Monograph

on

Cryptococcus and Cryptococcosis

In man, animals and birds

A guide for postgraduate students in developing countries

By

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Preface

I was lucky to meet scientists who participated in the history of Cryptococcus. The first was Dr. **Staib**, whom I visited in his laboratory in 1962 in Würzburg**.** I was impressed by his discovery of his bird seed agar and the brown effect of the seed extract on the colonies of *Cryptococcus neoformans*. He kindly gave me a handful of the Guizotia abyssinica seeds, which I took back with me to Cairo, kept in my desk until I gave it to the MS student El Shaboury in 1982, who used it in his survey of *Cryptococcus neoformans* in pigeon droppings in Lower Egypt. In 1963, Dr. Rieth in Hamburg gave me the honor to accompany Dr. CW **Emmons** during his visit to Hamburg. I was happy to meet the man who made the simple classification of dermatophytes in 1935 and who described outbreaks of mastitis in 1951 and isolated this organism for the first time from pigeon droppings in New York squares in 1955. In 1963 I visited Dr. **Vanbreuseghem** in Antwerp, who isolated *Cryptococcus gattii* for the first time from a leukemic patient. In the same year I visited the laboratory of Mrs **Kreger – Van Rij** in Delft, and I was very impressed by her yeast collection.

Almost 50 years have elapsed since I saw colonies of Cryptococcus during my postgraduate studies in Germany, when my students asked me to give them a lecture on Cryptococcus. I asked them to give me time to read and refresh my knowledge, to search the internet, and they helped me in collecting information and data. During this time I realized that researchers in the field of Cryptococcus have built a big family, have established working groups and made projects searching in all aspects of this fascinating organism leading to an explosive information concerning the organism. This changed my opinion to prepare a monograph rather than a simple lecture and try to make it in a panoramic form, spotting light on the main facets of Cryptococcus and some of the members of this big family of scientists working on this organism. I tried my best to get pictures of these eminent scientists, and I am indebted to Dr. **Perfect**, who sent me the picture of Dr **Sanfelice**.

During my trip in the world of Cryptococcus, I enjoyed reading the 25 years of **Bulmer** and the 100 years of **Mitchell** and **Perfect** concerning the history of Cryptococcus

This monograph is uploaded on my sites to be available without restrictions to all postgraduate students in the developing countries, particularly those who have no access to expensive books and cannot subscribe in journals or pay per page to read an article.

Mohamed Refai, October,2014

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1. Introduction

1.1. Cryptococcus in Egypt

a. Cryptococcus in birds and animals

Abdallah (1969) was the first to start working on Cryptococcus, when he isolated the organism from 2 goats suffering from mastitis. *Cryptococcus neoformans* was again isolated from the milk of one cow by Abdel Ghani *et al.* (1978) in their studies of mastitis in cattle. El-Far *et al.* (1987) isolated *C. neoformans* from 9 out of 60 milk samples from lactating Friesian cows. Asfour *et al.* (2009) collected 362 milk samples from cases of clinical, subclinical and recurrent mastitic buffaloes and cows from different dairy herds. Isolated yeasts from milk samples were *C. albicans*, *Cryptococcus neoformans, C. krusei*, and *C. guilliermondii.*

El Shaboury (1983) was the first to search for Cryptococcus organisms in pigeon droppings. Two hundred pigeon droppings, unmixed with soil, and collected from various governorates in Lower Egypt were cultured for yeasts. *C. neoformans* was recovered from 30 samples, *C. albidus* from 7 samples, *C. kuetzingii* from 4 samples and *C. gastricus* from 2 samples (Refai *et al*., 1983). Kotb (1986) isolated *C. neoformans* from droppings of chickens and canaries, fecal material of wild rats and from soil.

Mahmoud (2000) reported on the isolation of three isolates of *Cryptococcus neoformans* var. *gattii* from flowers of two *Eucalyptus camaldulensis* trees in the Qutur area and one tree from the Tanta area. Pigeon and sparrow droppings were also investigated for the occurrence of *C. neoformans* within the study area. Ninety five isolates of the *neoformans var*.. *neoformans* were recovered from 550 samples of avian droppings.

Abdel-Salam (2003) reported on the characterization of Cryptococcus serotypes A and A/D in samples from Egypt

Abou El-Yazid *et al* (200 7) isolated *C. neoformans* from 10 out of 424 samples of bird droppings (5 isolates from canaries, 3 from parrots and 2 from pigeons).

The biotyping of 9 Cryptococcus isolates recovered from pigeon droppings by El_hariri *et al.* (2007) revealed the identification of 5 isolates as *C. neoformans* and one isolate as *C. gattii*. The serotyping was done by serotype-specific primers,

which confirmed the *C. neoformans* isolate as serotype A and the *C. gattii* as serotype B

C. neoformans was isolated also from uteri, vaginal discharges, fresh cattle and buffalo semen as well as from frozen cattle semen (Kotb, 1990). Saleh (2005) isolated *C. neoformans* from 10 out of 455 vaginal swabs examined from different animal species.
Mycological examination of 200 milk and 200 pigeon droppings samples by Al-Arosi *et al*. (2006) revealed the isolation of *C. neoformans* from 13 milk samples and 20 pigeon droppings. Based on the growth of the isolates on CGB medium, 29 of the isolates were biotyped as *C. neoformans* and 4 as *C. gattii*. The serotyping using reciprocal hyperimmune sera by slide agglutination test confirmed the 29 isolates as serotype A and the 4 as serotype B.

b. Cryptococcosis in man:

Few studies were carried out on cryptococcosis in man in Egypt. Girgis *et al*. (1985) reported a fatal cryptococcal meningitis in four Egyptian patients suffering from meningitis. Soliman *et al.* (1995) reported 5 cases suffering from cryptococcal meningitis. A total of 1000 cerebrospinal fluid (CSF) samples were collected by Elias *et al.* (2009) from nine governorates of Egypt during 1998-2002. All CSF samples were cultured on brain heart infusion, Wickerham and Staib agar media for fungus isolation. CSF with suspected *Cryptococcus neoformans* infections were also tested by latex agglutination test for antigen detection. Species identification of selected isolates was carried out at the Mycotic Diseases Branch, CDC, Atlanta, Georgia, USA. Fungal agents were detected microscopically and by culture in 17 of 1000 (1.7%) CSF samples tested. Ten of 17 were identified as *C. neoformans var grubii* (serotype A). Saleh *et al.* (2011) isolated *C. neoformans* from throat and vaginal swabs from women rearing pigeons.

c. Experimental infection in mice

Abdelazez *et al.* (2011) studied the correlation of capsular size and pathogenicity of *C. neoformans* in experimentally infected mice. Various capsular sizes were induced by growing the standard strain of *C. neoformans* on media containing rosemary or thyme oil. Experimental infection of mice with the different phenotypes revealed that phenotype R with the large capsule caused signs of depression, loss of appetite and weight and the P. M. findings in this group of animals showed congested brain, haemorrhagic liver, congested lungs and spleen.

When the mice were injected with cinnamon extract 24 hours post infection with the R phenotype. All mice showed normal activity, normal P. M. findings and clean histopathological profile for internal organs

d. Other studies on Cryptococcus in Egypt:

Refai *et al.* (2005) formulated six new media from the extracts of seeds and leaves of different types of plants, namely, seeds of safflower, canola and cabbage as well as leaves of eucalyptus, tea and processed tobacco leaves. Water extracts of 50-80 grams of leaves or seeds were prepared by boiling for 30 minutes in one litre distilled water, the 20 grams of agar agar were added, mixed well and finally autoclaved at 121 ^{o}C for 15 minutes. Cryptococcus isolates developed brown colour on all these media.

The effect of camphor, rosemary, fennel, cinnamon, and thyme oils on the growth of the 4 standard serotypes of *C. neoformans* was studied on the media containing these oils. All serotypes showed weak growth on media containing camphor or fennel. Cinnamon inhibited the growth completely, while there was no inhibitory effect of thyme oil. The interesting finding was that camphor and rosemary oils increased the capsule size of *C. neoformans*. This finding may be useful in developing new media containing this oil for better development of the capsule (Alarousy *et al.*, 2011).

1.2. History and nomenclature of the genus Cryptococcus

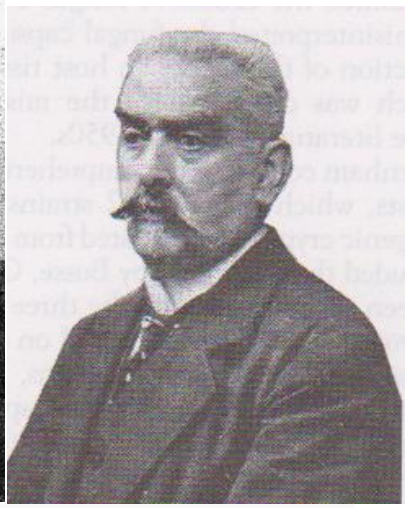
- **Kützing (1833)** described *Cryptococcus mollis* and based his description of the genus on this species. The name Cryptococcus was created from the Greek kryptus meaning 'hidden' for a group of yeasts that lacked the ability to produce endospores.
- Investigation of his original material has shown that a great part of the cells were yeasts of basidiomycetous nature. Therefore, *Cryptococcus mollis* was mentained as the type species for the genus.

Friedrich Kützing

Francesco San Felice

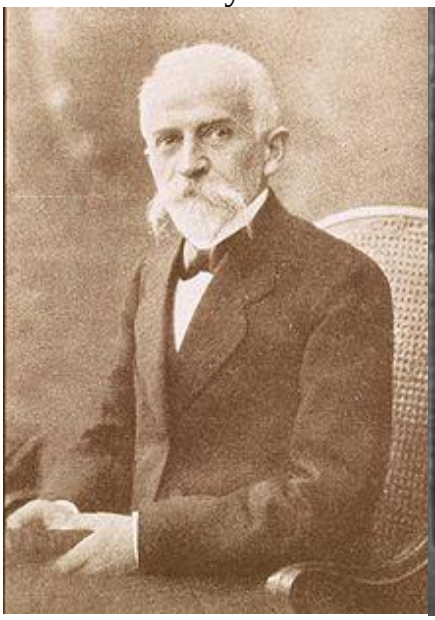
- **Sanfelice (1895)** isolated a yeast he named *Saccharomyces neoformans* from peche juice.
- **Busse and Buschke (1895)** incriminated *Saccharomyces neoformans* as a cause of fatal mycosis in a patient presenting tibial abscess.
- **Ferdinand Curtis (1896)** described a clinical disease caused by *Cryptococcus gattii*





OTTO BUSSE (1867 – 1922) Abraham Buschke (1868-1943) Ferdinand Curtis (1858-1937)

- **Klein (1901)** isolated a similar yeast from a case of mastitis, which he reported to be identical with strains of *C. neoformans* of human and plant origin.
- **Vuillemin (1901)** found cryptococci in a pulmonary lesion in a pig and named the organism *Cryptococcus hominis.* He emended the genus limiting it to ascosporogenous parasitic yeasts, since this time the name Cryptococcus has been widely used for such parasitic yeasts.




Jean Paul Vuillemin Von Hansemann

- **Frothingham (1902)** reported the isolation of *C. neoformans* from a myxomatous mass found in the lung of a horse.
- **Weiss (1902)** named this yeast *Torula neoformans.*

- **Von Hansemann (1905)** was the first, who observed the organism in association with a case of human meningitis.
- **Verse (1914)** recognized the clinical form of the human disease in a living patient, CNS involvement was characteristic
- **Guilliermond (1912)** applied the name Cryptococcus to the pathogenic types and Torula to those which were not pathogenic.
- **Stoddard and Cutler (1916)** reported additional cases and described the clinical and pathologic differences between cryptococcosis, blastomycosis, and other mycosis. They erroneously assumed that the capsules of the organisms were cysts in the tissue caused by digestive action of the fungus (histolytica) and named the organism *Torula histolytica.* torulosis or torula meningitis.
- **Benham (1935)** cleared up some of the confusion resulting from the use of the name blastomycosis and European blastomycosis for the disease caused by Busse – Buschke organism. She suggested retaining the name *Cryptococcus hominis*.
  She divided the genus into 4 groups based on the colony character, size and shape of the cells, fermentation of sugars and serological reactions.

RHODA W. BENHAM
1894—1957

Differential characteristics of cryptococcus groups

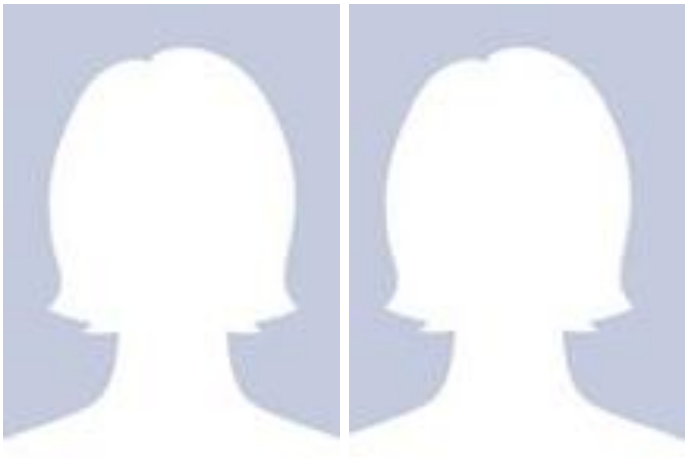
Group	Colony	Description of Cells	Gas Formation in Sugars	Agglutination			
				Serums			
				Group I	II	III	IV
1	Smooth or faintly convoluted, pasty white or cream.	Round or oval, usually 1.5 to 4.5 by 2.5 to 4.5 μ. Buds single or in chains or groups. No capsule.	+ or 0	++++	0	0	0
2	Convoluted, mucoid, cream to tan.	Round or oval, 3 to 6.5 μ by 4.5 to 6.5 μ. Thick wall, faint capsule.	0	0	++++	+	0
3	Smooth mucoid, cream to yellow or tan	Round, 4.5 to 6 μ. Thick wall, prominent capsule.	0	0	+ or 0	++++	0
4	Smooth or convoluted, pink to red.	Oval, usually 2.5 to 4 by 3 to 5 μ. Thick wall, faint capsule.	+ or 0	0	0	0	++++ or 0

- **Skinner (1950)** suggested the name Cryptococcus for the non-fermenting species and Torulopsis for the fermenting species.
- **Littman and Zimmerman (1950)** looked for all the recorded cases of Crytococcal meningitis and found 300 cases.
- **Emmons (1951)** isolated C. neoformans from the soil
- **Emmons (1952)** reported a severe outbreak of bovine cryptococcal mastitis

C.W. Emmons

Pigeons on a square

- **Lodder and Kreger – Van Rij (1952)** determined the priority of the *C. neoformans,* and this is now the accepted name, with *Cryptococcus hominis* and *Torula histolytica* falling into synonymy.



Jacomina Lodder, N. J. W. Kreger van Rij

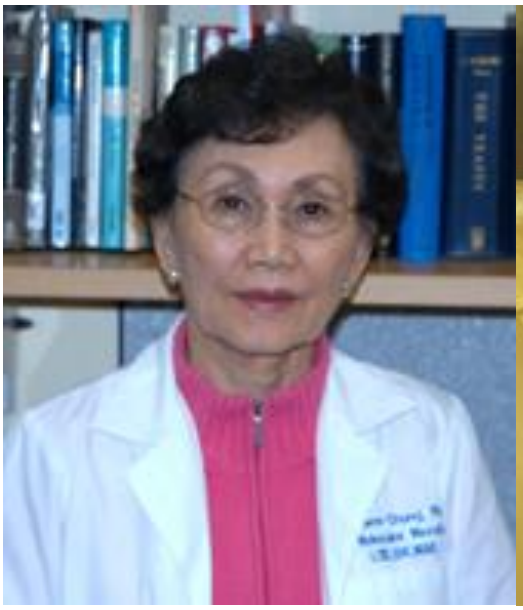
- **Emmons (1955)** isolated C. neoformans from the pigeon droppings

- **Staib** (**1962)** developed a new agar (Bird seed agar) that facilitates identification of *C. neoformans* in clinical end environmental samples

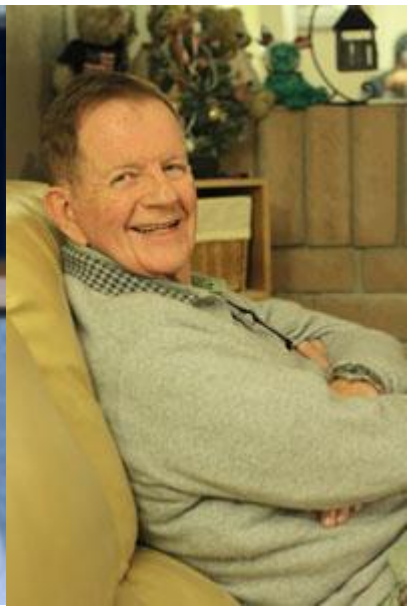
Friedrich Staib (1925-2011) Raymond Vanbreuseghem (1909-1993)

- **(1968)** Creation of cryptococcal polysaccharide antigen test. One of the best diagnostic and prognostic fungal test available to clinicians today

- **Pfafff and Spencer (1969)** placed those imperfect species, which assimilate inositol in Cryptococcus and those which lack the ability to assimilate inositol in Rhodotorula.
- **R. Vanbreuseghem and M. Takashio (1970)** isolated *C. gattii* for the first time from a leukemic patient.
- **G. S. Bulmer (1970)** proved that the cryptococcal capsule is antiphagocytic and therefore is a virulence factor.
- **Kauffman and Blumer (1970)** reported that cryptococcosis was the "awakening giant"
- **Kwon-Chung (1975)** found that there are two different mating types of cells and designated them α and a. "When she mated them on the agar plate, the sexual life cycle started. The result was thousands upon thousands of spores.



Kwon-Chung



Glenn S. Bulmer

- **Kwon-Chung, K. J., I. Polacheck, and J.E. Bennett. 1982**. Developed the CGB Agar, a diagnostic Medium for separation of *Cryptococcus neoformans* and *Cryptococcus gattii*
- **John Perfect (1983)** established the link between AIDS and cryptococcosis
- **Arturo Casadevall (1987)** reported on the generation of the first monoclonal antibodies to *C. neoformans* capsular polysaccharide


Arturo Casadevall John Perfect

- **(1990)** Cryptococcus was reported to be associated eucalyptus trees
- **(1993)** A capsular-conjugated vaccine was protective in mice
- **(1999-2001)** Cryptococcus gattii emerged on Vancouver Island, British Columbia (BC), Canada, among residents, visitors to the island, and domestic and wild animal populations.
- **Karen H. Bartlett (2004)** reported on spread of *Cryptococcus gattii* into Pacific Northwest Region of the United States

Cryptococcus gattii Working Group of the Pacific Northwest

Kausik Datta, Karen H. Bartlett, Rebecca Baer, Edmond Byrnes, Eleni Galanis, Joseph Heitman, Linda Hoang, Mira J. Leslie, Laura MacDougall,Shelley S. Magill, Muhammad G. Morshed, Kieren A. Marr

Karen H. Bartlett, Principle Investigator

- **Kwon-Chung KJ (2005):** made the classification of two pathogenic species. *C. neoformans* and *C. gattii* as separate species.
- **Loftus BJ (2005):** Genome sequence of *C. neoformans*

Brendan Loftus

Loftus BJ, Fung E, Roncaglia P, Rowley D, Amedeo P, Bruno D, Vamathevan J, Miranda M, Anderson IJ,Fraser JA, Allen JE, Bosdet IE, Brent MR, Chiu R, Doering TL, Donlin MJ, D'Souza CA, Fox DS, Grinberg V, Fu J, Fukushima M, Haas BJ, Huang JC, Janbon G, Jones SJ, Koo HL, Krzywinski MI, Kwon-Chung JK,Lengeler KB, Maiti R, Marra MA, Marra RE, Mathewson CA, Mitchell TG, Pertea M, Riggs FR, Salzberg SL,Schein JE, Shvartsbeyn A, Shin H, Shumway M, Specht CA, Suh BB, Tenney A, Utterback TR, Wickes BL,Wortman JR, Wye NH, Kronstad JW, Lodge JK, Heitman J, Davis RW, Fraser CM, Hyman RW.

- **Arturo Casadevall (2005)** Feasibility and prospects for a vaccine to prevent cryptococcosis.
- **Kausik Datta (2006)** reported on a vaccine for Cryptococcus neoformans: principles and caveats.

Kausik Datta

- **John Perfect (2008):** reported on the metabolic adaptation in *Cryptococcus neoformans* during early murine pulmonary infection

- **Del Poeta (2012):** found a drug that targets a sphingolipid on the surface of Cryptococcus that clears the infection in the brains of mice.
- **Fred Dietrich (2013)** Released the gene predictions for the serotype A isolate *Cryptococcus neoformans var. grubii* H99, based on a chromosome-based genome assembly achieved at the Duke Center for Genome Technology

1.3. Books on Cryptococcus

1.4. Reseach projects

1.4.1. Casadevall Laboratory Research Project

Arturo Casadevall, Albert Einstein College of Medicine

Many of the laboratory's projects seek to understand how hosts defend against C. neoformans and how the Cryptococcus organism's virulence contributes to disease. An antibody to fungal melanin made in the Casadevall laboratory is currently in evaluation for the treatment of melanoma, a type of skin cancer.

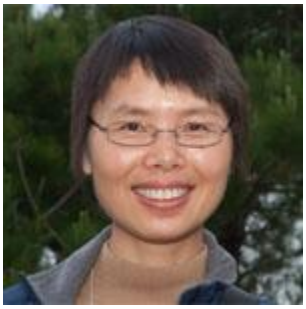
1.4.2. DUKE MYCOLOGY RESEARCH UNIT(DUMRU)

John Perfect

Dr. Perfect is engaged in molecular pathogenesis studies of *Cryptococcus neoformans*. In these studies, there is a merging of molecular biology manipulations of the pathogenic yeast with animal models and dissection of immune host factors.

1.4.3. Projects in Xiaorong Lin Lab

Department of Microbiology and Immunology, Texas A&M Health Science Center
BWF Investigator in Pathogenesis of Infectious Disease



Project 1: Morphogenesis, extracellular matrix, and development in fungi and their relationship with fungal pathogenesis

Project 2: Sexual reproduction and mitochondrial DNA inheritance in *Cryptococcus*

Xiaorong Lin, PI

1.4.4. Analysis of the Genome and Transcriptome of *Cryptococcus neoformans* var. *grubii*

Guilhem Janbon, PI
Institut Pasteur, Unité Biologie et Pathogénicité Fongiques. Département Génomes et Génétique, Paris, France

Research Team
Guilhem Janbon ,Kate L. Ormerod,Damien Paulet,Edmond J. Byrnes III,Vikas Yadav, Gautam Chatterjee,Nandita Mullapudi,Chung-Chau Hon,R. Blake Billmyre,François Brunel,Yong-Sun Bahn,Weidong Chen,Yuan Chen,Eve W. L. Chow,Jean-Yves Coppée,Anna Floyd-Averette,Claude Gaillardin,Kimberly J. Gerik,Jonathan Goldberg,Sara Gonzalez Hilarion,Sharvari Gujja,Joyce L. Hamlin,Yen-Ping Hsueh,Giuseppe Ianiri,Steven Jones,Chinnappa D. Kodira,Lukasz Kozubowski,Woei Lam,Marco Marra,Larry D. Mesner,Piotr A. Mieczkowski,Frédérique Moyrand,Kirsten Nielsen,Caroline Proux,Tristan Rossignol,Jacqueline E. Schein,Sheng Sun,Carolin Wollschlaeger,Ian A. Wood,Qiandong Zeng,Cécile Neuvéglise,Carol S. Newlon,John R. Perfect,Jennifer K. Lodge,Alexander Idnurm,Jason E. Stajich,James W. Kronstad,Kaustuv Sanyal, Joseph Heitman mail,James A. Fraser,Christina A. Cuomo mail,Fred S. Dietrich

1.4.5. Development of the Novel Antifungal VT-1129 for Cryptococcal Meningitis

The National Institutes of Health (NIH) - The Nation's Medical Research Agency

This project includes preclinical Investigational New Drug (IND)–enabling studies that will evaluate VT-1129 as a novel, oral, stand-alone drug candidate for the treatment of CM.

Elizabeth Ottinger, PI

1.4.6. Doering laboratory Research Projects

Department of Molecular Microbiology, Johns Hopkins University and Medical School
Washington University Medical School.

investigating unique aspects of cryptococcal biology with the dual goals of understanding basic processes and identifying potential drug targets.

The main focus of the work is the dominant virulence factor of *C. neoformans*, its polysaccharide capsule. They are examining how the capsule is synthesized, regulated, and associates with the cell, using approaches ranging from biochemistry and cell biology to molecular biology and genome analysis; They are interested in interactions between this fungal pathogen and its host.

Tamara Doering, PI

1.4.7. The cytoskeleton in human pathogenic fungi

Marie Kopecká, studied the yeast exoskeleton (cell wall ultrastructure in budding and fission yeasts, ß-1,3-glucan microfibril nature, biogenesis and ultrastructure) and the yeast cytoskeleton. She initiated the research of the cytoskeleton in the human patogenic yeasts Cryptococcus neoformans, Aureobasidium pullulans, Fellomyces fuzhouensis, Malassezia pachydermatis and other fungi.

Marie Kopecká, CSc.

1.4.8. Cryptococcal Meningitis Group, Research Centre of Infection and Immunity, St George's University of London, London, United Kingdom

Research team

Dr Tihana Bicanic, Dr Joseph Jarvis , Professor Tom Harrison

Laboratory studies focus on the genotype and phenotype of C. neoformans isolates, and on mechanisms of immunity in the human system. Interferon-gamma (IFN-γ) levels in the CSF were shown to be independently associated with rate of clearance of infection, providing direct in vivo evidence for the importance of this key cytokine in determining the outcome of cryptococccal infection in the human system. This finding led to a recently completed trial of adjunctive IFN-γ therapy, funded by the Wellcome Trust.

1.4.9. Center for Clinical Global Health Education at Johns Hopkins Bloomberg School of Public

The aims of this study are to 1) evaluate the burden of Cryptococcal disease among HIV-infected adults with low CD4 cell counts in Pune, India by conducting Cryptococcal antigen screening, and 2) determine whether a Cryptococcal screening strategy should be the standard of care in patients with advanced HIV disease who are admitted to medicine. Wards or are seen as new patients in the ART center.

Amita Gupta

1.4.10. Maurizio Del Poeta

Del Poeta
has found a drug that targets a sphingolipid on the surface of Cryptococcus that clears the infection in the brains of mice. He recently applied to start testing that drug on humans.

Del Poeta

1.4.11. Alfred Botha Research project

Alfred Botha

Department of Microbiology, Faculty of Science,
Stellenbosch university

The research of Alfred Botha is aimed at obtaining a better understanding of microbial ecology, especially the interactions of yeasts. Research projects include searching for natural habitats of the yeast***Cryptococcus neoformans***. In South Africa this opportunistic pathogen is annually responsible for thousands of deaths among those suffering from AIDS. However, very little is known about the natural habitats of *C. neoformans* and knowledge of this aspect will curb the spread of this yeast among the South African population. Currently, the interactions of *C. neoformans* with its natural environment are being studied

1.4.12. **Fred Dietrich**

IGSP's Center for Applied Genomics & Technology, Duke Univ. Medical Center

The project is aimed to answer the following questions:

Are there genes found in some *Cryptococcus neoformans*isolates but not in others? Are there regions of the genome or individual genes which are highly diverged between Cryptococcus isolates? Efforts are now underway at Stanford University to sequence the genome of the JEC21 strain of Cryptococcus. This is a strain that has been agreed upon by the community of Cryptococcus researchers as a reference strain. But how much do other Cryptococcus isolates differ from JEC21?

1.4.13. ISHAM Cryptococcus working group
Genotyping of Cryptococcus neoformans and Cryptococcus gattii

PROJECT 1

Cryptococcus gattii environmental survey: Europe and Mediterranean area

PROJECT 2

Genotyping of *Cryptococcus neoformans var. neoformans* (serotype D) isolates associated with cutaneous cryptococcosis

Coordinators

Massimo Cogliati,
Current Coordinator

Wieland Meyer,
Previous coordinator (2009-2012)

June Kwon-Chung,
Previous coordinator (2007-2009)

The working group is open to everybody who is interested on genotyping, molecular epidemiology and population genetics of the *C. neoformans/C. gattii* species complex. For membership please contact Massimo Cogliati (massimo.cogliati@unimi.it).

Members

- Dr. June Kwon-Chung, NIH/NAID, Bethesda, USA
- Prof. Wieland Meyer, University of Sydney, Westmead, Australia
- Dr. Luciana Trilles, FIOCRUZ, Rio de Janeiro, Brazil
- Prof. Anastasia Litvintseva, CDC, Atlanta, GA, USA
- Prof. Thomas Mitchell, Duke University Medical Center, Durham, NC, USA
- Dr. Ferry Hagen, CBS-KNAW, Utrecht, The Netherlands
- Dr. Teun Boekhout, CBS-KNAW, Utrecht, The Netherlands
- Dr. Massimo Cogliati, Università degli Studi di Milano, Milano, Italy
- Dr. Shawn Lockhart, CDC, Atlanta, GA, USA
- Dr. Françoise Dromer, Institut Pasteur, Paris, France
- Dr. Marie Desnos-Ollivier, Institut Pasteur, Paris, France
- Prof. Kirsten Nielsen,University of Minnesota, MN, USA
- Prof. Ariya Chindamporn, Chulalongkorn University, Bangkok, Thailand
- Dr. Popchai Ngamskulrungroj, Mahidol University, Bangkok, Thailand
- Prof. Elizabeth Castaneda, Instituto de Salud, Bogota, Colombia
- Dr. Patrica Escandon, Instituto de Salud, Bogota, Colombia
- Dr. Carolina Firacative, University of Sydney, Westmead, Australia
- Dr. Mara Diaz, RSMAS/University of Miami, Key Biscayne, FL, USA
- Prof. Salvatore Oliveri, Università degli Studi di Catania, Catania, Italy
- Prof. Simona Nardoni, Dipartimento di Scienze Veterinarie Università di Pisa, Pisa, Italy
- Prof. Giuseppe Criseo, University of Messina, Messina, Italy
- Prof. Cristina Macci, National Research Council (CNR), Pisa, Italy
- Prof. Ludovico Dipineto, University of Napoli Federico II, Napoli, Italy
- Dr. Luigi Vezzulli, Università di Genova, Genova, Italy
- Prof. Antonio Scopa, Università degli Studi della Basilicata, Potenza, Italy
- Prof. Vincenzo Pasquale, Università degli Studi di Napoli Parthenope, Napoli, Italy)
- Prof. Antonella De Donno, Università del Salento, Lecce, Italy
- Prof. Maria Teresa Montagna, Università degli Studi di Bari, Bari, Italy
- Dr. Stephane Ranque, Aix-Marseille University, Marseille, France
- Prof. Michele Mallié, Unité Mixte Internationale - Recherches Translationnelles sur l'infection à VIH et les Maladies Infectieuses,Montpellier, France
- Dr. Sebastien Bertout, Unité Mixte Internationale - Recherches Translationnelles sur l'infection à VIH et les Maladies Infectieuses, Montpellier, France
- Dr. Maria Francisca Colom, Universidad Miguel Hernández, Alicante, Spain
- Prof. Kathrin Tintelnot , Robert-Koch Institute, Berlin, Germany
- Prof. Maria Da Luz Martins, Instituto de Higiene e Medicina Tropical, Lisbon, Portugal
- Prof. Joao Jose Inacio Silva, Instituto Nacional de Investigação Agrária e Veterinária, Lisbon Portugal
- Prof. Ana Cristina Sampaio, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal
- Prof. Mehemet Macit Ilkit, University of Çukurova, Adana, Turkey
- Prof. Cagri Ergin, Pamukkale University, Denizli, Turkey
- Prof. Nilgün Çerikçioğlu, Marmara University,School of Medicine, Istanbul, Turkey
- Prof. Okan Tore, Uludag University, Bursa, Turkey
- Prof. Itzhack Polacheck, Hadassah-Hebrew University Medical Center, Jerusalem, Israel
- Prof. Emilija Mlinaric-Missoni, Croatian National Institute of Public Health, Zagreb, Croatia
- Prof. Aristea Velegraki, Medical School National and Kapodistrian University of Athens, Athens, Greece
- Dr. Serdar Susever, Cyprus Near East University, Nicosia, Cyprus
- Prof. Edmond Puca, University Hospital Center "Mother Teresa", Tirana, Albania
- Prof. Mohamed S. Ellabib, University of Tripoli, Tripoli, Lybia
- Prof. James Kronstad, University of British Columbia, Vancouver, BC, Canada
- Prof. Karen H. Bartlett, University of British Columbia, Vancouver, BC, Canada

1.5. Theses done by postgraduate students on Cryptococcus

1.5.1. Kirsten Nielsen

Research Interests:

Central Nervous System Penetration by *Cryptococcus neoformans*

We hypothesize that pheromone signaling plays a central role not only during mating of *C. neoformans* but also during entry into the CNS. By perturbing pheromone signaling during individual and coinfections with congenic alpha and **a**strains we can identify key components required for CNS penetration in this pathogen and provide a foundation for further treatment strategies to reduce cryptococcal meningitis.

1.5.2. Kyla Selvig

A major component of my thesis project is to analyze the role of the alkaline response transcription factor, Rim101, in altering the cell wall of *Cryptococcus neoformans* during infection. Without Rim101-regulated gene expression, *C. neoformans* becomes highly immunogenic and induces dramatic inflammation in both mouse and rabbit models of infection. Electron microscopy has shown that the *C. neoformans* cell wall structure is dramatically altered in a *rim101Δ* mutant. My goal is to identify the Rim101-mediated cell wall changed that prevent this immune recognition. To do this, I will compare the cell wall composition of WT and *rim101Δ* mutant cells. Specifically, I will use specialized chromatography and mass spectrometry to identify cell wall components that are altered on the*rim101Δ* mutant.

1.5.3. Makoto Inoue

Here, we show that the CD40 signaling in macrophages suppresses TNF-mediated inflammation and protect mice from lethal effects by acute fungal infection by *Cryptococcus neoformans*and septic shock. Ligation of CD40 on macrophages to inhibit TNF production is achieved by T cell contact to macrophages, suggesting negative regulation of innate inflammation by adaptive immunity. To exert the inhibitory effect of CD40 signaling, intracellular osteopontin (OPN) is essential as an adaptor molecule. Such suppressive role of OPN was observed in immunocompetent mice going through acute inflammation, but OPN works to enhance inflammation and immunity in immunocompromised mice. Appropriate "switching" of OPN's role from pro-inflammatory to anti-inflammatory, depending on the presence of adaptive immune cells, is therefore essential for hosts to cope with acute inflammation.

1.5.4. Marianna Feretzaki

In this study, we applied insertional mutagenesis via *Agrobacterium tumefaciens* trans-kingdom DNA delivery methods to identify mutants with a same-sex mating defect. In addition to factors known to be involved in sexual development (Crg1, Ste7, Mat2 and Znf2), three novel key regulators of sexual development were identified by our screen: Znf3, Spo11 and Ubc5. Znf3, a novel zinc finger transcription factor, orchestrates hyphal development during opposite- and same-sex mating and plays a key role in cell-cell fusion. Phenotypic and transcriptional analyses indicate that Znf3 governs mating by enhancing pheromone production. Virulence assays in murine models provide evidence that Znf3 is also required for virulence. Spo11 and Ubc5 are important factors of sporulation. Spo11 is a DNA topoisomerase IV, an ortholog that delivers DNA double strand breaks that provoke meiotic recombination, and Ubc5 a ubiquitin-conjugating enzyme. Both share significant identity with*Saccharomyces cerevisiae* Spo11 and Ubc5, key regulators of sporulation. We show that Spo11 and Ubc5 are required for sporulation during opposite- and same-sex mating in *C. neoformans* and are important factors for meiosis. These studies illustrate the power of unbiased genetic screens to reveal novel circuits that operate sexual reproduction.

1.5.5. Ricky Festa

developed a strategy that takes advantage of the toxic properties of copper using a small antifungal copper-binding molecule (8HQ) that is activated in the context of the oxidative burst by macrophages during infection. This protected form of the molecule (QBP) is aimed at preventing outright host copper imbalances. We have determined that when activated, QBP that is converted to 8HQ is fungicidal and the mode of action is most likely by bombarding the fungus with elevated copper levels. Furthermore, we have tested this concept in the context of *in vitro* infection as well as infection in the mouse lung, and have shown efficacy when QBP treatment was administered. The conditional activation of Cu ionophores by host innate immune cells manipulates and intensifies the hostile antimicrobial environment as a new approach to combat infectious disease.

1.5.6. Chen Ding:

Copper homeostasis as a virulence factor in systemic infection the human fungal pathogen *Cryptococcus neoformans*

We identified all of the genes induced either by Cu deficiency or excess in *C. neoformans*, including the previously known Ctr4 Cu importer, a new Cu importer, Ctr1, two metallothionein genes (MT1, MT2) and others. Surprisingly, both Cu inducible and Cu repressible genes are dependent on Cuf1 for their metalloregulation. To decipher which Cuf1 target genes contribute to virulence we generated Cuf1 target gene deletions and assayed survival to intra-nasal administration in mouse models of infection. We generated reporters of*C. neoformans* intracellular Cu status (high Cu or Cu deficiency) and are using live animal imaging to ascertain what Cu environment *C. neoformans* senses when it is first encountered by alveolar macrophages. Taken together, these studies will elucidate the contributions

1.5.7. Wilber Sabiiti

We show that adherence and internalization of cryptococci by brain microvascular endothelial cells is a rare event characterized by a small number of cryptococci, an indication that C. neoformans most likely uses multiple routes to traverse the BBB. Secondly, by studying clinical isolates from cerebral spinal fluid (CSF) of HIV-associated CM patients, we demonstrate that high rate of cryptococci uptake by macrophages is associated with patient fungal burden whilst the intracellular proliferation rate is inversely associated with TNF-α levels in the patient CSF. Interestingly, the high uptake – high fungal burden isolates were less encapsulated but more rapid melanin formers, traits known to modulate phagocytosis and protection from host-induced oxidative stress respectively. We therefore hypothesize that highly phagocytosed C. neoformans strains use phagocytes to disseminate faster to the brain resulting in high fungal burden.

1.5.8. Jamal Stie

After completing his Ph.D., he joined the laboratory of Dr. Deborah S. Fox at the Research Institute for Children in New Orleans, Louisiana, where his studies focused on blood-brain barrier invasion by the neurotropic fungal pathogen, Cryptococcus neoformans.

1.5.9. Ferry Hagen

Medical Molecular Microbiologist at Canisius-Wilhelmina Hospital
Nijmegen Area, Netherlands

- Low Diversity Cryptococcus neoformans Variety grubii Multilocus Sequence Types from Thailand Are Consistent with an Ancestral African Origin
- Activated dormant Cryptococcus gattii infection in a Dutch tourist who visited Vancouver Island (Canada): a molecular epidemiological approach
- The Search for the Natural Habitat of Cryptococcus gattii
- Antifungal susceptibility, serotyping, and genotyping of clinical Cryptococcus neoformans isolates collected during 18 years in a single institution in Madrid, Spain

1.5.10. Choo Khi Khi

Taylor's PhD Research Fellowship Recipient

School of Biosciences

Through an infection experiment coupled with Giemsa Staining and analysis through a compound microscope, we found that C.neoformans H99 adheres to host A549 alveolar epithelial cells as early as 2 hours post infection, and adherence is dependent on pathogen load and infection period. We have conducted a pathogen internalization assay by staining C.neoformans with Fluorescein Isothiocyanate (FITC) and quenching extracellular fluorescence signal with trypan blue. At 24 hours post infection, we noted that most of the pathogens merely adhered to the host cell, with some C.neoformans internalized into the host cell. Following that, we visualized the host filamentous actin under fluorescence microscope post staining of the host actin with Phalloidin coupled with FITC. The result revealed that actin reorganization was triggered upon adherence of the pathogen, particularly the formation of phagocytic cup in facilitating pathogen uptake. Hence, the host actin emodelling is hypothesized to be triggered by C.neoformans capsule or cell wall proteins to mediate its entry into host cells. We have extracted, enriched the cell wall/capsule proteins of C.neoformans and obtained a comprehensive protein profile of this fraction through 2-Dimensional Electrophoresis. In conclusion, our integrated approach of 2-Dimensional electrophoresis and fluorescence microscopy is hoped to provide new insights in the interaction of this pathogenic yeast with mammalian epithelial cells.

1.6. Theses on Cryptococcus done under my supervision at the Faculty of Veterinary Medicine, Cairo University:

1.6.1. Fatahalla Elshaboury

In his MS thesis (1983) he reported for the first report the isolation of Cryptococcus neoformans from pigeon droppings in Egypt. Two hundred pigeon droppings, unmixed with soil, and collected from various provinces in lower Egypt (Delta) were cultured to detect yeasts. Yeasts were isolated from 53.5% of the samples; Cryptococcus neoformans was recovered from 30 samples and Candida albicans from 19 samples. Other Cryptococcus and Candida species as well as species of Torulopsis and Rhodotorula were also encountered. Aspergillus fumigatus was isolated on 13 occasions.

1.6. 2. Hosam R Kotb

In his MS thesis (1986), he isolated *C. neoformans* from droppings of chickens and canaries, fecal material of wild rats and from soil. In his Ph D theses (1990), he isolated *C. neoformans* also from uteri, vaginal discharges, fresh cattle and buffalo semen as well as from frozen cattle semen. He prepared anticapsular monospecific antisera from the standard Cryptococcus serotypes A, D, C and B. and serotyped most of isolates recovered by the postgraduate students , as co-supervisor with me on theses done in our Department on Cryptococcus by Hala Saleh, Randa Alarousi and Sheimaa Abo-Elmagd.

1.6.3. Hala abdel Karemm Saleh

A total of 802 samples including human vaginal swabs, human throat swabs , animal vaginal swab, animal nasal swabs and soil samples were subjected to mycological isolation of yeasts. Cryptococcus neoformans was isolated from 20 human and animal throat and vaginal swabs as well as from soil samples.The dendogram analysis of RAPD pattern of C. neoformans isolates revealed a high similarity between most of the isolates that varied between 92 - 100 %.It was observed, that the strains isolated from different sites in the same species gave a high degree of similarity.

On the other hand, the similarity among animal and soil isolates was low (19- 30 %), although it was 92-93 % among the vaginal isolates of buffaloes, cows and soil samples.The inhibitory effect was studied on the growth of both *C. albicans C. neoformans* using some natural herbal oils as Camphor, Clove , Rosemary, Cinnamon, Garlic, Onion, and Mustard oils in addition to *L. termis* extract and Aloe juice extract , Bee honey and Sugar cane honey where , the data were valuable and recommended for treatment of diseases caused by them.

1.6.4. Mahmoud El-Hariri

Nine isolates of *Cr. neoformans* were isolated from 424 samples of pet birds droppings (1 pigeons,3 parrots and 5 canaries), All isolates developed BCE , not only on baird seed agar, but also on six media formulated from the extracts of seeds of safflower, canola and cabbags, and leaves of eucalyptus,tea and tobacco. , Biotyping of the 9 isolates of *Cryptococcus neoformans* recovered from bird droppings was carried out on CGB and GCP. Only one isolate was identified as *C. gattii,* 3 isolates as *Cryptococcus neoformans var neoformans* and 5 isolates as *Cryptococcus neoformans var grubii*.The reults were confirmed by PCR using specific primers. Several molecular approaches were optimized for universal amplification reaction the *Cr. neoformans* , *C. albicans M. canis* isolates. Polymorphism in intrageneic transcribed spacer regions length were selected for PCR identification by using universal two primer pairs (ITS3-ITS4) and (ITS1-ITS4). (ITS1-ITS4) produced a unique PCR product in all *M. canis* isolates while isolates of *Cr. neoformans* and *C. albicans* yielded PCR product of near size with that primer. Restriction fragments length polymorphism (RFLP) was applied on *Cr. neoformans* and *C. albicans* (ITS1-ITS4) amplicon by *Hae* III and *Bam HI*. *Hae* III generated polymorphic patterns between two fungal species. Inter simple sequence repeats (ISSR) by a single universal microsatelliate primer $(GACA)_4$ was also used. $(GACA)_4$ amplified a distinguishable banding profile for each species. All *Cr. neoformans* isolates were genotyped as *Cr. neoformans* var. *grubii* by ISSR and that finding was confirmed by two serovar specific primers for *Cr. neoformans*.

1.6.5. Randa Alarousy

Mycological examination of 200 milk and 200 pigepon samples revealed the isolation of *Cr. neoformans* (13 isolates from milk and 20 from pigeon droppings).Biotyping of the strains (n= 33) indicated that 29 (13 isolates from milk samples and 16 isolates from dropping samples) were *Cr. neoformans* and 4 were *Cr. gattii*. The serotyping of the isolates confirmed the 29 isolates as serotype A and the 4 isolates as serotype B. The study of the effect of oils indicated the inhibitory effect of camphor, fennel, and cinnamon oils on the growth of *Cryptococcus isolates,* while other oils caused marked increase in capsule size (camphor and rosemary oils) and there was no inhibitory effect of thyme oil.The isolates were studied for their phenotypic changes and virulence genes. In this study, four different phenotypes were under investigation, two varied in capsule diameter and other two varied in colony color and cell form,. The phenotypic switching was induced by certain volatile oils (rosemary and thyme). Capsule-associated genes,

The correlation of capsular size and pathogenicity of C. neoformans was studied in experimentally infected mice. Various capsular sizes were induced by growing the strains of C. neoformans on media containing rosemary or thyme oil. Experimental infection of mice with the different phenotypes revealed that phenotype R with the large capsule caused signs of depression, loss of appetite and weight and the P. M. findings in this group of animals showed congested brain, haemorrhagic liver, congested lungs and spleenomegally. when the mice were injected with cinnamon extract 24 hours post infection with the R phenotype. All mice showed normal activity, normal P. M. findings and clean histopathological profile for internal organs.

1.6.6. Sheimaa Abou-Elmagd

Master student

Out of 4527 samples of faecal samples, rectal swabs, nasal swabs, vaginal swabs, ear swabs, milk samples, conjunctival swabs, blood samples taken from buffaloes, cattle and sheep and chicken, of which 115 were from chickens and 34 from animals. Cryptococcus neoformans was isolated from faeces of cattle (15), buffaloes (13) and sheep (9). Of the 115 chicken isolates, 6 were isolated from the brain,10 from the crops, 6 from the trachea, 2 from the lungs, 2 from the proventriculus, 5 from the spleen, 10 from the kidneys and 6 from the intestine. Interestingly, during this work some isolates of *Cryptococcus neoformans* showed some sort of phenotypic switching represented in pink colour of the colonies. The application of RAPD-PCR using species-specific primers, indicated a significant degree in similarity when the genomes were compared for cluster relatedness on the basis of the pattern of polymorphism.

