48. <https://doi.org/10.1016/j.fgb.2015.02.009>
- [15] Hurst, S., Lyusen, C., Cooksey, G., Vugia, D.J., Litvintseva, A.P. and Lockhart, S.R. (2019) Molecular Typing of Clinical and Environmental Isolates of *Cryptococcus gattii* Species Complex from Southern California, United States. *Mycoses*, **62**, 1029-1034. <https://doi.org/10.1111/myc.12980>
- [16] Li, Y.H. and Liu, T.B. (2020) Zinc Finger Proteins in the Human Fungal Pathogen *Cryptococcus neoformans*. *International Journal Molecular Sciences*, **21**, E1361. <https://doi.org/10.3390/ijms21041361>
- [17] Sherrington, S.L., Kumwenda, P., Kousser, C. and Hall, R.A. (2018) Host Sensing by Pathogenic Fungi. *Advances in Applied Microbiology*, **102**, 159-221. <https://doi.org/10.1016/bs.aambs.2017.10.004>
- [18] Casadevall, A. and Pirofski, L.A. (2007) Accidental Virulence, Cryptic Pathogenesis, Martians, Lost Hosts, and the Pathogenicity of Environmental Microbes. *Eukaryotic Cell*, **6**, 2169-2174. <https://doi.org/10.1128/EC.00308-07>
- [19] Wang, Z.A., Li, L.X. and Doering, T.L. (2018) Unraveling Synthesis of the Cryptococcal Cell Wall and Capsule. *Glycobiology*, **28**, 719-730. <https://doi.org/10.1093/glycob/cwy030>
- [20] Nosanchuk, J.D., Stark, R.E. and Casadevall, A. (2015) Fungal Melanin: What Do We Know about Structure? *Frontiers in Microbiology*, **6**, 1463. <https://doi.org/10.3389/fmicb.2015.01463>
- [21] Ronne-Engström, E., Cesarini, K.G., Enblad, P., Hesselager, G., Marklund, N., Nilsson, P., Salci, K., Persson, L. and Hillered, L. (2001) Intracerebral Microdialysis in Neurointensive Care: The Use of Urea as an Endogenous Reference Compound. *Journal of Neurosurgery*, **94**, 397-402. <https://doi.org/10.3171/jns.2001.94.3.0397>
- [22] Ruane, P.J., Walker, L.J. and George, W.L. (1988) Disseminated Infection Caused by Urease-Negative *Cryptococcus neoformans*. *Journal of Clinical Microbiology*, **26**, 2224-2225. <https://doi.org/10.1128/JCM.26.10.2224-2225.1988>
- [23] Olszewski, M.A., Noverr, M.C., Chen, G.H., Toews, G.B., Cox, G.M., Perfect, J.R. and Huffnagle, G.B. (2004) Urease Expression by *Cryptococcus neoformans* Promotes Microvascular Sequestration, Thereby Enhancing Central Nervous System Invasion. *The American Journal of Pathology*, **164**, 1761-1771.

[https://doi.org/10.1016/S0002-9440\(10\)63734-0](https://doi.org/10.1016/S0002-9440(10)63734-0)