1.7. International conferences on Cryptococcosis

9 th International Conference on Cryptococcus and Cryptococcosis, Royal Tropical Institute (KIT), Amsterdam, The Netherlands

Programme

Friday 16 May 2014

09:00-10:30 **Session 1. Public Health Aspects of Cryptococcosis**

Chairs: Elisabeth Castañeda (Colombia) & Jacques Meis (the Netherlands)

09:00 Benjamin Park (USA) - Update on global burden of cryptococcosis

09:20 Nelesh Govender (South Africa) - Reducing cryptococcosis in Africa

09:40 Anuradha Chowdary (India) - Cryptococcosis in Asia

10:00 Marcia Lazera (Brazil) - Cryptococcosis in South America

10:20 Free communication: Volker Rickerts (Germany) - Cryptococcosis due to *Cryptococcus gattii* in Germany between 2004 and 2013

Session 2. Treatment and Resistance

Chairs: Tihana Bicanic (UK) & Itzhack Polacheck (Israel)

11:00-12:30

11:00 Bob Larsen (USA) - High dose fluconazole

11:20 Peter Pappas (USA) - Liposomal amphotericin

11:40 Angela Loyse (UK) - Antifungal drug access

12:00 Eddy Sionov (USA) - Mechanisms of resistance and heteroresistance

12:20 Free communication: Ji-Qin Wu (China) - Identification of amphotericin B as a P-glycoprotein substrate at the blood-brain barrier: in vitro and in vivo evidence

Chairs: Tania Sorrell (Australia) & Tom Harrison (UK)

12:30-14:00

John Perfect (USA) - Discussion of guidelines

Jeremy Day (UK) - Open access clinical trials in cryptococcal meningitis

14:00-15:30 **Session 3. Pathogenesis**

Chairs: Bettina Fries (USA) & Oscar Zaragoza (Spain)

14:00 Liping Zhu (China) - Human susceptibility to *Cryptococcus neoformans*

14:20 Liise-ann Pirofski (USA) - The role of B cells in immunity to cryptococcosis

14:40 Marcio Rodrigues (Brazil) - Secretory regulators and cryptococcal pathogenesis

15:00 Kirsten Nielsen (USA) - Role of titan cells during infection

15:20 Free communication: Greetje Vande Velde (Belgium) - The course of mouse pulmonary cryptococcosis visualized by multimodal imaging in live animals

Saturday 17 May 2014

ISHAM working group 1 [Breakfast session]

08:00-08:45 ***Chairs: María Francisca Colom Valiente (Spain) & Massimo Cogliati (Italy)***

Wieland Meyer (Australia) - The global MLST network

08:00 Ferry Hagen (Netherlands) - The global STR network

08:15 Massimo Cogliati (Italy) - Environmental survey of *Cryptococccus*

08:30 *neoformans* and *C. gattii* in European and Mediterranean area

Session 4. Genetics & molecular biology I

Chairs: Jennifer Lodge (USA) & Andrew Alspaugh (USA)

09:00-10:30 Andrew Alspaugh (USA) - Rim pathway-mediated adaptation to the host environment

09:00 Julie Djordjevic (Australia) - A novel branch of phospholipase C-mediated

09:20 signalling via inositol polyphosphate kinases is essential for fungal virulence

Damian Krysan (USA) - Novel antifungal targets

09:40 Maurizio Del Poeta (USA) - Role of sphingolipids in virulence

10:00 Free communication: Sara Gonzalez-Hilarion (France) - Nonsense-mediated

10:20 mRNA decay (NMD) in *Cryptococcus neoformans*

Session 5. Host – pathogen interaction I

Chairs: Tamara Doering (USA) & Marcio Rodrigues (Brazil)

June Kwon-Chung (USA) - The difference between *Cryptococcus gattii* and *C.*

11:00-12:30 *neoformans* is more striking than we thought

Bettina Fries (USA) - Replicative aging of *Cryptococcus neoformans* and its

11:00 implications for pathogenesis of chronic cryptococcosis

Alex Idnurm (USA) - Hypermutation, pseudophyhae and virulence in mammalian

11:20 and invertebrate hosts

Oscar Zaragoza (Spain) - Phenotypic variation during cryptococcosis

11:40 Free communication: Mitra Shourian (Canada) - The Cnes2 locus on mouse

12:00 chromosome 17 regulates host resistance to progressive cryptococcal infection

12:20 through pleiotropic effects on host immunity

14:00-15:30 **Session 6. The species complex**

Chairs: Joseph Heitman (USA) & Wieland Meyer (Australia)

14:00 Joe Heitman (USA) - Evolutionary genomics of the species complex

14:20 Matthew Fisher (UK) - Fungal Whole Genome Sequence Typing - it's not just for

14:40	bacteria
15:00	Christina Cuomo (USA) - Population genomic analysis of the human pathogenic fungus *Cryptococcus neoformans*
15:10	Free communication: Maureen Donlin (USA)- Cross talk between the cell wall integrity and cAMP/protein kinase A pathways in *C. neoformans*
15:20	Free communication: Candy Ramirez Zavaleta (USA) - Characterization of two drug transporters during late stages of sexual development in *Cryptococcus neoformans*
	Free communication: Kyla Selvig (USA) - The basics of the *Cryptococcus neoformans* alkaline response pathway: Adapting to a bitter host

Sunday 18 May 2014

	Session 7. Immunology I
	Chairs: Anna Vecchiarelli (Italy) & Robin May (UK)
	Guangxun Meng (China) - Function of the inflammasome in the host defense against *Cryptococcus neoformans*infection
09:00-10:30	Theo Geijtenbeek (Netherlands) - Fungal interactions with dendritic cells
	Kazu Kawakami (Japan) - Dendritic cells, toll-like receptors, PAMPs
09:00	Free communication: Karen Wozniak (USA) - Protection Against *C. neoformans* Pulmonary Infection in the Absence of Adaptive Immunity
09:20	Free communication: Daniel Piehler (Germany)- Characterization of T1/ST2-
09:40	expressing T helper 2 cells and innate lymphoid cells type 2 in pulmonary
10:00	cryptococcosis
	Free communication: Darin Wiesner (USA) - Specialized Regulatory T Cells
10:10	Suppress Pathologic Type-2 Helper T Cell Inflammation During Pulmonary Cryptococcosis
10:20	

11:00-12:30	**Session 8. Immunology II**
	Chairs: Liping Zhu (China) & Gottfried Alber (Germany)
11:00	Michael Olszewski (USA) - Interactions with macrophages
11:20	Floyd Wormley (USA) - Role of Th17 cells in cryptococcosis
11:40	Anna Vecchiarelli (Italy) - Immune modulation by capsular material of *C. neoformans*
12:00	
12:20	Robin May (UK) - Virulence and macrophages
	Free communication: Zachary Hadd (USA) - T-cell restricted Notch signaling

promotes immune protection against*C. neoformans* in the infected mice

Session 9. Virulence

Chairs: Christina Hull (USA) & Guilhem Janbon (France)

14:00-15:30 James Kronstad (Canada) - Iron metabolism and virulence
Simon Johnston (UK) - Cell-based and alternative virulence models
Hiten Madhani (USA) - New mechanisms of cryptococcal virulence

14:00 Xiaorong Lin (USA) - Morphology and virulence
14:20 Free communication: Wilber Sabiiti (UK) - Efficient phagocytosis and laccase
14:40 activity are associated with adverse clinical outcome of HIV-associated
15:00 Cryptococcal meningitis
15:20

Monday 19 May 2014

Session 10. Host pathogen interaction II

Chairs: Kirsten Nielsen (USA) & Kazuyoshi Kawakami (Japan)

09:00-10:30 Joe Jarvis (UK) - Protective immuno-response and immunotherapy
Graeme Meintjes (South Africa) - Immune Reconstitution Inflammatory Syndrome (IRIS)
Gottfried Alber (Germany) - Cryptococcus neoformans-related Immune Reconstitution Inflammatory Syndrome (IRIS): Analysis of pathomechanisms in a mouse model

09:00
09:20
09:40 Free communication: Robert Evans (UK)- The study of cryptococcal Phospholipase B and its role in virulence during macrophage infection
10:00 Free communication: Michael Davis (USA) - *Cryptococcus neoformans*-induced Iysosomal damage promotes intracellular survival and growth of the organism within macrophages
10:10
10:20 Free communication: Alexandre Alanio (France) - New tools to study the dynamics of *Cryptococcus neoformans*adaptation to host

11:00-12:30 Session 11. Genetics & molecular biology II

Chairs: Julie Djordjevic (Australia) & James Kronstad (Canada)

11:00 Christina Hull (USA) - Spores as infectious propagules
11:20 Tamara Doering (USA) - Capsule synthesis and regulation
11:40 Guilhem Janbon (France) - Introns in *Cryptococcus neoformans*

12:00 Jennifer Lodge (USA) - The dynamics of the cell wall
12:20 Free communication: John Panepinto (USA) - Post-transcriptional Mechanisms of Stress Adaptation in *Cryptococcus neoformans*

Cryptococcus Genomics workshop

14:15-16:45 ***Chairs: James Fraser (Australia) and Guilhem Janbon (France)***

14:00 Hiten Madhani (USA) - The state of the genome deletion collection
14:15 Gavin Sherlock (USA) - A manually curated gene set and community-based gene naming protocol for Cryptococcus
14:35 Rhys Farrer (USA) - Comparative genomics of C. gattii towards theevolution of its pathogenicity
14:55 Kate Ormerod (Australia) - The H99 family tree: Variation in the commonlaboratory reference strains of Cryptococcus
15:10 Jessica Brown (USA) - A chemical-genetic portrait of a human fungalpathogen
15:25 Blake Billmyre (USA) - Anatomy of an outbreak: Recombining clonalclusters comprise the VGII C. gattii population
15:40 David Engelthaler (USA) - A whole population comparativegenomics approach to understanding the emergence of Cryptococcus gattii in the American Pacific Northwest
15:55 Johanna Rhodes (UK) - Genomic approaches to inferring recombination

14:15-16:45 Clinical master class

14:15 Olivier Lortholary - Introduction
14:35 Graeme Meintjes (South Africa) - Clinical Masterclass case presentations
14:55 Liping Zhu (China) - Cryptococcus neoformans and C. gattii infection in an apparently immunocompetent host
15:15 Tania Sorrell (Australia) - Diagnosis and treatment cryptococcosis due to C. gattii

3 Short casus presentations:
15:35 from Indonesia - Retno Wahyuningsih
15:45 from Uganda - Henry Nabeta
15:55 from Russia - Tatiana Bogomolova

16:05 Round table discussion: All you wanted to know on management of cryptococcal infections
Olivier Lortholary (France) and Annemarie Brouwer (the Netherlands)

2. Taxonomy and nomenclature of the genus *Cryptococcus*

Cryptococcus (Latin for "hidden sphere") is a genus of fungi that grow in culture as yeasts. The sexual forms or teleomorphs of *Cryptococcus* species are filamentous fungi in the genus *Filobasidiella*. The name *Cryptococcus* is used when referring to the yeast states of the fungi. There are about 50 recognized species of *Cryptococcus*, but the taxonomy of the group is currently being re-evaluated with up-to-date methods. The majority of species live in the soil and are not harmful to humans.

2.1. Taxonomic classification

› Fungi
› Dikarya
› Basidiomycota
› Agaricomycotina
› Tremellomycetes
› Tremellales
› Tremellaceae
› Filobasidiella
› Filobasidiella/Cryptococcus

2.2. Cryptococcus species

- There are 322 yeasts, which were given the genus name Cryptococcus, as follows:

Cryptococcus farciminosus, Cryptococcus nigricans, Cryptococcus tsukubaensis, Cryptococcus phylloplanus ,Cryptococcus hominis,Cryptococcus costantinii, Cryptococcus breweri, Cryptococcus intermedius, Cryptococcus simplex, Cryptococcus amylolentus,Cryptococcus aquaticus, Cryptococcus curiosus, Cryptococcus curvatus,Cryptococcus huempii, Cryptococcus marinus, Cryptococcus podzolicus,Cryptococcus aggregatus, Cryptococcus albidosimilis, Cryptococcus ater, Cryptococcus bacillisporus, Cryptococcus baldrensis, Cryptococcus bhutanensis, Cryptococcus cellulolyticus,Cryptococcus cereanus , Cryptococcus cerebriloculosus, Cryptococcus consortionis, Cryptococcus copellii, Cryptococcus corallinus, Cryptococcus dimennae, Cryptococcus elinovii, Cryptococcus farinae, Cryptococcus ferigula, Cryptococcus friedmannii, Cryptococcus fuscescens,Cryptococcus gastricus, Cryptococcus genitalis, Cryptococcus gilchristi,Cryptococcus gilvescens, Cryptococcus glabratus, Cryptococcus glutinis,Cryptococcus guilliermondii, Cryptococcus guttulatus, Cryptococcus harteri, Cryptococcus hempflingii, Cryptococcus himalayensis,Cryptococcus hinnuleus, Cryptococcus hondurianus, Cryptococcus interdigitalis , Cryptococcus kartulisii, Cryptococcus kleinii, Cryptococcus kuetzingii, Cryptococcus lactativorus, Cryptococcus laryngitidis,Cryptococcus magnus, Cryptococcus ater, Cryptococcus ludwigii,Cryptococcus lupi, Cryptococcus macroglossiae, Cryptococcus mattletii,Cryptococcus mekundu, Cryptococcus mena, Cryptococcus meningitidis,Cryptococcus metaniger, Cryptococcus minor, Cryptococcus mucorugosus, Cryptococcus pararoseus, Cryptococcus pinoysimilis,Cryptococcus pleomorpha, Cryptococcus psicrofilicus, Cryptococcus psoriasis, Cryptococcus pulverulentus, Cryptococcus radiatus,Cryptococcus

rivoltae, Cryptococcus rubrorugosus, Cryptococcus skinneri,Cryptococcus skutetskyii, Cryptococcus socialis, Cryptococcus sulphureus,Cryptococcus terreus, Cryptococcus terricola, Cryptococcus tokishigei,Cryptococcus tyrolensis, Cryptococcus uvae, Cryptococcus victoriae,Cryptococcus vini, Cryptococcus wrightensis, Cryptococcus yarrowii,Cryptococcus hungaricus, Cryptococcus uniguttulatus, Cryptococcus castellanii, Cryptococcus favrei, Cryptococcus pulmonalis, Cryptococcus tonsillarum, Cryptococcus albus, Cryptococcus rhodozymus, Cryptococcus malassezii, Cryptococcus pachydermatis, Cryptococcus macerans,Cryptococcus bacillaris, Cryptococcus capillitii, Cryptococcus constantinii,Cryptococcus linguae-pilosae, Cryptococcus lithogenes, Cryptococcus neoformans, Cryptococcus ovalis, Cryptococcus plimmeri, Cryptococcus ruber, Cryptococcus stellatus, Cryptococcus aerius, Cryptococcus albidus,Cryptococcus candidus, Cryptococcus colliculosus, Cryptococcus dattilus,Cryptococcus flavus, Cryptococcus flavescens, Cryptococcus gropengiersseri, Cryptococcus heveanensis, Cryptococcus histolyticus,Cryptococcus holmii, Cryptococcus humicola, Cryptococcus infirmominiatus, Cryptococcus laurentii, Cryptococcus lipofer,Cryptococcus luteolus, Cryptococcus molischianus, Cryptococcus nasalis,Cryptococcus pulcherrimus, Cryptococcus sphaericus, Cryptococcus utilis,Cryptococcus bronchialis, Cryptococcus californicus, Cryptococcus diffluens, Cryptococcus fermentans, Cryptococcus liquefaciens,Cryptococcus melibiosum, Cryptococcus pseudoaerius, Cryptococcus rotundatus, Cryptococcus sanniei, Cryptococcus tonsillae, Cryptococcus dermatitidis, Cryptococcus dermatitis, Cryptococcus adeliensis,Cryptococcus arrabidensis, Cryptococcus chernovii, Cryptococcus cylindricus, Cryptococcus nodaensis, Cryptococcus oeirensis,Cryptococcus phenolicus, Cryptococcus saitoi, Cryptococcus uzbekistanensis, Cryptococcus wieringae, Cryptococcus nyarrowii,Cryptococcus haglerorum, Cryptococcus statzelliae, Cryptococcus heimaeyensis, Cryptococcus niccombsii, Cryptococcus capsulatus,Cryptococcus watticus, Cryptococcus nemorosus, Cryptococcus perniciosus, Cryptococcus allantoinivorans, Cryptococcus taeanensis,Cryptococcus antarcticus, Cryptococcus vishniacii, Cryptococcus kefyr,Cryptococcus conglobatus, Cryptococcus tephrensis, Cryptococcus subarcticus, Cryptococcus peneaus, Cryptococcus aureus, Cryptococcus carnescens, Cryptococcus daszewskae, Cryptococcus fragicola,Cryptococcus longus, Cryptococcus musci, Cryptococcus pseudolongus,Cryptococcus ramirezgomezianus, Cryptococcus surugaensis,Cryptococcus mycelialis, Cryptococcus taibaiensis, Cryptococcus foliicola,Cryptococcus taiwaniana, Cryptococcus amylolyticus, Cryptococcus armeniacus, Cryptococcus cistialbidi, Cryptococcus humicola,Cryptococcus huempii, Cryptococcus dactyliferus, Cryptococcus amylolentus, Cryptococcus podzolicus, Cryptococcus gammari,Cryptococcus nudans, Cryptococcus marinus, Cryptococcus jeanselmei,Cryptococcus membranogenes, Cryptococcus lactis, Cryptococcus cicadarum, Cryptococcus varians, Cryptococcus anguillulae, Cryptococcus inaequalis, Cryptococcus bernasconii, Cryptococcus burnieri,Cryptococcus cooperi, Cryptococcus eguttulatus, Cryptococcus fontoynontii, Cryptococcus fuscus, Cryptococcus gotoi, Cryptococcus gougerotii-ganceae, Cryptococcus gracilioides, Cryptococcus irritans,Cryptococcus krausii, Cryptococcus lacrymeatus, Cryptococcus mugera,Cryptococcus muris, Cryptococcus otae, Cryptococcus pyogenes,Cryptococcus cornealis, Cryptococcus dubius, Cryptococcus elongatus,Cryptococcus graciloides, Cryptococcus kayongosi, Cryptococcus mitis,Cryptococcus psichrophylicus, Cryptococcus quasilinguae-pilosae,Cryptococcus cavarae, Cryptococcus infiltrans, Cryptococcus septicus,Cryptococcus pettitii, Cryptococcus gotti-brazzolae, Cryptococcus natans,Cryptococcus sennae, Cryptococcus salmoneus, Cryptococcus alvearius,Cryptococcus clava, Cryptococcus anobii, Cryptococcus bainieri,Cryptococcus cerevisiae, Cryptococcus xanthogenicus, Cryptococcus niger, Cryptococcus nebulosus, Cryptococcus rogeri, Cryptococcus fermentum, Cryptococcus degenerans, Cryptococcus granulomatogenes,Cryptococcus cavarae, Cryptococcus mirandei, Cryptococcus mollis,Cryptococcus brunneus, Cryptococcus coccineus, Cryptococcus ovoidea,Cryptococcus corsellii, Cryptococcus epidermidis, Cryptococcus lesieurii,Cryptococcus myrmeciae, Cryptococcus neveuxii, Cryptococcus paraflavus, Cryptococcus parasitarius, Cryptococcus silvicola,Cryptococcus bestiolae, Cryptococcus dejecticola, Cryptococcus festucosus, Cryptococcus hondurianus, Cryptococcus gattii, Cryptococcus mirandei, Cryptococcus curvatus, Cryptococcus randhawai, Cryptococcus stepposus, Cryptococcus feraegula, Cryptococcus infirmo-miniatus,Cryptococcus kutzingii, Cryptococcus terricolus, Cryptococcus tonkini,Cryptococcus hessleri, Cryptococcus mujuensis, Cryptococcus harensis,Cryptococcus anemochoreius, Cryptococcus cuniculi, Cryptococcus ater,Cryptococcus glutinus, Cryptococcus sulfureus, Cryptococcus rajasthanensis, Cryptococcus carnescens, Cryptococcus arboriformis,Cryptococcus fagi, Cryptococcus cisti-albidi, Cryptococcus skutetzkyi,Cryptococcus filicatus, Cryptococcus flavus, Cryptococcus liquefaciens,Cryptococcus zeae, Cryptococcus keelungensis, Cryptococcus tepidarius,Cryptococcus tibetensis, Cryptococcus pseudaerius, Cryptococcus gilchristii, Cryptococcus gropengiesseri, Cryptococcus costantinii,Cryptococcus pinus, Cryptococcus shivajii, Cryptococcus cerealis,Cryptococcus spencermartinsiae, Cryptococcus agrionensis, Cryptococcus dermatitidis, Cryptococcus spaericus, Cryptococcus metallitolerans,Cryptococcus ibericus, Cryptococcus aciditolerans, Cryptococcus bromeliarum, Cryptococcus terrestris, Cryptococcus tepidarius,Cryptococcus farciminosus, Cryptococcus mangaliensis, Cryptococcus taiwanensis, Cryptococcus

cyanovorans, Cryptococcus yokohamensis,Cryptococcus nanyangensis, Cryptococcus psychrotolerans, Cryptococcus tronadorensis, Cryptococcus fonsecae, Cryptococcus frias, Cryptococcus lacticolor

- Many of these species have been moved to other genera, others have been considered synonyms and the number of accepted species has been much reduced to 37-50 species. However new species have been described, so that the number is increasing again and may reach up to 100 species.
- Of all species, *Cryptococcus neoformans* and *Cryptococcus gattii are* the major human and animal pathogen. However, other species as, *Cryptococcus laurentii*, *C. uniguttualus, C. gastricus, C. albidus,C. loteolus, C. adeliensis,C.humicola, C. magnus, C. diffluens*, *C. curvatus* and others have been known to occasionally cause moderate-to-severe disease.

2.2.1. Species accepted in this genus (doctorfungus):

1. *Cryptococcus aerius*
2. *Cryptococcus albidosimilis*
3. *Cryptococcus albidus*
4. *Cryptococcus albidus var. albidus*
5. *Cryptococcus albidus var. difluens*
6. *Cryptococcus amylolentus*
7. *Cryptococcus antarcticus*
8. *Cryptococcus aquaticus*
9. *Cryptococcus ater*
10. *Cryptococcus bhutanensis*
11. *Cryptococcus consortionis*
12. *Cryptococcus curvatus*
13. *Cryptococcus dimennae*
14. *Cryptococcus feraegula*
15. *Cryptococcus flavus*
16. *Cryptococcus friedmannii*
17. *Cryptococcus fuscescens*
18. *Cryptococcus gastricus*
19. *Cryptococcus gilvescens*
20. *Cryptococcus heveanensis*
21. *Cryptococcus huempii*
22. *Cryptococcus humicolus*
23. *Cryptococcus hungaricus*
24. *Cryptococcus kuetzingii*
25. *Cryptococcus laurentii*
26. *Cryptococcus luteolus*
27. *Cryptococcus macerans*
28. *Cryptococcus magnus*
29. *Cryptococcus marinus*
30. *Cryptococcus gattii*
31. *Cryptococcus neoformans var. grubii*
32. *Cryptococcus neoformans var. neoformans*
33. *Cryptococcus podzolicus*
34. *Cryptococcus skinneri*
35. *Cryptococcus terreus*
36. Cryptococcus uniguttulatus
37. Cryptococcus vishniacii
38. *Cryptococcus yarrowii*

2.2.2. Non-accepted species (doctorfungus):

1. ***Cryptococcus bacillisporus*** *(obsolete)*
   This obsolete species is a synonym of C. neoformans var. gattii
2. ***Cryptococcus capillitii*** *(obsolete)*
   This obsolete species is a synonym of Malassezia furfur
3. ***Cryptococcus capsulatus*** *(obsolete)*
   This obsolete species is a synonym of Histoplasma capsulatum var. capsulatum
4. ***Cryptococcus carollinus*** *(obsolete)*
   This obsolete species is a synonym of Rhodotorula mucilaginosa
5. ***Cryptococcus glabratus*** *(obsolete)*
   This obsolete species is a synonym of Candida glabrata

6. ***Cryptococcus ludwigi*** *(obsolete)*
   This obsolete species is a synonym of Rhodotorula mucilaginosa
7. ***Cryptococcus malassezi*** *(obsolete)*
   This obsolete species is a synonym of Malassezia furfur
8. ***Cryptococcus mena*** *(obsolete)*
   This obsolete species is a synonym of Rhodotorula mucilaginosa
9. ***Cryptococcus meningitidis*** *(obsolete)*
   This obsolete species is a synonym of C. neoformans
10. *C. bacillisporus is an obsolete synonym of Cryptococcus gattii .*
11. ***Cryptococcus pachydermatis*** *(obsolete)*
    This obsolete species is a synonym of Malassezia pachydermatis
12. ***Cryptococcus pararoseus*** *(obsolete)*
    This obsolete species is a synonym of Rhodotorula mucilaginosa
13. ***Cryptococcus radiatus*** *(obsolete)*
    This obsolete species is a synonym of Rhodotorula mucilaginosa
14. ***Cryptococcus ruber*** *(obsolete)*
    This obsolete species is a synonym of Rhodotorula mucilaginosa
15. ***Cryptococcus rubrorugosus*** *(obsolete)*
    This obsolete species is a synonym of Rhodotorula mucilaginosa
16. ***Cryptococcus sanniei*** *(obsolete)*
    This obsolete species is a synonym of Rhodotorula mucilaginosa
17. ***Cryptococcus simplex*** *(obsolete)*
    This obsolete species is a synonym of Rhodotorula mucilaginosa

2.2.3. Accepted *Cryptococcus* species according to *NCBI* (44 species and 7 varietes):

1. *Cryptococcus aciditolerans*
2. *Cryptococcus adeliensis*
3. *Cryptococcus aerius*
4. *Cryptococcus albidosimilis*
5. *Cryptococcus albidus*
   a. *Cryptococcus albidus var. albidus*
   b. *Cryptococcus albidus var. kuetzingii*
   c. *Cryptococcus albidus var. ovalis*
6. *Cryptococcus antarcticus*
   a. *Cryptococcus antarcticus var. circumpolaris*
7. *Cryptococcus cf. antarcticus DBVPG 5900*
8. *Cryptococcus arrabidensis*
9. *Cryptococcus ater*
10. *Cryptococcus bhutanensis*
11. *Cryptococcus chernovii*
12. *Cryptococcus consortionis*
13. *Cryptococcus cylindricus*
14. *Cryptococcus diffluens*
15. *Cryptococcus elinovii*
16. *Cryptococcus filicatus*
    a. *Cryptococcus filicatus var. filicatus*
    b. *Cryptococcus filicatus var. pelliculosus*
17. *Cryptococcus friedmannii*
18. *Cryptococcus fuscescens*

19. *Cryptococcus gastricus*
20. *Cryptococcus gilvescens*
21. *Cryptococcus aff. gilvescens IMUFRJ 51978*
22. *Cryptococcus aff. gilvescens IMUFRJ 51979*
23. *Cryptococcus himalayensis*
24. *Cryptococcus ibericus*
25. *Cryptococcus liquefaciens*
26. *Cryptococcus magnus*
27. *Cryptococcus aff. magnus A103*
28. *Cryptococcus aff. magnus A117*
29. *Cryptococcus metallitolerans*
30. *Cryptococcus oeirensis*
31. *Cryptococcus cf. oeirensis K95b*
32. *Cryptococcus phenolicus*
33. *Cryptococcus randhawii*
34. *Cryptococcus saitoi*
35. *Cryptococcus silvicola*
36. *Cryptococcus socialis*
37. *Cryptococcus stepposus*
38. *Cryptococcus terreus*
39. *Cryptococcus terricola*
40. *Cryptococcus cf. terricola Ice99-3TxM-Y12*
41. *Cryptococcus uzbekistanensis*
42. *Cryptococcus cf. uzbekistanensis EB93-4B-Y16*
43. *Cryptococcus vishniacii*
    a. *Cryptococcus vishniacii ANT03-052*
44. *Cryptococcus wieringae*

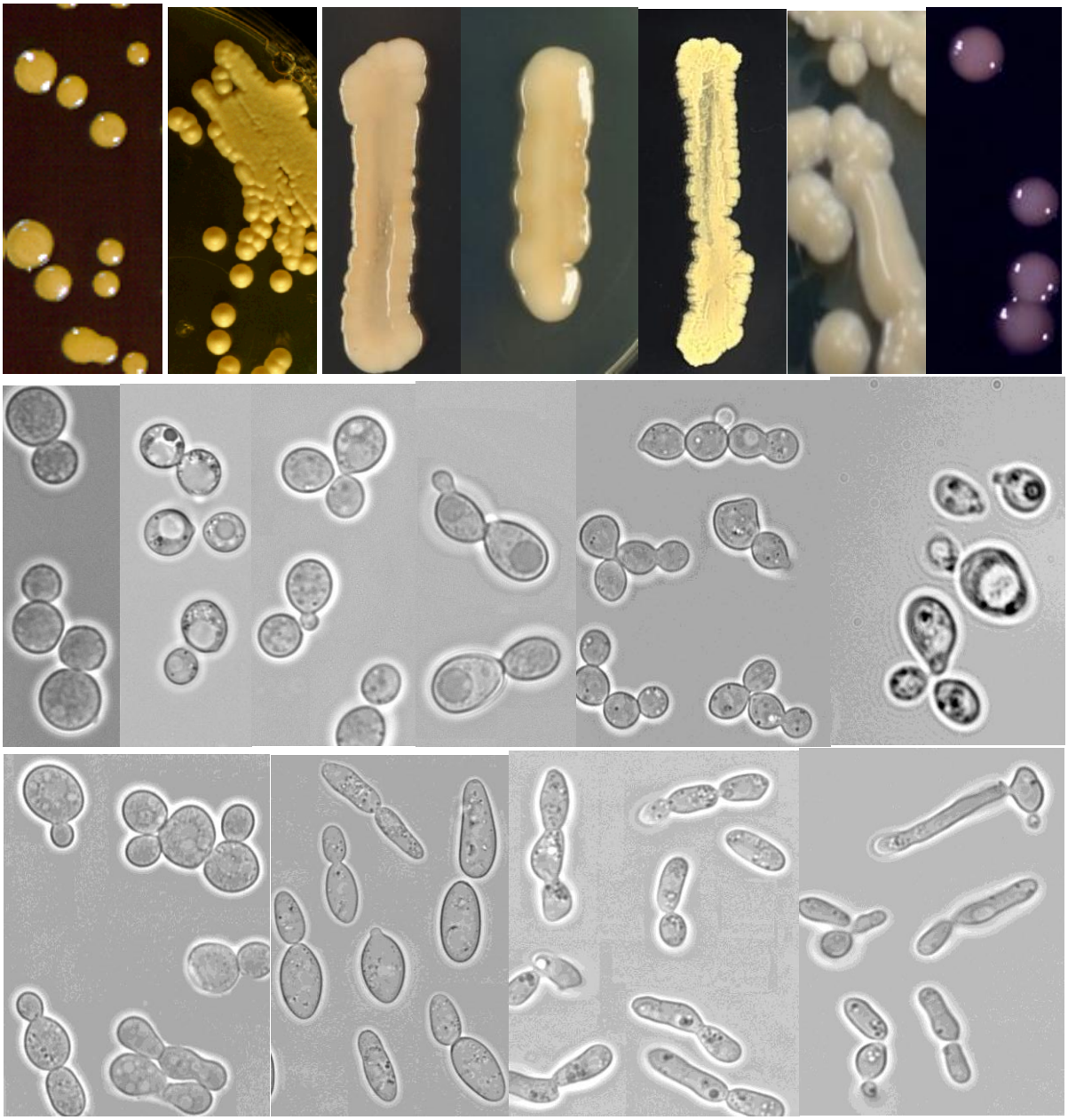
2.2.4. Newly described Cryptococcus species

1. *Cryptococcus allantoinivorans*
2. *Cryptococcus anemochoreius*
3. *Cryptococcus arboriformis*.
4. *Cryptococcus bestiolae*
5. *Cryptococcus bromeliarum*
6. *Cryptococcus cerealis*
7. *Cryptococcus cyanovorans*
8. *Cryptococcus taeanensis*
9. *Cryptococcus dejecticola*
10. *Cryptococcus festucosus*
11. *Cryptococcus foliicola*
12. *Cryptococcus haglerorum*
13. *Cryptococcus heimaeyensis*
14. *Cryptococcus keelungensis*
15. *Cryptococcus lacticolor*.
16. Cryptococcus nemorosus
17. *Cryptococcus nyarrowii*
18. *Cryptococcus paraflavus*
19. *Cryptococcus perniciosus*
20. *Cryptococcus pinus*
21. *Cryptococcus rajasthanensis*
22. *Cryptococcus statzelliae*
23. *Cryptococcus statzelliae*
24. *Cryptococcus taeanensis*
25. *Cryptococcus taibaiensis*
26. *Cryptococcus tephrensis*
27. *Cryptococcus thermophiles*
28. *Cryptococcus yokohamensis*
29. *Cryptococcus victoriae*
30. *Cryptococcus zeae*

3. Morphology of *Cryptococcus*

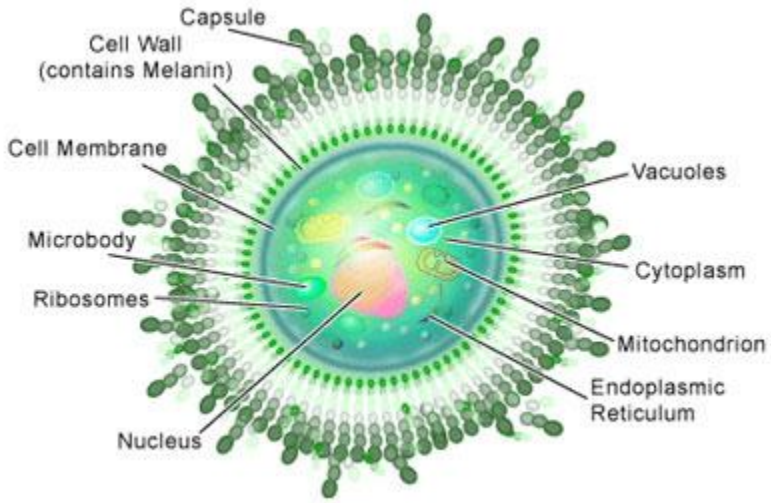
3.1. Macro- and microscopic morphology

On solid media most species have a slimy appearance, carotenoid pigments may be produced. The cells of *Cryptococcus* species are spheroidal, ovoidal, elongate, amoeboid or polymorphic. Cells of most strains are covered by a thin layer of glycoprotein capsular material that has a gelatin-like consistency and that, among other functions, serves to help extract nutrients from the soil. Pseudeomycelium is either not formed or rudimentary.



3.2. Ultrastructure

The electron microscopy of Cryptococcus cell reveals the presense of a capsule, cell wall, plasma membrane and nucleus, as well as other intracytoplasmic organelles. The capsule, sometimes thicker than cells, consists of either coiled, intertwining microfibrils or dots; the dot-type appears with or without radiating cilia-type filaments at the periphery. A clear zone often separats the capsule from the cell wall. The wall appears to be composed of multiple parallel layers of thin membraneous sheaths containing an inner dense dark zone and an outer lighter area. The plasma membrane is similar to that found in other yeasts. The nucleus shows a clear membrane and generally has disintegrated chromatin material, instead of a definite nucleolus. Mitochondria are consistent in size and shape. Lipid granules with glycogen in transition, large numbers of vacuoles, well preserved ribosomes, few plasmalemmosomes and endoplasmic reticulum are observed. Bud separation demonstrats a simulated break-off appearance from mother cells.



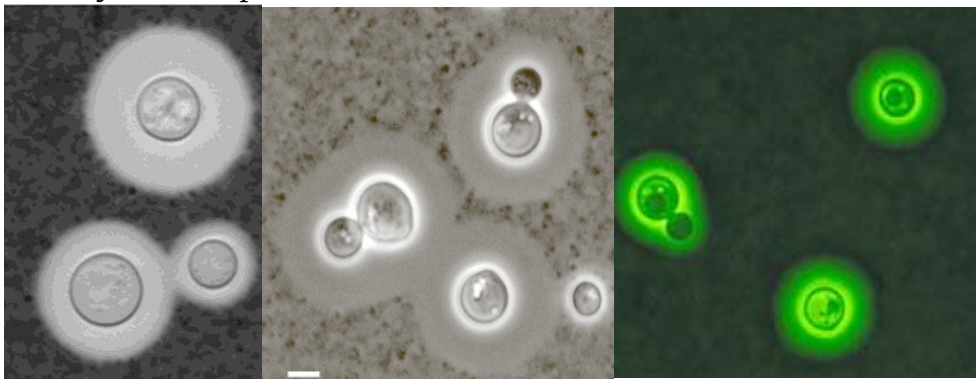
An idealized depiction of a C. neoformans cell. Note that melanin is found in the cell wall. The Basic Biology of C. neoforman. www.scq.ubc.ca

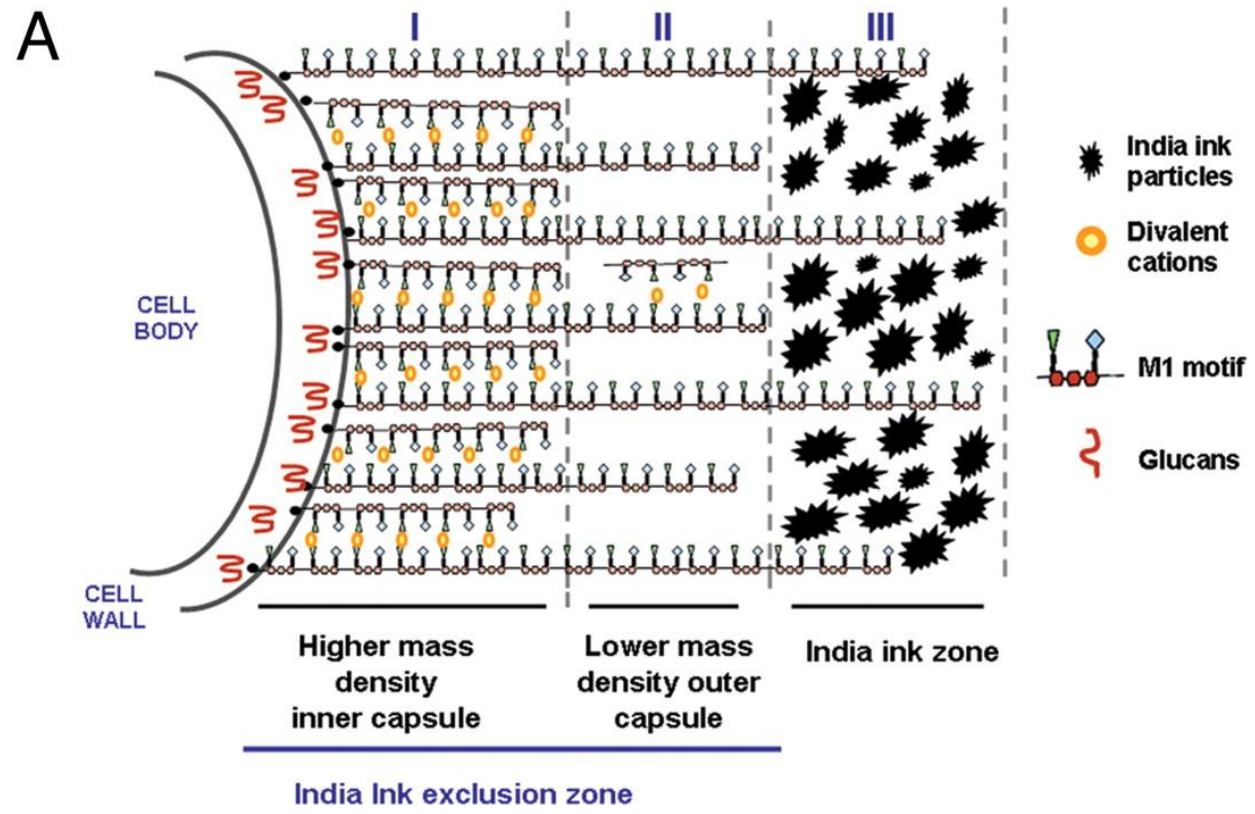
3.2.1. Capsule of *Cryptococcus neoformans*

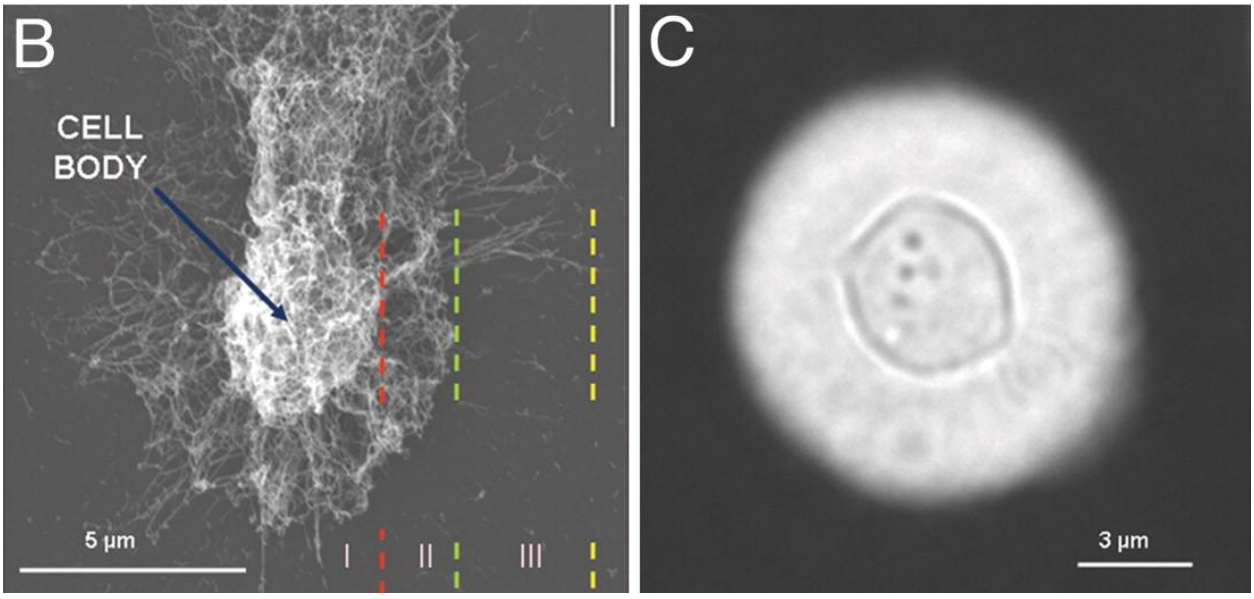
Sakaguchi ***et al.*** **(1993)** studied the ultrastructural of Cryptococcus neoformans by quick-freezing and deep-etching method. The ultrastructure of C. neoformans was

three-dimensionally demonstrated by the QF-DE method. The capsule was composed of fine meshworks of microfibrils (10-13 nm in diameter), which were directly attached to the cell walls. The capsule of the in vivo yeasts (yeast cells in the brain lesion) was thicker than that of the in vitro yeasts (yeast cells on agar culture). At the outer part of the cell wall, a particle-accumulating layer was observed. This layer in vivo was thicker than that in vitro. Occasionally, the yeast cells were ingested by phagocytes in the mouse brain. Although the cytoplasm of such yeast cells was destroyed, the capsular meshworks were well preserved. The ultrastructure of the capsule was the same both in cultured and phagocytized yeasts in the cystic lesions of the brains.

The capsule can vary in size between <1 um and >50 um, depending on growing conditions. The capsule consists for 88% of glucuronoxylomannan (GXM), for 10% of galactoxylomannan (GalXM) and for 2% of mannoproteins (MP) (89).
GXM is composed of an α-1,3-linked polymannose backbone with β-linked monomeric branches of xylose and glucuronic acid.
The four described serotypes A, D and AD, produced by *C. neoformans* and B and C, produced by *C. gattii*. are discriminated on the base of differences in GXM structure. The different serotypes have a common core-repeating unit, but differ in the degree of mannosyl substitution and the molar ratios of mannose, xylose, and glucuronic acid.
GalXM iscomposed of a backbone of an α- 1,6-linked polygalactose substituted with small side chains consisting of mannose, xylose and galactose. For individual strains there are differences in sugar composition in the GalXM, indicating that this polysaccharide is structurally heterogeneous.
MP is proposed to be consisting of a protein backbone heavily substituted by short oligosaccharides mainly containing mannose although significant amounts of galactose and xylose are present.



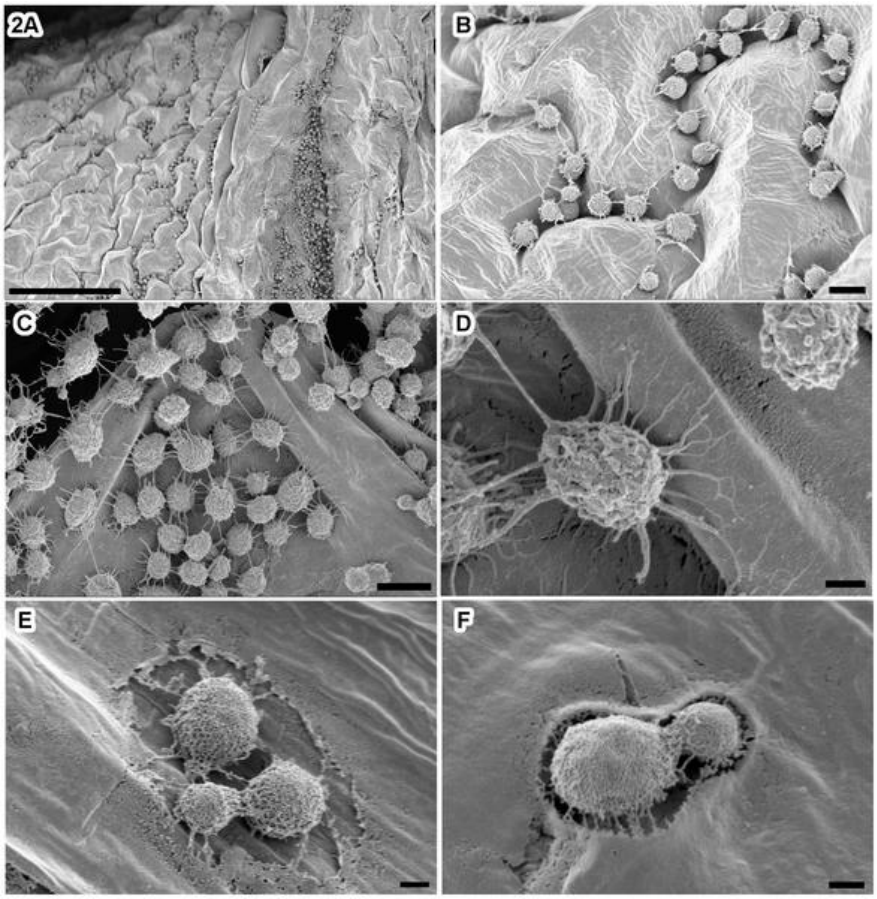




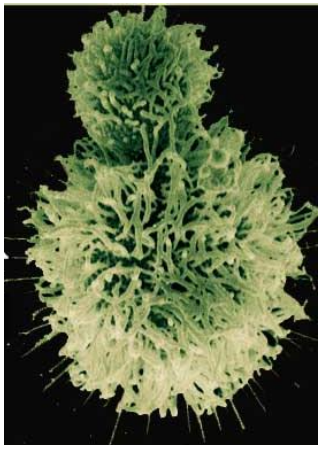
Proposed model for *Cryptococcus neoformans* architecture. Capsule assemblage model (*A*). (*B*) Scanning electron microscopic image of a *C. neoformans* cell where zones I and II of the capsule are apparent by the density of fibrils. (*C*) *C. neoformans* cell suspended in India Ink. According to the model proposed in *A*, zone II ends at the exclusion boundary of India Ink particles. There is a fuzzy edge to the capsule, which we represent as occurring within zone III. Frases et al. PNAS 2009 106 (4) 1228-1233; published ahead of print January 21, 2009,doi:10.1073/pnas.0808995106

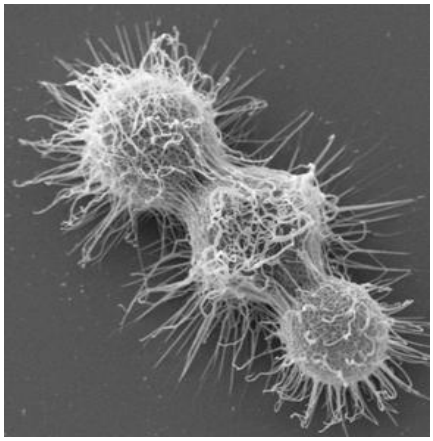
3.2.2. Extracellular fibrils

Spriger *et al.* (2010) used *Arabidopsis thaliana* plants and plant-derived substrates to model *C. gattii* in its natural habitat. Yeast cells readily colonized scratch-wounded plant leaves and formed distinctive extracellular fibrils (40–100 nm diameter ×500–3000 nm length). Extracellular fibrils were observed on live plants and plant-derived substrates by scanning electron microscopy (SEM) and by high voltage- EM (HVEM). Only encapsulated yeast cells formed extracellular fibrils as a capsule-deficient *C. gattii* mutant completely lacked fibrils. It is suggest that extracellular fibril formation could be a structural adaptation of *Cryptococcus* for cell-to-cell, cell-to-substrate and/or cell-to- phagocyte communications.