- [24] Pini, G., Faggi, E. and Campisi, E. (2017) Enzymatic Characterization of Clinical and Environmental *Cryptococcus neoformans* Strains Isolated in Italy. *Revista Iberoamericana de Micología*, **34**, 77-82. <https://doi.org/10.1016/j.riam.2016.04.005>
- [25] Lev, S., Desmarini, D., Li, C., Chayakulkeeree, M., Traven, A., Sorrell, T.C. and Djordjevic, J.T. (2013) Phospholipase C of *Cryptococcus neoformans* Regulates Homeostasis and Virulence by Providing Inositol Trisphosphate as a Substrate for Arg1 Kinase. *Infection and Immunity*, **81**, 1245-1255. <https://doi.org/10.1128/IAI.01421-12>
- [26] Cardoso, P.H.M., Silva, B.C.M., Santos, J.I., Santos, R.L.O., Leite-Jr., D.P., Auler, M.E., Ruiz, L.S., Silva, E.G., Moreira, D., Domaneschi, C., Baroni, F.A., Melhem, M.S.C., Martins, M.A. and Paula, C.R. (2018) Characterization of Clinical and Environmental Isolates of *Cryptococcus neoformans*/*Cryptococcus gattii* Complex Maintained in Yeast Culture Collection in São Paulo, Brazil. *Open Journal of Epidemiology*, **8**, 76-92. <https://doi.org/10.4236/ojepi.2018.82007>
- [27] Tone, T., Umeda, Y. and Makimura, K. (2016) Cross-Reactivity in Cryptococcus Antigen Latex Agglutination Test in Two Commercial Kits. *Medical Mycology*, **54**, 439-443. <https://doi.org/10.1093/mmy/myv115>
- [28] Khodadadi, H., Karimi, L., Jalalizand, N., Adin, H. and Mirhendi, H. (2017) Utilization of Size Polymorphism in ITS1 and ITS2 Regions for Identification of Pathogenic Yeast Species. *Journal of Medical Microbiology*, **66**, 126-133. <https://doi.org/10.1099/jmm.0.000426>
- [29] Escandón, P., de Bedout, C., Lizarazo, J., Agudelo, C.I., Tobón, A., Bello, S., Restrepo, A. and Castañeda, E. (2012) Criptococosis GCpeEdl. Cryptococcosis in Colombia: Results of the National Surveillance Program for the Years 2006-2010. *Biomedica*, **32**, 386-398. <https://doi.org/10.7705/biomedica.v32i3.707>
- [30] Rodríguez-Cerdeira, C., Arenas, R., Moreno-Coutiño, G., Vásquez, E., Fernández, R. and Chang, P. (2014) Systemic Fungal Infections in Patients with Human Immunodeficiency Virus. *Actas Dermo-Sifiliográficas*, **1**, 5-17. <https://doi.org/10.1016/j.adengl.2012.06.032>
- [31] Baddley, J.W., Forrest, G.N. and the AST Infectious Diseases Community of Practice (2013) Cryptococcosis in Solid Organ Transplantation. *American Journal of Transplantation*, **13**, 242-249. <https://doi.org/10.1111/ajt.12116>
- [32] Rajasingham, R., Smith, R.M., Park, B.J., Jarvis, J.N., Govender, N.P., Chiller, T.M., Denning, D.W., Loyse, A. and Boulware, D.R. (2017) Global Burden of Disease of HIV-Associated Cryptococcal Meningitis: An Updated Analysis. *The Lancet Infectious Diseases*, **17**, 873-881. [https://doi.org/10.1016/S1473-3099\(17\)30243-8](https://doi.org/10.1016/S1473-3099(17)30243-8)
- [33] Brizendine, K.D., Baddley, J.W. and Pappas, P.G. (2011) Pulmonary Cryptococcosis. *Seminars in Respiratory and Critical Care Medicine*, **32**, 727-734. <https://doi.org/10.1055/s-0031-1295720>
- [34] Valente, E.S., Lazzarin, M.C., Koech, B.L., da Rosa, R.V., de Almeida, R., de Oliveira, U.L., Neugebauer, M.G. and Sacco, A.G. (2015) Disseminated Cryptococcosis Presenting as Cutaneous Cellulitis in an Adolescent with Systemic Lupus Erythematosus. *Infectious Disease Reports*, **7**, 5743. <https://doi.org/10.4081/idr.2015.5743>
- [35] Shimoda, Y., Ohtomo, S., Arai, H., Ohtoh, T. and Tominaga, T. (2017) Subarachnoid Small Vein Occlusion Due to Inflammatory Fibrosis: A Possible Mechanism for Cerebellar Infarction in Cryptococcal Meningoencephalitis: A Case Report. *BMC Neurology*, **17**, 157. <https://doi.org/10.1186/s12883-017-0934-y>

- [36] Silva, E.G., Baroni, F.D., Viani, F.C., Ruiz, L.D., Gandra, R.F., Auler, M.E., Dias, A.L.T., Gambale, W. and Paula, C.R. (2006) Virulence Profile of Strains of *Cryptococcus neoformans* var. *grubii* Evaluated by Experimental Infection in BALB/c Mice and Correlation with Exoenzyme Activity. *Journal of Medical Microbiology*, **55**, 139-142. <https://doi.org/10.1099/jmm.0.46206-0>
- [37] Zaragoza, O., Alvarez, M., Telzak, A., Rivera, J. and Casadevall, A. (2007) The Relative Susceptibility of Mouse Strains to Pulmonary *Cryptococcus neoformans* Infection Is Associated with Pleiotropic Differences in the Immune Response. *Infection and Immunity*, **75**, 2729-2739. <https://doi.org/10.1128/IAI.00094-07>
- [38] Mylonakis, E., Ausubel, F.M., Perfect, J.R., Heitman, J. and Calderwood, S.B. (2002) Killing of *Caenorhabditis elegans* by *Cryptococcus neoformans* as a Model of Yeast Pathogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 15675-15680. <https://doi.org/10.1073/pnas.232568599>
- [39] Apidianakis, Y., Rahme, L.G., Heitman, J., Ausubel, F.M., Calderwood, S.B. and Mylonakis, E. (2004) Challenge of *Drosophila melanogaster* with *Cryptococcus neoformans* and Role of the Innate Immune Response. *Eukaryotic Cell*, **3**, 413-419. <https://doi.org/10.1128/EC.3.2.413-419.2004>
- [40] Mylonakis, E., Moreno, R., El Khoury, J.B., Idnurm, A., Heitman, J., Calderwood, S.B., Ausubel, F.M. and Diener, A. (2005) *Galleria mellonella* as a Model System to Study *Cryptococcus neoformans* Pathogenesis. *Infection and Immunity*, **73**, 3842-3850. <https://doi.org/10.1128/IAI.73.7.3842-3850.2005>
- [41] Matsumoto, Y., Miyazaki, S., Fukunaga, D.H., Shimizu, K., Kawamoto, S. and Sekimizu, K. (2012) Quantitative Evaluation of Cryptococcal Pathogenesis and Antifungal Drugs Using a Silkworm Infection Model with *Cryptococcus neoformans*. *Journal of Applied Microbiology*, **112**, 138-146. <https://doi.org/10.1111/j.1365-2672.2011.05186.x>
- [42] Torda, A., Kumar, R.K. and Jones, P.D. (2001) The Pathology of Human and Murine Pulmonary Infection with *Cryptococcus neoformans* var. *gattii*. *Pathology*, **33**, 475-478. <https://doi.org/10.1080/00313020120083197>
- [43] Cheng, P.Y., Sham, A. and Kronstad, J.W. (2009) *Cryptococcus gattii* Isolates from the British Columbia Cryptococcosis Outbreak Induce Less Protective Inflammation in a Murine Model of Infection than *Cryptococcus neoformans*. *Infection and Immunity*, **77**, 4284-4294. <https://doi.org/10.1128/IAI.00628-09>
- [44] Silva, E.G., Paula, C.R., Baroni, F.A. and Gambale, W. (2012) Voriconazole, Combined with Amphotericin B, in the Treatment for Pulmonary Cryptococcosis Caused by *C. neoformans* (Serotype A) in Mice with Severe Combined Immunodeficiency (SCID). *Mycopathologia*, **173**, 445-449. <https://doi.org/10.1007/s11046-011-9499-2>
- [45] Silva, E.G., Silva, M.S., Paula, C.R. and Ruiz, L.S. (2016) Effect of GXM (*Glucuronoxylomannan*) on the Inflammatory Response in Lung Infection Caused by *Cryptococcus neoformans* (Serotype A) in Immunodeficient Murine Model (BALB/c-SCID). *Journal of Medical Microbiology & Diagnosis*, **5**, 4-6.
- [46] Silva, E.G., Silva, M.S., Paula, C.R., Ruiz, L.S. and Dias, A.L.T. (2018) Modulatory Effect of Voriconazole on the Production of Proinflammatory Cytokines in Experimental Cryptococcosis in Mice with Severe Combined Immunodeficiency. *Journal de Mycologie Médicale*, **28**, 106-111. <https://doi.org/10.1016/j.mycmed.2017.11.008>
- [47] Casadevall, A., Coelho, C. and Alanio, A. (2018) Mechanisms of *Cryptococcus neoformans*-Mediated Host Damage. *Frontiers in Immunology*, **9**, 855. <https://doi.org/10.3389/fimmu.2018.00855>
- [48] Feldmesser, M., Kress, Y., Novikoff, P. and Casadevall, A. (2000) *Cryptococcus neoformans* Is a Facultative Intracellular Pathogen in Murine Pulmonary Infection.

Infection and Immunity, **68**, 4225-4237.