SEM images of infected leaves showed that *C. gattii* colonized wound site and crevices along leaf surfaces (A, B), extracellular fibrils were visible projecting from *C. gattii* cells, connecting yeast cells to each other and to the leaf surface (C, D). Note a yeast cell at higher magnification with prominent extracellular fibrils extending to other yeast cells and to the leaf tissue (D). Higher magnification of *C. gattii*- inoculated leaf surface revealed formation of 'leaf halo; and 'pocket' in *A. thaliana* in some instances (E, F). Scale bar equals 100 µm (A), 5.0 µm (B, C), and 1.0 µm (D, E, F).
Spriger *et al.* (2010), PLoS ONE 5(6): e10978. doi:10.1371/journal.pone.0010978





Scanning electron micrograph of. encapsulated *C neoformans* shows fibrillar appearance of the polysaccharide capsule; X 9,000. polysaccharide capsule; X 8,000. W. Cleare.

3.2.3. Cell wall

- **α-1,3-Glucan:** the association of the capsule with the cell requires a specific component of the cell wall, alpha-1,3-glucan. Post-transcriptional inhibition of alpha-1,3-glucan synthase expression, using double-stranded RNA interference, yields cells that are unable to assemble a capsule although they generate its polysaccharide components. The resulting cryptococci are slow-growing and acapsular. This finding demonstrates a novel mode of polysaccharide attachment and an important application of RNA interference in fungi.

- **β-1,3-Glucan:** Continued synthesis of glucan linkages is required for fungal viability. Fungal 1,3-*β*-D-glucan also has immunomodulatory effects. For example, 1,3-*β*-D-glucan competitively inhibits macrophage ingestion of acapsular *C. neoformans* it was detected at high levels in csf in cryptococcal meningitis

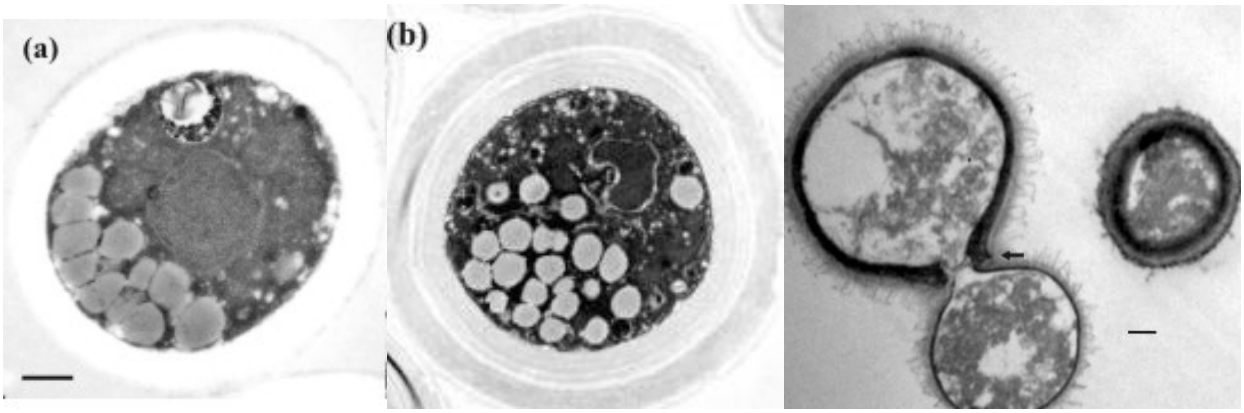
- **β-1,6-Glucan:** is a major component of the cell wall of Cryptococcus . **Beta**(**1**-- **6**)-**glucan** interconnects manno- protein, **beta**(**1**--)3-glucan, and chitin. The latest research confirms the immunomodulatory effect of **beta**-**glucan 1**-3, **1**-**6** in humans. ..

- **Chitin/Chitosan:** chitin is an essential component of Cryptococcus cell wall that contributes to its strength and integrity. It is a linear polymer of β-(1,4)-linked *N*-acetylglucosamine (GlcNAc) and is formed from cytoplasmic pools of UDP-GlcNAc. Chitosan produced by the enzymatic removal of acetyl groups from nascent chitin polymers has been implicated as an important component of the vegetative cell wall. chitosan may be an essential factor for the proper maintenance of cell wall integrity in *C. neoformans*

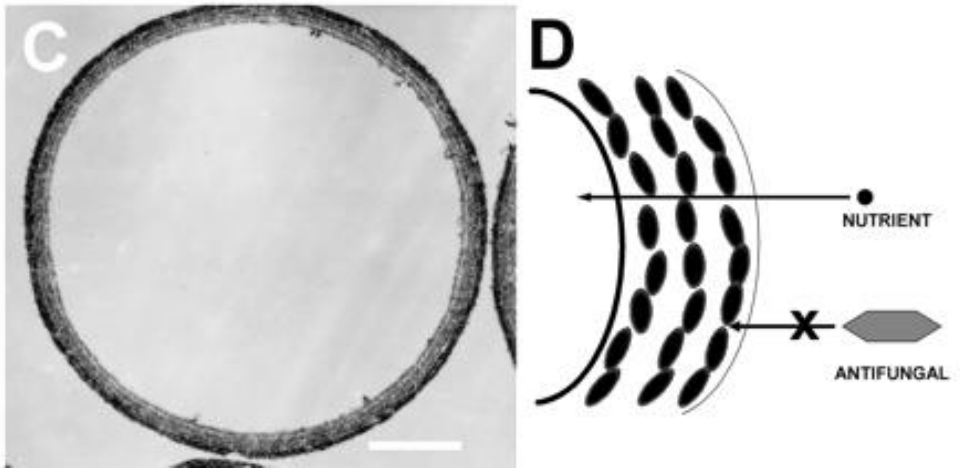
- **Cell wall manoproteins:** MPs are minor components of the capsule and the cell wall. Immunogold electron microscopy located most mannoprotein in the inner cell wall. Chemical analysis of purified cell walls showed the lack of mannose, xylose, and galactose residues. These data indicate that cryptococcal mannoprotein recovered from the cultural supernatant is a nonstructural element of the cell wall. The most important MPs areP
    - **Phosphplipase B:** Secreted phospholipase B (PLB1), which contains three enzyme activities in the one protein, is necessary for the initiation of pulmonary infection by *Cryptococcus neoformans* and for dissemination from the lung via the lymphatics and blood. Adhesion to lung epithelium is the first step in this process.
    - **Laccase:** The cryptococcal enzyme laccase is a well-characterized virulence factor, which oxidizes both iron and polyphenols, producing a protective melanin cell wall coat and modifies prostaglandin synthesis. The enzyme laccase catalyses the formation of melanin by oxidizing L-DOPA, initiating a series of presumably spontaneous reactions that ultimately leads to the polymerization of the pigment in the yeast cell wall. There, melanin protects the cell from a multitude of environmental and host assaults. Thus, the ability of C. neoformans to produce pigments from a variety of available substrates is likely to confer a survival advantage.
    - **Cellulase and others**

- **Melanin**

Electron microscopic study made by **Mandal *et al* (2007)** on melanin-lacking and melanin-producing Cryptococcus neoformans clinical isolates revealed the presence of melanin as an electron dense layer in the cell wall of the melanin-producing isolates.



TEM of melanin-lacking (a) and melanin-producing (b) C. neoformans cells gron in L-DOPA medium. C: The melanin layer is disrupted at the budding sites in the mother cells and the separated daughter cells had complete, melanized cell walls. Mandal. Braz. J. Microbiol. vol.38 no.4 São Paulo Oct./Dec. 2007



(C) Transmission electron micrograph of a cross-section of a *C. neoformans* "ghost" showing that the particle is formed of concentric layers of melanin. Bar, 1 μm. (D) Depiction of the melanin granules comprising the melanin layers, demonstrating how the packing of the granules results in pores that obstruct the passage of large molecules, such as amphotericin B or caspofungin. Obstruction of antifungal molecules can occur by virtue of a reduced melanin pore size or melanin binding. **Joshua D. Nosanchuk1,* and Arturo Casadevall.,2006**

3.2.4. The F-actin cytoskeleton

The F-actin cytoskeleton of *Cryptococcus neoformans* is known to comprise actin cables, cortical patches and cytokinetic ring. **Kopecka *et al.* (2013)** described a perinuclear F-actin collar ring around the cell nucleus, by fluorescent microscopic imaging of rhodamine phalloidin-stained F-actin. Perinuclear F-actin rings may function as 'funicular cabin' for the cell nucleus, and actin cables as intracellular 'funicular' suspending nucleus in the central position in the cell and moving nucleus along the polarity axis along actin cables.

4. Biochemical characteristics of *Cryptococcus* species

Biochemically, the members of the genus *Cryptococcus* are unable to ferment sugars, but do assimilate different sugars, inositol and produce urease. Carotenoid pigment production is extremely variable. Cryptococcus differs from *Rhodotorula* by assimilating inositol, and from Candida glabrata by assimilating inositol and being urease positive.

4.1. Biochemical characteristics of 12 Cryptococcus species according to Benham (1935).

Organism	Growth at 37 C	Path. for Mice	KNO_3 Assimilation	Nitrate to Nitrite	Carbon Assimilation				
					Glucose	Maltose	Sucrose	Lactose	Galactose
1. *Cryptococcus neoformans*	+	+	0	0	+	+	+	0	+
2. *Cryptococcus neoformans*	+	+	0	0	+	+	+	0	+
3. *Cryptococcus neoformans*	+	+	0	0	+	+	+	0	+
4. *Cryptococcus Laurentii*	0	0	0	0	+	+	+	+	+
5. *Cryptococcus luteolus*	0	0	0	0	+	+	+	0	+
6. *Cryptococcus mucorugosus*	0	0	+	+	+	+	+	+	+
7. *Cryptococcus albidus*	0	0	+	+	+	+	+	0	0
8. *Cryptococcus diffluens*	0	0	+	+	+	+	+	0	0
9. *Cryptococcus neoformans* var. *innocuus*	0	0	+	+	+	+	+	0	0
10. *Cryptococcus neoformans* var. *innocuus*	0	0	+	+	+	+	+	0	0
11. *Cryptococcus neoformans* var. *innocuus*	0	0	+	+	+	+	+	0	0
12. *Cryptococcus neoformans* var. *innocuus*	0	0	+	+	+	+	+	0	0

4.2. Biochemical characteristics of Cryptococcus spesies according to Rieth (1979)

Fermentation					Species	Assimilation									
D	G	S	M	L		D	G	S	M	L	R	T	m	C	X
(Nitrate positive species)															
–	–	–	–	–	1. Cr. terreus	+	(+)	–	(+)	+	–	+	–	+	+
–	–	–	–	–	2. Cr. kuetzingii	+	–	+	–	–	+	+	–	+	+
–	–	–	–	–	3. Cr. infirmo-miniatus	+	(+)	+	+	–	+	(+)	–	+	(+)
–	–	–	–	–	4. Cr. macerans	+	(+)	+	+	(+)	+	+	–	+	+
–	–	–	–	–	5. Cr. albidus	+	(+)	+	+	+	+	+	–	+	+
(Nitrate negative species)															
–	–	–	–	–	6. Cr. lactativorus	+	–	–	–	–	–	–	–	–	+
–	–	–	–	–	7. Cr. skinneri	+	(+)	–	–	–	–	+	–	+	+
–	–	–	–	–	8. Cr. melibiosum	+	+	–	–	(+)	–	–	+	(+)	–
–	–	–	–	–	9. Cr. gastricus	+	+	–	+	–	–	+	–	+	+
–	–	–	–	–	10. Cr. dimennae	+	+	+	–	+	+	+	–	+	+
–	–	–	–	–	11. Cr. uniguttulatus	+	(+)	+	+	–	(+)	–	–	(+)	+
–	–	–	–	–	12. Cr. neoformans	+	+	+	+	–	(+)	+	–	(+)	+
–	–	–	–	–	13. Cr. hungaricus	+	+	+	+	–	+	+	(+)	+	+
–	–	–	–	–	14. Cr. luteolus	+	+	+	+	–	+	+	(+)	+	+
–	–	–	–	–	15. Cr. ater	+	+	+	+	+	+	+	–	+	+
–	–	–	–	–	16. Cr. laurentii	+	+	+	+	+	+	(+)	+	+	+
–	–	–	–	–	17. Cr. flavus	+	+	+	+	+	+	+	+	+	+

4.3. Biochemical characteristics of the species in the genus Cryptococcus (Kreger-van Rij (1984)

	Assimilation									Color of culture	St prod	37°C
	Su	Ma	La	Ce	Me	St	Rh	Er	Mz			
Nitrate-positive species:												
Cr. albidus var. *albidus*	+	+/w	v	+	v	v	v	–		hyaline	+/w	–(+)
Cr. albidus var. *aerius*	+	+	+	+	+	v	v	–		hyaline	–	–
Cr. elinovii	–	+	+	+	–	+	+	–	+	hyaline	+	–
Cr. infirmo-miniatus	+	+	+ s/w	+	–	+	v	–		red	+	–
Cr. kuetzingii	+	–	v	+	–	–	–	–		hyaline	+	–
Cr. macerans	+	+/w	+/w	+	+w/–	+s	–(+)	+		red	+	–
Cr. terreus	–	+/w	+	+	–	+/w	+	–	–	hyaline	+	–
Nitrate-negative species:												
Cr. ater	+	+	+	+	–	+/w	+	–		hyaline[1]	+	–
Cr. dimennae	+	–	+	+	–	–	+	–		hyaline	+	–
Cr. flavus	+	+	+w	+	+	+w	+w	+		sl. yellow	–	–
Cr. gastricus	–	+	+	+	–	+	+	–		hyaline	+	–
Cr. heveanensis	+	+	+	+	–	+w	+	+		hyaline	+	–
Cr. hungaricus	+	+	v	+	v	+w	+/w	–(+)		red	+	–
Cr. laurentii	+	+	+	+	+	+/w	+(w)	+		hyaline	+	v
Cr. luteolus	+	+	–/w	+	+	+w	+/w	+		hyaline	+	–
Cr. magnus	+	+	+	+	–	+w	–	–		hyaline	+w	–
Cr. melibiosum	–	–	+	+	+	–	–	–		hyaline	+w	+w
Cr. neoformans var. *neoformans*	+	+	–	+/w	–	v	+	v		hyaline	+/w	+
Cr. neoformans var. *gattii*	+	+	–	+/w	–	v	+	v		hyaline	+/w	+
Cr. skinneri	–	–	–	+	–	–	+	–/w		hyaline	+w	–
Cr. uniguttulatus	+	+	–	–	–	+w	+/w	–		hyaline	+/w	–
Trem. aurantia	+	+	+	+	+w	+	+	+w		hyaline	+	–
Trem. encephala	+	+	+	+	+	+	+	–		hyaline	+	–
Trem. foliacea	–	+w	–	–	–	–	+	+w		hyaline	+	+w
Trem. subanomala	+	+	+	+	+w	+	+	–		hyaline	+	–
Holterm. corniformis	–	+	–	+	–	+w	+	–		hyaline	+	–

Su = sucrose, Ma = maltose, La = lactose, Ce = cellobiose, Me = melibiose, St = soluble starch, Rh = L-rhamnose
Er = erythritol, Mz = melezitose, St prod = starch formation, CGB = CGB agar turns blue.
[1] After several weeks growth dark brown or black on certain media.

5. Antigenic structure of *Cryptococcus* species (Ikeda *et al*., 2000;)

The antigenic structures of 34 species in the genus*Cryptococcus* were determined by using type strains and eight factor sera prepared from adsorption experiments with *Cryptococcus neoformans* serotypes. These antigenic factors were shared by 19 species. The strains used could be divided into eight serological groups. The patterns of groups 1, 2, 3, 5, and 6 were the same as the patterns of *C. neoformans* serotypes A, D, A-D, B, and C, respectively. The species belonging to group 4 reacted to factor sera 1, 2, and 3. Group 7 contained one species that reacted only to factor serum 1. The 15 species in group 8 did not react to any of the factor sera used. Compared to the reported molecular phylogenetic tree, the serological and phylogenetic data were correlated in the *Filobasidium* lineage. All the members of the albidus clade in the *Filobasidium* lineage had antigens 1, 2, and 3, and all the strains in the magnus clade belonged to serogroup 8. Moreover, intraspecies diversity was examined using strains of *C. curvatus*, *C. humicolus*, and*C. laurentii*.

5.1. Determination of the antigenic formulas of *Cryptococcus* species

- Two milliliters of anti-*C. neoformans* serum and 1 ml of heat-killed packed cells are mixed.
- The suspension is then incubated at 37°C for 2 h and then at 4°C overnight.
- After centrifugation, the supernatant is tested for antibody by the slide agglutination test.
- To determine the antigenic formulas of *Cryptococcus* species, equal volumes of factor serum and heat-killed cell suspension are mixed on a glass slide and rotated for 5 min, and then the results of agglutination are observed.
- The formation of aggregates within 5 min is considered positive. Smaller clumps are recorded as weakly positive. PSS is used for a negative control.

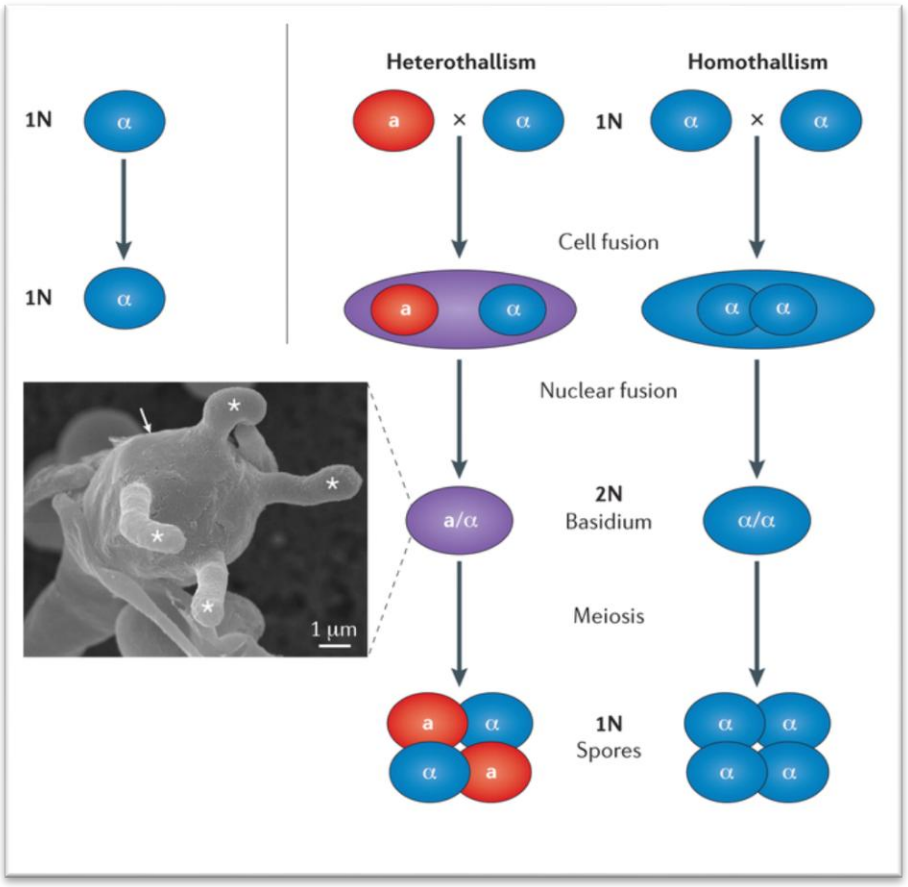
5. 2. Antigenic formulas of *Cryptococcus* species

Group and species	Slide agglutination with factor sera[a]								Serotype
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
1 *C. albidus*	+	+	+	−	−	−	+	−	A
C. consortionis	+	+	+	−	−	−	+w	−	
C. feraegula	+	+	+	−	−	−	+	−	
C. friedmannii	+	+	+	−	−	−	+w	−	
C. yarrowii	+	+	+	−	−	−	+w	−	
2 C. humicolus	+	+	+	−	−	−	−	+w	D
3 C. curvatus	+	+	+	−	−	−	+	+	A-D
4 C. albidosimilis	+	+	+	−	−	−	−	−	
C. antarcticus	+	+	+	−	−	−	−	−	
C. bhutanensis	+	+	+	−	−	−	−	−	
C. kuetzingii	+	+	+w	−	−	−	−	−	
C. luteolus	+	+	+w	−	−	−	−	−	
C. terreus	+	+	+w	−	−	−	−	−	
C. vishniacii	+	+	+	−	−	−	−	−	
5 C. amylolentus	+	+	−	+	+	−	−	−	B
6 C. aerius	+	−	−	+	−	+	−	−	C
C. macerans	+	−	−	+	−	+	−	−	
7 C. dimennae	+	−	−	−	−	−	−	−	
8 C. aquaticus	−	−	−	−	−	−	−	−	
C. ater	−	−	−	−	−	−	−	−	
C. flavus	−	−	−	−	−	−	−	−	
C. fuscescens	−	−	−	−	−	−	−	−	
C. gastricus	−	−	−	−	−	−	−	−	
C. gilvescens	−	−	−	−	−	−	−	−	
C. heveanensis	−	−	−	−	−	−	−	−	
C. hungaricus	−	−	−	−	−	−	−	−	
C. huempii	−	−	−	−	−	−	−	−	
C. laurentii	−	−	−	−	−	−	−	−	
C. magnus	−	−	−	−	−	−	−	−	
C. marinus	−	−	−	−	−	−	−	−	
C. podzolicus	−	−	−	−	−	−	−	−	
C. skinneri	−	−	−	−	−	−	−	−	
C. uniguttulatus	−	−	−	−	−	−	−	−	

[a] +, positive; +w, weakly positive; −, negative.

6. Reproduction of Cryptococcus

- *Cryptococcus neoformans* cells are haploid (1N) and can divide asexually or enter a heterothallic or homothallic mating cycle.
- In heterothallic mating, pheromone signalling between **a** cells and α cells results in cell–cell fusion. Nuclei do not fuse but form a filamentous dikaryon. The tips of the filamentous cells differentiate into basidia, in which nuclear fusion and meiosis occur. Additional rounds of mitotic division produce multiple, haploid basidiospores, which results in the formation of four long chains.



Sexual and asexual reproduction in Cryptococcus neoformans. :.www.nature.com

7. Genetics of Cryptococcus

- **Janbon *et al.* (2014)** sequenced the genome and performed an RNA-Seq-based analysis of the *C. neoformans* var. *grubii* transcriptome structure. They determined the chromosomal locations, analyzed the sequence/structural features of the centromeres, and identified origins of replication.
- The genome was annotated based on automated and manual curation.
- More than 40,000 introns populating more than 99% of the expressed genes were identified. Most of these introns are located in the coding DNA sequences (CDS), over 2,000 introns in the untranslated regions (UTRs) were also identified. Poly(A)-containing reads were employed to locate the polyadenylation sites of more than 80% of the genes.
- Examination of the sequences around these sites revealed a new poly(A)-site-associated motif (AUGHAH). In addition, 1,197 miscRNAs were identified. These miscRNAs can be spliced and/or polyadenylated, but do not appear to have obvious coding capacities.
- This genome sequence enabled a comparative analysis of strain H99 variants obtained after laboratory passage. The spectrum of mutations identified provides insights into the genetics underlying the micro-evolution of a laboratory strain, and identifies mutations involved in stress responses, mating efficiency, and virulence.

7.1.The genome of *C. neoformans* var. *grubii*

- The nuclear genome is approximately 19 Mb in size, which is organized into 14 chromosomes predicted to encode 6,967 protein-coding genes. More than 98% of these protein-coding genes contain short introns. These introns add to the complexity of genome through alternative splicing, exon skipping or truncation/extension at their 5′ or 3′ ends. Therefore, experimental approaches are required in order to refine these computationally derived gene models.
- Genome annotation predicted 6,967 protein-coding genes from its nuclear genome and 13 protein-coding genes from its mitochondrial genome. Of the 6,980 annotated protein-coding genes, 2,200 genes

were annotated based only on computational predictions and lacked any experimental evidence.

- The proteomic study has provided experimental validation, for the first time, for 746 protein-coding genes, covering 33% of genes which did not have any experimental evidence at the RNA or protein level. On the other hand, of the remaining 4,780 genes that had cDNA evidence, 2,928 (61%) gene products were identified.

7.2. Molecular typing of Cryptococcus neoformans/ Cryptococcus gattii (Cogliati, 2013)

7.2.1. Molecular typing techniques:

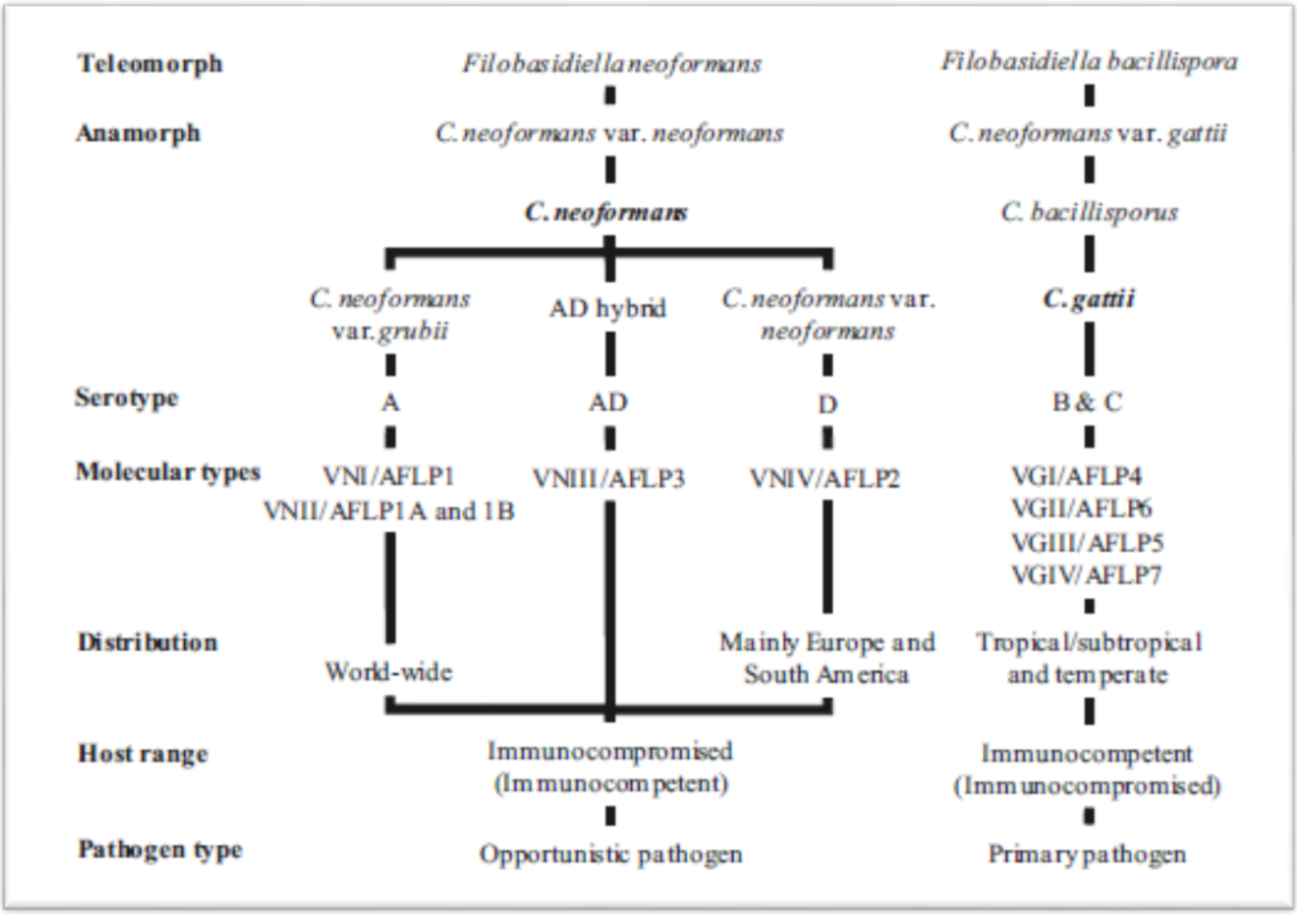
1. Multilocus enzyme electrophoresis (MLEE)
2. DNA fingerprinting
3. Random amplification of polymorphic DNA (RAPD)
4. PCR fingerprinting amplified fragment length polymorphism (AFLP)
5. Restriction fragment length polymorphism (RFLP) of *PLB1* gene
6. Restriction fragment length polymorphism (RFLP) of *GEF1* gene
7. Restriction fragment length polymorphism (RFLP) of *URA5* gene
8. Sequencing of ITS1-5.8S-ITS2 rDNA region
9. Sequencing of intergenic spacer region (IGS)
10. Sequencing of multilocus sequence typing (MLST)
11. Sequencing of multilocus microsatellite typing (MLMT)
12. Matrix-assisted laser desorption ionization-time of flight mass spectrometry-based method (MALDI-TOF) analysis

- Numerous molecular techniques have been applied to *C. neoformans* and *C. gattii* strains,
- Only the following methods were proved to produce comparable results:
  PCR Fingerprinting, AFLP, and MLST. M13 PCR Fingerprinting and *URA5* RFLP:

7.3.Distribution of eight molecular types in *C. neoformans* and *C. gattii*:

1. The molecular types VNI, VNII, VNIII and VNIV were recognized among the *C. neoformans* isolates,
2. The molecular types VGI,VGII, VGIII, and VGIV, were found among *C. gattii* isolates.

Genotyping of the Cryptococcus neoformans/C. gattii species complex



Meyer and Trilles, 2010, Australian Biochemist, vol 41, p 12

Taxonomic, molecular, epidemiological and clinical correlation within C. neoformans/C. gattii species complex, Meyer and Trilles, 2010. Aust. Biochem. P 13

	C. neoformans var. *grubii*			AD hybrid	*C. neoformans* var. *neoformans*	*C. gattii*			
Serotype	A	A	A	AD	D	B/C	B/C	B/C	B/C
PCR-fingerprinting molecular type **Reference 7** **Reference 18**	VNI VN6 (VN5)	VNII	VNII VN7	VNIII VN3/VN4	VNIV VN1 (VN2)	VGI	VGII	VGIII	VGIV
AFLP genotype **Reference 9** **Reference 12**	AFLP1 VNI	AFLP1A/ AFLP1B VNB	AFLP1A/ AFLP1B VNII	AFLP3	AFLP2	AFLP4A/ AFLP4B	AFLP6	AFLP5A/ AFLP5B/ AFLP5C	AFLP7
***URA5* RFLP type** **Reference 7**	VNI	VNII	VNII	VNIII	VNIV	VGI	VGII	VGIII	VGIV
***PLB1* RFLP type** **Reference 10**	A1		A2	A3	A4	A5	A6	A7	A8
ITS genotype **Reference 11**	ITS1	ITS1	ITS1	ITS1/ITS2	ITS2	ITS3/ITS7	ITS4	ITS5	ITS6
IGS genotype **Reference 21**	1A/1B	1A	1C	2C	2A/2B/2C	4	3	5	6

7.4. Global Molecular Epidemiology of *Cryptococcus neoformans* and *Cryptococcus gattii*: (Massimo Cogliati, An Atlas of the Molecular Types, Scientifica, 2013)

1. The analysis of data collected shows that about 68,811 *C. neoformans*/*C. gattii* isolates were reported in the world till now. The majority of the isolates were reported from Asia and Africa followed by Central and South America, Europe,North America and Oceania.

2. The countries where the isolates were prevalently isolated are South Africa, China, USA, and Brazil.

3. Data are completely lacking from many countries of Africa, Asia and Eastern Europe.

4. United States is the country where the environment was more extensively surveyed, followed by Brazil, Australia, Colombia, and India

5. *C. neoformans*/*C. gattii* ratio is variable for each continent being
    - 68 : 1 in Europe,
    - 33 : 1 in Africa,
    - 7.6 : 1 in Asia,
    - 4.5 : 1 in Central and South America,
    - 3.5 : 1 in North America, and
    - 1 : 1.5 in Oceania, *C. gattii* is the prevalent species isolated.

6. Molecular type was determined for 8,077 isolates (12%) representing only a part of the world countries. Molecular data are absent from large parts of Africa, Asia, Eastern Europe, as well as from United Kingdom, Ireland, Norway, and Finland.
    - **VNI** is the prevalent molecular type worldwide except in Australia and Papua New Guinea.
    - **VNII** is a rare molecular type which is reported from all the continents, except from Europe
    - **VGI** is the prevalent molecular type in Australia and Papua New Guinea. VGI molecular type was also found in 13.2% of the Asian, in 7% of the North American, in 4% of the Central and South American, and in 3.4% of the European isolates,while only four VGI strains have been reported from Africa.
    - **VGII** molecular type has its main reservoirs in Brazil (266 isolates), Colombia (*nn n nnn*), Australia (*nn n nnn*), and Puerto Rico (*nn n nn*). These data confirm the hypotheses suggested by other authors that the Vancouver outbreak could be originated from Australia or from South America
    - VGIII molecular type has been prevalently detected in Latin American countries, including Mexico and Sothern California

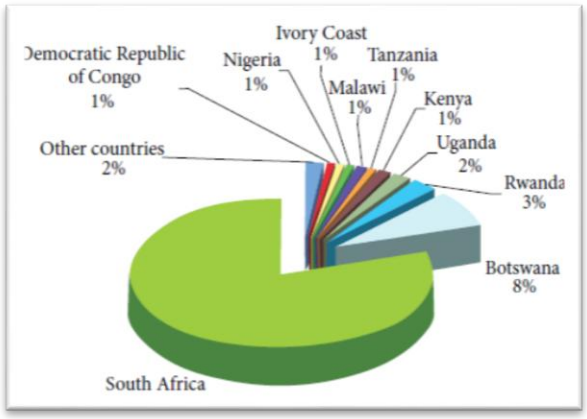
7.4.1. Africa

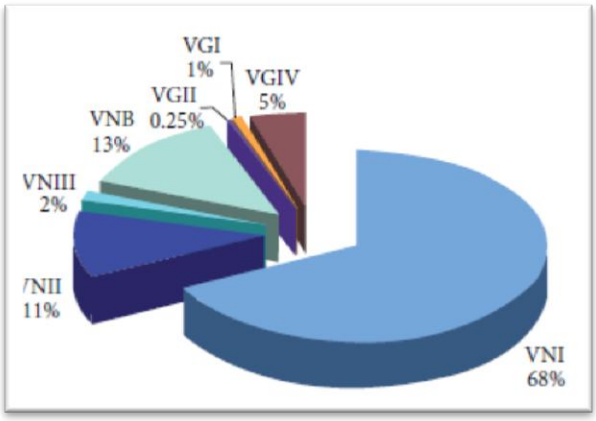
- Isolation of 19,753 *C. neoformans* and *C. gattii* strains was reported from 25 of the 58 African countries and mainly from South Africa (79%).
- *C. neoformans* was not only isolated from pigeon and other birds excreta but also from soil and house dust, as well as from trees such as *Eucalyptus camaldulensis*, mopane tree, and baobab
- *C. gattii* was isolated from soil, *Eucalyptus camaldulensis*, and other trees.
- The majority of the studies reported only the species of the isolates (68%), 19% were reported as *Cryptococcus* species complex, and 11% as variety or serotype
- Molecular typing techniques were applied to identify 2% of the isolates.
    - VNI. VNII and VNIII (68%) were reported only from Uganda and South Africa.

- VNB (14%) was reported in Botswana, South Africa, Rwanda, and Republic Democratic of Congo.
- VGIV was isolated in Botswana and Malawi. Only one isolate belonging to
- VGII (Only one isolate) was reported from Senegal
- VGI (Only 4 isolates) were reported from Republic Democratic of Congo, respectively, whereas
- VNIV was totally absent among the African isolates

Ecology and molecular epidemiology of C. neoformans and C. gattii

Countries	Animal source	Environmental Source	Notes
South Africa, Botswana, Malawi, Senegal, DR Congo; cryptococcal isolates found in 25 out of 58 countries (including Uganda, Rwanda, Tanzania, Zimbabwe, Egypt, Morocco, Tunisia, Algeria, Ghana, Mali, Ivory coast, Ethiopia, Nigeria *et al*.) but species, serotype, genotype info extremely sparse.	Mainly Human	soil, Eucalyptus trees, and almond tree	**VGI 1%**.VGII 0.25% VGIV 5% Of total isolates. **CG** appears to be relatively rare, and less prevalent than CN. Paucity of serotype and molecular identification data (11% and 2% respectively).





7.4.2. Asia

Of all the Asian countries , China, India, and Thailand, together, mainly contributed to the study reporting the 80% of the Asian isolates.

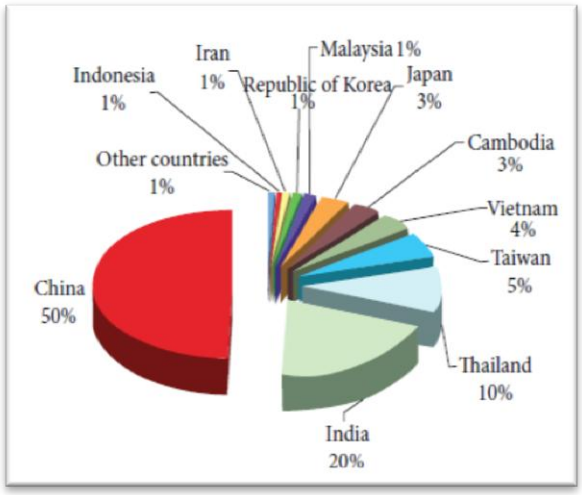
- Six percent of the isolates were recovered from the environment or from animals in Turkey, Israel, Iran, India, Nepal, China, Thailand, Malaysia, Taiwan, Republic of Korea, and Japan.
- In most of the environmental surveys, *C. gattii* was isolated from tree samples, namely, from *Syzygium cumini*, *Mimusops elengi*, *Azadirachta indica*, *Acacia*

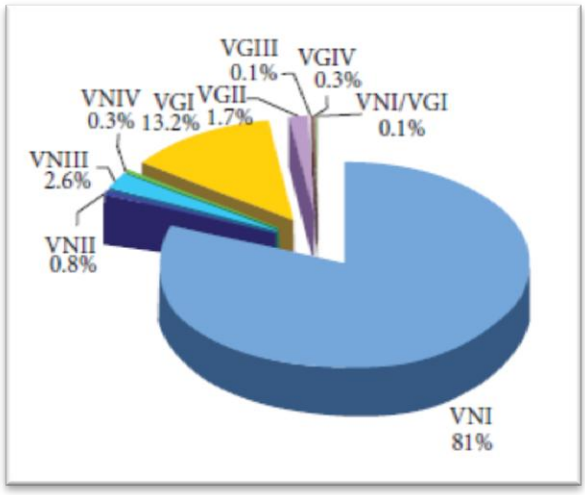
nilotica, *Cassia fistola*, *Manikara hexandra*, *Polyalthia longifolia*, *Eucalyptus camaldulensis*, *Tamarindus indica*, *Cassia marginata*, and *Mangifera indica*

- Only ten isolates from an animal source were recovered from koalas living in two different zoos in Japan.
- *C. neoformans* was prevalently isolated from pigeon and other birds excreta and less frequently from trees such as *Eucalyptus* tree, *Tamarindus arjuna*, *Tamarindus indica*, *Cassia fistola*, *Syzygium cumini*, and *Ficus religiosa*, as well as from some vegetables and fruit (tomato, carrot, banana, eggplant, papaya, apple, and guava).
- Among animals, few *C. neoformans* isolates were isolated from cat and dog and one from a bandicoot.
- *C. neoformans* was the species prevalently isolated in Asia, more common than C. gattii. Molecular types:

    - VNI (81%) in all the Asian countries
    - VNIII was reported in China, India and Thailand
    - VNIV was reported in China and in India
    - VGI (13.2%) in all the Asian countries
    - VGII (low %) in all the Asian countries, except for Israel and Taiwan.
    - VGIII seems to be very rare or absent in Asia since only one isolate was detected in Republic of Korea
    - VGIV molecular type

Countries	Animal source	Environmental Source	Notes
China, India, Thailand (~80% of Asian isolates); also, Republic of Korea, Japan, Taiwan, Malaysia, Vietnam. Cryptococcal isolates found in Turkey, Iran, Nepal, Cambodia, Philippines, Singapore, Indonesia, Bangladesh, Pakistan, Saudi Arabia, Oman, Kuwait, Qatar, and so forth, but species/ serotype/ genotype identification data available are rather poor.	Koala AND Human	Portugese Plum , Spanish Cherry, Neem, Acacia, Golden Shower, Manilkara , Indian Mast tree, Eucalyptus, Tamarind, Cassia Rose/Red Shower Mango;Bark	**VGI 13.2%** VGII 1.7% VGIII 0.1% VGIV 0.3% Of total cryptococcal isolates; **CG** less prevalent than CN.



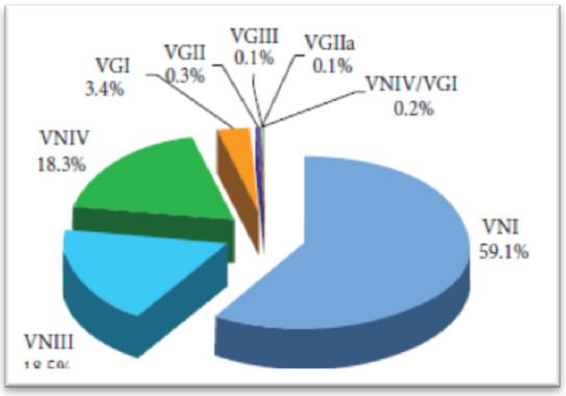


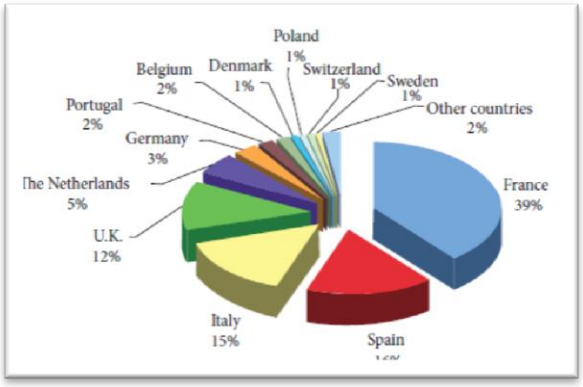
7.4.3. Europe

The majority of the isolates were reported from France, Spain, Italy, and United Kingdom.

- *C. neoformans* was not only isolated from pigeon and other birds excreta, but also from bat guano and red fox faeces.
- Veterinary isolates include strains recovered from cat, dog, magpie, and some isolates from striped grass mouse and degu living in a zoo.
- Few *C. neoformans* strains were isolated from trees, namely, from *Eucalyptus camaldulensis* and oak tree.
- Most of the *C. gattii* natural isolates were from *Eucalyptus camaldulensis*, Douglas tree, carob tree, and stone pine .
- *C. gattii* animal infections were reported in a ferret and in some goats.
- C. neoformans molecular types:
  - VNI molecular type (59%)
  - VNIII (18.5%)
  - VNIV (18.3).
- *C. gattii* molecular types:
  - VGI is the prevalent molecular type, being 43 isolates reported from Portugal, Spain, Italy, and The Netherlands.
  - VGII, few isolates were reported from Greece, Switzerland, The Netherlands, and Denmark.
  - VGIII, only one isolate was found in Greece

Countries	Animal source	Environmental Source	Notes
Denmark, Netherlands, Italy, Spain, Portugal, Greece, Switzerland	Ferrets, Goats Human	Eucalyptus tree, Douglas fir, Carob tree, and Stone pine	**VGI 3.4%**,VGII 0.3% VGIII 0.1%, **CG** appears less prevalent than CN.



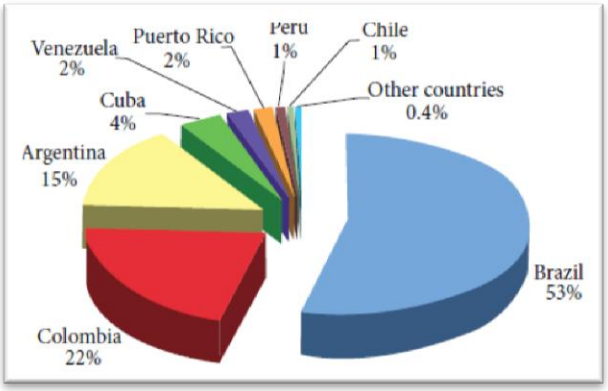


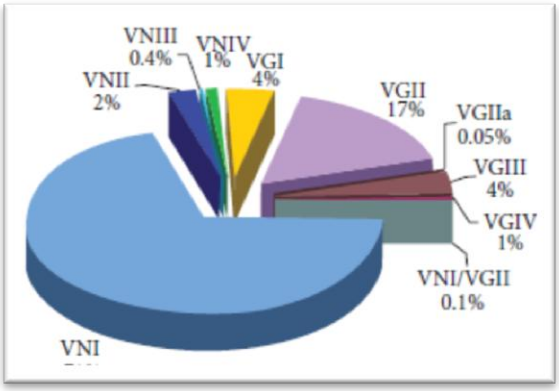
7.4.4. Central and South America

C. neoformans was recognized in 6,665 and *C. gattii* in 1,464 isolates.

- In Brazil: all the molecular types, except for VGIV, are represented.
  - VNI and VGII were the most prevalent
  - VNII, VNIV, VGI, and VGIII occurred in a lower percentage. Two
  - VNIII (isolates of 2)
  - VNI/VGII hybrid (one isolate)
- In Colombia : similar to Brazil, except for
  - VGIII, which occurred in a higher percentage than VNII, VGI,
  - VGIV and VNIV, that was recognized only in one isolate.
  - VNIII AD hybrids seem to be absent in Colombia.
- In Argentina : VGI molecular type is the prevalent
- In Cuba, 198 VNI isolates were detected, whereas only one isolate was VGI. In Puerto Rico, only *C. gattii* isolates (16 VGII and one VGIV) were reported.
- In Chile, all the four *C. neoformans* molecular types were reported but no *C. gattii* isolates were found.

Countries	Animal source	Environmental Source	Notes
Guatemala, Honduras, Aruba, Cuba, Puerto Rico, Venezuela, Brazil, Peru, Colombia, Argentina, Uruguay; regional differences in genotype distribution: e.g. VGI major in Argentina, VGII in Brazil, Puerto Rico, and Colombia	Cheetah, goat, some Psittacine birds Human	Eucalyptus, almond tree, kassod tree, pottery tree, jungle tree, Red flowering gum , soil, dust, and psittacine bird excreta	VGI 4% **VGII 17.5%** VGIII 4% VGIV 1% Impressive 77% rate of identification of all cryptococcal isolates.





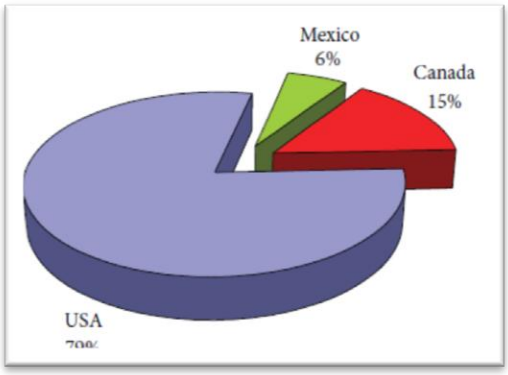
7.4.5. USA, Mexico and Canada

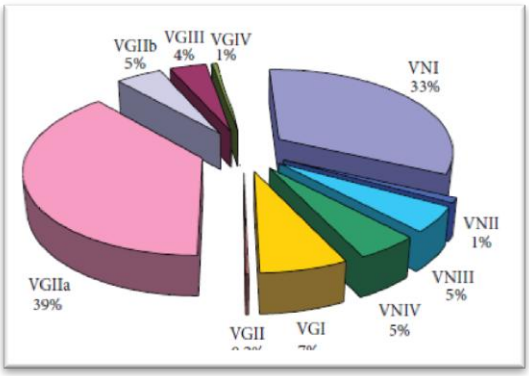
- A total of 7,922 *C. neoformans* and *C. gattii* isolates were reported from the USA (79%), Canada (15%), and Mexico (6%). Eighty percent of the isolates were from clinical sources, whereas 20% were recovered from the environment and animals.
- Pigeon droppings were the main source for *C. neoformans* isolation, although, in Mexico, it was also isolated from fruit and vegetables . Three *C. neoformans* isolates (two in Canada and one in the United States) caused infection in ferret.
- Isolation of *C. gattii* from the environment and from animals was widely reported from Canada during the monitoring of the Vancouver Island *C. gattii* outbreak. Soil, trees, and animals living in Vancouver Island (dogs, cats, horses, ferrets, and birds) resulted colonized or infected with this pathogen.
- Outside Canada, VGIIa isolates were found in the environment and animals (air, water, soil, tree, cats, dogs, alpacas, and parrots) in Oregon and Washington State, and one VGI strain was isolated from *Eucalyptus camaldulensis* in Mexico.

- Almost half of the isolates (49%) reported from North America were identified only as *Cryptococcus* species complex, 10% were identified at species level, while variety or serotype was reported for 21%.
- Molecular type was determined in 20% of the isolates (707). Despite the fact that *C. neoformans* was more frequently isolated in North America than *C. gattii* (3,148 *versus* 885 isolates, resp.), 39% of the isolates identified by molecular techniques belong to VGIIa molecular type.

    - The extensive effort produced to discover the cause of Vancouver Island outbreak which, at present, includes 473 VGIIa, 57 VGIIb, and 70 VGI isolates.
    - A recent study has reported the infection of a Canadian patient with an interspecies VNI/VGI AB hybrid strain.
    - VNI was the prevalent molecular type in both the United States and Mexico
    - VNII, VNIII, VNIV, and VGI are also present in lower percentages.
    - VGII and VGIIa *C. gattii* molecular types were reported from the Northwest Pacific Coast of United States
    - VGIII was reported more frequently from Mexico and Southern Californi.
    - Five VGIV isolates were reported from Mexico, although this molecular type is absent in Canada and in the United States.

Countries	Animal source	Environmental Source	Notes
US, Canada, Mexico; fair coverage, about 51% of total cryptococcal isolates have species, serotype, genotype info.	Cat, dog, bird, ferret, llama, alpaca, elk, goat, sheep, horse, porpoise Humans	Air, water, soil; Douglas Fir, Eucalyptus trees	**VGI 7%** **VGIIa 39%** **VGIIb 5%** **VGIIc (Note: 9 found in Oregon, % not reported)** **VGIII 4%** **VGIV 1%**





7.4.6. Oceania

- A total of 2,518 *Cryptococcus* species complex isolates were reported from four countries of Oceania: Australia, New Zealand, Papua New Guinea, and Hawaii Islands.
- Most of the strains were isolated in Australia representing 85.4% of the isolates reported. Sixty-five percent of the isolates were from clinical source, whereas 35% were from environmental and veterinary source.
- *C. neoformans* was isolated from:

    - cat, dog, horse, koala, ferret, *Potorous gilbertii*,
    - *Eucalyptus camaldulensis* and pine needles,

- *C. gattii* was isolated from :

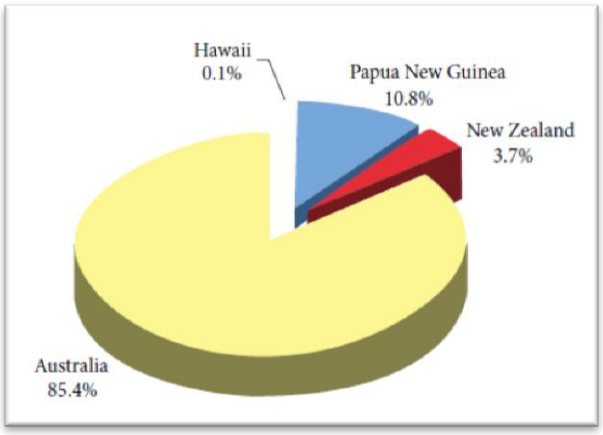
    - kiwi, cat, dog, horse, sheep, cow, koala, quokka, cockatoo, ferret, *Potorous tridactylus*, echidna, African grey parrot, and dolphin,
    - *Eucalyptus camaldulensis*, *Eucalyptus tereticornis*, *Syncarpia glomulifera*, insect frass, olive seedlings, and plant debris.

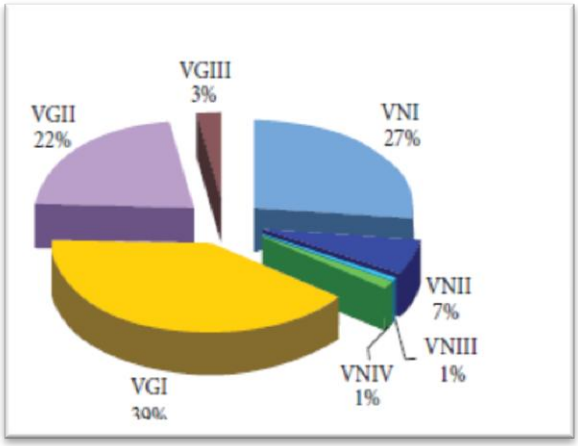
- Identification at species level was performed for 62% of the isolates, variety or serotype was identified in 7%, and molecular type in 22%.

    - Only a small percentage of the isolates (9%) was identified as *Cryptococcus* species complex.(A total of 1,328 *C. gattii* and 900 *C. neoformans* isolates were reported. Among the isolates identified at molecular type level,
    - VGI represented the more frequently isolated molecular type (39%)

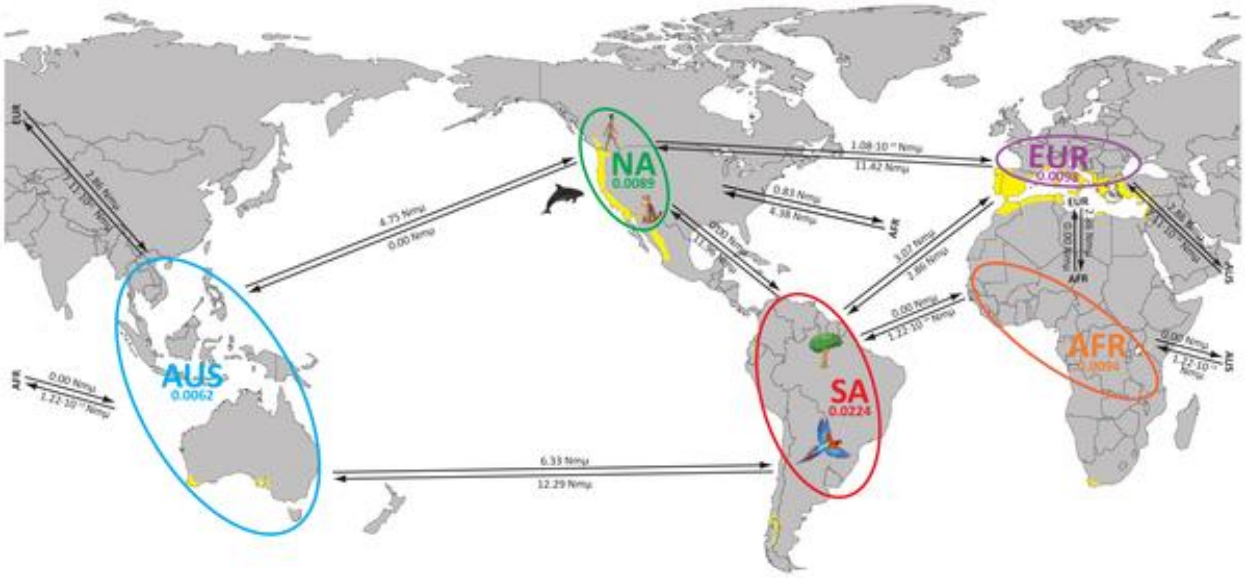
- followed by VNI (27%) and VGII (22%),
- the other molecular types were less frequent. .

Countries	Animal source	Environmental Source	Notes
Australia, New Zealand, Papua New Guinea, and Hawaii Islands	kiwi, cat, dog, horse, sheep, cow, koala, quokka, cockatoo, ferret, long-nosed potoroo, echidna, parrot, dolphin Humans	Species of Eucalyptus, Turpentine tree, insect frass, olive seedlings, and plant debris	**VGI 39%** VGII 22% VGIII 3% Only 22% of 1328 **CG**isolates tested for serotype and genotype; more **CG** than CN.





8. Population sizes and global migration patterns of C. gattii.



Hagen F, Ceresini PC, Polacheck I, Ma H, et al. (2013) Ancient Dispersal of the Human Fungal Pathogen Cryptococcus gattii from the Amazon Rainforest. PLoS ONE 8(8): e71148. doi:10.1371/journal.pone.0071148

It is clear that *Cryptococcus gattii* (CG), the deadly fungal pathogen and a native of tropical and subtropical regions of the world, has stealthily charted itself a course of world domination, starting with the Pacific Northwest of North America.

8.1. Population sizes of *C. neoformans*/*C. gattii* isolates

- The present combined analysis shows that about 68,811 *C. neoformans*/*C. gattii* isolates were reported in the world till now. The majority of the isolates were reported from Asia and Africa followed by Central and South America, Europe, North America and Oceania.
- The countries where the isolates were prevalently isolated are South Africa, China, USA, and Brazil.

- On the contrary, data are completely lacking from many countries of Africa, Asia and Eastern Europe.
- United States is the country where the environment was more extensively surveyed, followed by Brazil, Australia, Colombia, and India
- *C. neoformans*/*C. gattii* ratio is variable for each continent being
  - 68 : 1 in Europe,
  - 33 : 1 in Africa,
  - 7.6 : 1 in Asia,

- 4.5 : 1 in Central and South America,
- 3.5 : 1 in North America, and
- 1 : 1.5 in Oceania, where *C. gattii* is the prevalent species isolated.

- Molecular type was determined for 8,077 isolates (12%) representing only a part of the world countries. Molecular data are absent from large parts of Africa, Asia, Eastern Europe, as well as from United Kingdom, Ireland, Norway, and Finland.
  - **VNI** is the prevalent molecular type worldwide except in Australia and Papua New Guinea.
  - **VNII** is a rare molecular type which is reported from all the continents, except from Europe
  - **VGI** is the prevalent molecular type in Australia and Papua New Guinea. VGI molecular type was also found in 13.2% of the Asian, in 7% of the North American, in 4% of the Central and South American, and in 3.4% of the European isolates,while only four VGI strains have been reported from Africa.
  - **VGII** molecular type has its main reservoirs in Brazil (266 isolates), Colombia (*nn n nnn*), Australia (*nn n nnn*), and Puerto Rico (*nn n nn*). These data confirm the hypotheses suggested by other authors that the Vancouver outbreak could be originated from Australia or from South America
  - **VGIII** molecular type has been prevalently detected in Latin American countries, including Mexico and Sothern California (134 isolates).

8.2. The possibilities about transmission of *Cryptococcus gattii* –

- **C. *gattii*** may have spread as a result of human activity, human and avian migration, and other natural means of dispersal; and
- Slow, but sustained, elevation of global temperatures and corresponding changes in the long-term climate of different regions may have created micro-climates suitable for **C. *gattii*** growth within zones with a temperate climate ordinarily considered inhospitable to the fungus.

- From the currently available epidemiological evidence, more than one **C. *gattii*** clusters are criss-crossing the world, causing infections simultaneously in different parts.

- **C. *gattii*** VGIIa and VGIIb subtypes have been designated, respectively, the major and minor genotypes responsible for the outbreak in North America. However, the genetically distinct VGI, which is the predominant subtype in several parts of the world, has also been causing infections in parts of US, of Asia, of the Mediterranean region (Italy and Spain, for example), and in the Netherlands (which has the temperate climate of North Europe).

- Many of the clinical cases are 'autochthonous' (locally sourced **C. *gattii*** , not travel related); for instance, in Netherlands, the **C. *gattii*** recovered post mortem in 1957 from a woman's lungs has been found to be VGI, and recent studies in the Berg en Dal forests have isolated the same genotype. VGI, same broad genotype but some with fine distinctions, have been found from patients in California, Southwest Georgia, Northern Florida, and Rhode Island in the US, whereas a VGIII cluster is known amongst HIV positive patients in Southern California since 2011. Delhi and Bangalore in India have recently reported VGIII and VGIV from patients and environment. Argentina and Colombia have both recently reported the first environmental isolation of VGIII.

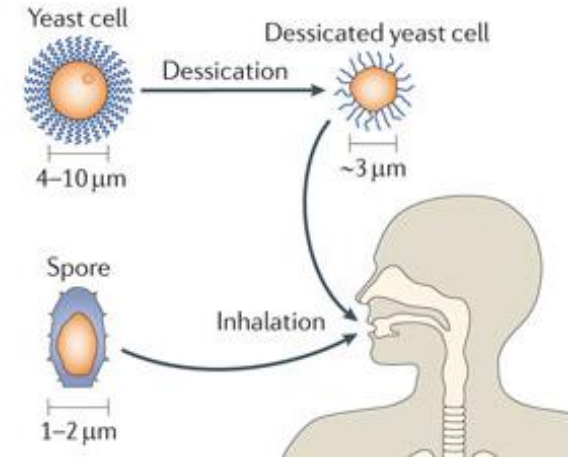
9. Pathogenicity

Virulence factors increase the degree of pathogenicity of a microbe. *Cryptococcus* has a number of virulence factors; however, the severity of the host's disease results from a combination of several virulence factors superimposed on the host's innate and immune resistance status. Virulent isolates of *Cryptococcus* must be able to

1. produce small particles that can get into the alveolar spaces,
2. grow at 37^{o}C at a pH of 7.3 to 7.4 in an atmosphere of approximately 5% CO_2,
3. have an intact calcineurin pathway,
4. must be an -mating type.
5. The ability to produce a large capsule and shed great amounts of capsular material into the body fluids makes the organism highly virulent.
6. Other factors, such as melanin, mannitol, superoxide dismutase, protease, and phospholipase production, may enhance the pathogenicity of *C. neoformans*.
7. The effectiveness of many of these cryptococcal virulence factors depends on the status of the host's defensive mechanisms.

9.1. The Infectious Particle

- To enter the alveolar spaces of the lungs and establish pulmonary infection, an organism must produce viable forms smaller than 4 µm in diameter.
- The typical vegetative form of *Cryptococcus* is the yeast form with a cell diameter of 2.5 µm to 10 µm. The organism can also undergo sexual reproduction, and since it is a basidiomycete (*Filobasidiella*), it forms basidiospores, which are approximately 1.8 µm to 3 µm in diameter. Although the exact nature of the infectious particles of *Cryptococcus* is not known, they are presumed to be the dehydrated yeast cells or basidiospores of the appropriate size range to get into the lungs.
- Once inside the lungs, the yeast cells become rehydrated and acquire the characteristic polysaccharide capsule. In the case of basidiospores, they would convert to encapsulated blastoconidia.



9.2. Growth in vivo

- To cause infection, a *Cryptococcus* isolate must grow at 37°C in an atmosphere of approximately 5% CO_2 and at a pH of 7.3 to 7.4.
- To survive at 37°C, the organism must have an intact gene that encodes the *Cryptococcus* calcineurin A catalytic subunit.
- Calcineurin is a serine-threonine specific phosphatase that is activated by Ca^{2+}-calmodulin and appears to be a basic requirement for *Cryptococcus* survival at 37°C in the host and consequently is a necessary factor for the pathogenicity of the organism.

9.3. Capsule

Cryptococcus capsule is a key virulence factor for *Cryptococcus;* acapsular mutants are typically avirulent, whereas encapsulated isolates have varying degrees of virulence.

i. Chemotactic effects on leukocytes

- Some properties of the *Cryptococcus* capsule enable the host to more effectively clear the organism from tissues; however, others protect the organism from host defenses.
- The capsules are chemotactic for neutrophils.
- Complement is fixed by cryptococcal capsules by the alternative pathway and this process produces chemotactic peptides such as C5a.
- Chemotaxis of leukocytes induced by either of these mechanisms would be advantageous to the host.

ii. Effects of complement interactions

- Complement fixing by *Cryptococcus* in tissue would result in chemotactic factor production and attraction of leukocytes into the infection site.
- Once in the infected tissue, the leukocytes would interact with and kill the organism.
- As complement is fixed, C3b and C3bi are deposited on the surface of the cryptococci. which facilitate binding of the cryptococci to CR3 receptors on leukocytes which in turn enhance the opportunity for the leukocytes to kill the cryptococci either extracellularly or after phagocytosis.
- The organism can also be opsonized by antibodies to GXM
    - The capsule may block the Fc portion of the antibody and prevent it from binding to Fc receptors on the phagocytic host cells.
    - If the capsule is very large, the organism is protected.
    - The cryptococci could deplete complement in the host, creating an environment that favors the cryptococci.

iii. Effects on phagocytosis

- Encapsulated *Cryptococcus* cells are not phagocytized or killed by neutrophils, monocytes, or macrophages to the same degree as acapsular mutants.

- Encapsulated *Cryptococcus* cells have a stronger negatively charged surface than acapsular cells. The high negative charge could cause electrostatic repulsion between the organism and the negatively charged host effector cells and reduce intimate cell-cell interactions required for clearance of the cryptococci.

iv. Altered antigen presentation

- The inability of macrophages to ingest the encapsulated organisms could also diminish antigen presentation to T cells and consequently reduce immune responses.
- encapsulated isolates cannot stimulate proliferative responses in T cells because of the reduced secretion of interleukin-1 (IL-1) by the antigen-presenting cells stimulated with the encapsulated yeasts..

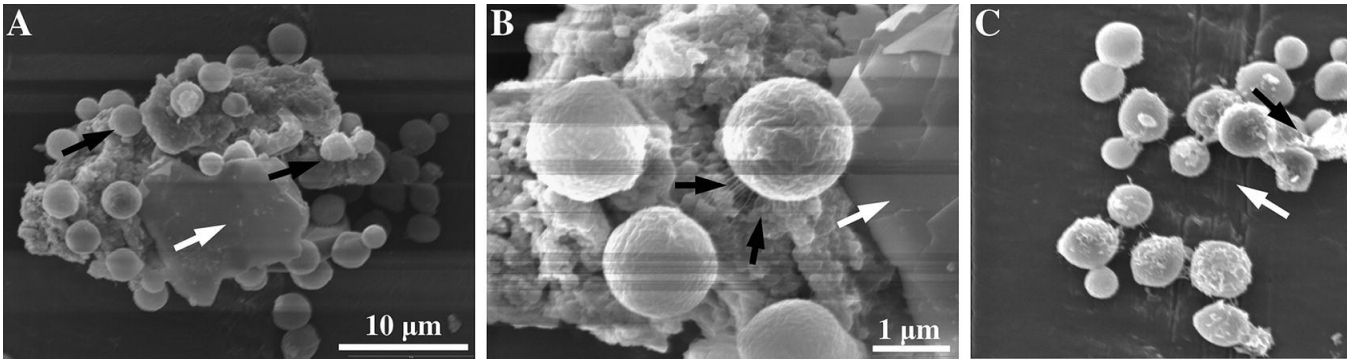
v. Effects on Cytokine Production

- The capsule blocks phagocytosis. Consequently, any cytokines induced by the phagocytic process would not be induced by the encapsulated *Cryptococcus* cell.
- TNFα is necessary for the induction of the protective immune response against *C. neoformans* . Consequently, the lack of or reduced production of TNFα in infections with highly encapsulated isolates of *Cryptococcus* would prevent the induction of protective immunity, resulting in progressive disease.
- High levels of IL-10 would preferentially stimulate the induction of a T-helper 2 (Th2) response rather than a Th1 response in the T cells . Consequently, increased levels of IL-10 would dampen induction of the protective immune response.

9.4. Biofilm formation

Martinez and Casadevall (2007) described the characteristics of *C. neoformans* biofilm development.

- Chemical analysis of the exopolymeric material revealed sugar composition consisting predominantly of xylose, mannose, and glucose, indicating the presence of other polysaccharides in addition to glucurunoxylomannan.
- Biofilms are less susceptible to heat, cold, and UV light exposition than their planktonic counterparts.

- GXM release is essential for cryptococcal biofilm formation, a strategy that has been associated with chronic infections as a result of acquired resistance to host immune mechanisms and antimicrobial therapy.
- *C. neoformans* forms biofilms on polystyrene plates and prosthetic medical devices, including ventriculoatrial shunt catheters, which are increasingly used to manage intracranial hypertension associated with cryptococcal meningoencephalitis.
- Biofilm formation represents the most common mode of growth of microorganisms in nature, a state that presumably allows microbial cells to both survive hostile environments and disperse to colonize new niches.
- 

SEM images of a mature *C. neoformans* B3501 biofilm formed on polyvinyl catheters in vitro revealed the strong attachment of cryptococcal cells to the substrate. Black and white arrows denote polysaccharide and polyvinyl substrate, respectively.
Martinez and **Casadevall** (2007)

9.5. Cryptococcal products (antigens)

In disseminated cryptococcosis, measurable levels of cryptococcal products are present in the body fluids of the patients , namely GXM, galactoxylomannan (GalXM) and mannoproteins (MP).

a. Cryptococcal antigens have adverse effects on host defenses

The direct relationship of cryptococcal antigen levels in body fluids and the severity of disease suggests that the cryptococcal antigens in the host's circulatory system or spinal fluid may have adverse effects on host defenses.

b. Cryptococcal antigens have effects on leukocyte migration

It has been recently demonstrated in the mouse model that intravascular cryptococcal products inhibit the migration of leukocytes from the bloodstream into an inflammatory site. If leukocytes are inhibited from entering tissues, the organism is not effectively eliminated, and the disease is more severe.

c. Cryptococcal antigens have effects on induction of immunomodulatory cells

- Cryptococcal antigens injected into the bloodstream of experimental animals can induce regulatory T cells, which dampen or ablate the anticryptococcal humoral as well as cell-mediated immune responses. Some clinical correlates support the concept that the antigenemia seen in disseminated cryptococcosis downmodulates the immune responses.
- Experimental animal models demonstrate convincingly that cryptococcal antigens given intravenously can induce immunoregulatory T cells that downmodulate the anticryptococcal CMI response.The available data strongly support the notion that cryptococcal products in the circulation induce an array of immunoregulatory T cells, which depress the anticryptococcal immune responses and protection.

d. Cryptococcal antigens have effects on intracranial pressure

- High cryptococcal antigen concentrations could change the osmolality of the CSF, thereby affecting its outflow and adsorption and increasing intracranial pressure.
- The increased pressure may cause headaches, visual loss, and early death.
- It is also possible that release of mannitol by *Cryptococcus* contributes to increased intracranial pressure in cryptococcal meningitis patients.

9.6. Melanin synthesis

- Pathogenic isolates of *C. neoformans* differ from none pathogenic isolates and other *Cryptococcus* species in the organism's ability to form a brown to black pigment on a medium (such as birdseed or caffeic acid agar) that contains diphenolic compounds. This pigment is a melaninlike compound produced by *C. neoformans* isolates with phenoloxidase activity:

    - Melanin protects *C. neoformans* from damage by hypochlorite and permanganate but not by hydrogen peroxide.
    - *C. neoformans* can produce sufficient levels of melanin to effectively protect the organism from oxidative compounds produced by macrophages.
    - It is hypothized that *C. neoformans* uses catecholamines in the brain to make melanin, thereby protecting the organism from oxidative damage by scavenging free radicals.
    - It is suggested that the laccase (phenoloxidase) encoded by *CNLAC1* is a potential virulence factor of *C. neoformans*.
    - It is suggested that melanin deposition in the cell wall may inhibit opsonization by specific antibodies
    - Melanin is an antioxidant, melanin production may help *C. neoformans* survive in the host in other ways.
    - Melanized yeast cells are less susceptible to amphotericin B than nonmelanized yeast cells.

9.7. Mannitol Production

- Production of the hexitol D-mannitol may contribute to survival of *C. neoformans* in the host.
- Two means by which mannitol production may contribute to *C. neoformans* pathogenesis.

    1. high concentrations of D-mannitol in the CNS may contribute to brain edema.
    2. mannitol is a potent scavenger of hydroxyl radicals, and cryptococcal-produced D-mannitol may help protect the organism from oxidative damage.

- Mannitol production by *C. neoformans* correlates with increased resistance to heat stress, osmotic stress, and damage by reactive oxygen intermediates, as well as increased pathogenicity of this fungal agent.

9.8. Superoxide Dismutase

- SOD may participate in free radical scavenging at this higher temperature in vivo,
- however, there is no evidence that SOD production serves as a virulence factor for *C. neoformans*.

9.9. Proteases

- *C. neoformans* proteases possibly serve as virulence mechanisms by initiating invasion of host tissues;
- however, more studies with isogenic strains of *C. neoformans* are required before proteases can be listed as virulence factors.

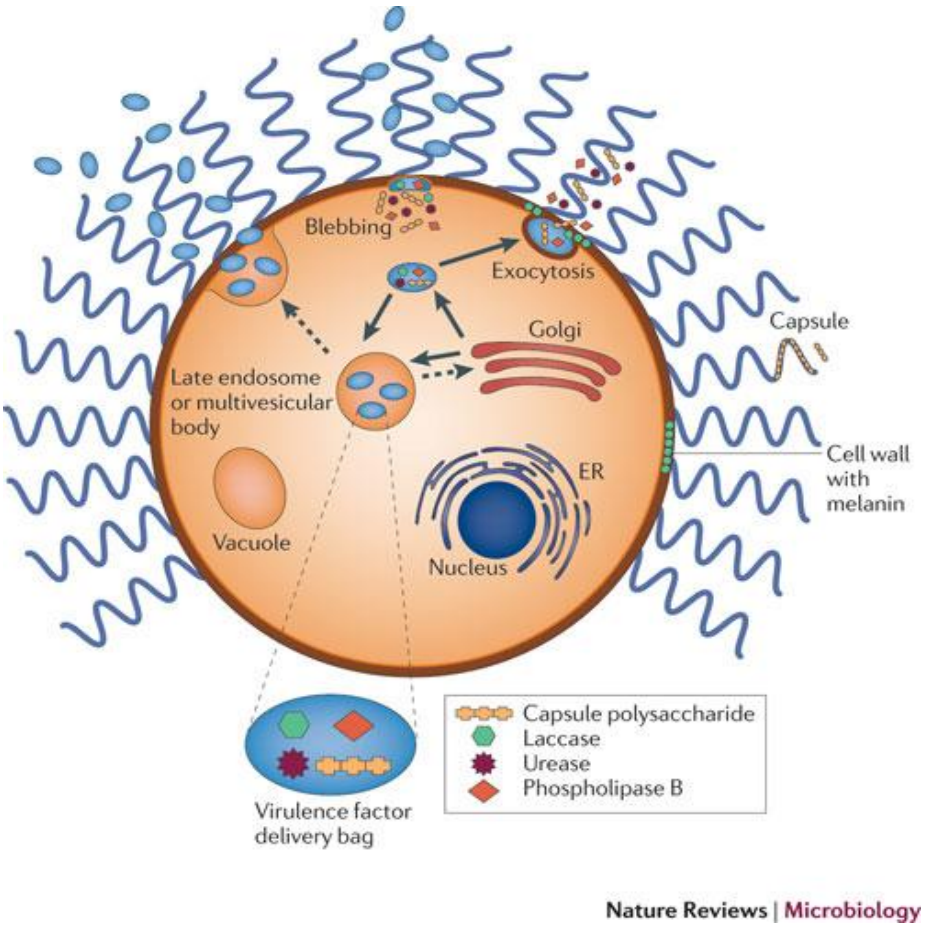
9.10. Phospholipases

- *C. neoformans* produces phospholipase, lysophospholipase, and lysophospholipase-transacylase enzymes
- Analysis of four strains with varying levels of phospholipase activity indicated a correlation between phospholipase activity and virulence in BALB/c mice.
- Extracellular phospholipase activity produced by *C. neoformans* may disrupt mammalian cell membranes and allow the yeast cells to penetrate into host tissues ;
- however, further investigations are necessary to establish the role, if any, of these types of products in the virulence of *C. neoformans*.

9.11. Secretion of cryptococcal virulence factors

The ability of Cryptococcus neoformans and Cryptococcus gattii to influence the intracellular environment of macrophages during fungal proliferation, expulsion and transfer between cells is probably dependent on exported fungal factors.

- Secretion is clearly necessary for cell surface delivery of known virulence factors such as
    - the polysaccharide capsule,
    - the enzyme laccase (which synthesizes melanin) and
    - the enzymes phospholipase B and urease.
- Many of these factors are delivered, at least in part, by membrane-bound, extracellular vesicles that have been observed to traverse the cell wall.
- These so-called 'virulence factor delivery bags' may be released by the fusion of multivesicular bodies with the plasma membrane — thus suggesting a role for Golgi–endosome trafficking — and/or by blebbing from the plasma membrane (capturing cytosolic material to form vesicles).
- There is some evidence both for a role for exocytosis in the export of internally-synthesized capsule polysaccharide, and for regulation of secretion by the cyclic AMP signalling pathway.
- The extracellular vesicles are produced by *C. neoformans* during infection of the mouse macrophage-like cell line J774.16, and similar vesicles that contain capsule polysaccharide accumulate in the cytoplasm of infected macrophages *in vivo* and *in vitro*.
- These phagocytic cells show permeabilization of the phagosomal membrane, and it is tempting to speculate that cryptococcal vesicles and/or exported enzymes (for example, phospholipase B) play a role in this modificatio.
- The extracellular vesicles from the supernatants of fungal cultures are internalized by macrophage-like cells (the mouse cell line RAW 264.7), and these vesicles also induce a dose-dependent production of nitric oxide, tumour necrosis factor, interleukin-10 and transforming growth factor-β by the mouse cells.



9.12. Regulation of Virulence *(Kronstadet al.. 2011)*

- The gene GPA1, which encodes a G-protein α-subunit, is involved in the regulation of these virulence factors as well as in *C. neoformans* mating.
- **Disruption of *GPA1* resulted in**:
    - defects in mating in response to nitrogen starvation,
    - capsule production in response to iron limitation, and
    - melanin synthesis in response to glucose starvation.
    - *gpa1* mutants were much less virulent in a rabbit model of cryptococcal meningitis.

- **Reconstitution of the *gpa1* mutant** with wild-type*GPA1* restored:
    - mating,
    - capsule production,
    - melanin synthesis, and
    - virulence.

- Addition of cyclic AMP also restored these phenotypes, which suggests that *C. neoformans GPA1* regulates these factors by sensing the nutritional signals of the environment and regulating cyclic AMP metabolism in the organism.

10. Description of important Cryptococcus species

10.1. Cryptococcus neoformans (San Felice) Vuillemin, 1901

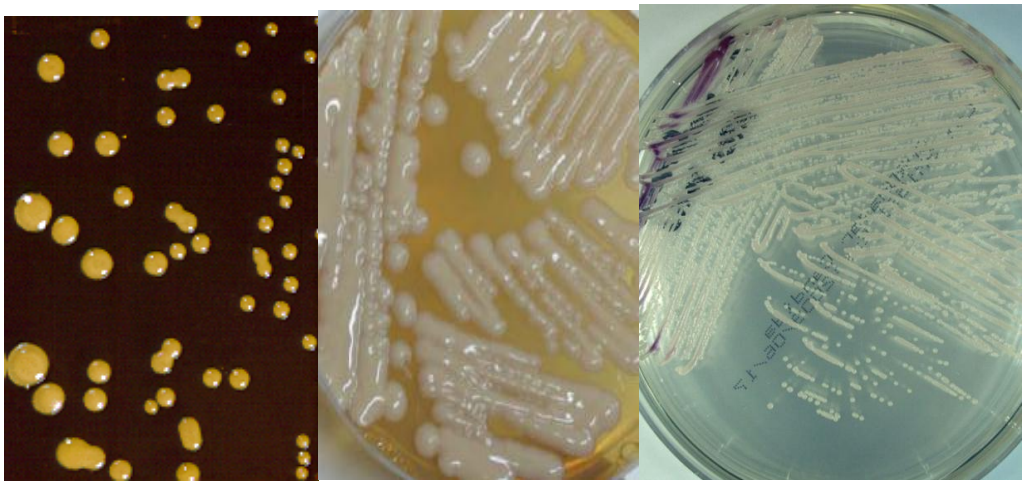
Synonyms:

1. *Saccharomyces neoformans San Felice, Annali Ig. Sperim.: 241 (1895)*
2. *Cryptococcus neoformans (San* Felice) Vuill., Revue Générale des Sciences Pures et Appliquées 12: 747 (1901)
3. *Torula neoformans* (San Felice) J.D. Weis, Journal of Medical Research 7 (1902)
4. *Blastomyces neoformans* (Vuill.) Arzt, Archiv Dermatolo und Syphilis 145: 311 (1924)
5. *Torulopsis neoformans* (San Felice) Redaelli, Rivista di Biologia 13: 171-235 (1931)
6. *Debaryomyces neoformans* (San Felice) Redaelli, Cif. & Giordano, Boll. Sez. Ital. Soc. Int. Microbiol.: 24 (1937)
7. *Lipomyces neoformans* (San Felice) Cif., Manuale de Micologica Medica 2: 214 (1960)
8. *Atelosaccharomyces busse-buschki* Beurm. & Gougerot
9. *Cryptococcus cerebriloc*ulosus Freeman & Weidman [MB#456237]
10. *Torulopsis neoformans var. sheppei* A. Giord. [MB#456608]
11. *Saccharomyces lithogenes* San Felice, Cblatt Bakt.., Parasitk. Infektkrankhe., Abt.I. Origi.521-526 (1896)
12. *Cryptococcus hominis* Vuill., Revue Générale des Sci. Pures et Appl, 12: 735 (1901)
13. *Saccharomyces hominis* Costantin, Bulle. Soc. Mycol. de France 17: 145-148 (1901)
14. *Saccharomyces plimmeri* Costantin, Bulle. Soc. Mycol. de France 17: 147 (1901)
15. *Torula klein* J.D. Weis, Journal of Medical Research 7 (1902)
16. *Torula plimmeri* J.D. Weis, Journal of Medical Research 7 (1902)
17. *Cryptococcus kleinii* E. Cohn, Les Champignons parasites de l'homme et des animaux: 114 (1904)
18. *Atelosaccharomyces breweri* Verdun, Précis Parasitol. (1912)
19. *Cryptococcus guilliermondii* Beauverie & Lesieur, Journal de Physiologie et Pathologie Général 14 (1912)
20. *Torula histolytica* J.L. Stoddart & Cutler, Studies from the Rockefeller Institute for Medical Research (1916)
21. *Cryptococcus constantinii* Mello & L.G. Fern. (1918)
22. *Torula nasalis* F.C. Harrison, Transactions Royal Society of Canada 22: 187-225 (1928)
23. *Cryptococcus psicrofilicus* Niño, Bol. Inst. Clín. Quirúrg. Univ. B. Aires: 94 (1930)
24. *Torulopsis neoformans var. neoformans* (1931) [MB#425188]
25. *Torulopsis constantinii* (Mello & L.G. Fern.) F.P. Almeida (1933) [MB#251557]
26. *Cryptococcus hondurianus* Castell., Medical Press and Circular: 438-443 (1933)
27. *Cryptococcus hominis var. hondurianus* Castell., J.Trop, Med. Hyg. 36: 297-321 (1933)
28. *Cryptococcus meningitidis* C.W. Dodge, Medical mycology.: 333 (1935)

29. *Cryptococcus neoformans var. grubii* Franzot et al., J. Clin. Microbiol. 37: 839 (1999)

Morphology

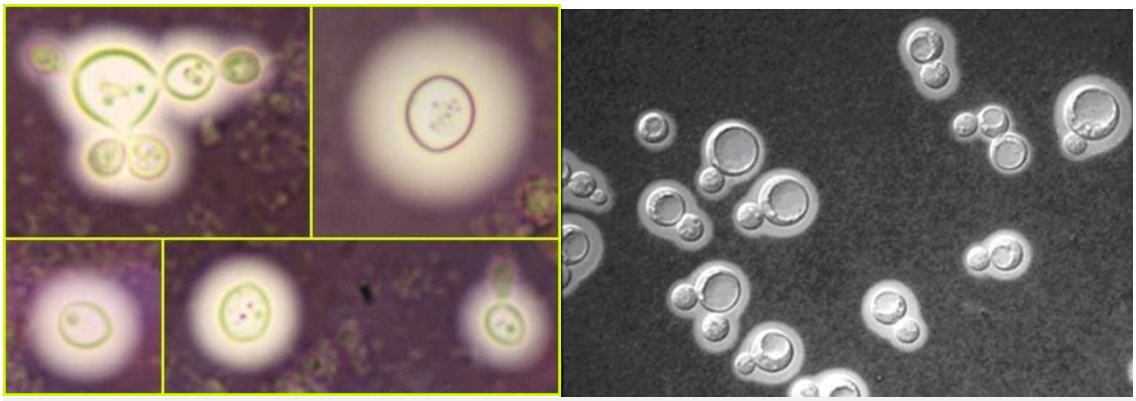
Colonies of *Cryptococcus neoformans* are fast growing, soft, glistening to dull, smooth, usually mucoid, and cream to slightly pink or yellowish brown in color. The growth rate is somewhat slower than *Candida* and usually takes 48 to 72 h. It grows well at 25°C as well as 37°C. Ability to grow at 37°C is one of the features that differentiates *Cryptococcus neoformans* from other *Cryptococcus* spp. However, temperature-sensitive mutants that fail to grow at 37°C in vitro may also be observed . At 39-40°C, the growth of *Cryptococcus neoformans* starts to slow down.



Cryptococcus neoformans colonies

Micromorphology

On cornmeal tween 80 agar, *Cryptococcus neoformans* produces round, budding yeast cells. No true hyphae are visible. Pseudohyphae are usually absent or rudimentary. The capsule is best visible in India ink preparations. The thickness of the capsule is both strain-related and varies depending on the environmental conditions. Upon growth in 1% peptone solution, production of capsule is enhanced



Various capsule sizes of Cryptococcus neoformans. Cryptococcal cells of various capsule sizes (all images under same magnificationwww.scilogs.com Left: ww.ppdictionary.com

10. 1. 1. *Cryptococcus neoformans var. neoformans* (1952)

Synonyms:

1. Saccharomyces neoformans San Felice, Annali Ig. Sperim.: 241 (1895)
2. Cryptococcus neoformans (San Felice) Vuill., Revue Générale des Sciences
3. Pures et Appliquées 12: 747 (1901) [MB#119294]
4. Torula neoformans (San Felice) J.D. Weis, J Medical Research 7 (1902)
5. Blastomyces neoformans (Vuill.) Arzt, Archiv Dermat Syphilis 145: 311 1924)
6. Torulopsis neoformans (San Felice) Redaelli, Riv di Biol 13: 171-235 (1931)
7. Debaryomyces neoformans (San Felice) Redaelli, Cif. & Giordano, Boll. Sez. Ital. Soc. Int. Microbiol.: 24 (1937) [MB#272141]
8. Lipomyces neoformans (San Felice) Cif., Man Micol Medica 2: 214 (1960)
9. Atelosaccharomyces busse-buschki Beurm. & Gougerot
10. Cryptococcus cerebriloculosus Freeman & Weidman [MB#456237]
11. Torulopsis neoformans var. sheppei A. Giord. [MB#456608]
12. Saccharomyces lithogenes San Felice, Centbl Bakt, Parasitk Infektkr, Abt I. Orig : 521-526 (1896)
13. Cryptococcus hominis Vuill., Rev Génér Sci Pures et Appl 12: 735 (1901)
14. Saccharomyces hominis Costantin, Bull Soc Mycol de Fr 17: 145-148 (1901)
15. Saccharomyces plimmeri Costantin,Bull de la Soc Mycol de Fr 17: 147 (1901)
16. Torula klein J.D. Weis, Journal of Medical Research 7 (1902) [MB#456572]
17. Torula plimmeri J.D. Weis, Journal of Medical Research 7 (1902)
18. Cryptococcus kleinii E. Cohn, Les Champignons parasites de l'homme et des animaux: 114 (1904)
19. Atelosaccharomyces breweri Verdun, Précis Parasitol. (1912) [MB#456180]
20. Cryptococcus guilliermondii Beauverie & Lesieur, J Physiol Pathol Gén 14 (1912)
21. Torula histolytica J.L. Stoddart & Cutler, (1916)
22. Cryptococcus constantinii Mello & L.G. Fern. (1918) [MB#456239]
23. Torula nasalis F.C. Harrison, Trans Royal Soc Canada 22: 187-225 (1928)
24. Cryptococcus psicrofilicus Niño, Bol. Inst. Clín. Quirúrg. Univ. B. Aires: 94 (1930)
25. Torulopsis neoformans var. neoformans (1931) [MB#425188]
26. Torulopsis constantinii (Mello & L.G. Fern.) F.P. Almeida (1933) [MB#251557]
27. Cryptococcus hondurianus Castell., Med Press Circular: 438-443 (1933)
28. Cryptococcus hominis var. hondurianus Castell., J Trop Med Hyg36: 2,(1933)
29. Cryptococcus meningitidis C.W. Dodge, Medical mycology. 333 (1935)
30. Cryptococcus neoformans var. grubii Franzot et al., J. Clin. Microbiol (1999)

10.1. 2. ***Cryptococcus neoformans var. grubii*** Franzot et al., J.Clin. Microbiol. 37: 839 (1999)

Synonyms:

1. *Atelosaccharomyces busse-buschki* Beurm. & Gougerot [MB#456181]
2. *Cryptococcus cerebriloculosus* Freeman & Weidman [MB#456237]
3. *Torulopsis neoformans var. sheppei* A. Giord. [MB#456608]
4. *Saccharomyces neoformans* San Felice, Annali Ig. Sperim.: 241 (1895)
5. *Saccharomyces lithogenes* San Felice, Ceblatt Bakt, Parasit Infekt krank, Abt I. Origi: 521-526 (1896)
6. *Cryptococcus hominis* Vuill., Revue Générale des Sciences Pures et Appliquées 12: 735 (1901)
7. *Saccharomyces hominis* Costantin, Bull de la Société Mycologique de France 17: 145-148 (1901)
8. *Saccharomyces plimmeri* Costantin, Bulletin de la Société Mycologique de France 17: 147 (1901)
9. *Torula klein* J.D. Weis, Journal of Medical Research 7 (1902)
10. *Torula plimmeri* J.D. Weis, Journal of Medical Research 7 (1902)
11. *Cryptococcus kleinii* E. Cohn, Les Champ parasites de l'homme et des animaux: 114 (1904)
12. *Atelosaccharomyces breweri* Verdun, Précis Parasitol. (1912) [MB#456180]
13. *Cryptococcus guilliermondii* Beauverie & Lesieur, J Physiologie et Pathologie Général 14 (1912)
14. *Torula histolytica* J.L. Stoddart & Cutler, the Rockefeller Institute for Medical Research (1916)
15. *Cryptococcus constantinii* Mello & L.G. Fern. (1918) [MB#456239]
16. *Torula nasalis* F.C. Harrison, Transactions of the Royal Society of Canada 22: 187-225 (1928)
17. *Cryptococcus psicrofilicus* Niño, Bol. Inst. Clín. Quirúrg. Univ. B. Aires: 94 (1930)
18. *Torulopsis neoformans var. neoformans* (1931) [MB#425188]
19. *Torulopsis constantinii* (Mello & L.G. Fern.) F.P. Almeida (1933)
20. *Cryptococcus hondurianus* Castell., Medical Press and Circular: 438-443 (1933)
21. *Cryptococcus hominis var. hondurianus* Castell., J.Trop.Med.Hyg. 36: 297-321 (1933)
22. *Cryptococcus meningitidis* C.W. Dodge, Medical mycology. 333 (1935)
23. *Cryptococcus neoformans var. grubyi:* Separate varietal status for Cryptococcus neoformans serotype A. Franzot, S.P.; Salkin, I.F.; Casadevall, A. 1999. J.Clin. Microbiol. 37:838-840
24. *Cryptococcus bacillisporus* Kwon-Chung & J.E. Benn., Inter.J. Syst. Bacteriol. 28: 618 (1978)
25. *Cryptococcus neoformans var. gattii* Vanbreus. & Takashio, Ann de la Soc Belge de Méd Trop 50 (6): 701 (1970)
26. *Cryptococcus neoformans var. shanghaiensis* W.Q. Liao et al., Chinese Med Journal: 287 (1983)
27. *Cryptococcus neoformans var. gattii* Vanbreus. & Takashio ex De Vroey & Gatti, Mycoses 32 (12): 675 (1989)
28. *Cryptococcus bacillisporus sp. nov.:* serotype B-C of Cryptococcus neoformans. Kwon-Chung, K.J.; Bennett, J.E.; Theodore, T.S. 1978.Intern.J.Syst. Bacteriol.. 28:616-620