<https://doi.org/10.1128/IAI.68.7.4225-4237.2000>

- [49] Snelgrove, R.J., Edwards, L., Williams, A.E., Rae, A.J. and Hussell, T. (2006) In the Absence of Reactive Oxygen Species, T Cells Default to a Th1 Phenotype and Mediate Protection against Pulmonary *Cryptococcus neoformans* Infection. *The Journal of Immunology*, **177**, 5509-5516. <https://doi.org/10.4049/jimmunol.177.8.5509>
- [50] Charlier, C., Nielsen, K., Daou, S., Brigitte, M., Chretien, F. and Dromer, F. (2009) Evidence of a Role for Monocytes in Dissemination and Brain Invasion by *Cryptococcus neoformans*. *Infection and Immunity*, **77**, 120-127. <https://doi.org/10.1128/IAI.01065-08>
- [51] Sorrell, T.C., Juillard, P.G., Djordjevic, J.T., Kaufman-Francis, K., Dietmann, A., Milonig, A., Combes, V. and Grau, G.E. (2016) Cryptococcal Transmigration across a Model Brain Blood-Barrier: Evidence of the Trojan Horse Mechanism and Differences between *Cryptococcus neoformans* var. *grubii* Strain H99 and *Cryptococcus gattii* Strain R265. *Microbes and Infection*, **18**, 57-67. <https://doi.org/10.1016/j.micinf.2015.08.017>
- [52] Sabiiti, W., Robertson, E., Beale, M.A., Johnston, S.A., Brouwer, A.E., Loyse, A., Jarvis, J.N., Gilbert, A.S., Fisher, M.C., Harrison, T.S., May, R.B. and Bicanic, T. (2014) Efficient Phagocytosis and Laccase Activity Affect the Outcome of HIV-Associated Cryptococcosis. *Journal of Clinical Investigation*, **124**, 2000-2008. <https://doi.org/10.1172/JCI72950>
- [53] Koguchi, Y. and Kawakami, K. (2002) Cryptococcal Infection and Th1-Th2 Cytokine Balance. *International Reviews of Immunology*, **21**, 423-438. <https://doi.org/10.1080/08830180213274>
- [54] Arora, S., Olszewski, M.A., Tsang, T.M., McDonald, R.A., Toews, G.B. and Huffnagle, G.B. (2011) Effect of Cytokine Interplay on Macrophage Polarization during Chronic Pulmonary Infection with *Cryptococcus neoformans*. *Infection and Immunity*, **5**, 1915-1926. <https://doi.org/10.1128/IAI.01270-10>
- [55] Stevens, T.L., Bossie, A., Sanders, V.M., Fernandez-Botran, R., Coffman, R.L., Mosmann, T.R. and Vitetta, E.S. (1988) Regulation of Antibody Isotype Secretion by Subsets of Antigen-Specific Helper T Cells. *Nature*, **334**, 255-258. <https://doi.org/10.1038/334255a0>
- [56] Guery, J.C., Galbiati, F., Smirolto, S. and Adorini, L. (1996) Selective Development of T Helper (Th)2 Cells Induced by Continuous Administration of Low Dose Soluble Proteins to Normal and Beta(2)-Microglobulin-Deficient BALB/c Mice. *Journal of Experimental Medicine*, **183**, 485-497. <https://doi.org/10.1084/jem.183.2.485>
- [57] Smith, K.D., Achan, B., Hullsiek, K.H., McDonald, T.R., Okagaki, L.H., Alhadab, A.A., Akampurira, A., Rhein, J.R., Meya, D.B., Boulware, D.R., Nielsen, K. and ASTRO-CM/COAT Team (2015) Increased Antifungal Drug Resistance in Clinical Isolates of *Cryptococcus neoformans* in Uganda. *Antimicrobial Agents and Chemotherapy*, **59**, 7197-7204. <https://doi.org/10.1128/AAC.01299-15>
- [58] Kneale, M., Bartholomew, J.S., Davies, E. and Denning, D.W. (2016) Global Access to Antifungal Therapy and Its Variable Cost. *Journal of Antimicrobial Chemotherapy*, **71**, 3599-3606. <https://doi.org/10.1093/jac/dkw325>
- [59] Khan, A.A., Alanazi, A.M., Jabeen, M., Khan, S. and Malik, A. (2018) Additive Potential of Combination Therapy against Cryptococcosis Employing a Novel Amphotericin B and Fluconazole Loaded Dual Delivery System. *European Journal of Pharmaceutical Sciences*, **119**, 171-178. <https://doi.org/10.1016/j.ejps.2018.04.015>
- [60] Perfect, J.R., Dismukes, W.E., Dromer, F., Goldman, D.L., Graybill, J.R., Hamill,

R.J., Harrison, T.S., Larsen, R.A., Lortholary, O., Nguyen, M.H., Pappas, P.G., Powderly, W.G., Singh, N., Sobel, J.D. and Sorrell, T.C. (2010) Clinical Practice Guidelines for the Management of Cryptococcal Disease: 2010 Update by the Infectious Diseases Society of America. *Clinical Infectious Diseases*, **50**, 291-322. <https://doi.org/10.1086/649858>