Physiological data

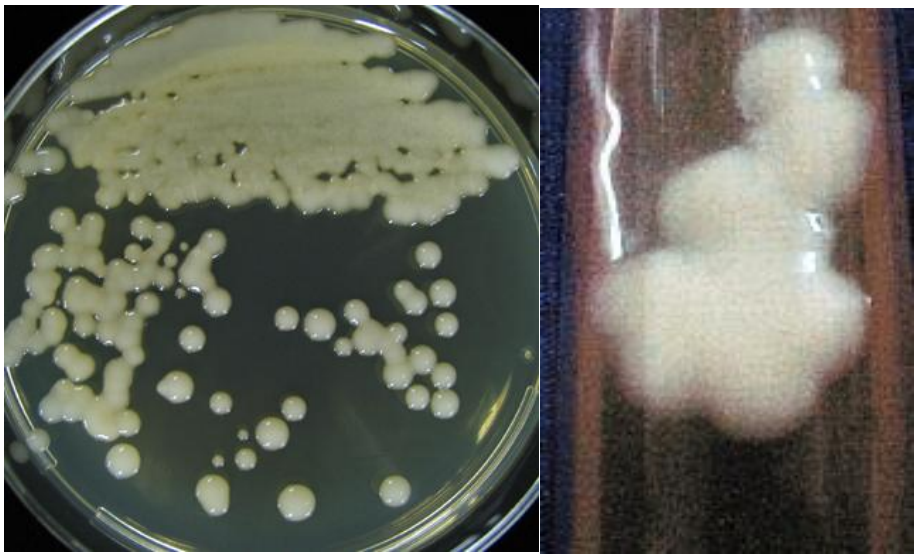
C1 D-Glucose+	C20 Melezitose+	C39 Succinated
C2 D-Galactose+	C21 Inulind	C40 Citrate-
C3 L-Sorbose+	C22 Starch+	C43 Propane 1,2 diold
C4 D-Glucosamine+	C23 Glycerol-	C44 Butane 2,3 diol-
C5 D-Ribose+	C24 Erythritol+	C45 Quinic acid-
C6 D-Xylose+	C25 Ribitol+	C46 D-glucarate+
C7 L-Arabinose+	C26 Xylitol+	C47 D-Galactonated
C8 D-Arabinose+	C27 L-Arabinitol+	N1 Nitrate-
C9 L-Rhamnose+	C28 D-Glucitol+	N2 Nitrite-
C10 Sucrose+	O3 Acetic acid 1%-	N3 Ethylamine+
C11 Maltose+	C29 D-Mannitol+	N4 L-Lysine+
C12 a,a-Trehalose+	C30 Galactitol+	N5 Cadaverine-
C13 Me a-D-Glucoside+	C31 myo-Inositol+	N6 Creatine-
C14 Cellobiose+	C32 D-Glucono-1,5-lactone+	N7 Creatinine+
C15 Salicin+	C33 2-Keto-D-Gluconate+	N8 Glucosamine-
C16 Arbutin+	C35 D-Gluconate-	N9 Imidazole-
C17 Melibiose-	C36 D-Glucuronate+	N10 D-Tryptophan-
C18 Lactose-	C37 D-Galacturonate+	V1 w/o vitamins-
C19 Raffinose+	C38 DL-Lactate	O1 Cycloheximide 0.01%-

10.2. *Cryptococcus gattii* (Vanbreusghem & Takashio) Kwon-Chung & Boekhout, Taxon 51 (4): 806 (2002)

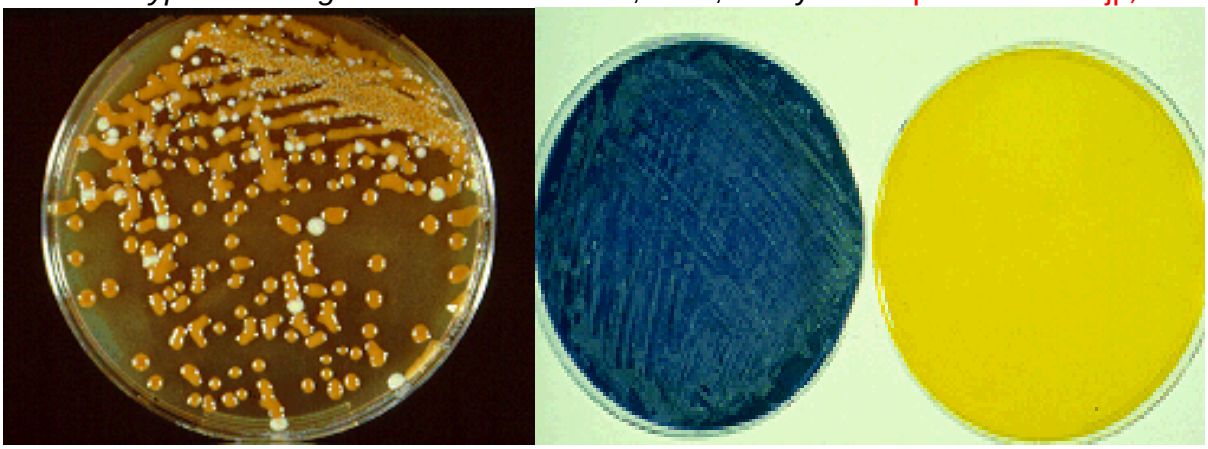
Teleomorph: *Filobasidiella bacillispora*

Synonyms:

1. *Cryptococcus neoformans var. gattii* Vanbreuseghem& Takashio, Annal. de la Soci. Belge de Méd.Trop. 50 (6): 701 (1970)
2. *Cryptococcus neoformans var. gattii* Vanbreuseghem & Takashio ex De Vroey & Gatti, Mycoses 32 (12): 675 (1989)
3. *Cryptococcus bacillisporus* Kwon-Chung & J.E. Benn., Intern.J.Syste. Bacteriol.28: 618 (1978)
   Cryptococcus neoformans var. shanghaiensis W.Q. Liao et al., Chinese Med. J.: 287 (1983)



Cryptococcus gattii colonies. YPGA*, 25℃, 5 days www.pf.chiba-u.ac.jp,



Bird seed agar plate, brown colonies of C. gattii www.mycology.adelaide.edu.au, CGB agar turns blue for *Cryptococcus gattii.*

Physiological data

C1 D-Glucose+	C22 Starch+	C44 Butane 2,3 diol-
C2 D-Galactose+	C23 Glycerold	C45 Quinic acid-
C3 L-Sorbose+	C24 Erythritold	C46 D-glucarate+
C4 D-Glucosamined	C25 Ribitol+	C47 D-Galactonated
C5 D-Ribose+	C26 Xylitol+	N1 Nitrate-
C6 D-Xylose+	C27 L-Arabinitol+	N2 Nitrite-
C7 L-Arabinose+	C28 D-Glucitol+	N3 Ethylamine+
C8 D-Arabinose+	C29 D-Mannitol+	N4 L-Lysine-, d, w
C9 L-Rhamnose+	C30 Galactitol+	N5 Cadaverine-
C10 Sucrose+	C31 myo-Inositol+	N6 Creatine-
C11 Maltose+	C32 D-Glucono-1,5-	N7 Creatinine+
C12 a,a-Trehalose+	lactone+	N8 Glucosamine-
C13 Me a-D-Glucoside+	C33 2-Keto-D-Gluconate+	N9 Imidazole-
C14 Cellobiose+	C35 D-Gluconate+	N10 D-Tryptophan-
C15 Salicind	C36 D-Glucuronate+	V1 w/o vitamins-
C16 Arbutin+	C37 D-Galacturonate+	V2 w/o myo-Inositol+
C17 Melibiose-	C38 DL-Lactate-	V3 w/o Pantothenate+
C18 Lactose-	C39 Succinate+	V4 w/o Biotin+
C19 Raffinose+	C40 Citrate+	V5 w/o Thiamin-
C20 Melezitose+	C43 Propane 1,2 diol-	V6 w/o Biotin & Thiamin
C21 Inulind		

10.3. *Cryptococcus albidus*

Synonyms:

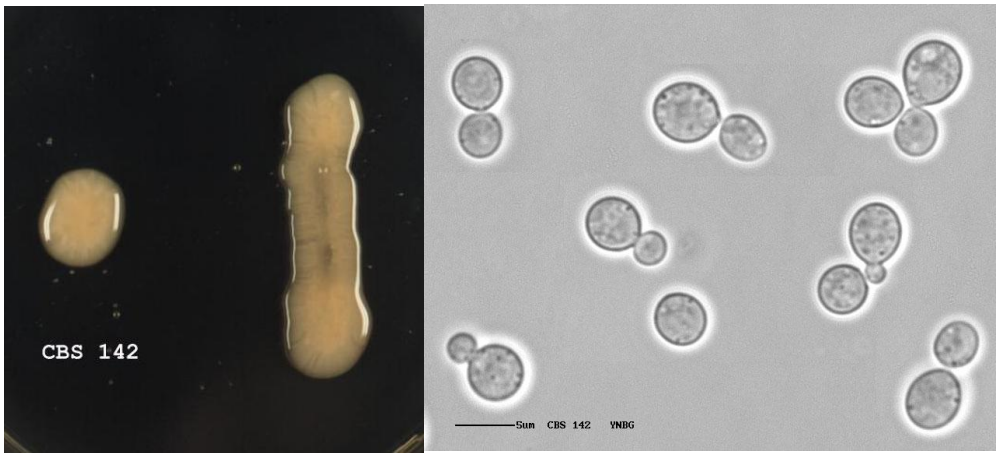
1. *Torulopsis albida var. albida* [MB#424433]
2. *Torula albida* Saito, Journal of Japanese Botany 1: 43 (1922) [MB#256207]
3. *Torulopsis albida* (Saito) Lodder, Verhandelingen Koninklijke Nederlandse Akademie van Wetenschappen Afdeling Natuurkunde 32: 163 (1934)
4. *Torulopsis neoformans var. albida* (Saito) W. Kaufman, Zentralblatt für Bakteriologie und Parasitenkunde Abteilung 2 106: 442 (1944) [MB#351847]
5. *Cryptococcus albidus* (Saito) C.E. Skinner, The American Midland Naturalist 43: 249 (1950)
6. *Rhodotorula albida* (Saito) Galgoczy & E.K. Novák, Acta Microbiol. Acad. Sci. Hung. 12 (2): 155 (1965)
7. *Torula alpina* Grüss ex Lodder [MB#167763]
8. *Cryptococcus pseudoaerius* (Zsolt) Kock.-Krat. [MB#456251]
9. *Torulopsis ptarmiganii* J. Hedrick [MB#456612]
10. *Torulopsis acris var. granulosa* Marcilla & Feduchy [MB#456592]
11. *Torulopsis dattila var. armeniaca* Sarukhanyan [MB#456597]
12. *Torulopsis dattila var. armeniensis* Saruch. [MB#456598]
13. *Torulopsis rotundata* Redaelli (1925) [MB#254463]
14. *Torulopsis nadaensis* Saito & Oda, Journal of the Brewing Society of Japan 12: 167 (1934)
15. *Cryptococcus mucorugosus* Benham, Journal of Infectious Diseases 57: 255-274 (1935) [MB#456246]
16. *Cryptococcus neoformans var. innocuous* Benham, Transactions of the New York Academy of Sciences 17: 418-429 (1955)
17. *Torulopsis pseudaeria* Zsolt, Antonie van Leeuwenhoek 24: 213 (1958) [MB#306946]
18. *Torulopsis pseudoaeria* Zsolt, Antonie van Leeuwenhoek 24: 213 (1958) [MB#499927
19. *Cryptococcus genitalis* Castell., Derm. Trop.: 140 (1963)

Morphology

The colonies are cream-colour to pale pink, with the majority of colonies being smooth with a mucoid appearance. Some of the colonies may be rough and wrinkled, but this is a rare occurrence. This species is very similar to *Cryptococcus neoformans*, but can be differentiated because it is phenol oxidase-negative, and, when grown on Niger or birdseed agar, *C. neoformans* produces melanin, causing the cells to take on a brown color while the *C. albidus* cells stay cream-color.

Microscopically, *C. albidus* has an ovoid shape, and when viewed with India ink it is apparent that a capsule is present. This species also reproduces through budding. The formation of pseudohyphae has not been seen. *C. albidus* is able to use glucose, citric acid, maltose, sucrose, trehalose, salicin, cellobiose, and inositol, as well as many other compounds, as sole carbon sources. This species is also able to use potassium nitrate as a nitrogen source. *C. albidus* produces urease, as is common for *Cryptococcus* species. *Cryptococcus albidus* is very easily mistaken for other *Cryptococcus* species, as well as species from other genus of yeast, and as such should be allowed to grow for a minimum of seven days before attempting to identify this species.

Cryptococcus albidus var. albidus is a variety of *C. albidus* that has been considered unique. It differs from *Cryptococcus neoformans* because of its ability to assimilate lactose, but not galactose. This species is also considered unique because its strains have a maximum temperature range from between 25°C and 37°C.



10.3.1. *Cryptococcus albidus var albidus*

physiological data :

C1 D-Glucose+	C24 Erythritol-, d, w	N3 Ethylamine-
C2 D-Galactose-	C25 Ribitold	N4 L-Lysine-, +
C3 L-Sorbose-, d, w	C26 Xylitol+, d, w	N5 Cadaverine+
C4 D-Glucosamine-	C27 L-Arabinitol-, d, w	N6 Creatine-
C5 D-Ribose+, d, w	C28 D-Glucitol+	N7 Creatinine-
C6 D-Xylose+	C29 D-Mannitol+	N8 Glucosamine-
C7 L-Arabinose+, d, w	C30 Galactitol-, d, w	N9 Imidazole-
C8 D-Arabinose-, +	C31 myo-Inositol+, d, w	N10 D-Tryptophan-, d, w
C9 L-Rhamnose-, +	C32 D-Glucono-1,5-lactone-, +	V1 w/o vitamins-
C10 Sucrose+	C33 2-Keto-D-Gluconate+	V2 w/o myo-Inositol+
C11 Maltose+	C35 D-Gluconate-, +	V3 w/o Pantothenate+
C12 a,a-Trehalose+, d, w	C36 D-Glucuronate+	V4 w/o Biotin+
C13 Me a-D-Glucoside-, d, w	C37 D-Galacturonate-	V5 w/o Thiamin-
C14 Cellobiose+	C38 DL-Lactate+, d, w	V6 w/o Biotin & Thiamin-
C15 Salicin+	C39 Succinate+, d, w	V7 w/o Pyridoxine+
C16 Arbutin+, d, w	C40 Citrate+	V8 w/o Pyridoxine & Thiamin-
C17 Melibiose-	C43 Propane 1,2 diol-	V9 w/o Niacin+
C18 Lactose-, +	C44 Butane 2,3 diol-	V10 w/o PABA+
C19 Raffinose+	C45 Quinic acid-, +	O1 Cycloheximide 0.01%-
C20 Melezitose+	C46 D-glucarate+	O2 Cycloheximide 0.1%-
C21 Inulin-	C47 D-Galactonate	O3 Acetic acid 1%-
C22 Starch-, +	N1 Nitrate+	O6 10% NaCl-, d, w
C23 Glycerol-, +	N2 Nitrite+	O7 16% NaCl

10.3.2. *Cryptococcus albidus var ovalis*

physiological data :

C1 D-Glucose+	C20 Melezitose+	C40 Citratew
C2 D-Galactose-, d, w	C21 Inulin-	C43 Propane 1,2 diol-
C3 L-Sorbose-	C22 Starch-	C44 Butane 2,3 diol-
C4 D-Glucosamine-	C23 Glycerol+, d, w	C45 Quinic acid+
C5 D-Ribosed	C24 Erythritol-	C46 D-glucarate+
C6 D-Xylose+	C25 Ribitold	C47 D-Galactonate-
C7 L-Arabinose+	C26 Xylitold	N1 Nitrate+
C8 D-Arabinosed	C27 L-Arabinitol-	N2 Nitrite+
C9 L-Rhamnosed	C28 D-Glucitol+	N3 Ethylamine-
C10 Sucrose+	C29 D-Mannitol+	N4 L-Lysine+
C11 Maltose+	C30 Galactitol-	N5 Cadaverine+
C12 a,a-Trehalose+	C31 myo-Inositol+	N6 Creatine-
C13 Me a-D-Glucoside-	C32 D-Glucono-1,5-lactone+, d, w	N7 Creatinine-
C14 Cellobiose+	C33 2-Keto-D-Gluconate+	N8 Glucosamine-
C15 Salicin+	C35 D-Gluconated	N9 Imidazole-
C16 Arbutin+	C36 D-Glucuronate+	N10 D-Tryptophan-
C17 Melibiose-	C37 D-Galacturonate-	V1 w/o vitamins-
C18 Lactose+, d, w	C38 DL-Lactatew	O1 Cycloheximide 0.01%-
C19 Raffinose+	C39 Succinate+	O3 Acetic acid 1%-

Cases of *C. albidus* infection have increased in humans during the past few years, and it has caused ocular and systemic disease in those with immuno-incompetent systems, for example, patients with AIDS, leukemia, or lymphoma. While systemic infections have been found with increasing regularity in humans, it is still relatively rare in animals.

C. albidus was found in a genital infection in one horse, and in cases of fatal disseminated cryptococcosis in a dog and a cat. Both C. albidus and bacterial pneumonia were thought to have contributed to the death of a California sea lion. This organism was also found in the eye of a horse with keratitis; however, several bacteria were also detected, and its contribution to the condition is uncertain.

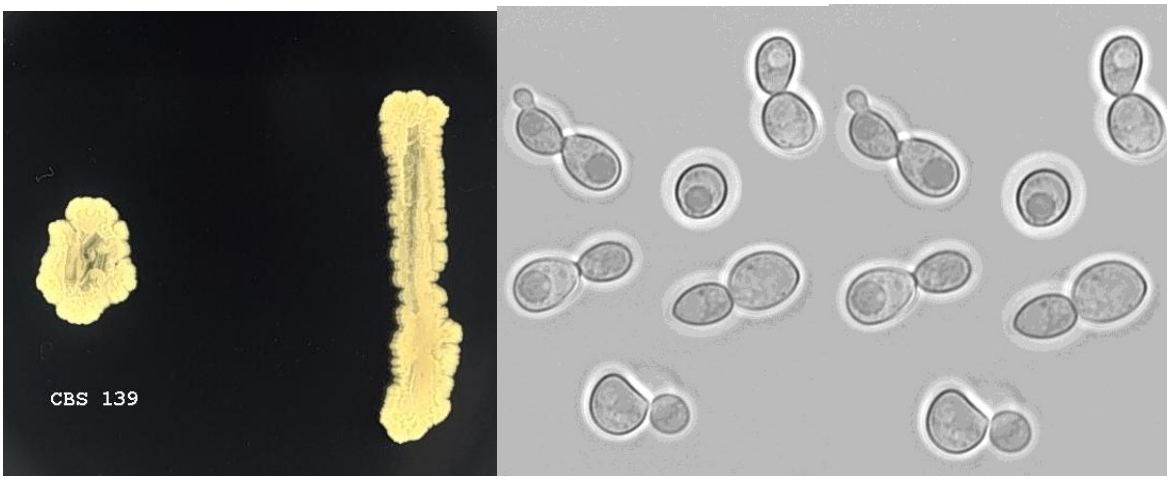
10.4. *Cryptococcus laurentii* (Kuff.) C.E. Skinner, The American Midland Naturalist 43: 249 (1950)

Synonyms:

1. *Torula laurentii* Kuff., Bulletin de la Societé Royale des Sciences Medicales et Naturelles de Bruxelles 1: 1-31 (1920)
2. *Torulopsis laurentii* (Kuff.) Lodder, Verhandelingen Koninklijke Nederlandse Akademie van Wetenschappen Afdeling Natuurkunde 32: 160 (1934) [MB#269102]
3. Cryptococcus laurentii var. laurentii (1952) [MB#429219]
4. *Rhodotorula laurentii* (Kuff.) T. Haseg., Banno & Yamauchi, J Gen Appl Microbiol Tokyo 6 (3): 212 (1960)
5. Rhodotorula nitens Mackenzie & Auret, Journal of General Microbiology 31 (2): 171 (1963)

Morphology

Colonies are yellowish to orange, sometimes pink in colour. Colony texture is smooth. Budding cells are round, oval or somewhat cylindrical.



Physiological data :

C1 D-Glucose+	C24 Erythritol-, +	N2 Nitrite-, +
C2 D-Galactose+	C25 Ribitol-, +	N3 Ethylamine-, +
C3 L-Sorbose-, +	C26 Xylitol+, d, w	N4 L-Lysine-, +
C4 D-Glucosamine-, +	C27 L-Arabinitol+, d, w	N5 Cadaverine-, +
C5 D-Ribose+, d, w	C28 D-Glucitol+, d, w	N6 Creatine-, +
C6 D-Xylose+	C29 D-Mannitol+, d, w	N7 Creatinine-, +
C7 L-Arabinose-, +	C30 Galactitol-, +	N8 Glucosamine-, +
C8 D-Arabinose-, +	C31 myo-Inositol+, d, w	N9 Imidazole-
C9 L-Rhamnose+, d, w	C32 D-Glucono-1,5-lactone-, +	N10 D-Tryptophan-, +
C10 Sucrose+	C33 2-Keto-D-Gluconate+	V1 w/o vitamins-, +
C11 Maltose+	C34 5-Keto-D-Gluconate+	V2 w/o myo-Inositol+
C12 a,a-Trehalose+, d, w	C35 D-Gluconate+	V3 w/o Pantothenate+
C13 Me a-D-Glucoside+, d,w	C36 D-Glucuronate+	V4 w/o Biotin+
C14 Cellobiose+	C37 D-Galacturonate-, +	V5 w/o Thiamin-, d, w
C15 Salicin+, d, w	C38 DL-Lactate-, +	V6 w/o Biotin & Thiamin-, d, w
C16 Arbutin+, d, w	C39 Succinate+, d, w	V7 w/o Pyridoxine+
C17 Melibiose+	C40 Citrate+, d, w	V8 w/o Pyridoxine & Thiamin-, d, w
C18 Lactose+	C43 Propane 1,2 diol-, +	V9 w/o Niacin+
C19 Raffinose-, +	C44 Butane 2,3 diol-	V10 w/o PABA+
C20 Melezitose+	C45 Quinic acid-, +	O1 Cycloheximide 0.01%-, +
C21 Inulin-, d, w	C46 D-glucarate-, +	O2 Cycloheximide 0.1%-, +
C22 Starch-, +	C47 D-Galactonate-, +	O3 Acetic acid 1%-
C23 Glycerol-, +	N1 Nitrate-	O6 10% NaCl-, +
		O7 16% NaCl-

C. laurentii was detected a dog with panniculitis and osteomyelitis. This organism was also found in the stomach of aborted equine fetuses.

10. 5. *Cryptococcus uniguttulatus* (Zach) Phaff & Fell, The Yeasts: a taxonomic study: 1140 (1970)

Synonyms:

1. *Eutorulopsis uniguttulata* Zach, Arch. Derm. Syph., Berlin: 688 (1934) [MB#281144]
2. *Cryptococcus neoformans var. uniguttulatus* (Zach) Lodder & Kreger, The Yeasts: a taxonomic study: 373 (1952)

Cryptococcus uniguttulatus (*Filobasidium uniguttulatus* is a teleomorph) was the first non-*neoformans Cryptococcus* to infect a human. It was isolated from ventricular fluid from a patient having had a neurosurgical procedure. This species was found to be very sensitive to amphotericin B at the minimum inhibitory dose. This species was first isolated from a human nail.

Physiological data

C1 D-Glucose+	C23 Glycerold	N1 Nitrate-
C2 D-Galactosed	C24 Erythritol-	N2 Nitrite-
C3 L-Sorbose-	C25 Ribitold	N3 Ethylamine-
C4 D-Glucosamine-	C26 Xylitol-	N4 L-Lysine+
C5 D-Ribose-	C27 L-Arabinitol-	N5 Cadaverine-
C6 D-Xylose+	C28 D-Glucitol+	N6 Creatine-
C7 L-Arabinose+	C29 D-Mannitold	N7 Creatinine-
C8 D-Arabinose-	C30 Galactitol-	N8 Glucosamine-
C9 L-Rhamnose+	C31 myo-Inositol+	N9 Imidazole-
C10 Sucrose+	C32 D-Glucono-1,5-lactone-	N10 D-Tryptophan-
C11 Maltose+	C33 2-Keto-D-Gluconate+	V1 w/o vitamins-
C12 a,a-Trehalosed	C35 D-Gluconate+	V2 w/o myo-Inositol+
C13 Me a-D-Glucoside+	C36 D-Glucuronate+	V3 w/o Pantothenate+
C14 Cellobiose-	C37 D-Galacturonate-	V4 w/o Biotin+
C15 Salicind	C38 DL-Lactated	V5 w/o Thiamin-
C16 Arbutind	C39 Succinated	V6 w/o Biotin & Thiamin-
C17 Melibiose-	C40 Citrated	V7 w/o Pyridoxine+
C18 Lactose-	C43 Propane 1,2 diol-	V8 w/o Pyridoxine & Thiamin-
C19 Raffinose+	C44 Butane 2,3 diol-	V9 w/o Niacin+
C20 Melezitose+	C45 Quinic acid-	V10 w/o PABA+
C21 Inulin-	C46 D-glucarate-	O1 Cycloheximide 0.01%-
C22 Starchw	C47 D-Galactonate-	O3 Acetic acid 1%-

10.6. *Cryptococcus adeliensis* Scorzetti Scorzetti, G.; Petrescu, I.; Yarrow, D.; Fell, J.W. 2000.

Morphology

Cryptococcus adeliensis is a species of Cryptococcus that when plated on agar produces colonies that are cream colored, with a smooth, glossy appearance. The colonies frequently appear to have a soft texture. The optimal growth range for this species is at 25 degrees Celsius.

Physiological data

C1 D-Glucose+	C28 D-Glucitol+, d, w	C57 Ethylene glycol-
C2 D-Galactosed	C29 D-Mannitol-, +	C58 Tween 40+
C3 L-Sorbose-	C30 Galactitol-, d, w	C59 Tween 60w
C4 D-Glucosamine-	C31 myo-Inositol+, d, w	C60 Tween 80-
C5 D-Ribose-, d, w	C32 D-Glucono-1,5-lactone-	N1 Nitrate+, d, w
C6 D-Xylose+	C33 2-Keto-D-Gluconate+	N2 Nitrite+, d, w
C7 L-Arabinose+	C34 5-Keto-D-Gluconatew	N3 Ethylamine-
C8 D-Arabinose-, d, w	C35 D-Gluconate-, +	N4 L-Lysine-, d, w
C9 L-Rhamnosed	C36 D-Glucuronate+, d, w	N5 Cadaverine+, d, w
C10 Sucrose+	C37 D-Galacturonate-	N6 Creatine-
C11 Maltose+	C38 DL-Lactate-	N7 Creatinine-
C12 a,a-Trehalose+, d, w	C39 Succinate-, d, w	N8 Glucosamine-
C13 Me a-D-Glucoside-, d, w	C40 Citrate-, +	N9 Imidazole-
C14 Cellobiose+, d, w	C43 Propane 1,2 diol-	N10 D-Tryptophan-, d, w
C15 Salicin-, +	C44 Butane 2,3 diol-	N11 D-Prolinew
C16 Arbutin+, d, w	C45 Quinic acid-	N12 Putrescine-
C17 Melibiose-, d, w	C46 D-glucarate+, d, w	V1 w/o vitamins-
C18 Lactosed	C47 D-Galactonate-	O1 Cycloheximide 0.01%-, +
C19 Raffinose+	C48 Palatinosew	O2 Cycloheximide 0.1%-
C20 Melezitose+, d, w	C49 Levulinate-	O3 Acetic acid 1%-
C21 Inulin-, d, w	C50 L-Malic acid-	O6 10% NaCl-
C22 Starch+	C51 L-Tartaric acid-	O7 16% NaCl-
C23 Glycerol-, d, w	C52 D-Tartaric acid-	O8 Growth at pH=3w
C24 Erythritol-	C53 meso-Tartaric acid-	O9 Growth at pH=9.5-
C25 Ribitol-, d, w	C54 Galactaric acid-	
C26 Xylitol+, d, w	C55 Uric acidw	
C27 L-Arabinitol-, d, w	C56 Gentobiosew	

Cryptococcus adeliensis sp. nov. (CBS 8351) was described based on phenotypic characteristics and molecular sequence analysis of the D1/D2 large subunit and internal transcribed spacer regions of the ribosomal DNA. Molecular comparisons include species closely related to Cryptococcus albidus and several species isolated from the Antarctic. C. adeliensis, which has a cold-adapted xylanase, was isolated from Terre Adelie, Antarctica. ATCC 34633, which has a mesophilic xylanase, was identified as Cryptococcus albidosimilis.

Cryptococcus adeliensis has been reported to cause meningitis in a German patient with acute myeloid leukemia. *Cryptococcus adeliensis* has been isolated from a lung biopsy of a male adult suffering from a progressive lung disease and from the oral cavity from an HIV-infected 8-year-old-human. Finally,*Cryptococcus curvatus* has been reported from cerebrospinal fluid of a 30-year-old HIV-infected

male patient. Clinical isolates of this latter species from the CBS culture collection originated from sputum, urine, and feces. It was isolated from pigeon droppings. *C. adeliensis* can be misidentified as *C. albidus* due to the high variability of phenotypic markers of the latter.

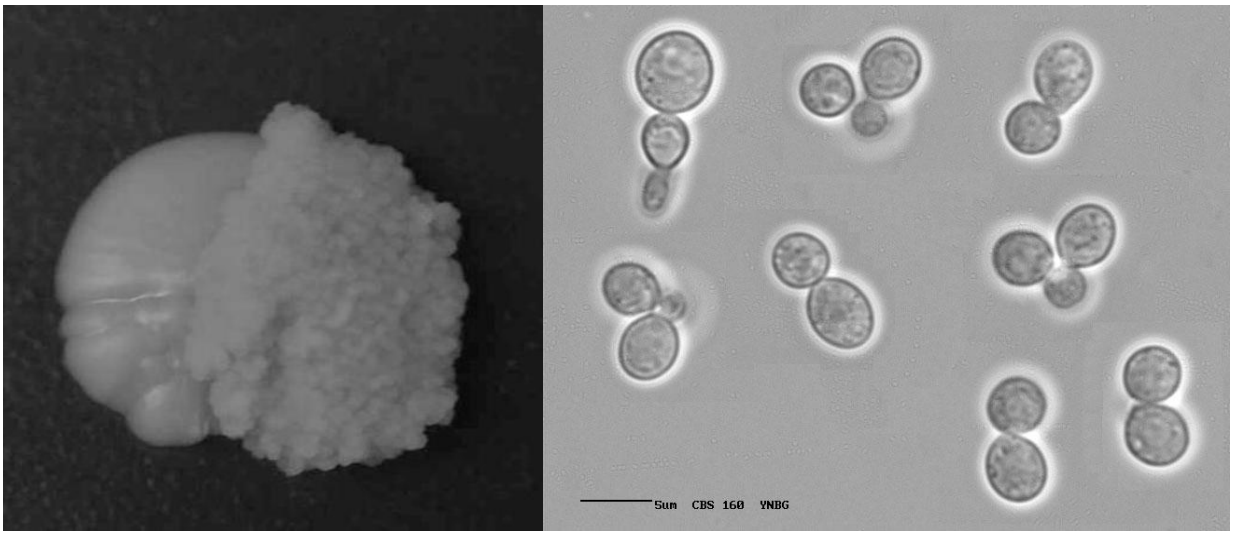
10.7. *Cryptococcus diffluens* (Zach) Lodder & Kreger, The Yeasts: a taxonomic study: 391 (1952

Synonyms:

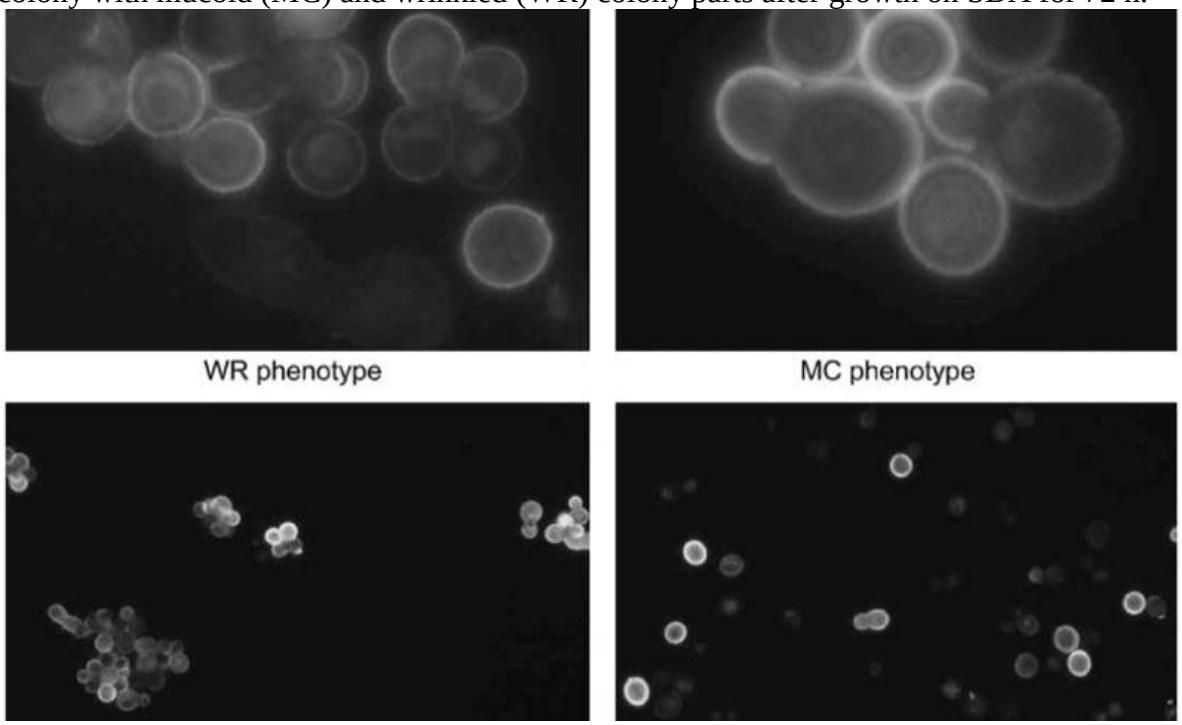
1. *Torulopsis diffluens* Zach, Arch. Dermatol. Syph.: 690 (1934) [MB#456599]
2. *Cryptococcus diffluens var. diffluens* (1954) [MB#426064
3. *Rhodotorula diffluens (Zach) T. Haseg., Banno & Yama.J. Gen. Appl. Microbiol. Tokyo 6 (3): 212 (1960)*
4. *Cryptococcus albidus var. diffluens* (Zach) Phaff & Fell, The Yeasts: a taxonomic study: 1099 (1970)
5. *Torula diffluens* (Zach) A.V. Martini (1991) [MB#263182]
6. *Torula gelatinosa* Saito, Journal of Japanese Botany 1: 42 (1922) [MB#265849]
7. *Torulopsis albida var. japonica* Lodder (1934) [MB#256210]
8. *Cryptococcus diffluens var. uruguaiensis* Artag.-All. & L.A. Queiroz, Publicação No. 663 Instituto de Micologia Universidade do Recife: 1-9 (1970)

Morphology

Two basic colony morphologies, mucoid and wrinkled develop on solid media. The colony morphologies are unstable. The wrinkled cell population is more heterogenous with respect to cell size and grow in clumps, whereas the mucoid cell size is more homogenous and less clumps are observed. The capsule of the mucoid colony phenotype is larger than the capsule of the wrinkled yeast cells (1.06 μm ± 0.4 vs 0.74 μm ± 0.19, P= 0.003).



colony with mucoid (MC) and wrinkled (WR) colony parts after growth on SDA for 72 h.



Indirect immunofluorescens with Mab 2H1 of wrinkled (WR) and mucoid (MC) cells respectively (upper panel: 100× and lower panel: 40×).

Physiological data :

C1 D-Glucose+	C24 Erythritol-	N3 Ethylamine+
C2 D-Galactose-, d, w	C25 Ribitold	N4 L-Lysine-, +
C3 L-Sorbose+, d, w	C26 Xylitol+, d, w	N5 Cadaverine+
C4 D-Glucosamine-	C27 L-Arabinitol-, +	N6 Creatine-
C5 D-Ribose+, d, w	C28 D-Glucitol+	N7 Creatinine-
C6 D-Xylose+	C29 D-Mannitol-, +	N8 Glucosamine-
C7 L-Arabinose+	C30 Galactitol-	N9 Imidazole-
C8 D-Arabinose-, +	C31 myo-Inositol+	N10 D-Tryptophan-, +
C9 L-Rhamnose+	C32 D-Glucono-1,5-lactone+	V1 w/o vitamins-
C10 Sucrose+	C33 2-Keto-D-Gluconate+	V2 w/o myo-Inositol+
C11 Maltose+	C35 D-Gluconate+	V3 w/o Pantothenate+
C12 a,a-Trehalose+	C36 D-Glucuronate+, d, w	V4 w/o Biotin+
C13 Me a-D-Glucoside+	C37 D-Galacturonate-, d, w	V5 w/o Thiamin-
C14 Cellobiose+	C38 DL-Lactate-, +	V6 w/o Biotin & Thiamin-
C15 Salicin+	C39 Succinate+	V7 w/o Pyridoxine+
C16 Arbutin+	C40 Citrate+	V8 w/o Pyridoxine & Thiamin-
C17 Melibiose-	C43 Propane 1,2 diol-	V9 w/o Niacin+
C18 Lactose-	C44 Butane 2,3 diol-	V10 w/o PABA+
C19 Raffinose+	C45 Quinic acid+	O1 Cycloheximide 0.01%-, +
C20 Melezitose+	C46 D-glucarate-	O2 Cycloheximide 0.1%-
C21 Inulin-	C47 D-Galactonate-, d, w	O3 Acetic acid 1%-
C22 Starch+	N1 Nitrate+	O6 10% NaCl-
C23 Glycerol-, d, w	N2 Nitrite+	O7 16% NaCl-

Cryptococcus diffluens was reported by Suqita et al.(2003) to colonize the skin of patients with atopic dermatitis. Kantarcioğlu et al. ( ) a case of subcutaneous cryptococcosis due to *Cryptococcus diffluens* in a patient with sporotrichoid lesions.

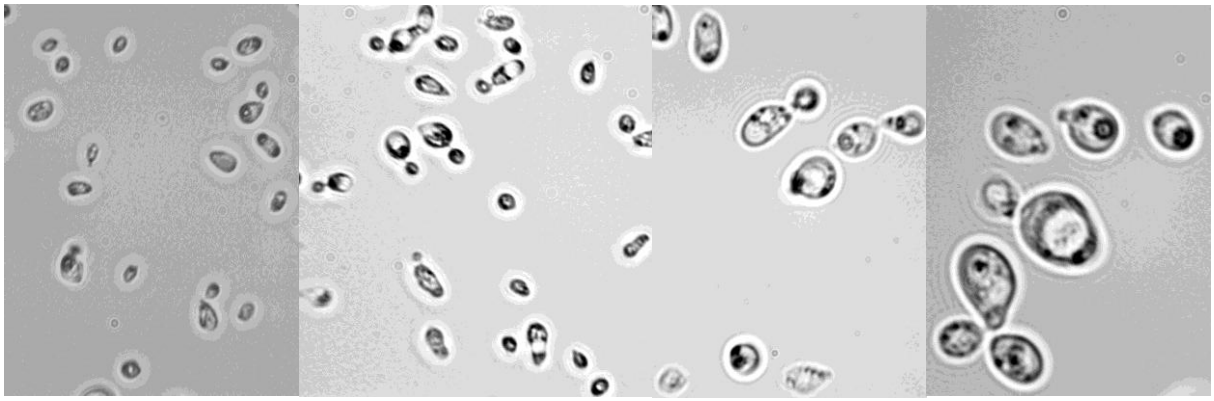
10.8. *Cryptococcus chernovii*

Physiological data

C1 D-Glucose+	C21 Inulin-	C43 Propane 1,2 diol-
C2 D-Galactose+	C22 Starch+	C44 Butane 2,3 diol-
C3 L-Sorbose+	C23 Glycerold	C45 Quinic acid-
C4 D-Glucosamined	C24 Erythritol-	C46 D-glucarate-
C5 D-Ribose-	C25 Ribitol-, d, w	C47 D-Galactonate-
C6 D-Xylose+	C26 Xylitol+, d, w	N1 Nitrate+
C7 L-Arabinose+	C27 L-Arabinitol-	N2 Nitrite+
C8 D-Arabinose-	C28 D-Glucitol+	N3 Ethylamine+
C9 L-Rhamnose+	C29 D-Mannitol+	N4 L-Lysine-
C10 Sucrose+	C30 Galactitold	N5 Cadaverine+
C11 Maltose+	C31 myo-Inositol+	N6 Creatine-
C12 a,a-Trehalose+	C32 D-Glucono-1,5-lactone-	N7 Creatinine-
C13 Me a-D-Glucoside+	C33 2-Keto-D-Gluconate+	N8 Glucosamine-
C14 Cellobiose+	C35 D-Gluconate-	N9 Imidazole-
C15 Salicin+	C36 D-Glucuronate+	N10 D-Tryptophan-
C16 Arbutin+	C37 D-Galacturonate-	V1 w/o vitamins-
C17 Melibiose-	C38 DL-Lactate-	O1 Cycloheximide 0.01%+
C18 Lactose+	C39 Succinate+	O2 Cycloheximide 0.1%-
C19 Raffinose+	C40 Citrate+	O3 Acetic acid 1%
C20 Melezitose+		

?? reported the isolation of Cryptococcus magnus and Cryptococcus chernovii in cultures inoculated with nasal specimens of pediatric cancer patients with acute lymphoblastic leukemia. reported previously.

10.9. Cryptococcus magnus



physiological data

C1 D-Glucose+	C24 Erythritol-	N2 Nitrite-
C2 D-Galactose+, d, w	C25 Ribitol-, d, w	N3 Ethylamine+
C3 L-Sorbose-, d, w	C24 Erythritol-	N4 L-Lysine+
C4 D-Glucosamine-, +	C25 Ribitol-, d, w C26 Xylitol+	N5 Cadaverine+
C5 D-Ribose-, +	C27 L-Arabinitol-, d, w	N6 Creatine-
C6 D-Xylose+	C28 D-Glucitol+, d, w	N7 Creatinine-
C7 L-Arabinose+, d, w	C29 D-Mannitol+, d, w	N8 Glucosamine-, +
C8 D-Arabinose-, d, w	C30 Galactitol-, d, w	N9 Imidazole-
C9 L-Rhamnose-, +	C31 myo-Inositol+	N10 D-Tryptophan-
C10 Sucrose+	C32 D-Glucono-1,5-lactone-, d, w	V1 w/o vitamins-
C11 Maltose+	C33 2-Keto-D-Gluconate+	V2 w/o myo-Inositol+
C12 a,a-Trehalose+	C34 5-Keto-D-Gluconate+	V3 w/o Pantothenate+
C13 Me a-D-Glucoside+	C35 D-Gluconate-, +	V4 w/o Biotin+
C14 Cellobiose+	C36 D-Glucuronate+, d, w	V5 w/o Thiamin-
C15 Salicin+	C37 D-Galacturonate-	V6 w/o Biotin & Thiamin-
C16 Arbutin+	C38 DL-Lactate-, d, w	V7 w/o Pyridoxine+
C17 Melibiose-	C39 Succinate+	V8 w/o Pyridoxine & Thiamin-
C18 Lactose+	C40 Citrate+, d, w	V9 w/o Niacin+
C19 Raffinose+	C43 Propane 1,2 diol-	V10 w/o PABA+
C20 Melezitose+, d, w	C44 Butane 2,3 diol-	O1 Cycloheximide 0.01%-
C21 Inulin-, +	C45 Quinic acid+	O2 Cycloheximide 0.1%-
C22 Starch+	C46 D-glucarate+, d, w	O3 Acetic acid 1%-
C23 Glycerol-	C47 D-Galactonate-	O6 10% NaCl-
	N1 Nitrate-	O7 16% NaCl-

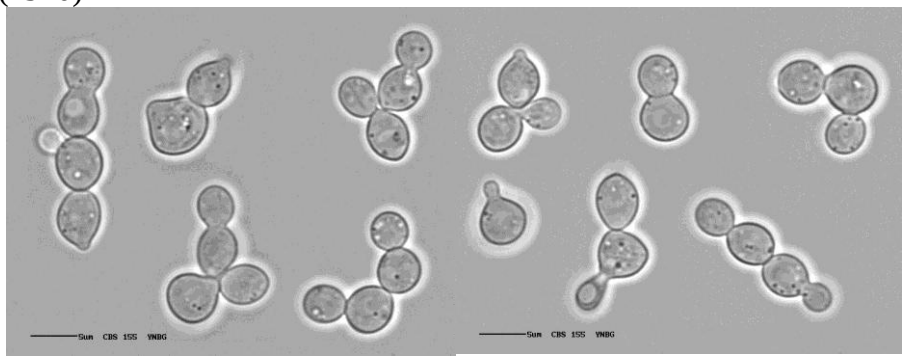
Poth et al. (2010) described an uncommon case of cryptococcosis in an apparently immunocompetent cat caused by *Cryptococcus magnus*. An amputation of the complete left foreleg and excision of the ipsilateral cervical lymph node were performed in a young-adult male Domestic Shorthair cat due to suspicion of a tumor. Granulomatous dermatitis, panniculitis, myositis, and lymphadenitis were diagnosed histologically. Intralesional, numerous round-to-ovoid yeast cells showing no capsule were detected within macrophages using special staining methods.

C. magnus was the causative agent in an immunocompetent cat that had a recurrent painful mass lesion of the foreleg, together with enlargement of the regional lymph node. Convulsions, seen in this cat during the early stage of treatment, suggested CNS involvement but resolved with continuing therapy. C. magnus was also isolated from the ear of a cat with otitis externa, but the inflammation was attributed to Aspergillus fumigatus, and the role of C. magnus is uncertain.

10. 10. *Cryptococcus aerius* (Saito) Nannizzi, Com. fatta R. Accad. Fisiocrit. Siena: (1927)

Synonyms:

1. *Torulopsis aeria var. aeria* [MB#494232]
2. *Torula aerius* Saito (1922) [MB#255932]
3. Torula aeria Saito, Journal of Japanese Botany 1: 41 (1922) [MB#536550]
4. *Torulopsis aeria* (Saito) Lodder, Verhandelingen Koninklijke Nederlandse Akademie van Wetenschappen Afdeling Natuurkunde 32: 161 (1934)
5. *Paratorulopsis aeria* (Saito) E.K. Novák & Zsolt, Acta Botanica Academiae Scientiarum Hungarica 7: 141 (1961) [MB#335633]
6. *Cryptococcus albidus var. aerius* (Saito) Phaff & Fell, The Yeasts: a taxonomic study: 1093 (1970)



Physiological data :

C1 D-Glucose+	C24 Erythritol-	N3 Ethylamine-, d, w
C2 D-Galactose+	C25 Ribitol+, d, w	N4 L-Lysine+, d, w
C3 L-Sorbose+, d, w	C26 Xylitol+	N5 Cadaverine+, d, w
C4 D-Glucosaminew	C27 L-Arabinitol+	N6 Creatine-
C5 D-Ribose+, d, w	C28 D-Glucitol+	N7 Creatinine-
C6 D-Xylose+	C29 D-Mannitol+	N8 Glucosamine-
C7 L-Arabinose+	C30 Galactitol+	N9 Imidazole-
C8 D-Arabinose+, d, w	C31 myo-Inositol+	N10 D-Tryptophan-, d, w
C9 L-Rhamnose+	C32 D-Glucono-1,5-lactone-, +	V1 w/o vitamins+, d, w
C10 Sucrose+	C33 2-Keto-D-Gluconate+	V2 w/o myo-Inositol+
C11 Maltose+	C35 D-Gluconate+	V3 w/o Pantothenate+
C12 a,a-Trehalose+	C36 D-Glucuronate+	V4 w/o Biotin+
C13 Me a-D-Glucoside+, d, w	C37 D-Galacturonate-	V5 w/o Thiamind
C14 Cellobiose+	C38 DL-Lactate-, d, w	V6 w/o Biotin & Thiamind
C15 Salicin+	C39 Succinate+, d, w	V7 w/o Pyridoxine+
C16 Arbutin+, d, w	C40 Citrate+	V8 w/o Pyridoxine & Thiamind
C17 Melibiose+	C43 Propane 1,2 diol-	V9 w/o Niacin+
C18 Lactose+	C44 Butane 2,3 diol-	V10 w/o PABA+
C19 Raffinose+	C45 Quinic acid+	O1 Cycloheximide 0.01%-, d, w
C20 Melezitose+	C46 D-glucarate+	O2 Cycloheximide 0.1%-
C21 Inulin-	C47 D-Galactonate-, d, w	O3 Acetic acid 1%-
C22 Starch+, d, w	N1 Nitrate+	O6 10% NaCl-
C23 Glycerol-, d, w	N2 Nitrite+	O7 16% NaCl-

Cryptococcus aerius is an obligate aerobe, that has been previously isolated from soil samples and samples of sand. Fonseca A., Scorzeti G., and Fell J. (2000) Diversity in the yeast Cryptococcus albidus and related species as revealed by ribosomal DNA sequence analysis. Can. J. Microbiol. 46:7-27
It has a growth temperature range between 20Â°C and 35Â°C. This species secretes amylase at the end of its exponential phase, and it is believed to produce the most amylase at 30Â°C between pH 4.5 and pH 6. It is believed that the amylases that are produced by *C. aerius* are able to digest raw starch, and this ability to break down raw starch has been studied extensively, because the ability to find microorganisms that can break down raw starch has become increasingly important as the production of materials such as liquid fuel and chemicals using starch has become more prominent. This species ability to break down starch is greatly improved when it is cocultured with *Saccharomyces cerevisiae*. *C. aerius* is able to use glucose, galactose, maltose and starch as sole carbon sources, and it is able to use nitrate and nitrite as sole nitrogen sources.

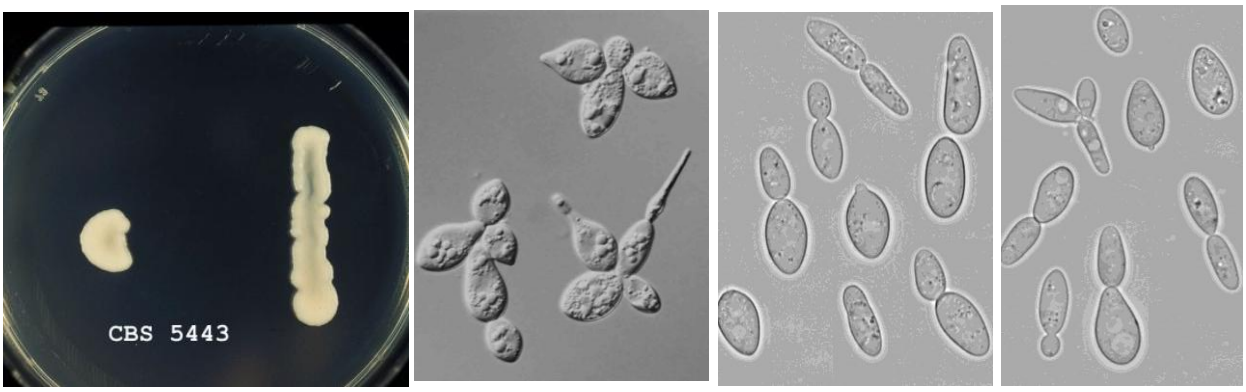
10. 11. *Cryptococcus aquaticus* (E.B.G. Jones & Slooff) Rodr. Mir. & Weijman, (1988)

Synonyms:

1. *Candida aquatica* E.B.G. Jones & Slooff, Antonie van Leeuwenhoek 32 (2): 223 (1966)
2. *Vanrijia aquatica* (Jones & Slooff) R.T. Moore (1980) [MB#493616]
3. *Vanrija aquatica* (E.B.G. Jones & Slooff) R.T. Moore, Botanica Marina 23 (6): 367 (1980)
4. *Mrakiella aquatica* (E.B.G. Jones & Sloof) Margesin & Fell, International Journal of Systematic and Evolutionary Microbiology 58 (12) (2008) [MB#514705]

Cryptococcus aquaticus is a species of *Cryptococcus* that is found in extreme climates, and has an optimal growth temperature of 9°C, but it can grow at temperatures as low as 2°C. When plated on agar it produces smooth, butyrous colonies with a cream coloration. When grown in liquid media it takes approximately 76 hours to reach stationary phase and is very sensitive to decreases in pH.

Cryptococcus aquaticus typically has an oval or cylindrical shape, but it can be dumb-bell shaped at the microscopic level. *C. aquaticus* reproduces through bipolar mypodial budding.



physiological data :

C1 D-Glucose+	C24 Erythritol-	N3 Ethylamine-
C2 D-Galactose+	C25 Ribitol+	N4 L-Lysine+
C3 L-Sorbose+	C26 Xylitol+	N5 Cadaverine-
C4 D-Glucosamine-	C27 L-Arabinitol-	N6 Creatine-
C5 D-Ribose-, d, w	C28 D-Glucitol+	N7 Creatinine-
C6 D-Xylose+	C29 D-Mannitol+	N8 Glucosamine-
C7 L-Arabinose+	C30 Galactitol-	N9 Imidazole-
C8 D-Arabinose-	C31 myo-Inositol-	N10 D-Tryptophan-
C9 L-Rhamnose-	C32 D-Glucono-1,5-lactone+	V1 w/o vitamins-
C10 Sucrose+	C33 2-Keto-D-Gluconate+	V2 w/o myo-Inositol+
C11 Maltose+	C35 D-Gluconate+	V3 w/o Pantothenate+
C12 a,a-Trehalose+	C36 D-Glucuronate+	V4 w/o Biotin-
C13 Me a-D-Glucoside-	C37 D-Galacturonated	V5 w/o Thiamin-
C14 Cellobiose+	C38 DL-Lactate-	V6 w/o Biotin & Thiamin-
C15 Salicin+	C39 Succinate+	V7 w/o Pyridoxine+
C16 Arbutin+, d, w	C40 Citrate+	V8 w/o Pyridoxine & Thiamin-
C17 Melibiose+	C43 Propane 1,2 diol-	V9 w/o Niacin+
C18 Lactose+, d, w	C44 Butane 2,3 diol-	V10 w/o PABA+
C19 Raffinose+	C45 Quinic acid-	O1 Cycloheximide 0.01%-
C20 Melezitose+	C46 D-glucarate+	O2 Cycloheximide 0.1%-
C21 Inulin-	C47 D-Galactonate-	O3 Acetic acid 1%-
C22 Starch+	N1 Nitrate+	O6 10% NaCl-
C23 Glycerold	N2 Nitrite+	O7 16% NaCl-

Cryptococcus aquaticus is somewhat unique in the *Cryptococcus* family in that it can weakly ferment D-glucose, D-galactose, maltose and melezitose. This species is DBB+. *C. aquaticus* has been studied because of its ability to produce pecticase. It produces polycalacturonase, but not isoenzymes of polygalacturonase, and it has increased activity in the presence of glucose.

This species of *Cryptococcus* has an interested trait in that it produces mycocins, which are proteinacous toxins that either kill or inhibit the ability of fungi that are

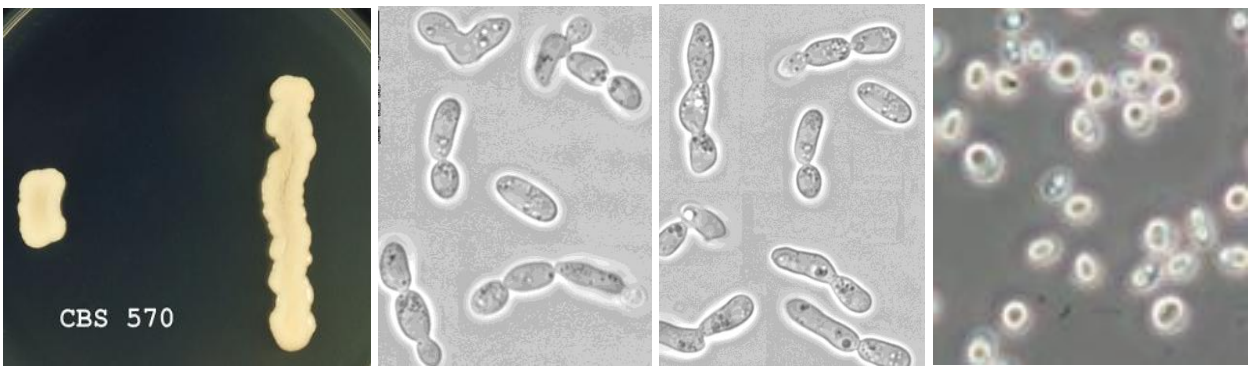
in the same taxonomic rank or in a related taxonomic rank. The mycocin that *C. aquaticus* produces was only able to kill Cystofilobasidiales clade basidiomycetes, and was unable to kill yests in any phyla outside of basidiomycetes.

10.12. *Cryptococcus curvatus* (Diddens & Lodder) Golubev, Mikologiya i Fitopatologiya 15 (6): 467 (1981)

Synonyms:

1. *Candida heveanensis var. curvata* Diddens & Lodder, Die anaskosporogenen Hefen, II Hälfte: 486 (1942) [MB#346021]
2. *Candida curvata* (Diddens & Lodder) Lodder & Kreger-van Rij, The Yeasts: a taxonomic study: 576 (1952)
3. *Azymocandida curvata* (Diddens & Lodder) E.K. Novák & Zsolt, Acta Botanica Academiae Scientiarum Hungarica 7: 134 (1961)
4. *Apiotrichum curvatum* (Diddens & Lodder) Arx & Weijman, Antonie van Leeuwenhoek 45: 554 (1979)
5. *Vanrijia curvata* (Diddens & Lodder) R.T. Moore (1980)
6. *Cryptococcus curvatus* (Diddens & Lodder) Golubev, Taxonomy and identification of yeast fungi of the genus Cryptococcus: 26 (1980)
7. *Vanrija curvata* (Diddens & Lodder) R.T. Moore, Botanica Marina 23 (6): 367 (1980)

Cryptococcus curvatus is a fungus species. It is an extremophile found in cold-seep sites. It is oleaginous, and uses the sugars in cellulose for the growth and production of storage triglycerides. This species has been extensively studied in relationship to lipids. It can uptake both glucose and xylose simultaneously. When grown in old oil with high levels of polymerized triglyceride, the cell wall transforms from being smooth to having hair or wart-like protuberances which are believed to assist in lipid uptake



Cryptococcus curvatus with intracellularly formed "single cell oil"

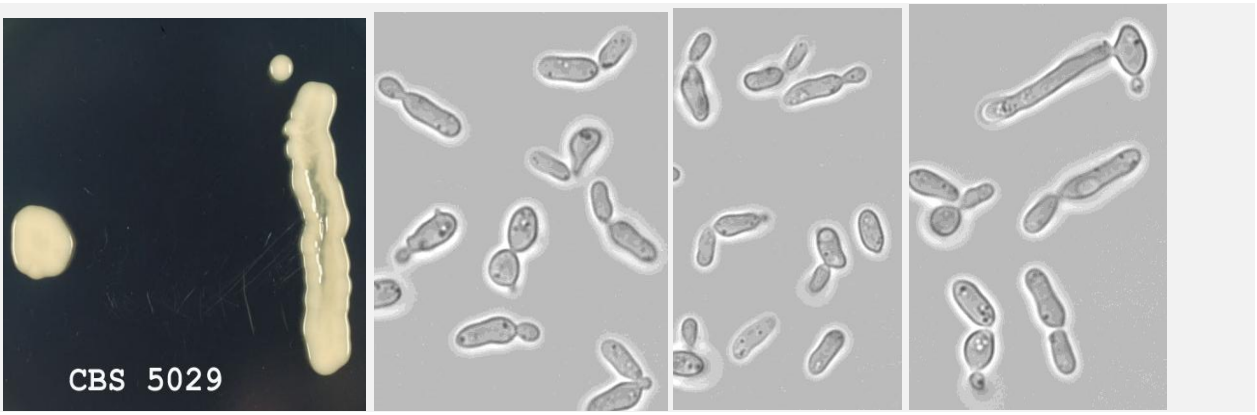
Physiological data :

C1 D-Glucose+	C25 Ribitol+, d, w	N4 L-Lysine+
C2 D-Galactose+	C26 Xylitol+, d, w	N5 Cadaverine+
C3 L-Sorbose-, +	C27 L-Arabinitol-, +	N6 Creatine-
C4 D-Glucosamine-, +	C28 D-Glucitol-, +	N7 Creatinine-
C5 D-Ribose+	C29 D-Mannitol-, +	N8 Glucosamine-
C6 D-Xylose+	C30 Galactitol-, +	N9 Imidazole-
C7 L-Arabinose-, +	C31 myo-Inositol-, +	N10 D-Tryptophan-
C8 D-Arabinose-, +	C32 D-Glucono-1,5-lactone-, +	V1 w/o vitamins-, +
C9 L-Rhamnose-, +	C33 2-Keto-D-Gluconate+	V2 w/o myo-Inositol+
C10 Sucrose+	C34 5-Keto-D-Gluconate+	V3 w/o Pantothenate+
C11 Maltose+, d, w	C35 D-Gluconate+	V4 w/o Biotin-
C12 a,a-Trehalose+, d, w	C36 D-Glucuronate+	V5 w/o Thiamin-
C13 Me a-D-Glucoside-, +	C37 D-Galacturonate-	V6 w/o Biotin & Thiamin-
C14 Cellobiose+	C38 DL-Lactate+, d, w	V7 w/o Pyridoxine+
C15 Salicin+	C39 Succinate+	V8 w/o Pyridoxine & Thiamin-
C16 Arbutin+	C40 Citrate+, d, w	V9 w/o Niacin+, d, w
C17 Melibiose-	C43 Propane 1,2 diol+	V10 w/o PABA+
C18 Lactose+, d, w	C44 Butane 2,3 diol-, +	O1 Cycloheximide 0.01%-, +
C19 Raffinose+	C45 Quinic acid-, +	O2 Cycloheximide 0.1%-, d, w
C20 Melezitose-, +	C46 D-glucarate-	O3 Acetic acid 1%-
C21 Inulin-	C47 D-Galactonate-, +	O6 10% NaCl-
C22 Starch-, +	N1 Nitrate-	O7 16% NaCl-
C23 Glycerol+	N2 Nitrite-, +	
C24 Erythritol+, d, w	N3 Ethylamine+, d, w	

Cryptococcus curvatus has been reported from cerebrospinal fluid of a 30-year-old HIV-infected male patient [11]. Clinical isolates of this species from the CBS culture collection originated from sputum, urine, and feces [12].

10. 13. *Cryptococcus skinneri* Phaff & Carmo Souza, Antonie van Leeuwenhoek 28: 205 (1962)

Cryptococcus skinneri is species of yeast which forms white and cream colored colonies which are smooth with a mucoid texture. No hyphae are present and it reproduces by budding. The individual cells are round and oval in shape. The formation of starch and urea has been observed. It will grow at 25 degrees Celsius up to 30 degrees Celsius in some cases.



Physiological data :

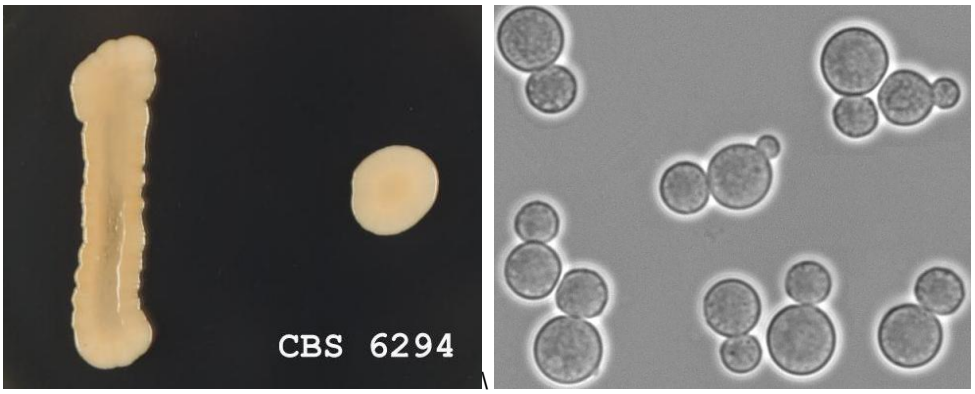
C1 D-Glucose+	C25 Ribitol+, d, w	N4 L-Lysine+
C2 D-Galactose-, +	C26 Xylitol+, d, w	N5 Cadaverine-
C3 L-Sorbose-, +	C27 L-Arabinitol+, d, w	N6 Creatine-
C4 D-Glucosamine-, d, w	C28 D-Glucitol+	N7 Creatinine-
C5 D-Ribose+, d, w	C29 D-Mannitol+	N8 Glucosamine-, +
C6 D-Xylose+	C30 Galactitol+, d, w	N9 Imidazole-
C7 L-Arabinose+, d, w	C31 myo-Inositol+, d, w	N10 D-Tryptophan-
C8 D-Arabinose+, d, w	C32 D-Glucono-1,5-lactone+	V1 w/o vitamins-
C9 L-Rhamnose+	C33 2-Keto-D-Gluconate+	V2 w/o myo-Inositol+
C10 Sucrose-	C35 D-Gluconate+	V3 w/o Pantothenate+
C11 Maltose-	C36 D-Glucuronate+	V4 w/o Biotin+
C12 a,a-Trehalose+	C37 D-Galacturonate+	V5 w/o Thiamin-
C13 Me a-D-Glucoside-	C38 DL-Lactate-, +	V6 w/o Biotin & Thiamin+
C14 Cellobiose+, d, w	C39 Succinate+	V7 w/o Pyridoxine+
C15 Salicin-, d, w	C40 Citrate+	V8 w/o Pyridoxine & Thiamin-
C16 Arbutin-, d, w	C43 Propane 1,2 diol-	V9 w/o Niacin+
C17 Melibiose-	C44 Butane 2,3 diol-	V10 w/o PABA+
C18 Lactose-, d, w	C45 Quinic acid-	O1 Cycloheximide 0.01%-, d, w
C19 Raffinose-	C46 D-glucarate+, d, w	O2 Cycloheximide 0.1%-
C20 Melezitose-	C47 D-Galactonate+	O3 Acetic acid 1%-
C21 Inulin-	N1 Nitrate-	O6 10% NaCl-
C22 Starch-	N2 Nitrite-	O7 16% NaCl-
C23 Glycerol+, d, w	N3 Ethylamine-	
C24 Erythritol-		

10. 14. *Cryptococcus terreus* Di Menna, Journal of General Microbiology 11: 195 (1954)

Synonyms:

1. *Cryptococcus himalayensis* Goto & Sugiy., Canadian Journal of Botany 48 (12): 2099 (1970) [MB#312343]
2. *Cryptococcus elinovii* Golubev, Mikologiya i Fitopatologiya 13: 466 (1979) [MB#312339

Cryptococcus terreus is a fungus species. It is unique within its genus because it can use glucose, lactose, galactose and potassium nitrate. The cells are oval in shape with mucous capsules. The culture when grown start of cream color but turned tan with a "tough" surface skin. No mycelium present. This species is very similar to C. albidus with the notable exception of not being able to ferment sucrose.



Physiological data

C1 D-Glucose+	C19 Raffinose-	C39 Succinate+
C2 D-Galactose+	C20 Melezitose+	C40 Citrate+
C3 L-Sorbose+	C21 Inulin	C43 Propane 1,2 diol
C4 D-Glucosamined	C22 Starch+	C44 Butane 2,3 diol
C5 D-Ribose+	C23 Glycerol+, d, w	C45 Quinic acid+
C6 D-Xylose+	C25 Ribitol+	C46 D-glucarate+
C7 L-Arabinose+	C26 Xylitold	C47 D-Galactonate
C8 D-Arabinose+	C27 L-Arabinitol+	N1 Nitrate+
C9 L-Rhamnose+	C28 D-Glucitol+	N2 Nitrite+
C10 Sucrose-	O3 Acetic acid 1%-	N3 Ethylamine-
C11 Maltose+	C29 D-Mannitol+	N4 L-Lysine+
C12 a,a-Trehalose+	C30 Galactitol+	N5 Cadaverine+
C13 Me a-D-Glucosided	C31 myo-Inositol+	N6 Creatine-
C14 Cellobiose+	C32 D-Glucono-1,5-lactone-, d, w	N7 Creatinine-
C15 Salicin+	C33 2-Keto-D-Gluconate+	N8 Glucosamine-
C16 Arbutin+	C35 D-Gluconate+	N9 Imidazole-
C17 Melibiose-	C36 D-Glucuronate+	N10 D-Tryptophan-
C18 Lactose+	C37 D-Galacturonate+, d, w	V1 w/o vitamins+
	C38 DL-Lactate-	O1 Cycloheximide 0.01%-

10. 15. *Cryptococcus flavescence*

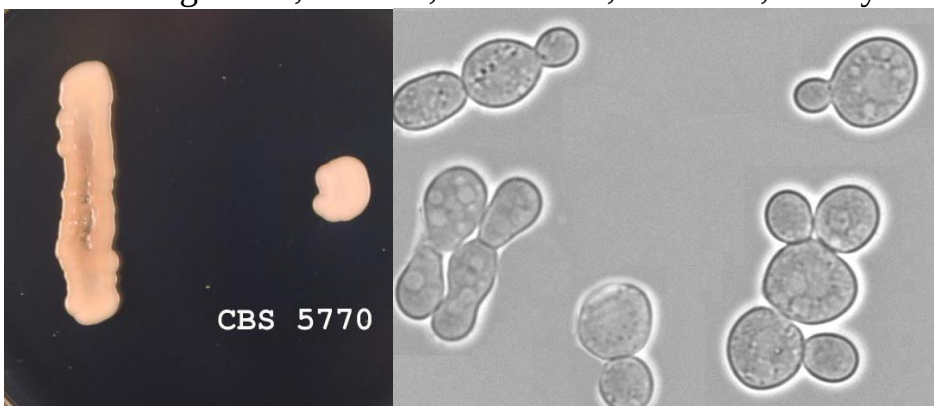
Physiological data :

C1 D-Glucose+	C19 Raffinose+	N1 Nitrate-
C2 D-Galactose+	C20 Melezitose+	N2 Nitrite-
C3 L-Sorbose-	C21 Inulin-	N3 Ethylamine+
C4 D-Glucosamine-	C22 Starch-	N4 L-Lysine+
C5 D-Ribose+	C23 Glycerol-	N5 Cadaverine+
C6 D-Xylose+	C24 Erythritold	N6 Creatine-
C7 L-Arabinose+	C25 Ribitol+	N7 Creatinine-
C8 D-Arabinose+	C26 Xylitol+	N8 Glucosamine-
C9 L-Rhamnose+	C27 L-Arabinitol+	N9 Imidazole-
C10 Sucrose+	C28 D-Glucitol+	N10 D-Tryptophan-
C11 Maltose+	C29 D-Mannitol+	V1 w/o vitamins+
C12 a,a-Trehalose+	C30 Galactitol+	O1 Cycloheximide 0.01%-, d, w
C13 Me a-D-Glucoside+	C31 myo-Inositol+	O2 Cycloheximide 0.1%-
C14 Cellobiose+	C32 D-Glucono-1,5-lactone+	O3 Acetic acid 1%-
C15 Salicin+	C33 2-Keto-D-Gluconate+	C40 Citrate+, d, w
C16 Arbutin+	C35 D-Gluconate+	C43 Propane 1,2 diol-
C17 Melibiose+	C36 D-Glucuronate+	C44 Butane 2,3 diol-
C18 Lactose+	C37 D-Galacturonate-	C45 Quinic acid-
	C38 DL-Lactate-, d, w	C46 D-glucarate+, d, w
	C39 Succinate+	C47 D-Galactonate+

Cryptococcus flavescens has been isolated from cerebrospinal fluid. A subcutaneous infection with C. flavescens in a dog appeared as abscessed lesions on the muzzle, jaw and eyelid.

10.16. *Cryptococcus vishniacci*

Cryptococcus vishniacci was isolated from soil samples in the Antarctica. It grows at 4 degrees Celsius and below but not at 26 degrees Celsius and above. Visually it is characterized as a cream colored mass, lacking psedomycelia. It is non-fermentative and uses glucose, maltose, melezitose, trehalose, and xylose



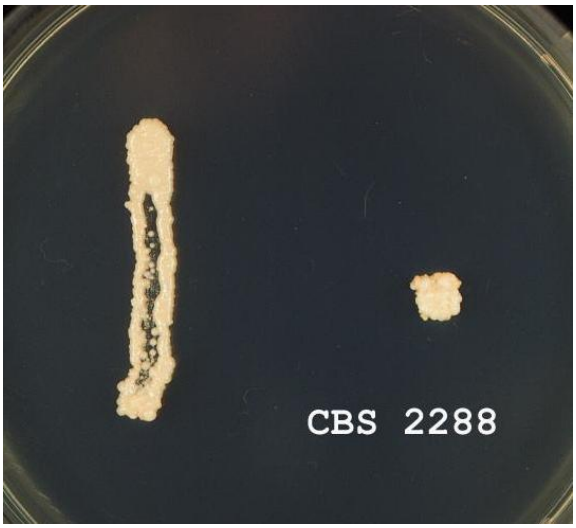
10.17. *Cryptococcus friedmannii* Vishniac, Mycologia 77 (1): 150 (1985)

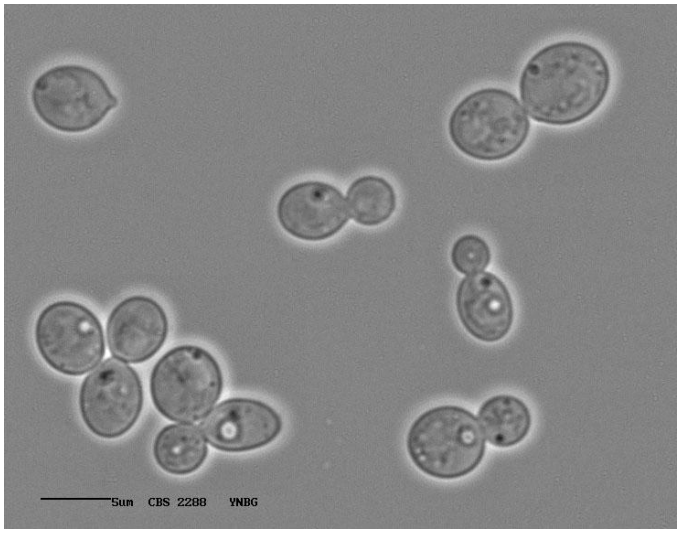
Cryptococcus friedmannii is a psychrophilic basidioblastomycete characterized by cream-colored colonies of cells with smooth, layered walls, budding monopolarly, producing amylose and extracellular proteinase, utilizing nitrate and D-alanine (inter alia) as nitrogen sources and L-arabinose, arbutin, cellobiose, D-glucuronate, maltose, melezitose, salicin, soluble starch, trehalose, and D-xylose as carbon sources. This species differs from all other basidiomycetous yeasts in possessing the following combination of characters: amylose production (positive), assimilation of cellobiose (positive), D-galactose (negative), myo-inositol (negative), D-mannitol (negative), and sucrose (negative).

Physiological data :

C1 D-Glucose+	C21 Inulin-	C44 Butane 2,3 diol-
C2 D-Galactose-	C22 Starch+	C45 Quinic acid-
C3 L-Sorbose-	C23 Glycerol-	C46 D-glucarate-
C4 D-Glucosamine-	C24 Erythritol-	C47 D-Galactonate-
C5 D-Ribose-	C25 Ribitol-	N1 Nitrate+
C6 D-Xylose+	C26 Xylitol-	N2 Nitrite+
C7 L-Arabinose+	C27 L-Arabinitol-	N3 Ethylamine-
C8 D-Arabinose-	C28 D-Glucitol-	N4 L-Lysine+
C9 L-Rhamnose-	C29 D-Mannitol-	N5 Cadaverine+
C10 Sucrose+, d, w	C30 Galactitol-	N6 Creatine-
C11 Maltose+	C31 myo-Inositol-	N7 Creatinine-
C12 a,a-Trehalose+	C32 D-Glucono-1,5-lactone-	N8 Glucosamine-
C13 Me a-D-Glucosided	C33 2-Keto-D-Gluconate+	N9 Imidazole-
C14 Cellobiose+	C35 D-Gluconated	N10 D-Tryptophan+
C15 Salicin+	C36 D-Glucuronate+	V1 w/o vitaminsd
C16 Arbutin+	C37 D-Galacturonate-	O1 Cycloheximide 0.01%-
C17 Melibiose-	C38 DL-Lactate-	O2 Cycloheximide 0.1%-
C18 Lactose-	C39 Succinate-	O3 Acetic acid 1%-
C19 Raffinose-	C40 Citrate+, d, w	O6 10% NaCl-
C20 Melezitose+	C43 Propane 1,2 diol-	O7 16% NaCl-

10. 18. *Cryptococcus gastricus* Reiersöl & Di Menna, Antonie van Leeuwenhoek 24: 28 (1958)

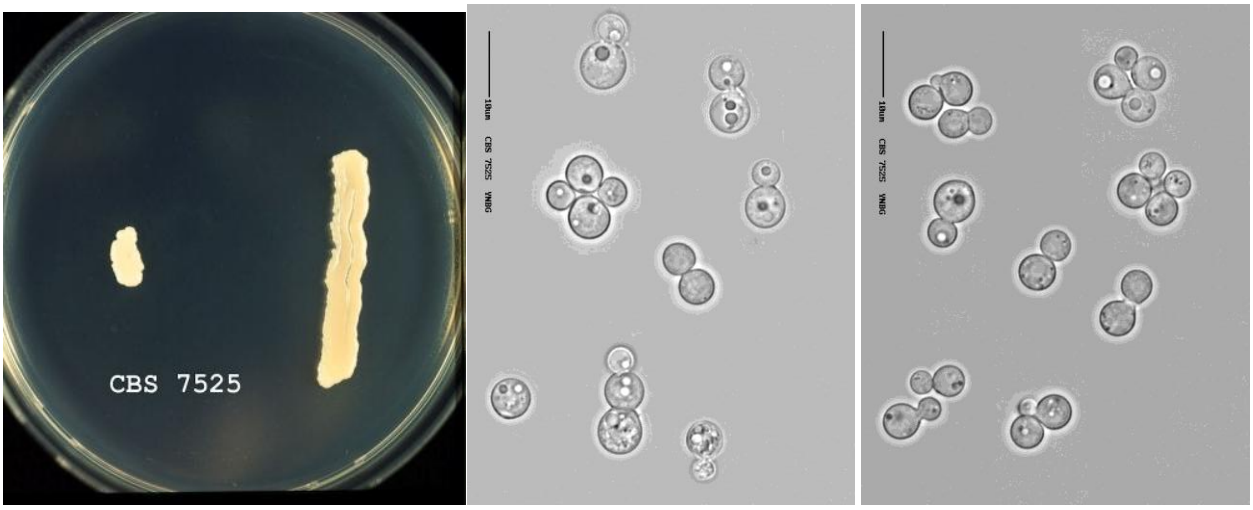




Physiological data

C1 D-Glucose+	C24 Erythritol-	N3 Ethylamine-
C2 D-Galactose+	C25 Ribitol-, d, w	N4 L-Lysine+, d, w
C3 L-Sorbose-	C26 Xylitol-, d, w	N5 Cadaverine+, d, w
C4 D-Glucosamine-	C27 L-Arabinitol-	N6 Creatine-
C5 D-Ribose-, d, w	C28 D-Glucitol+, d, w	N7 Creatinine-
C6 D-Xylose+	C29 D-Mannitol+	N8 Glucosamine-
C7 L-Arabinose+	C30 Galactitol-	N9 Imidazole-
C8 D-Arabinose-, d, w	C31 myo-Inositol+	N10 D-Tryptophan-
C9 L-Rhamnose+, d, w	C32 D-Glucono-1,5-lactone-	V1 w/o vitamins-
C10 Sucrose-	C33 2-Keto-D-Gluconate+	V2 w/o myo-Inositol+
C11 Maltose+	C35 D-Gluconate+, d, w	V3 w/o Pantothenate+
C12 a,a-Trehalose+	C36 D-Glucuronate+	V4 w/o Biotin+, d, w
C13 Me a-D-Glucoside-, d, w	C37 D-Galacturonate-	V5 w/o Thiamin-
C14 Cellobiose+	C38 DL-Lactate-	V6 w/o Biotin & Thiamin-
C15 Salicin+	C39 Succinate+	V7 w/o Pyridoxine+
C16 Arbutin+, d, w	C40 Citrate-	V8 w/o Pyridoxine & Thiamin-
C17 Melibiose-	C43 Propane 1,2 diol-	V9 w/o Niacin+
C18 Lactose-, +	C44 Butane 2,3 diol-	V10 w/o PABA+
C19 Raffinose-	C45 Quinic acid-	O1 Cycloheximide 0.01%-
C20 Melezitose+	C46 D-glucarate-	O2 Cycloheximide 0.1%-
C21 Inulin-	C47 D-Galactonate-	O3 Acetic acid 1%-
C22 Starch+	N1 Nitrate-	O6 10% NaCl-
	N2 Nitrite-	O7 16% NaCl-

10.19. *Cryptococcus gilvescens* Chernov & Babeva, Mikrobiologiya 57: 1032 (1988)



Physiological data :

C1 D-Glucose+	C25 Ribitol-	N3 Ethylamine-
C2 D-Galactose+	C26 Xylitol-	N4 L-Lysine+
C3 L-Sorbose-	C27 L-Arabinitol-	N5 Cadaverine+
C4 D-Glucosamine-	C28 D-Glucitold	N6 Creatine-
C5 D-Ribose-	C29 D-Mannitol+, d, w	N7 Creatinine-
C6 D-Xylose+	C30 Galactitol-	N8 Glucosamine-
C7 L-Arabinose+	C31 myo-Inositol+	N9 Imidazole-
C8 D-Arabinose-	C32 D-Glucono-1,5-lactone+	N10 D-Tryptophan-
C9 L-Rhamnose-, d, w	C33 2-Keto-D-Gluconate+	V1 w/o vitamins-
C10 Sucrose-	C34 5-Keto-D-Gluconate-	V2 w/o myo-Inositol+
C11 Maltose+	C35 D-Gluconated	V3 w/o Pantothenate+
C12 a,a-Trehalose+	C36 D-Glucuronate+	V4 w/o Biotin+
C13 Me a-D-Glucoside-	C37 D-Galacturonate-	V5 w/o Thiamin-
C14 Cellobiose+	C38 DL-Lactate-	V6 w/o Biotin & Thiamin-
C15 Salicin+	C39 Succinate+	V7 w/o Pyridoxine+
C16 Arbutin+, d, w	C40 Citrate-	V8 w/o Pyridoxine & Thiamin-
C17 Melibiose-	C43 Propane 1,2 diol-	V9 w/o Niacin+
C18 Lactose+	C44 Butane 2,3 diol-	V10 w/o PABA+
C19 Raffinose-	C45 Quinic acid-	O1 Cycloheximide 0.01%-, d, w
C20 Melezitose+	C46 D-glucarate-	O2 Cycloheximide 0.1%-
C21 Inulin-	C47 D-Galactonate-	O3 Acetic acid 1%-
C22 Starch+	N1 Nitrate-	O6 10% NaCl-
C23 Glycerol+, d, w	N2 Nitrite-	O7 16% NaCl-
C24 Erythritol-		

10. 20. *Cryptococcus ate*r Castell., Journal of Tropical Medicine and Hygiene 63: 27 (1960) [MB#329355]

Synonyms:

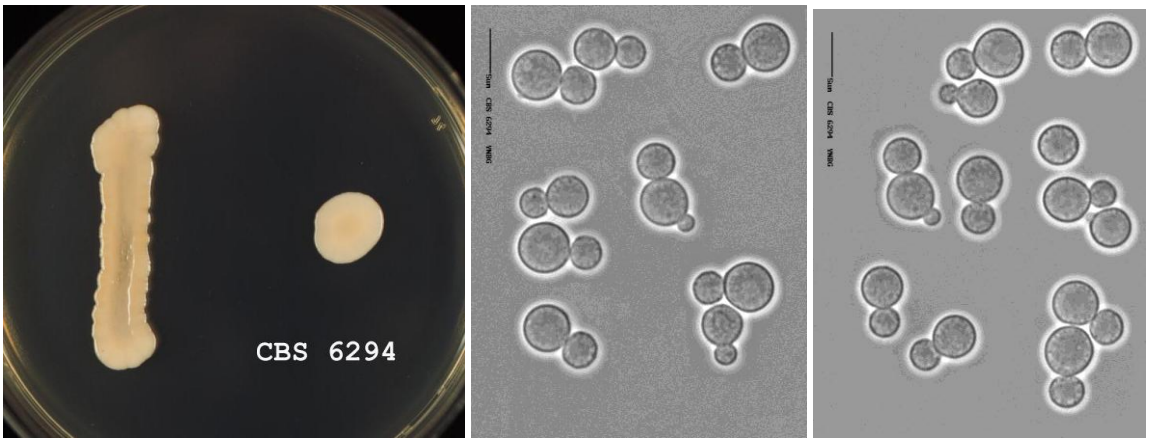
1. *Melanocryptococcus ater* (Castell.) Della Torre & Cif., Atti dell'Istituto Botanico della Università e Laboratorio Crittogamico di Pavia 21 (5): 5-13 (1964) [MB#283133]
2. *Cryptococcus laurentii f. ater* Castell. ex W.B. Cooke, Mycopathologia et Mycologia Applicata 30 (3-4): 351 (1966) [MB#372731]
3. *Cryptococcus ater* (Castell. ex W.B. Cooke) Rodr. Mir., Antonie van Leeuwenhoek 47 (1981)
4. *Rhodotorula lini Wieringa*, Netherlands Journal of Agricultural Science 4: 204-209 (1956) [MB#456466]
5. *Rhodotorula macerans* Frederiksen, Friesia 5 (3-5): 237 (1956) [MB#305279]
6. *Cryptococcus hungaricus var. gallicus* Saëz, Bulletin Mensuel de la Société Linnéenne de Lyon 42: 220 (1973) [MB#352769]

Cryptococcus ater is a species of *Cryptococcus* that has some unique characteristics. When grown on agar it typically produces cream colonies, however when grown on neopeptone agar slants, the colonies turn olive green after approximately four weeks. This species also turns nearly black when grown on Diamalt agar slants and on Gorodkova agar after approximately three months. When grown in liquid media it develops sediment and a weak ring. On the microscopic level the cells appear globose to ovate and are capsulated. Occasionally the cells have been seen to create chains of four to five cells. When grown, it does not require vitamins, but its growth is weakened by the presence of ammonium sulfate. It is able to assimilate alpha-methyl-D-glucoside, Ca-2-keto-gluconate, cellobiose, D-arabinose, D-mannitol, D-sorbitol, D-xylose, galactose, glucose, K-5-keto-gluconate- K-gluconate, lactose, L-arabinose, L-rhamnose, maltose, melezitose, i-inositol, raffinose, salicin and trehalose. This species has been isolated from ulcers in a leg.

10.21. Cryptococcus bhutanensis Goto & Sugiy., Canadian Journal of Botany 48 (12): 2097 (1970)

Cryptococcus bhutanensis is a fungus species. It was isolated from soil in Bhutan. The cell is encapsulated with an extended ovoid shape. when the cell buds, it creates birth scars, and the neck of the new yeast fits inside of the bud scar neck. The new cell typically only buds from the birth scar present from where it budded off the parent cell. Over half of the dividing cells in *C. bhutanensis* cultures the cell

walls were holoblastic, meaning that the new cell wall was continuous with the old cell wall on the parent cell; the other portion of dividing cells in *C. bhutanensis* cultures divide enteroblastically, meaning that only the innter layer of the new cell wall is continuous with the inner layer of the parental cell wall. After the cells bud off they produce a collar on the parent cell. One interesting thing of note with *C. bhutanensis* is that mitosis is not intranuclear. This species does not produce urease



Yeasts physiological data :

C1 D-Glucose+	C23 Glycerol-	N1 Nitrate+
C2 D-Galactose+, d, w	C24 Erythritol-	N2 Nitrite+
C3 L-Sorbosed	C25 Ribitol-	N3 Ethylamine+
C4 D-Glucosamine-	C26 Xylitold	N4 L-Lysine+
C5 D-Ribose-, d, w	C27 L-Arabinitold	N5 Cadaverine+
C6 D-Xylose+	C28 D-Glucitol+	N6 Creatine-
C7 L-Arabinose+	C29 D-Mannitol+	N7 Creatinine-
C8 D-Arabinosed	C30 Galactitol-	N8 Glucosamine-
C9 L-Rhamnose-, +	C31 myo-Inositol-	N9 Imidazole-
C10 Sucrose+	C32 D-Glucono-1,5-lactone+	V1 w/o vitamins-
C11 Maltose+	C33 2-Keto-D-Gluconate+	V2 w/o myo-Inositol+
C12 a,a-Trehalose+	C35 D-Gluconate-, +	V3 w/o Pantothenate+
C13 Me a-D-Glucoside+	C36 D-Glucuronate+	V4 w/o Biotin+
C14 Cellobiose+	C37 D-Galacturonate-	V5 w/o Thiamin-
C15 Salicin+	C38 DL-Lactate-	V6 w/o Biotin & Thiamin-
C16 Arbutin+	C39 Succinate+	V7 w/o Pyridoxine+
C17 Melibiose-	C40 Citrate+, d, w	V8 w/o Pyridoxine &
C18 Lactose-, +	C43 Propane 1,2 diol-	Thiamin-
C19 Raffinose-, +	C44 Butane 2,3 diol-	V9 w/o Niacin+
C20 Melezitose+	C45 Quinic acid+	V10 w/o PABA+
C21 Inulin-	C46 D-glucarate+	O1 Cycloheximide 0.01%-
C22 Starch+	C47 D-Galactonate-	O2 Cycloheximide 0.1%-
	O7 16% NaCl-	O3 Acetic acid 1%-

10.22. *Cryptococcus consortionis* Vishniac, Intern. J. Syst. Bacteriolo. 35: 120 (1985)

Cryptococcus consortionis is a fungus species. It produces colonies that are cream colored with a glistening, mucoid appearance. When grown in liquid media, this species requires constant agitation. This species growth range is from 4°C to 23°C, with growth at 23°C occurring very slowly. On the microscopic level, *C. consortionis* appears ovoid, with a thin capsule. Sexual reproduction does not occur in this species, but it asexually reproduces through budding at the birth scar site. Very occasionally, the cells have been observed to produce three celled pseudomycelia. *C. consortionis* does not ferment. This species produces Amylose, but it is the only basidioblastomycete which does so but is unable to also assimilate cellobiose, D-galactose, mannitol, myo-inositol and nitrate. *C. consortionis* is DBB positive. This species required thiamine for proper growth, and its growth is slowed by small amounts of cycloheximide. *C. consortionis* does not produce urease, and does not produce melanin on DOPA.

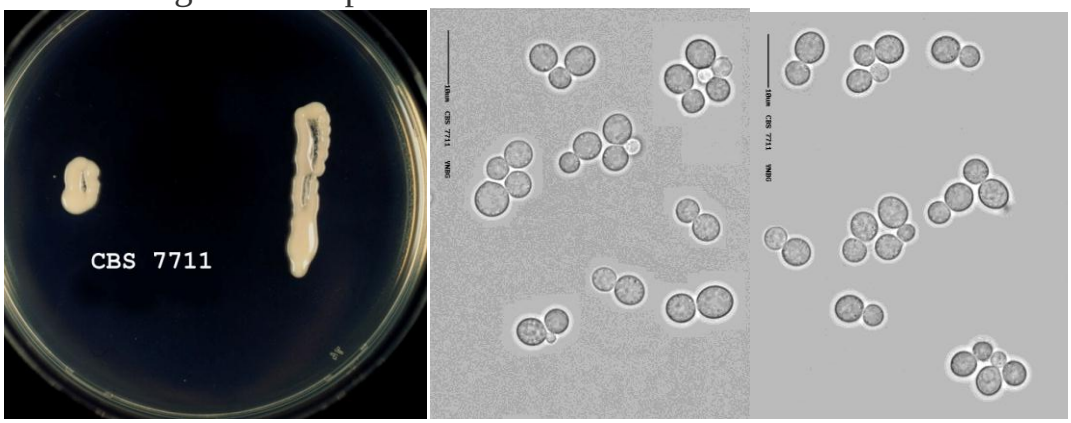
10.23. *Cryptococcus uzbekistanensis*

physiological data :

C1 D-Glucose+	C21 Inulin-	C43 Propane 1,2 diol-
C2 D-Galactose-	C22 Starch+	C44 Butane 2,3 diol-
C3 L-Sorbosed	C23 Glycerol-	C45 Quinic acid-
C4 D-Glucosamine-	C24 Erythritol-	C46 D-glucarate+
C5 D-Ribose+	C25 Ribitol-	C47 D-Galactonate-
C6 D-Xylose+	C26 Xylitold	N1 Nitrate+
C7 L-Arabinose+	C27 L-Arabinitol-	N2 Nitrite+
C8 D-Arabinosed	C28 D-Glucitol+	N3 Ethylaminew
C9 L-Rhamnose+	C29 D-Mannitol	N4 L-Lysine+
C10 Sucrose+	C30 Galactitol-	N5 Cadaverine+
C11 Maltose+	C31 myo-Inositol+	N6 Creatine-
C12 a,a-Trehalose+	C32 D-Glucono-1,5-lactone-	N7 Creatinine-
C13 Me a-D-Glucoside+	C33 2-Keto-D-Gluconate+	N8 Glucosamine-
C14 Cellobiose+	C35 D-Gluconate+	N9 Imidazole-
C15 Salicin+	C36 D-Glucuronate+	N10 D-Tryptophan+
C16 Arbutin+	C37 D-Galacturonate-	V1 w/o vitamins-
C17 Melibiose-	C38 DL-Lactate-	O1 Cycloheximide 0.01%-
C18 Lactose-	C39 Succinate+	O2 Cycloheximide 0.1%-
C19 Raffinose+, d, w	C40 Citrate+	O3 Acetic acid 1%
C20 Melezitose+		

10.24. *Cryptococcus albidosimilis* Vishniac & Kurtzman, International Journal of Systematic Bacteriology 42: 550 (1992)

Cryptococcus albidosimilis is a species that has been isolated from soil in Antarctica. When plated on agar it produces colonies that are shining white. The colonies appear to be mucosoid in appearance when plated on agar. When grown in liquid media, the yeast fails to grow well unless the media is constantly agitated. This species is considered mesophilic, with optimal growth temperature is at 25°C, with a maximum growth temperature.

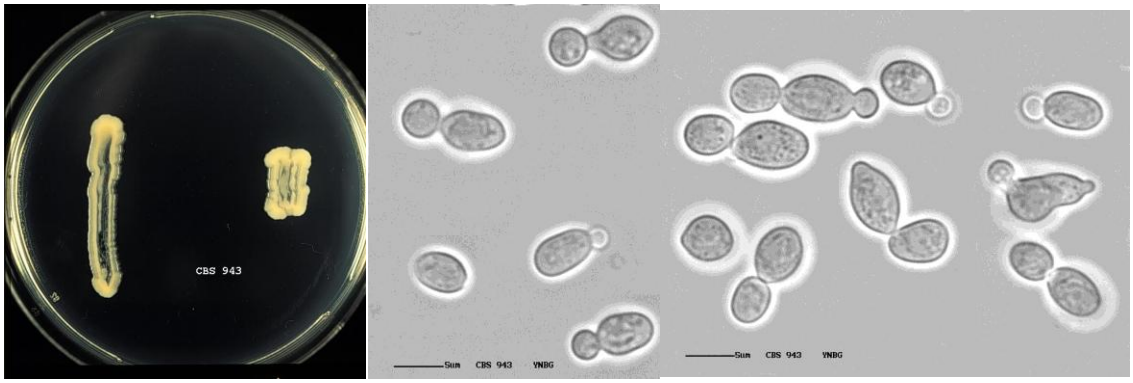


physiological data

C1 D-Glucose+	C24 Erythritol-, d, w	N3 Ethylamine+
C2 D-Galactose+, d, w	C25 Ribitol-, d, w	N4 L-Lysinew
C3 L-Sorbose-, d, w	C26 Xylitol+	N5 Cadaverine+
C4 D-Glucosamine-	C27 L-Arabinitol+	N6 Creatine-
C5 D-Ribosed	C28 D-Glucitol+	N7 Creatinine-
C6 D-Xylose+	C29 D-Mannitol+	N8 Glucosamine-
C7 L-Arabinose+	C30 Galactitol-	N9 Imidazole-
C8 D-Arabinose-	C31 myo-Inositol+	N10 D-Tryptophan-
C9 L-Rhamnose+	C32 D-Glucono-1,5-lactone-	V1 w/o vitamins-
C10 Sucrose+	C33 2-Keto-D-Gluconate+	V2 w/o myo-Inositol+
C11 Maltose+	C35 D-Gluconated	V3 w/o Pantothenate+
C12 a,a-Trehalose+	C36 D-Glucuronate+	V4 w/o Biotin+
C13 Me a-D-Glucoside+	C37 D-Galacturonate-	V5 w/o Thiamin-
C14 Cellobiose+	C38 DL-Lactate-	V6 w/o Biotin & Thiamin-
C15 Salicin+	C39 Succinate+	V7 w/o Pyridoxine+
C16 Arbutin+	C40 Citrate+	V8 w/o Pyridoxine & Thiamin-
C17 Melibiose-	C43 Propane 1,2 diol-	V9 w/o Niacin+
C18 Lactose+	C44 Butane 2,3 diol-	V10 w/o PABA+
C19 Raffinose-	C45 Quinic acid+	O1 Cycloheximide 0.01%-
C20 Melezitose+	C46 D-glucarate-, d, w	O2 Cycloheximide 0.1%-
C21 Inulin-	C47 D-Galactonate+, d, w	O6 10% NaCl-
C22 Starch+	N1 Nitrate+	O7 16% NaCl-
C23 Glycerol-	N2 Nitrite+	

10.25. *Cryptococcus luteolus* (Saito) C.E. Skinner et al., The American Midland Naturalist 43: 249 (1950)

Synonyms : *Torula luteola* Saito, Journal of Japanese Botany 1: 44 (1922)
Chromotorula luteola (Saito) F.C. Harrison, Transa. Royal SocCanada 22: 202 (1928)
Torulopsis luteola (Saito) Lodder, Verhand. Konink. Nederlandse Akad. van Wetenschappen Natuurkunde 32: 169 (1934)
Rhodotorula luteola (Saito) T. Haseg et al., J. Gen. Appl. Microbiol. Tokyo 6 (3): 212 (1960)
Hannaella luteola (Saito) F.Y. Bai & Q.M. Wang, FEMS Yeast Research 8 (5): 805 (2008)

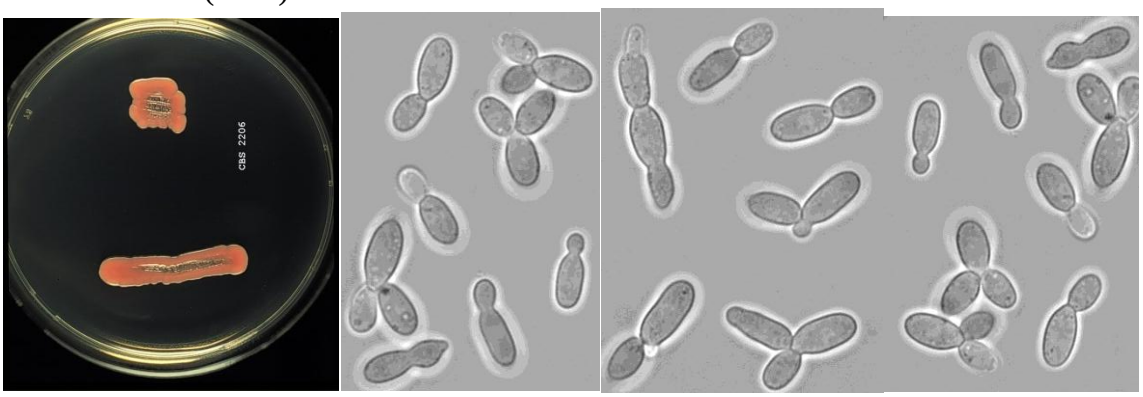


Physiological data

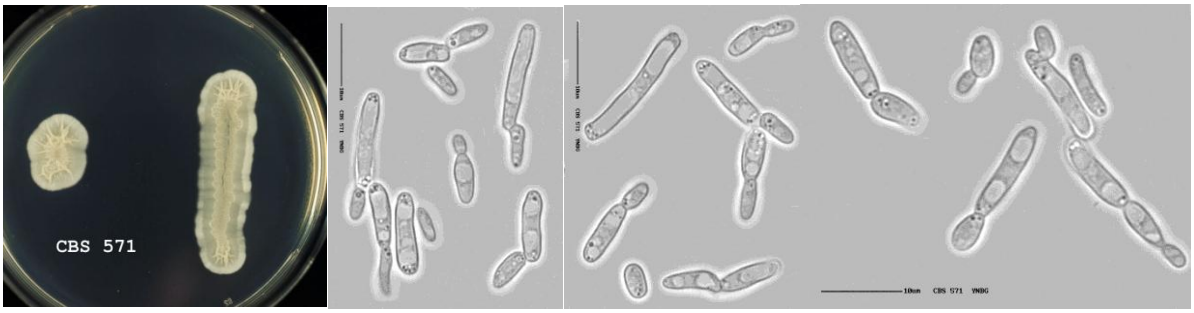
C1 D-Glucose+	C24 Erythritol+	N4 L-Lysine+
C2 D-Galactose+	C25 Ribitol-	C46 D-glucarate+
C3 L-Sorbose-, d, w	C26 Xylitol+, d, w	C47 D-Galactonate+
C4 D-Glucosamine-	C27 L-Arabinitol-	N1 Nitrate-
C5 D-Ribose-, d, w	C28 D-Glucitol-, d, w	N5 Cadaverine+
C6 D-Xylose+	C29 D-Mannitol+	N6 Creatine-
C7 L-Arabinose+	C30 Galactitold	N7 Creatinine-
C8 D-Arabinose+	C31 myo-Inositol+	N8 Glucosamine-
C9 L-Rhamnose+	C32 D-Glucono-1,5-lactoned	N9 Imidazole-
C10 Sucrose+	C33 2-Keto-D-Gluconate+	N10 D-Tryptophan+
C11 Maltose+	O3 Acetic acid 1%-	V1 w/o vitamins-
C12 a,a-Trehalose+	C35 D-Gluconate+	V2 w/o myo-Inositol+
C13 Me a-D-Glucoside+	C36 D-Glucuronate+	V3 w/o Pantothenate+
C14 Cellobiose+	C37 D-Galacturonate-	V4 w/o Biotin+
C15 Salicin-	C38 DL-Lactate-, d, w	V5 w/o Thiamin-
C16 Arbutin-	C39 Succinate+	V6 w/o Biotin & Thiamin-
C17 Melibiose+	C40 Citrate+	V7 w/o Pyridoxine+
C18 Lactose-	C43 Propane 1,2 diol+, d, w	V8 w/o Pyridoxine & Thiamin-
C19 Raffinose+	C44 Butane 2,3 diol-	V9 w/o Niacin+
C20 Melezitose+	C45 Quinic acid-	V10 w/o PABA+
C21 Inulin-	N2 Nitrite+, d, w	O1 Cycloheximide 0.01%d
C22 Starch-	N3 Ethylamine+	O2 Cycloheximide 0.1%-
C23 Glycerold		

10.26. *Cryptococcus macerans* (Frederiksen) Phaff & Fell, The Yeasts: a taxonomic study: 1127 (1970)

Synonyms:
Rhodotorula macerans Frederiksen, Friesia 5 (3-5): 237 (1956)
Rhodotorula lini Wieringa, Netherlands Journal of Agricultural Science 4: 204-209 (1956)
Cryptococcus ater Castell., Journal of Tropical Medicine and Hygiene 63: 27 (1960)
Cryptococcus laurentii f. ater Castell. ex W.B. Cooke, Mycopathologia et Mycologia Applicata 30 (3-4): 351 (1966)
Cryptococcus hungaricus var. gallicus Saëz, Bulletin Mensuel de la Société Linnéenne de Lyon 42: 220 (1973)



10.27. *Cryptococcus humicola* (Dasz.) Golubev, Mikologiya i Fitopatologiya 15 (6): 467 (1981)
Synonyms:
Torula humicola Dasz., Bulletin de la Société Botanique de Genève 4: 289 (1912)
Mycotorula humicola (Dasz.) F.C. Harrison, Trans. Royal Soc.Canada 22: 217 (1928)
Candida humicola (Dasz.) Diddens & Lodder, Die anaskosporogenen Hefen, II Hälfte: 266 (1942) [MB#284769]
Candida humicola (Dasz.) Knüsel, Archiv für Mikrobiologie 27 (3): 250 (1957)
Azymoprocandida humicola (Dasz.) E.K. Novák & Zsolt, Acta Bot. Acad. Sci. Hun. 7: 132
Apiotrichum humicola (Dasz.) Arx & Weijman, Antonie van Leeuwenhoek 45: 553 (1979)
Vanrijia humicola (Dasz.) R.T. Moore (1980)
Vanrija humicola (Dasz.) R.T. Moore, Botanica Marina 23 (6): 368 (1980)
Cryptococcus humicola (Dasz.) Golubev, Taxonomy and identification of yeast fungi of the genus Cryptococcus: 26
Asterotremella humicola (Dasz.) Prillinger, Lopandic & Sugita, J. Gen. Appl. Microbiol. Tokyo 53 (3): 173 (2007)
Sachsia suaveolens Lindner, Mikroskopische Betriebskontrolle in den Sachsia suaveolens Lindner ex Lindner, Wochenschr. Brauerei: 1 (1906)



Yeasts physiological data

C1 D-Glucose+	C23 Glycerol-, +	N8 Glucosamine-, +
C2 D-Galactose-, +	C24 Erythritol-, +	N9 Imidazole-
C3 L-Sorbose-, +	C25 Ribitol-, +	N10 D-Tryptophan-, +
C4 D-Glucosamine-, +	C26 Xylitol-, +	V1 w/o vitamins-, +
C5 D-Ribose+, d, w	C27 L-Arabinitol-, +	V2 w/o myo-Inositol+
C6 D-Xylose+	C28 D-Glucitol-, +	V3 w/o Pantothenate+
C7 L-Arabinose-, +	C29 D-Mannitol+, d, w	V4 w/o Biotin+
C8 D-Arabinose+, d, w	C30 Galactitol-, +	V5 w/o Thiamin-, d, w
C9 L-Rhamnose+, d, w	C31 myo-Inositol+	V6 w/o Biotin & Thiamin-, d, w
C10 Sucrose+	C32 D-Glucono-1,5-lactone-, +	V7 w/o Pyridoxine+
C11 Maltose+, d, w	C33 2-Keto-D-Gluconate-, +	V8 w/o Pyridoxine & Thiamin-, d, w
C12 a,a-Trehalose+, d, w	C34 5-Keto-D-Gluconate+C35 D-Gluconate+	V9 w/o Niacin+
C13 Me a-D-Glucoside-, +	C36 D-Glucuronate+	V10 w/o PABA+
C14 Cellobiose+	C37 D-Galacturonate-, +	O1 Cycloheximide 0.01%-, +
C15 Salicin-, +	C38 DL-Lactate-, +	O2 Cycloheximide 0.1%-, +
C16 Arbutin+, d, w	C39 Succinate-, +	O3 Acetic acid 1%-
C17 Melibiose-, +	C40 Citrate-, +	O6 10% NaCl-, +
C18 Lactose-, +	C43 Propane 1,2 diol-, +	O7 16% NaCl
C19 Raffinose-, +	C44 Butane 2,3 diol-, +	N1 Nitrate-
C20 Melezitose-, +	C45 Quinic acid-, +	N2 Nitrite-, +
C21 Inulin-	C46 D-glucarate-, +	N3 Ethylamine+
C22 Starch-, +	C47 D-Galactonate+, d, w	N4 L-Lysine-, +
		N5 Cadaverine

11. Cryptococcosis

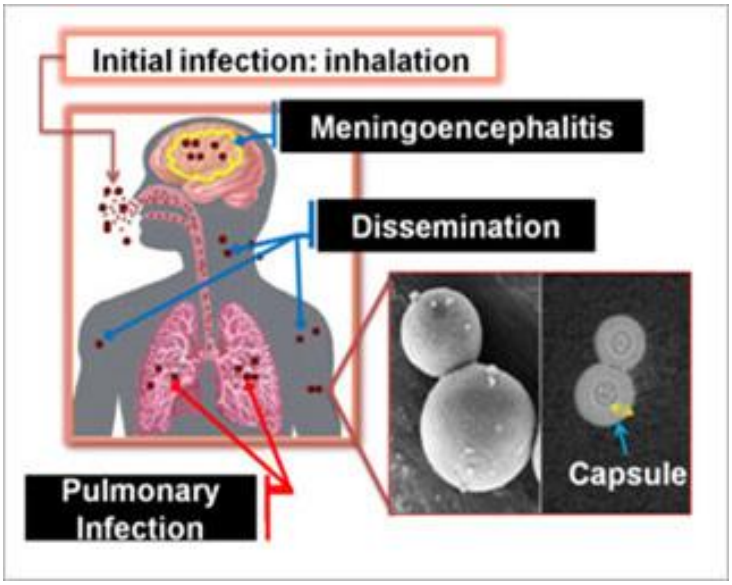
(Fungal pneumonia, Torulosis, European Blastomycosis, Busse-Buschke's Disease)

11.1. Definition:

Cryptococcosis is a chronic, subacute to acute pulmonary, systemic or meningitic disease, initiated by the inhalation of basidiospores and/or desiccated yeast cells of *Cryptococcus neoformans*. Primary pulmonary infections have no diagnostic symptoms and are usually subclinical. On dissemination, the fungus usually shows a predilection for the central nervous system, however skin, bones and other visceral organs may also become involved. Although *C. neoformans* and C. gattii are regarded as the two principle pathogenic species, *C. albidus*, *C. laurentii* and other species have on occasion also been implicated in human infection.

11.2. Pathogenesis

Outside the host, *C. neoformans* is believed to exist as a poorly or moderately encapsulated spherical to oval structure with a diameter ranging from 2 to 10 µm. Human infection is believed to occur via inhalation and colonization of the distal alveolar spaces of the lung by infectious propagules consisting of desiccated yeast or basidiospores.



❖ The pathogenesis of *C. neoformans* infection is mediated by four main virulence factors that allow it to survive within the host environment; these include :

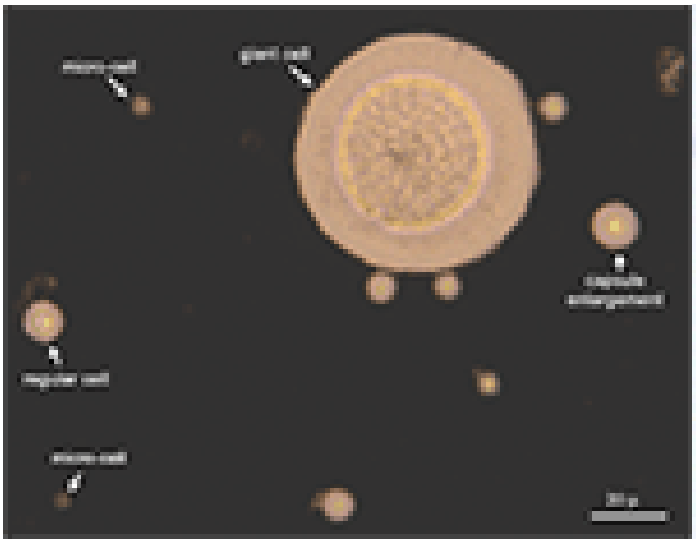
1. The ability to grow at 37°C
    - The innate ability of *C. neoformans* to grow at 37°C is an essential, calcineurin-dependent characteristic that allows the fungus to proliferate and cause disease.

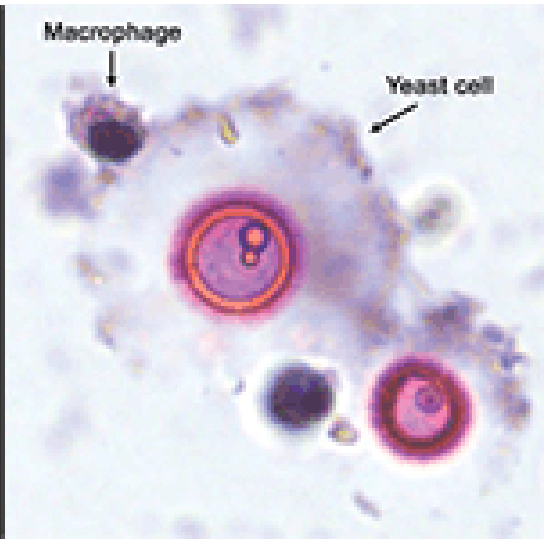
2. Synthesis of an extracellular capsule
    - The expression of a unique polysaccharide capsule, the most virulence factor possessed by *C. neoformans*, is enhanced at body temperature.

    - The capsular structure allows *C. neoformans* to resist opsonization and phagocytosis;

    - Shedding of capsule during infection may also subvert the immune response by altering the host chemokine and cytokine secretion profile.

3. Production of melanin
    - The ability of *C. neoformans* to produce melanin from diphenolic compounds via the enzyme laccase allow the yeast to resist environmental and host oxidative stress, particularly generated by ultraviolet (UV) light and phagocytic cells
4. Secretion of extracellular proteases.
    - The production of extracellular proteases by *C. neoformans* aids in hematogenous dissemination from the lungs to various organs including the brain, skin, kidney, and liver.





Left:Heterogeneity of fungal population extracted from the lungs of infected mice. The yeast cells isolated from the lungs of infected mice were isolated and suspended in India Ink to visualize the capsule. As shown, multiple forms of *C. neoformans* (regular cells, cells with enlarged capsule, fungal giant/titan cells, and microforms) are present during infection. Medical Microbiology: June 2012 varuncnmicro.blogspot.com

Right: Interaction between macrophages and cryptococcal giant/titan cells. Fungal giant/titan cells obtained from infected mice were co-incubated *in vitro* with RAW264.7 macrophage-like cells. As shown in the figure, the macrophages can recognize and bind to the fungal giant/titan cells, but they cannot internalize the fungal cells due to their enormous size. Oscar Zaragoza. Multiple disguises for the same party: the concepts of morphogenesis and phenotypic variations in *Cryptococcus neoformans*-Front. Microbiol., 06 September 2011 | doi: 10.3389/fmicb.2011.00181

11.3. Immunity to Cryptococcus infection

i. The Innate Response

- Cells of the innate immune system can rapidly recognize *C. neoformans* upon infection.
    - ➢ Toll-like receptors, mannose receptors, b-glucan receptors and the complement system can all contribute to rapid recognition of *Cryptococcus* and activation of innate responses.
    - ➢ Cryptococcal products/antigens can be detected by different cell types including professional antigen-presenting cells (APCs; such as dendritic cells [DCs] and macrophages), natural killer cells, other leukocyte subsets and endothelial and epithelial cells.

- Cells of the innate immune system can effectively control the growth of avirulent cryptococcal strains.
    - Macrophages, DCs, natural killer cells and neutrophils can kill cryptococci.
- Cells of the innate immune system may be insufficient to clear the infection, however, they are important for the generation of signals necessary for the development of the adaptive immune response.Furthermore,
- Cells of the innate immune system continue to contribute to the host defenses during the adaptive phase of the immune response.

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ii. T-cell-mediated Immunity

- The successful clearance of *C. neoformans* relies on the development of $CD4^+$ Th1 immunity
- $CD4^+$ T lymphocytes that become Th1 cells produce the cytokines (IFN-γ and IL-2)
- $CD4^+$ T lymphocytes that become Th2 cells produce the cytokines (IL-4, IL-5 and IL-13).
- The development of $CD8^+$ Tc1 and $CD8^+$ Tc2 cells mirrors the development of Th1 and Th2 cells and results in a similar cytokine profile in these cells.
- Th17 arm of the immune response may contribute to anti-cryptococcal protection.

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iii. The Role of Macrophages

Macrophages are crucial players in the anti-cryptococcal host defenses during the afferent and efferent phase of the immune response. In either phase of the immune response, macrophages have the potential to either benefit or impair the outcome of host–cryptococcal response.

- **Alveolar macrophages** represent the first line of host defence.
    - Macrophages are able to bind, ingest and, with appropriate stimulation, kill yeast cells.
    - Macrophage phagocytosis of *C. neoformans* can occur through:
        - antibody, and complement receptors (serum-dependent-phagocytosis) or

- mannose , and glucan receptors (serum-independent-phagocytosis).

❖ **The development of a protective Th1 type CMI**

➢ The production of Th1- and macrophage-activating cytokines TNF-α, IL1, IL-12, IFN-γ, and GM-CSF are required to:

- eradicate the infection,
- control cryptococcal dissemination from the lungs, and
- eliminate subsequent invasion in the brain.

❖ **In cases the cryptococci escape local (impaired) innate and adaptive immunity and gain access to the bloodstream**

➢ they meet **polymorphonuclear cells and monocytes** in the vascular compartment, which can phagocytose and kill the organism.cells with the help of opsonins, like complement

❖ **In cases the cryptococci escape phagocytosis** by the polymorphonuclear cells and monocytes through developing large –sized capsule

➢ they dessiminate to other organs with predilection for the brain.

iv. Mechanisms of Cryptococcal Persistence

a. Metabolic adaptation to physiological host conditions

- The thermal stability of cryptococcal strains is linked to functional calcineurin A, encoded by the CNA1 gene.
    - Calcineurin is a Ca^{2+}-calmodulin-regulated protein phosphatase that is activated by a stress response.
    - Calcineurin dephosphorylates a group of proteins that allow for C. neoformans growth at 37°C.
    - Calcineurin signaling also results in induction of metabolic and oxidative stress genes required for survival under stress conditions.

b. Evasion of immune recognition

- The cryptococcal capsule is one of the most important microbial factors for immune evasion,
    - The cryptococcal capsule possesses a number of characteristics and factors that interfere with immune recognition.
    - Capsule expression is one of the major determinants of cryptococcal virulence.
    - The capsule can mediate evasion of immune recognition by a variety of mechanisms,

c. Physical barrier/steric hindrance.

Cryptococcal capsule is made of polysaccharides rich in mannan residues with strong hydrophilic properties.

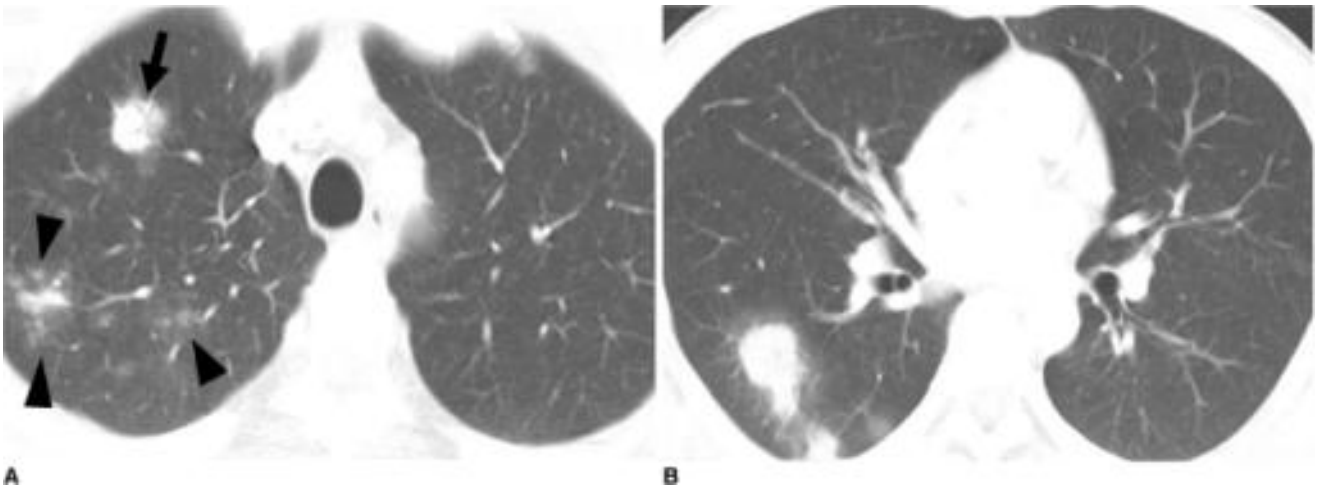
- These polysaccharides form a dense gelatinous buffer zone that separates the *C. neoformans* cell from exogenous factors and prevents the recognition of other cryptococcal cellular components that could be 'sensed' by the immune system as danger signals (by pattern recognition receptors).
- It also provides a barrier that prevents efficient antibody binding and resultant activation of complement via the classical pathway.

11.4. Clinical manifestations in man:

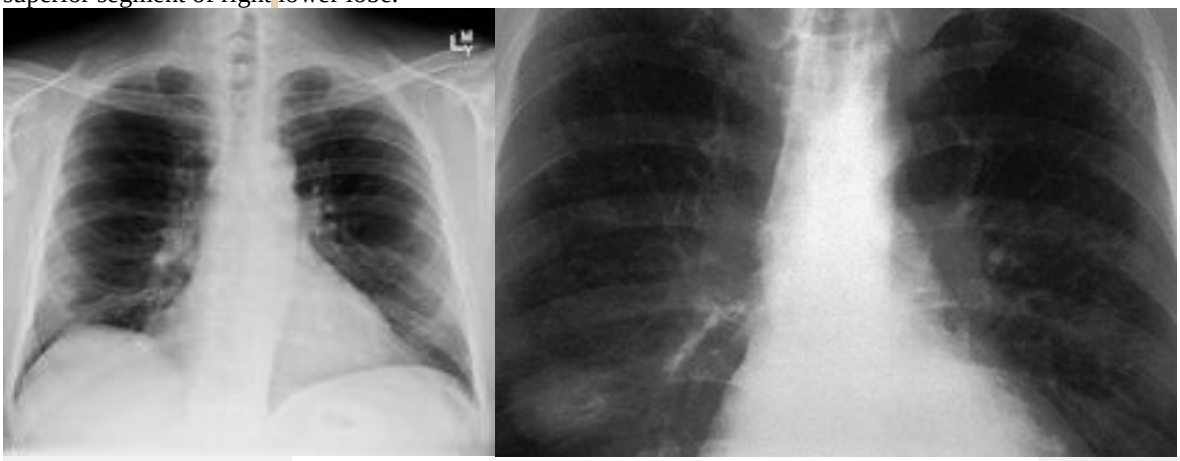
11.4.1. Pulmonary Cryptococcosis:

- **Asymptomatic carriage of *Cryptococcus*** has been reported from the respiratory tract, especially sputum and from skin in healthy people as a result of normal environmental exposure. In addition, patients with chronic lung disease, such as bronchitis and bronchiectasis, may also have asymptomatic colonization, with *Cryptococcus* being isolated from their sputum over many years.
- **Subclinical cryptococcosis** may result of environmental exposure, normal individuals may experience a self-limiting pneumonia with accompanying sensitization. Most primary infections of this type have no diagnostic symptoms and are usually discovered only by routine chest x-ray. When present, symptoms include cough, low-grade fever and pleuritic pain.
- **Invasive pulmonary cryptococcosis** may occur in some patients when primary infections may not readily resolve in some patients, leading to a more chronic pneumonia progressing slowly over several years. Patients may become pyrexic and have an accompanying cough, however many pulmonary lesions are often asymptomatic, especially when chronic granulomas are formed. Chronic pulmonary cryptococcosis also increases the risk of dissemination to the central nervous system.
- Radiographic features. In general there are several CT patterns that can be seen:
    - clustered nodular pattern - most prevalent
    - solitary pulmonary nodular
    - scattered nodular
    - bronchopneumonic
    - single mass - rare

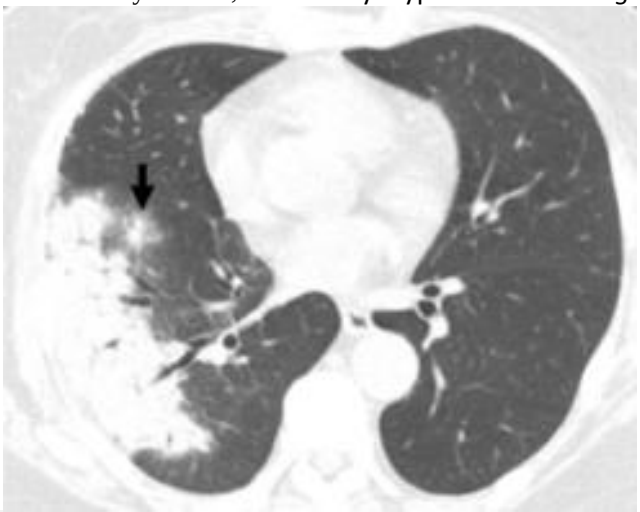
  The most common CT findings in immunocompetent patients with pulmonary cryptococcosis are pulmonary nodules. The nodules are most often multiple, smaller than 10 mm in diameter, and well defined with smooth margins. The nodules usually involve less than 10% of the parenchyma and tend to be distributed peripherally in the middle and upper zones. Where there are multiple nodules, they are usually bilateral . Associated cavitation may be seen in up to 40% of cases



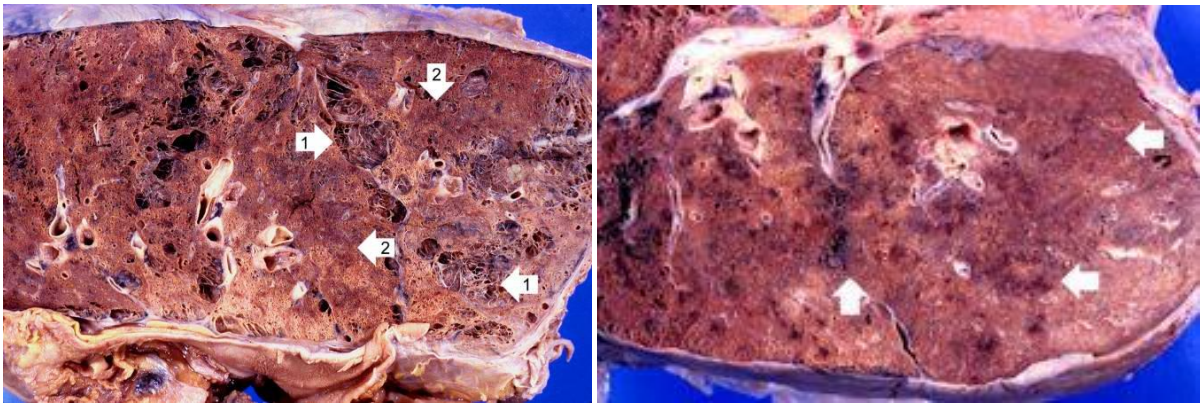
Pulmonary cryptococcosis with scattered nodular pattern in 38-year-old man who had no underlying disease. openi.nlm.nih.gov. A. Lung window of transverse CT scan obtained at level of great vessels shows nodule (arrow) with surrounding ground-glass opacity (halo sign) and centrilobular small nodules (arrowheads) in right upper lobe.B. CT scan obtained at level of basal trunk demonstrates nodules with halo sign in superior segment of right lower lobe.



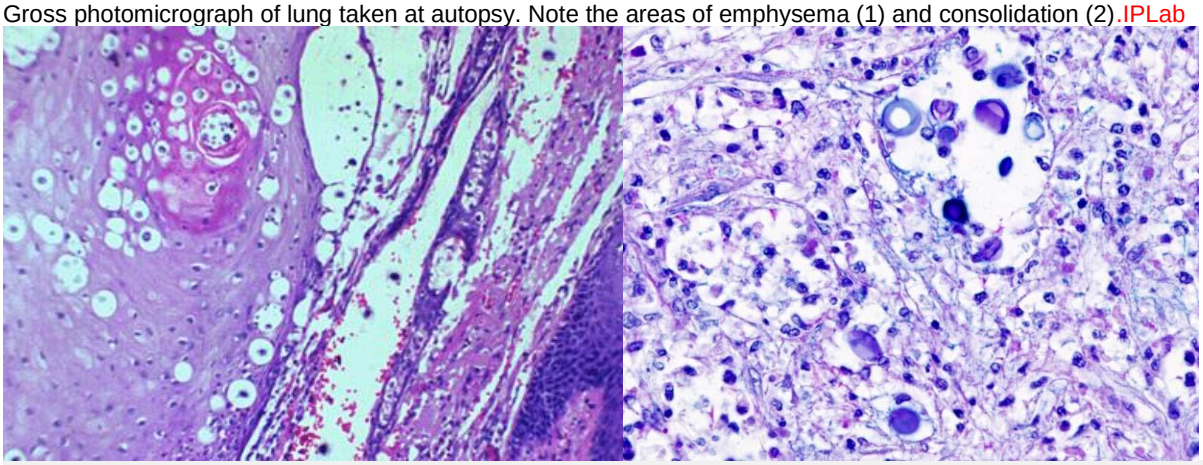
Pulmonary cryptococcosis with cavitatory nodule., Pulmonary cryptococcosis- *Large nodule* , Radiopaedia.

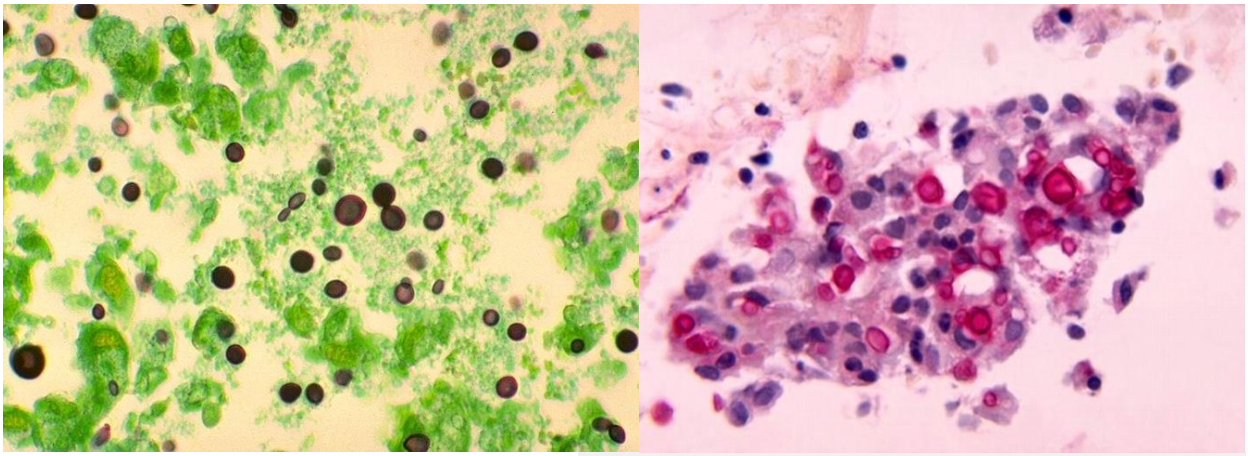


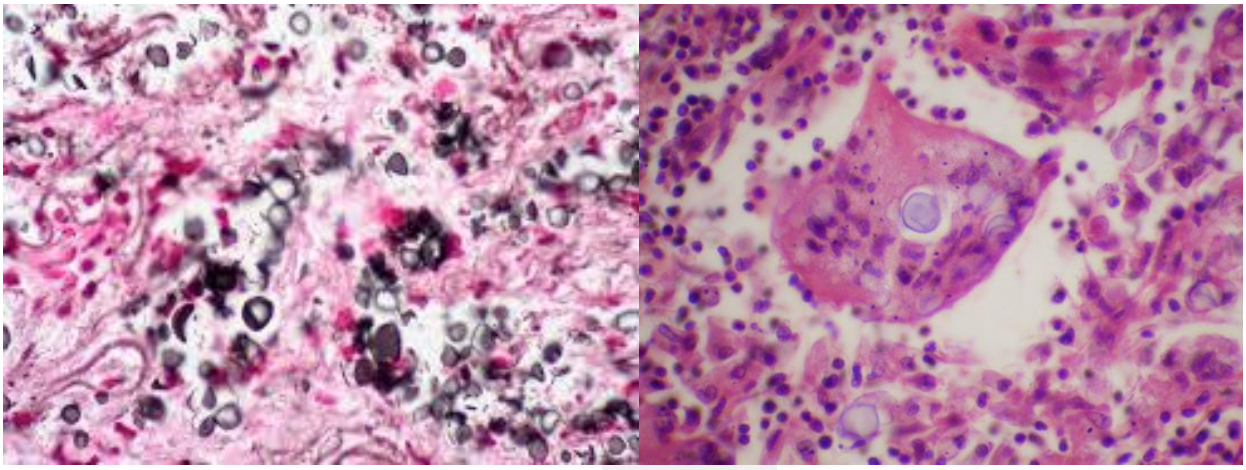
Cryptococcosis of bronchopneumonic pattern in 72-year ...A. Lung window of transverse CT scan obtained at level of right inferior pulmonary vein shows subpleural consolidation and nodule (arrow) with surrounding ,openi.nlm.nih.gov



Gross photomicrograph of lung taken at autopsy. Note the areas of emphysema (1) and consolidation (2).IPLab


A: Pulmonary cryptococcal infection [right upper lobe]. Histopathology: Histopathology. Tissue section stained by haematoxylin and eosin (H&E),Mycology on line .B: Pulmonary cryptococcosis (3) Alcian blue-PAS, Wikimedia


A: Cryptococcosis of lung Methenamine silver stain., pathmicro.med.sc.edu. B: cryptococcosis of the lung using mucicarmine stain. apredbook.aappublications.org

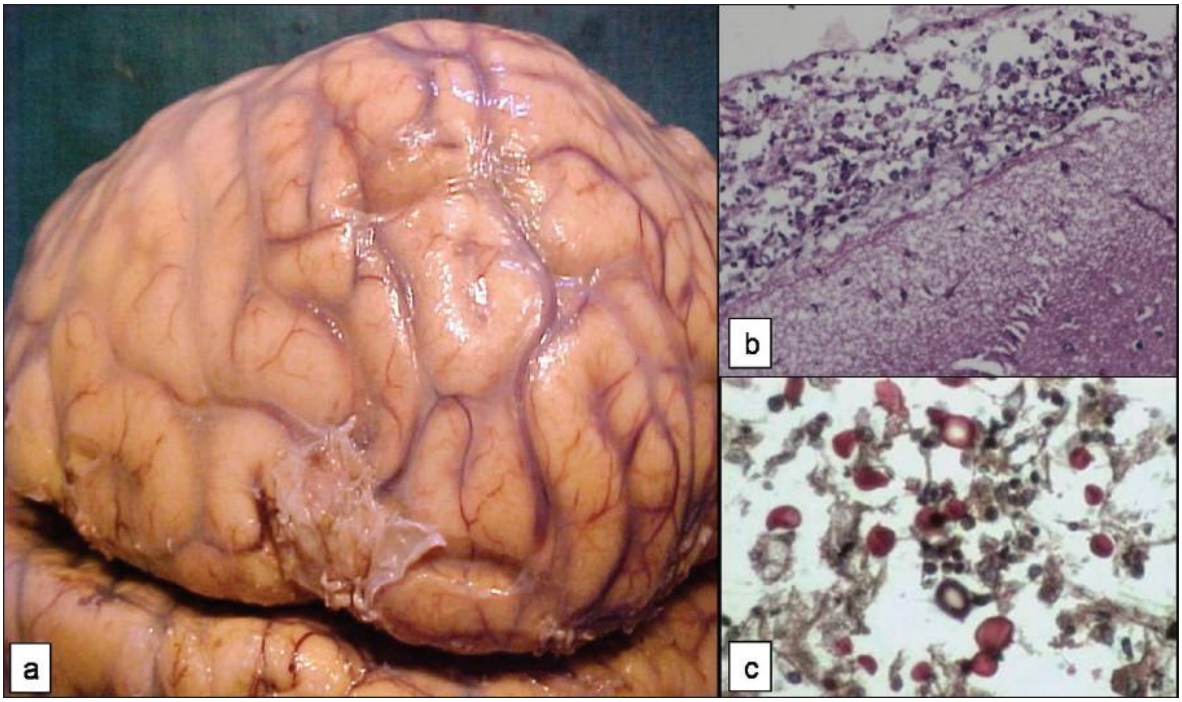


Cryptococcosis, lung, GMS stain (euthman) ckrhivemind.net Cryptococcosis, lung, H&Eو
granuloma.homestead. com

11.4.2. Central Nervous System:

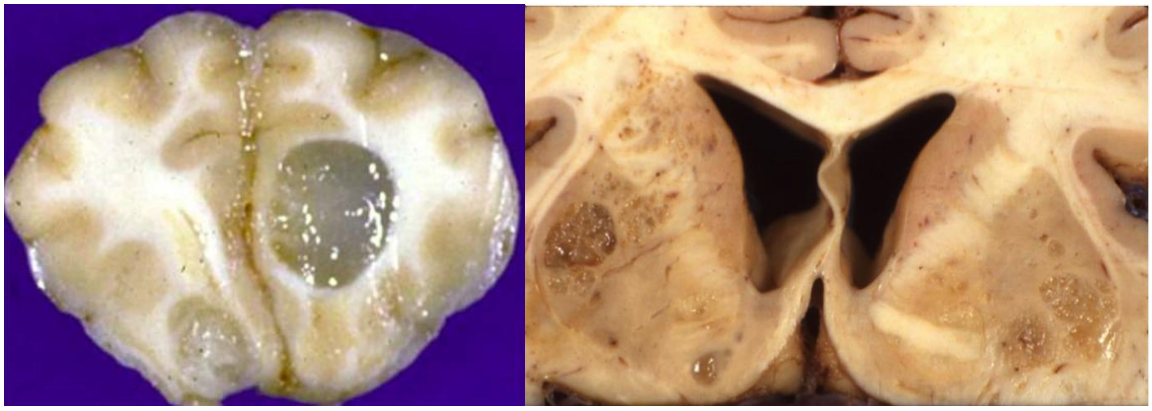
Dissemination to the brain and meninges is the most common clinical manifestation of cryptococcosis and includes meningitis, meningoencephalitis or expanding cryptococcoma.

i. Meningitis is the most common clinical form, accounting for up to 85% of the total number of cases, however the clinical signs are rarely dramatic. Symptoms usually develop slowly over several months, and initially include headache, followed by drowsiness, dizziness, irritability, confusion, nausea, vomiting, neck stiffness and focal neurological defects, such as ataxia. Diminishing visual acuity and coma may also occur in later stages of the infection. Acute onset cases may also occur, especially in patients with widespread disease, and these patients may deteriorate rapidly and die in a matter of weeks.

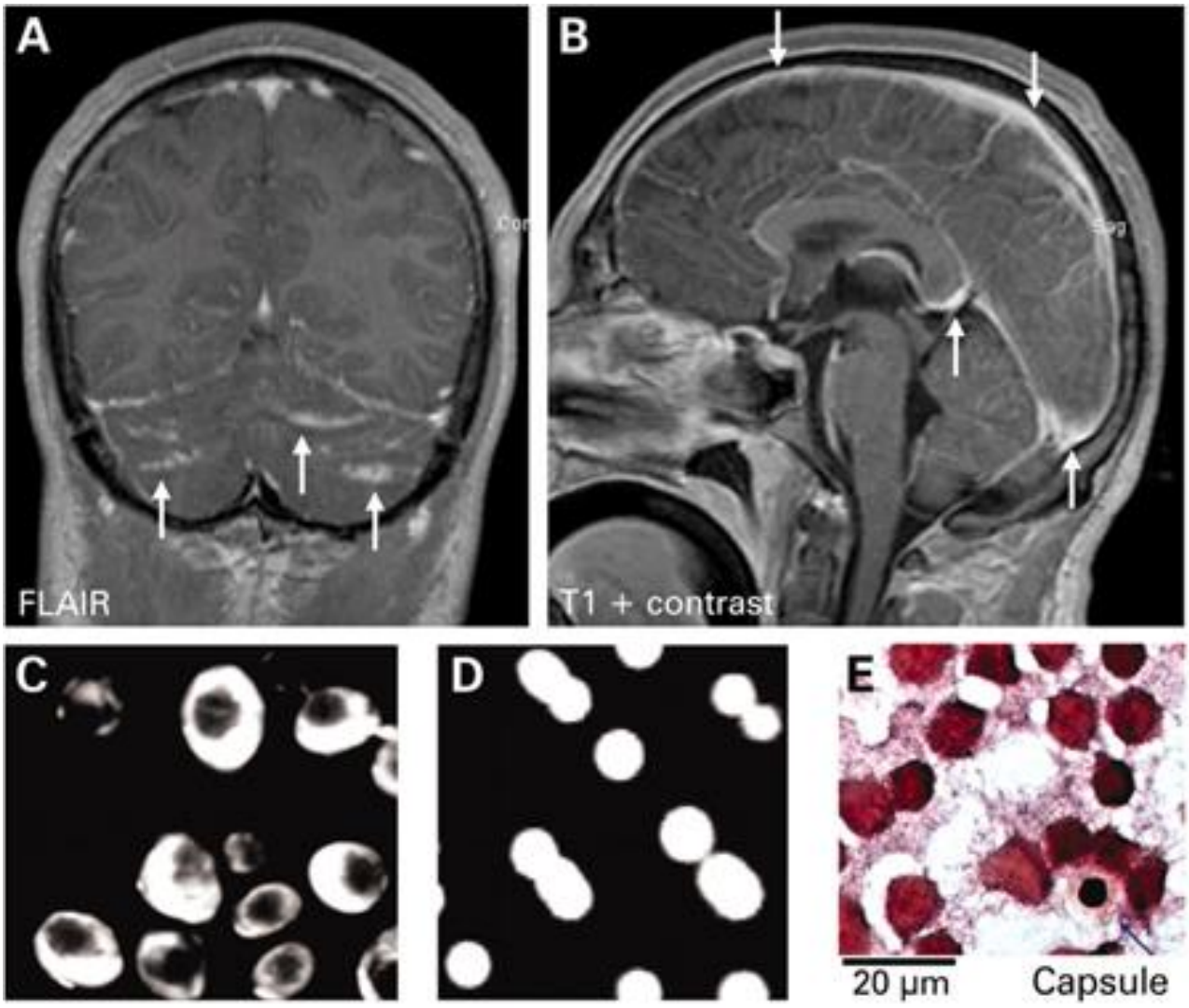


(a) Gross photograph of the brain showing opaque meninges in cryptococcal meningitis; (b) dense lymphomononuclear inflammation over meninges with scattered yeast like fungal organisms. (H and E; ×400); (c) yeast like forms of Cryptococcus highlighted by mucicarmine stain (×400). Uppin et al. Indian J Pathol Microbiol 2011;54:344-9

ii. Meningoencephalitis due to invasion of the cerebral cortex, brain stem and cerebellum is an uncommon, rapid fulminate infection, often leading to coma and death within a short time. Symptoms include slow response to treatment and signs of cerebral edema or hydrocephalitis, especially papilledema.



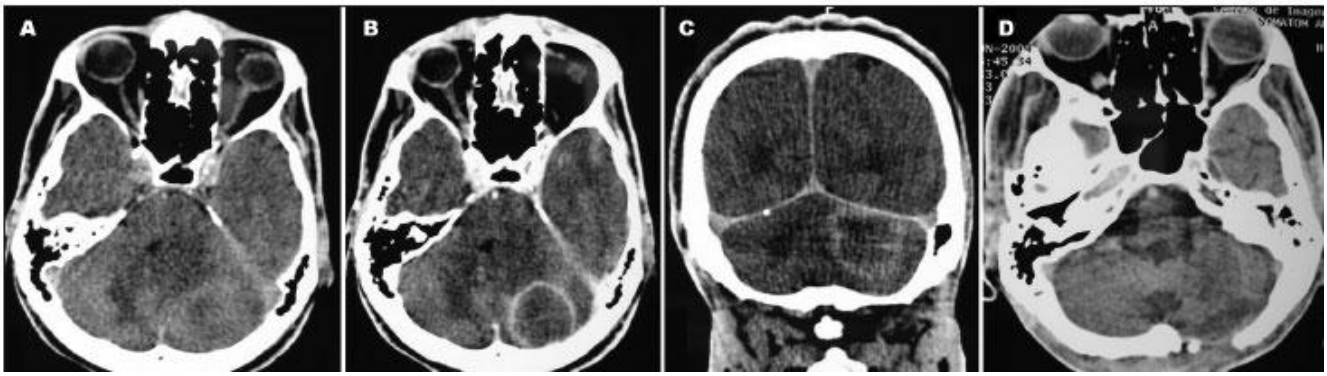
Left:Cryptococcal meningeoencephalitis: Cryptococcus neoformans. -cystic granulomatous lesions typical of Cryptococcus infections. Right: Cystic encapsulated yeast masses "Soap Bubbles" due to accumulated cryptococcal capsular polysaccharide. ww.studyblue.com



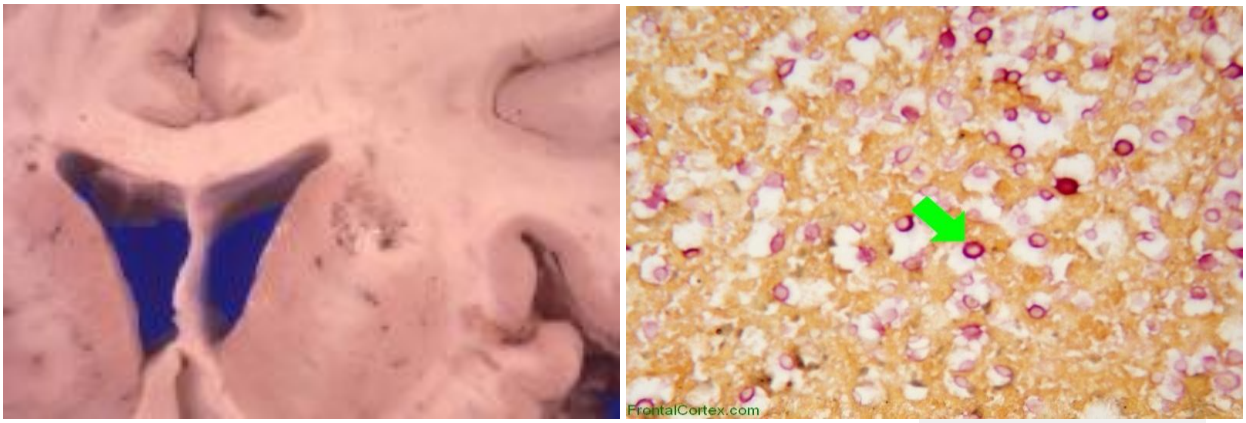
Cryptococcal meningoencephalitis. (A) Cranial MRI shows cerebellar hyperintensities (arrows) in FLAIR sequences (fluid-attenuated inversion recovery) and (B) meningeal contrast enhancement (arrows) in T1 weighted MRI. (C) Indian ink stain, (D) fungal culture, and (E) Gram stain of cerebrospinal fluid were positive. Braun J. Headache, personality changes and fine motor disturbances. BMJ Case Reports. 2009;

iii. Cryptococcoma

Cryptococcoma is a rare entity, characterized by localized, solid, tumor-like masses, usually found in the cerebral hemispheres or cerebellum, or more rarely in the spinal cord. Symptoms are consistent with an expanding intracranial mass and include headache, drowsiness, nausea, vomiting, mental changes, slurred speech, double vision, unsteadiness of gait, coma, paralysis and hemiparesis. These symptoms may mimic cerebral neoplasm which may delay a true diagnosis



Cerebellar cryptococcoma simulating metastatic neoplasm . The lesion presented with surrounding edema, which caused fourth ventricle displacement Arq. Neuro-Psiquiatr. vol.67 no.2a São Paulo June 2009

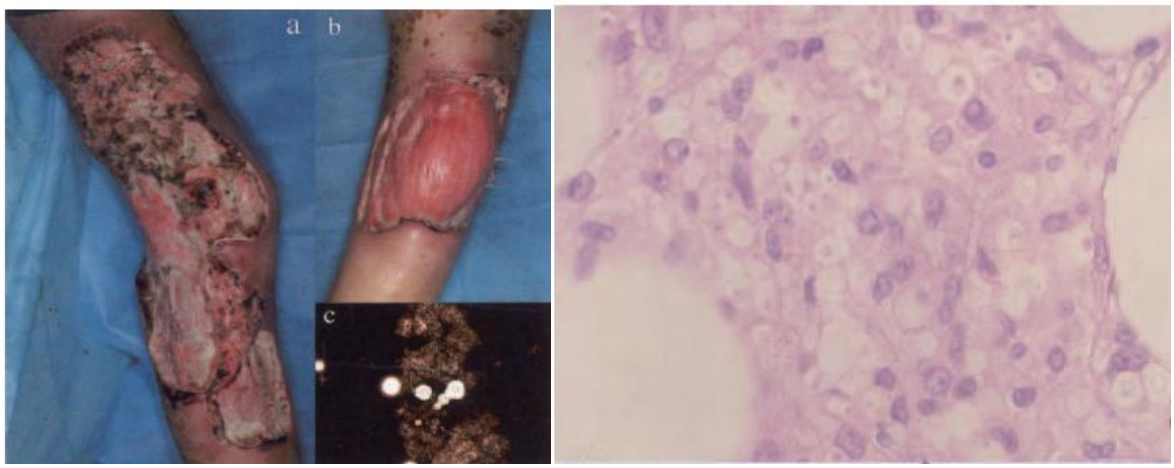


Left: Coronal section of brain showing a cryptococcoma in the basal ganglia. / Right: a high-power image of a cryptococcoma (mass in the brain) emedicine.medscape.com

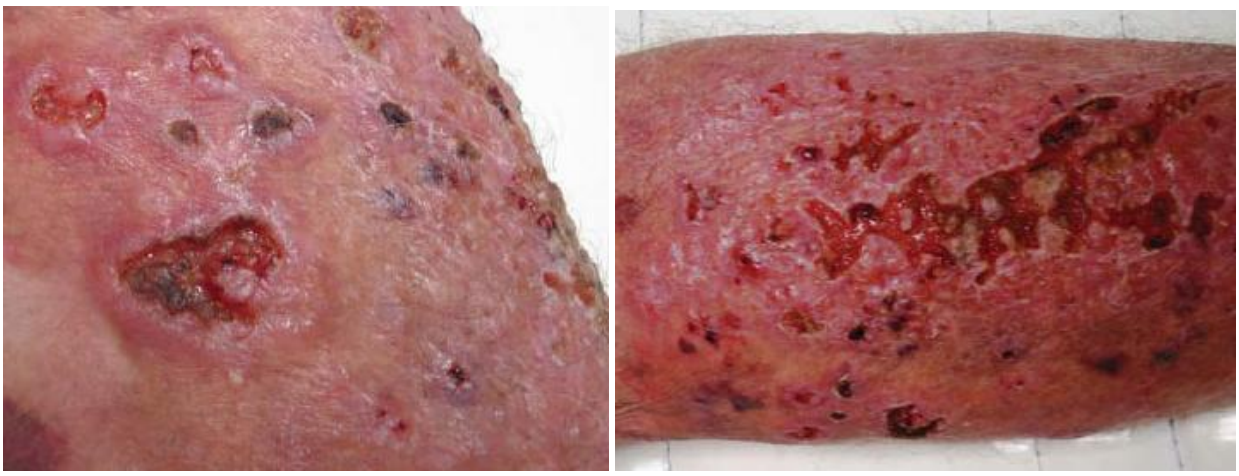
11.4.3. Cutaneous Cryptococcosis:

In patients with AIDS, skin manifestations represent the second most common site of disseminated cryptococcosis. Lesions often occur on the head and neck and may present as papules, nodules, plaques, ulcers, abscesses, cutaneous ulcerated plaques, herpetiform lesions, lesions simulating both molluscum contagiosum and Kaposi's sarcoma. Anal ulceration may also occur.

i. Primary cutaneous cryptococcosis in the form of ulcerated lesions or cellulitis occasionally occurs, especially in immunosuppressed patients. These lesions may resolve spontaneously or with systemic antifungal treatment. However, all patients with skin lesions should be monitored carefully for possible dissemination to the central nervous system.

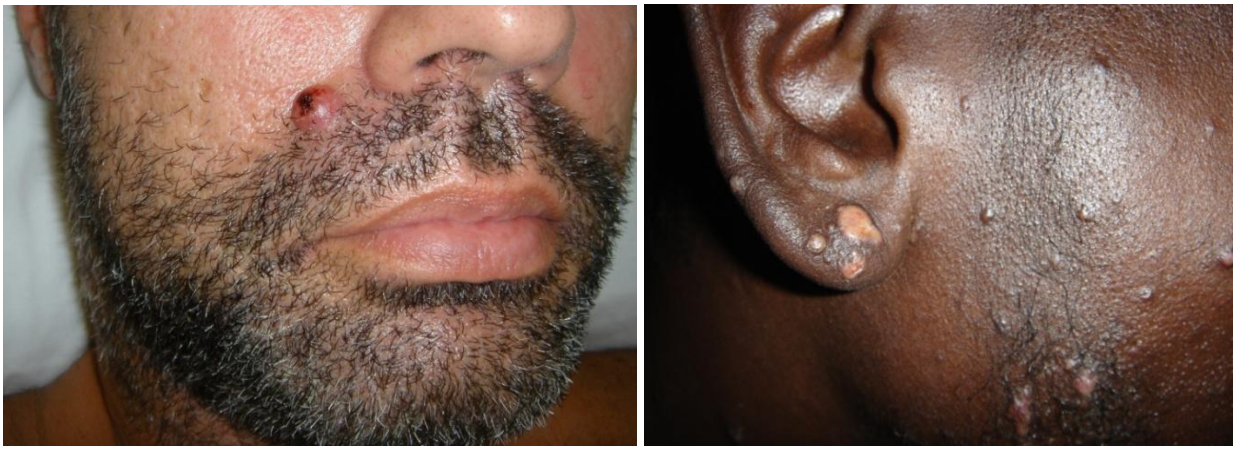


Large, deep ulcers on the left (a) and right (b) legs. Cryptococci were found in the smear of pus , Oval or round budding cells surrounded by haloes with lymphocytes. Kobayashi et al. (2003), Acta Derm Venereol. 84,320-321

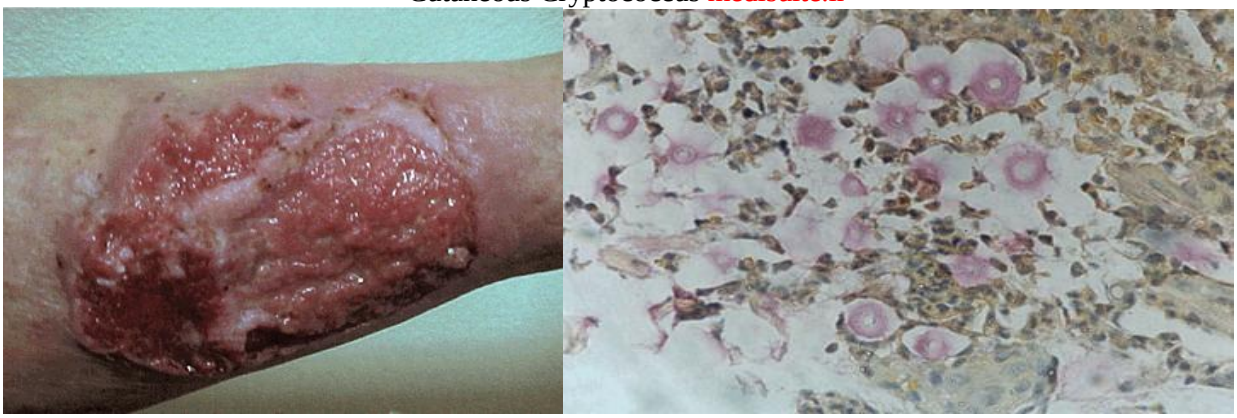


Primary cutaneous cryptococcosis showing nodular ulcerated and crusty lesions

ii. Secondary cutaneous cryptococcosis occurs in up to 15% of patients with disseminated cryptococcosis and often indicate a poor prognosis. Lesions usually begin as small papules that subsequently ulcerate, but may also present as abscesses, erythematous nodules, or cellulitis.



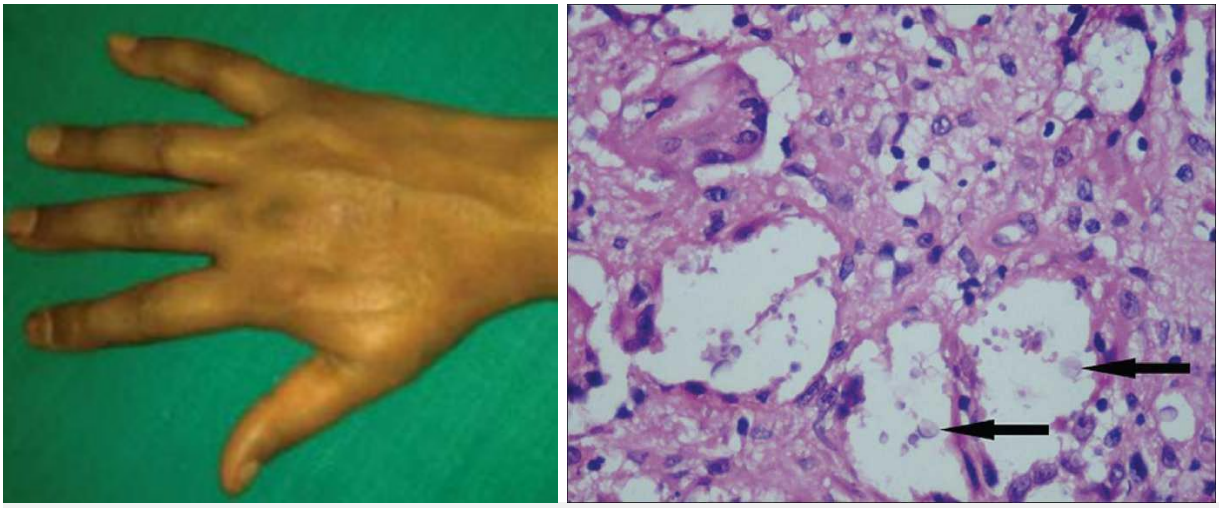
Cutaneous Cryptococcus medisuite.ir



Left arm cryptococcosis with extensive ulceration Skin lesion positive for C. neoformans,mucicarmin stain
LACAZ, Carlos da Silva et al. *Rev. Inst. Med. trop. S. Paulo* [online]. 2002, .44, .4. 225-228 .

11.4.4. Cryptococcal infection of the bone and joints

Cryptococcal infection of the bone and joints can present with osteolytic lesions or evidence of acute/chronic arthritis, respectively, and there has been some association for these infections with sarcoidosis.



Cryptococcal abscess and osteomyelitis of the proximal phalan of the hand Jain K, Mruthyunjaya, Ravishankar R .

11.4.5. Cryptococcal peritonitis

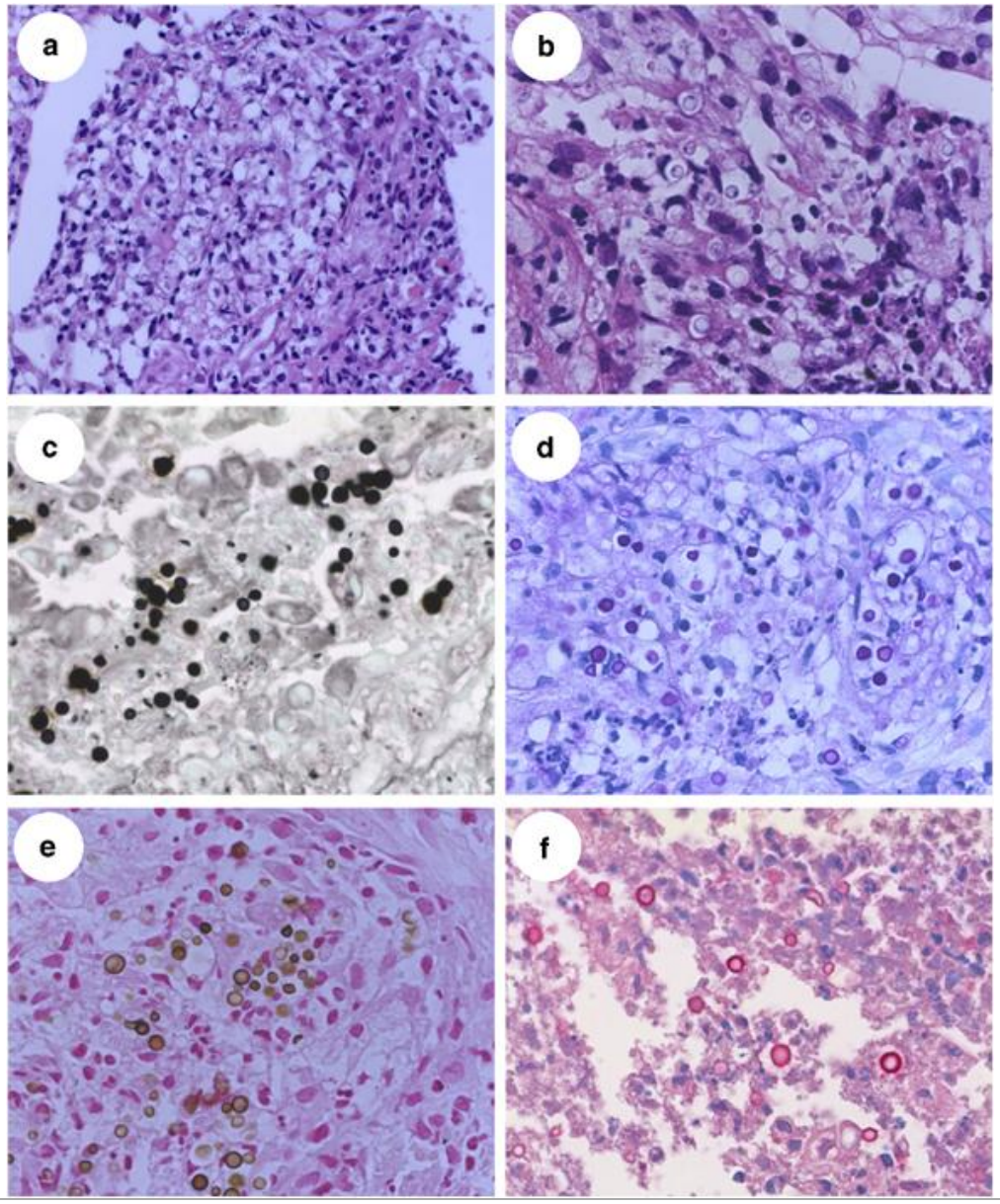
Cryptococcal peritonitis has been well- described. It may present in the ascites of chronic liver disease or in the peritoneal dialysate of patients with chronic renal disease.

11.4.6. Cryptococcal pyelonephritis

Cryptococcuria occurs and even pyelonephritis is described but the urinary tract site is less common than one would expect with severely disseminated disease and may simply reflect the length of routine culture methods.

11.4.7. Prostatic cryptococcosis

Prostatic cryptococcosis is generally asymptomatic and has been considered a site for occult or sequestered infection that is protected from antifungal treatments. Latent prostatic cryptococcosis has been uncovered in the blood after prostatic surgery_ and even serum prostatic-specific antigen has been elevated with chronic infection of the prostate with cryptococcus.



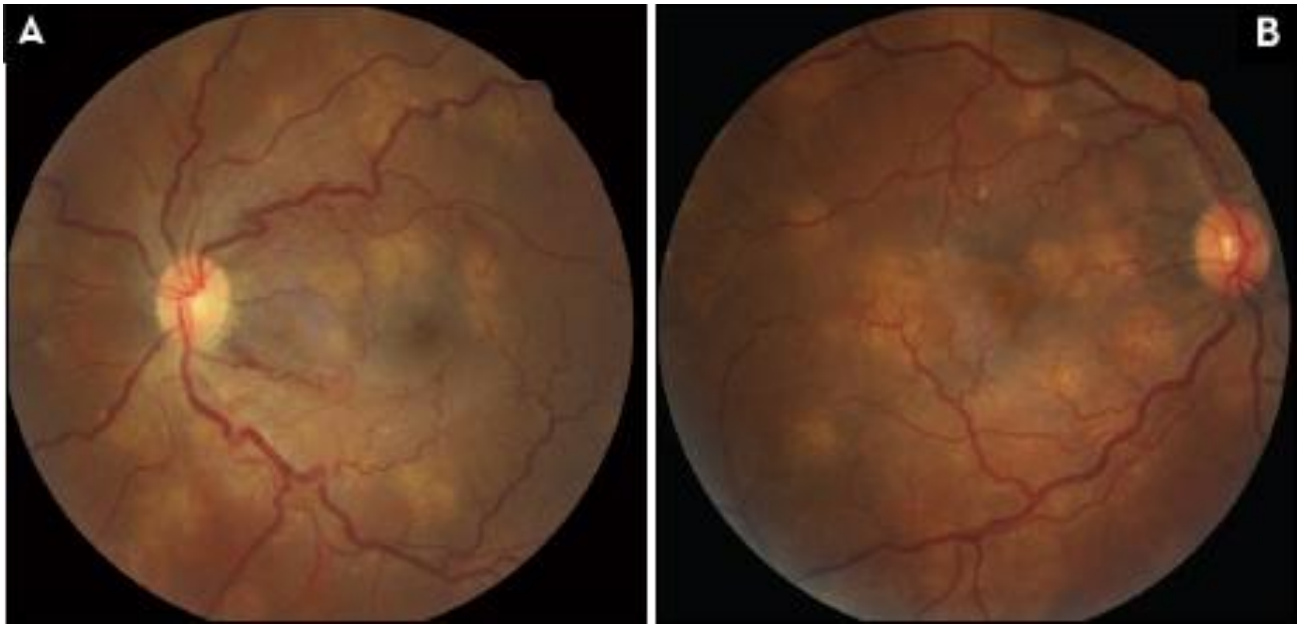
Histology of the biopsy specimen of the prostate. (a) Granulomatous lesion of the prostate (HE, × 200). (b) High magnification of the granuloma. Small round organisms are scattered in granulomas (HE, × 400). (c) Grocott's methenamine silver demonstrates numerous yeast-form pathogens in the granulomatous lesion. (d) The pathogen is clearly stained with periodic acid-Schiff with diastase treatment. (e) Fontana-Masson stain demonstrates melanin in the cell wall. (f) The mucoid capsule of the pathogen is stained with mucicarmine.

Wada etal.. ***Prostate Cancer and Prostatic Diseases*** **(2008)** 11, **203–206**

11.4.8. Ocular cryptococcosis

- Ocular cryptococcosis has been reported in up to 45% of patients with cryptococcosis and thus becomes a significant site for disease .
- The most common manifestations are ocular palsies, papilledema and blindness but in severely immunosuppressed patients retinal lesions with or without vitritis may appear.
- Catastrophic loss of vision without evidence of endophthalmitis also occurs.
- Visual loss which is a serious sequelae of cryptococcal meningoencephalitis can be caused by three pathogenic processes:
    1. The first process is caused by infiltration of the optic nerve and produces a rapid visual loss with few effective treatments.
    2. The second process is invasion of the retinal area and extension into the vitreous body and this also has high a rate of permanent visual loss but may be more responsive to antifungal therapy.
    3. The third process is caused by increased intracranial pressure and leads to a slower visual loss.

- In an early case series, approximately half of patients with cryptococcal meningitis experienced visual symptoms, most commonly blurred vision but also double vision, orbital pain, and photophobia. In the majority of cases, such symptoms are a secondary phenomenon caused by increased intracranial pressure causing papilledema or sixth cranial nerve palsy.
- A more recent series from Rwanda of 80 patients with cryptococcosis and AIDS demonstrated papilledema in 32% of cases. Spread of infection into the intraocular space is less common.
- Intraocular *Cryptococcus* may take the form of chorioretinitis, multifocal choroiditis, neuroretinitis, vitritis, or endophthalmitis. The organism seems most commonly to reach the eye hematogenously via the choriocapillaris due to its high volume of blood flow, but it may also enter through the retinal circulation or by direct extension along the leptomeninges of the optic nerve.



Color fundus photos of the left (a) and right (b) eyes reveal multiple deep yellow-white plaques prior to therapy. Christopher J. Brady, and Carl D Regillo, Wills Eye Institute in Philadelphia

11.4.9. Cryptococcal endocarditis

Despite frequent cryptococcemia, the reported cases of cryptococcal endocarditis are less than a dozen and this includes both native and prosthetic valve endocarditis.

11.4.10. Other cryptococcal involvements:

- Cryptococcual myositis,
- Cryptococcual genital-lesions,
- Cryptococcual hepatitis,
- Cryptococcual thyroiditis,
- Cryptococcual adrenal mass,
- Cryptococcual gingivitis,
- Cryptococcual salivary gland involvement,
- Cryptococcual neck masses,
- Cryptococcual oesophagitis,
- Cryptococcual biliary tract involvement,
- Cryptococcual enteritis,
- Cryptococcual mastitis,
- Cryptococcual breast masses,
- Cryptococcual lymphadenopathy.

11.5. Clinical manifestation of cryptococcosis in animals

- Cryptococcosis in animals is a systemic fungal infection of worldwide significance that usually initially infects the nasal cavity, paranasal tissues, or lungs. It can then disseminate, most commonly to the skin, eyes, or central nervous system.
- **Organs most commonly affected** :
    1. **Respiratory tract**

        Respiratory infections (seen in > 80% of cases) are characterized by sneezing, nasal discharge (pus-filled, bloody, or clear), swelling underneath the skin in the nasal area, mouth lesions, and swelling of lymph nodes
    2. **Central nervous system**

        Neurological signs vary with the location of the lesion and can include depression, poor movement coordination, seizures, partial paralysis, and blindness.
    3. **Eyes**

        Eye abnormalities predominately affect the retina, choroid, and optic nerve. Clinical signs can range from dilated, unresponsive pupils and blindness to chorioretinitis, anterior uveitis, and retinal damage. While the prognosis for survival with the ocular form of Cryptococcus is fair to good using triazole antifungals, the prognosis for return of vision is guarded to poor due to retinal damage
    4. **Nares**

        Asymptomatic colonization of the nares has been reported in cats, dogs and koalas. Organisms have also been detected in the nares of horses and wild squirrels, but whether this was caused by colonization or environmental exposure is still unknown.

- **Cryptococcosis has been described in a many species of mammals and marsupials:**
    - Relatively common in cats, and it has been described in other felids, especially cheetahs (Acinonyx jubatus).
    - Clinical cases have also been reported in dogs, ferrets, guinea pigs, horses, donkeys, cattle, sheep, goats, water buffalo, pigs and South American camelids (llamas, alpacas and vicunas).
    - Captive wild animals: cryptococcosis has been documented in red foxes (Vulpes vulpes), mink, a civet, gazelles, tapirs (Tapirus spp.), collared peccaries (Tayassu tajacu), mouflon sheep (Ovis

musimon), tree shrews (Tupaia tana and Tupaia minor), elephant shrews (Macroscelides proboscides), a striped grass mouse (Lemniscomys barbarus), potoroos (Potorous spp.), koalas (Phascolarctos cinereus), wallabies, non-human primates and wild marine mammals.

- **Host ranges of *C. neoformans* and *C. gattii* :**

  Both organisms are known to affect cats, dogs, ferrets and cheetahs. Many cases of cryptococcosis in horses seem to be caused by *C. gattii*. This organism also infects koalas in Australia, with consequences ranging from asymptomatic colonization to severe illness. In addition, *C. gattii* has been found in clinical cases from goats, llamas, alpacas, tapirs and marine mammals.
- Other species of Cryptococcus that may occasionally cause illness in mammals include:
  - *C. albidus* (horses and a dog),
  - *C. magnus* (cats),
  - *C. laurentii* (dog, equine fetuses) and
  - *C. flavescens* (dog).

Cryptococcosis in domestic animals residing in Western Australia

(11-year-period,**McGill *et al.*, 2009)**

- Cryptococcosis was identified in 155 animals: 72 cats, 57 dogs, 20 horses, three alpacas, two ferrets and a sheep.
- Cats were five to six times more likely to develop this disease than dogs, and three times more likely than horses . Amongst the feline cohort, Ragdoll and Birman breeds were over-represented
- Horses were almost twice as likely as dogs to become infected., while in dogs several pedigree breeds were similarly overrepresented.
- Dogs and horses tended to develop disease at an early age (one to five years), while cats were presented over a much wider range of ages.
- In cats and dogs the upper respiratory tract was the most common primary site of infection, while horses and alpacas tended to have lower respiratory involvement.
- The most striking finding of the study was the high frequency with which *C. gattii* was identified, with infections attributable to this species

comprising 5/9 cats, 11/22 dogs, 9/9 horses and 1/1 alpaca, where appropriate testing was conducted.

- Preliminary molecular genotyping suggested that most of the *C. gattii* infections in domestic animals (9/9 cases) were of the VGII genotype. This contrasts the situation on the eastern seaboard of Australia, where disease attributable to *C. gattii* is less common and mainly due to the VGI genotype. *C. gattii* therefore appears to be an important cause of cryptococcosis in Western Australia.

11.5.1. Cryptococcosis in Cats

Pennisi et al. (2013) mentioned that, cryptococcosis is the most common systemic fungal disease in cats; it is caused by by *C neoformans* or *C gattii* and the infection is indistinguishable clinically. The disease can present in nasal, central nervous system (which can derive from the nasal form or occur independently), cutaneous and systemic forms.

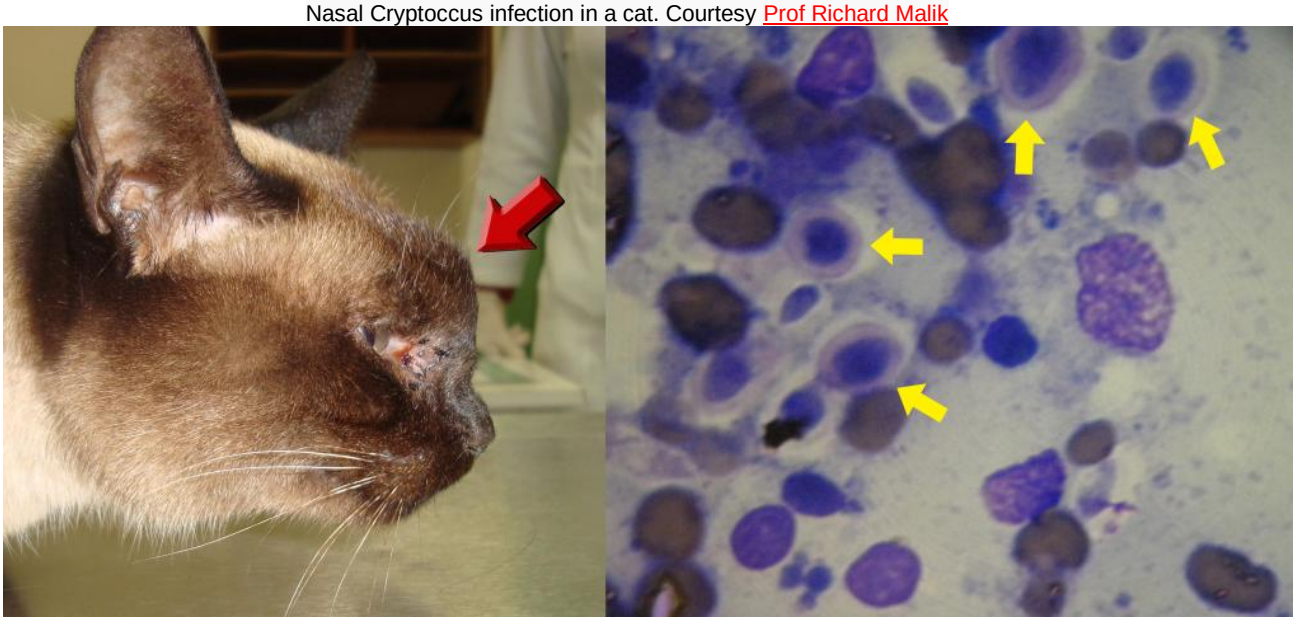
Cardoso et al. (2013) described a case of a nasal granuloma in a cat due to C. *gattii*. The confirmation of the specie *Cryptococcus gattii* and its molecular type were performed using the PCR-RFLP molecular techniques. The isolated strain was identified as *C. gattii* type VGII and was susceptible to all antifungal drugs tested. The characterization and molecular investigation of this microorganism are relevant because they could help better understand the epidemiology of the infection and to guide us to treat properly the disease.

- **In cats**, cryptococcosis can be either focal or disseminated, affecting a single organ system or many.

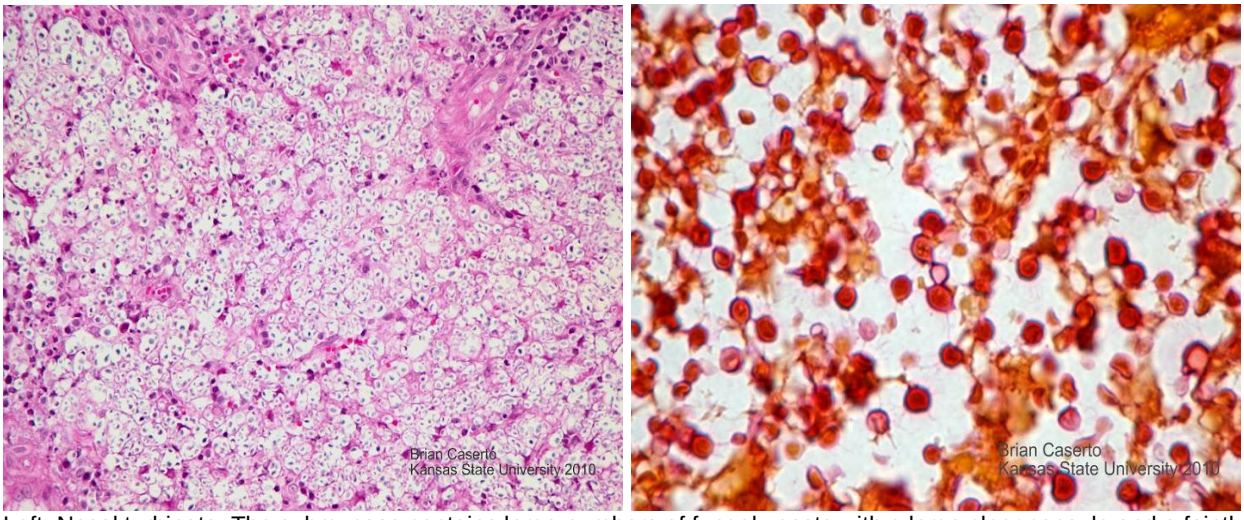
- **The clinical signs**
  - Can begin insidiously, and may gradually become more severe over weeks or months.
  - Fever may be absent, and if present, is often mild.
  - Other nonspecific signs can include lethargy, anorexia and weight loss.
  - Cats with localized infections, including those in the nasal cavity, do not necessarily have constitutional signs.

- **Localized upper respiratory disease**
    - Unilateral or bilateral chronic rhinitis or sinusitis is the most common form of cryptococcosis in cats.
    - Frequently seen clinical signs include sneezing, snoring or snorting, dyspnea, nasal deformities and/ or a mucopurulent, serous or serosanguineous nasal discharge. Polyp-like masses sometimes protrude from one or both nostrils.

Nasal Cryptoccus infection in a cat. Courtesy Prof Richard Malik

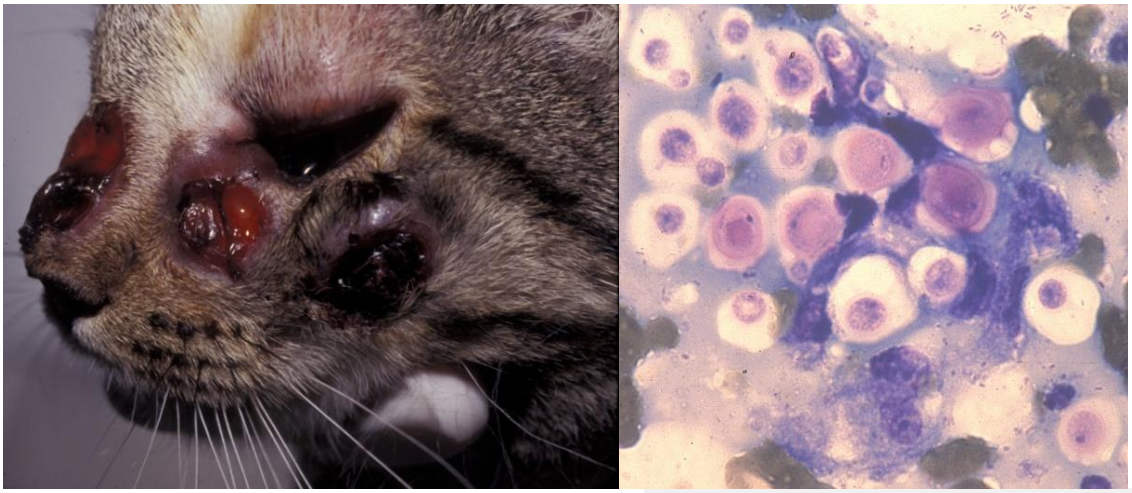


Feline cryptococcosis. left: a cat presenting a nasal masse (red arrow). right: Cytology by fine needle aspirate of the nasal ...www.intechopen.com



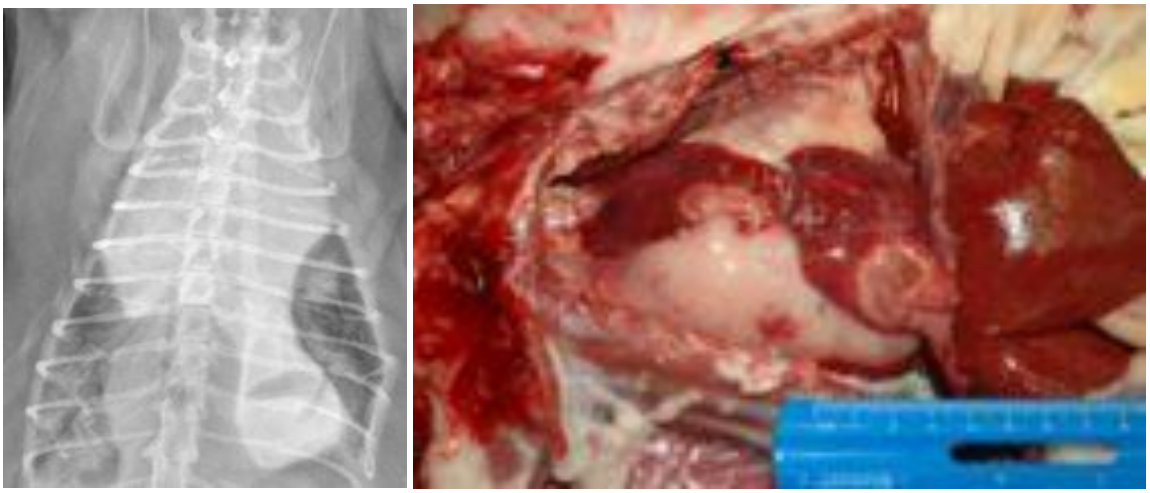
Left: Nasal turbinate: The submucosa contains large numbers of fungal yeasts with a large clear capsule and a faintly basophilic nucleus. Right: Mucicarmine stain of feline nasal turbinates with Cryptococcus neoformans: The cell walls stain red and the capsule is clear. Vet.Path.Forum

- **Cutaneous or subcutaneous swellings and nodules**
  - may be seen on the face, particularly the bridge of the nose, side of the face, upper lip or nostril.
  - Some of these lesions may ulcerate. In addition, the submandibular lymph nodes are often enlarged. With time, infections involving the nasal cavity can spread to adjacent structures.
  - Ulcerative or proliferative lesions may develop on **the tongue, gingiva or palate**. Extension to the **ear** can result in otitis media and vestibular signs.
  - Cutaneous involvement usually appears as fluctuant or firm papules and nodules. Some skin lesions may ulcerate, but there is little or no pruritus. Generalized skin disease suggests disseminated cryptococcosis. Direct inoculation of organisms into the skin can occasionally cause solitary lesions.



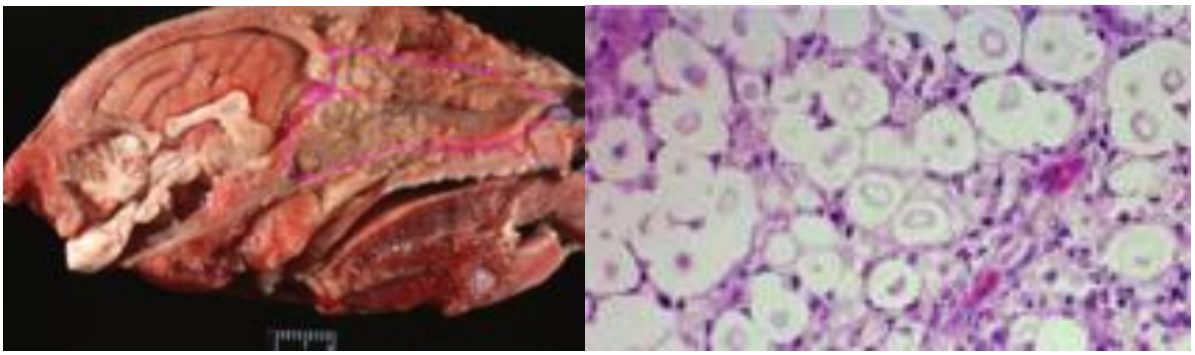
Cat cryptococcosis. multiple foci of ulcerative dermatitis; aspirate from a cutaneous lesion contains numerous Cryptococcus neoformans yeast organisms surrounded by a nonstaining capsulewww.vetnext.com

- **Lower respiratory disease**
  - can also occur in cats, although it is less common than upper respiratory lesions.
  - Syndromes may include pneumonia, pleuritis and mediastinal masses.

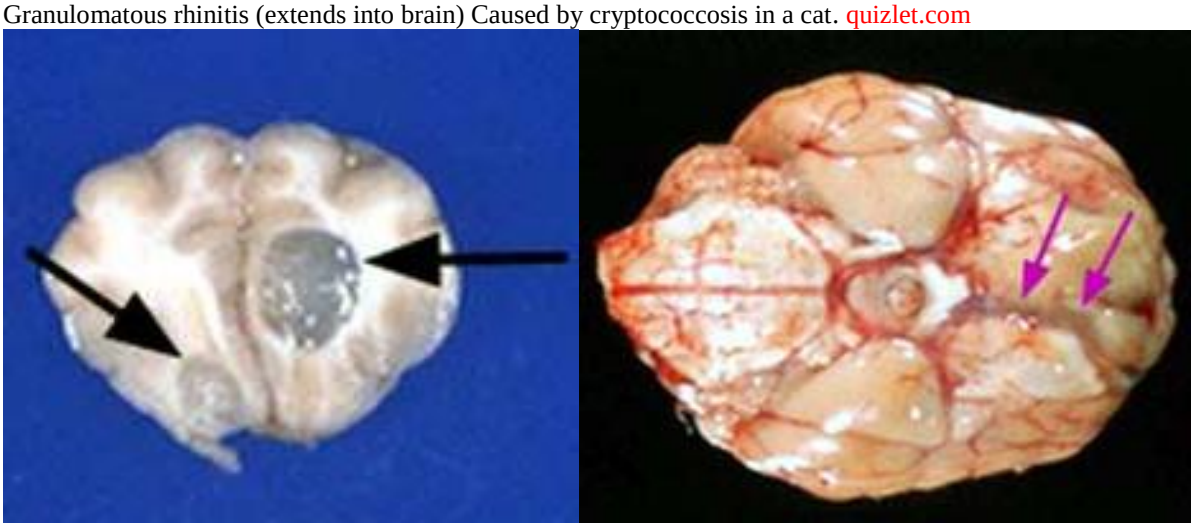


Lateral thoracic radiograph of a 12-year-old Siamese with pulmonary cryptococcus. There is a soft tissue mass in the cranial mediastinum as well as in the dorsal portion of the mid thorax causing ventral and caudal displacement of the tracheal carina. There is also severe atelectasis of all lung lobes. Right:Post-mortem of a 12-year-oldSiamese with pulmonary cryptococcus. Courtesy Prof Allison Zwingenberger

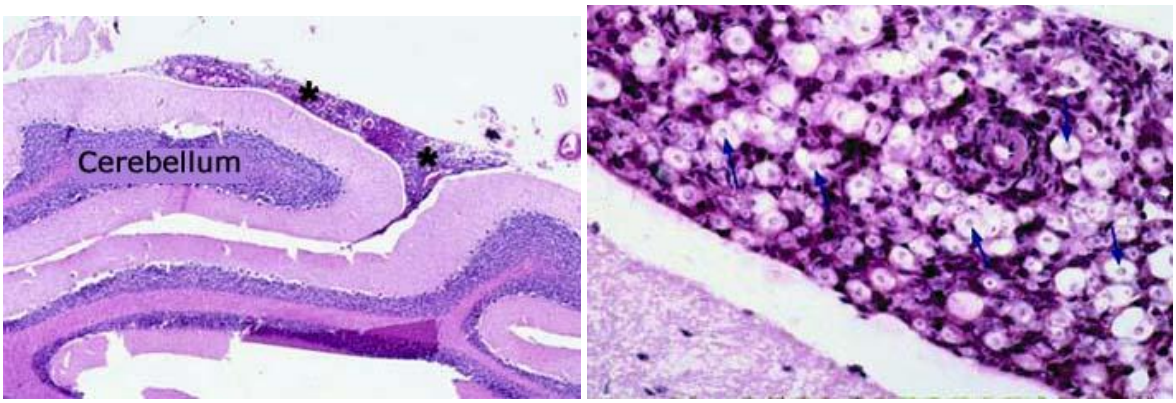
- **CNS involvement is common in cats**
  - both focal mass lesions (cryptococcomas) and cryptococcoal meningitis may be seen.
  - The neurological signs can be mild to severe, with various presentations such as a change in temperament or behavior, depression, disorientation, vestibular signs (e.g., head tilt, circling, nystagmus), head pressing, ataxia, paresis or paralysis, tremors, seizures, abnormal pupillary responses and blindness.
  - Meningitis may appear as pain over the thoracolumbar spine or pelvic limbs, but hyperesthesia and nuchal rigidity are uncommon. Deficits of cranial nerves 5 to 12 are often found.
  - The CNS is sometimes involved even if there are few or no obvious neurological signs. In one case, the only sign was unusual sleepiness.



Granulomatous rhinitis (extends into brain) Caused by cryptococcosis in a cat. quizlet.com



Note the two circumscribed areas in this cat brain that are very gelatinous in appearance. This gelatinous appearance is typical for *Cryptococcus* and is due to the mucinous capsule surrounding each organism.



Left, note the cellularity of the meninges (*) overlying the cerebellum in this case of cryptococcosis. Right: a higher magnification of the cellular infiltration.which is granulomatous to pyogranulomatous.

- **Ocular lesions**
  - Chorioretinitis, optic neuritis, panophthalmitis, retinal hemorrhages and iridocyclitis have been reported. T
  - Small transparent focal retinal detachments with a minimal inflammatory response may be seen.
  - Ocular lesions often accompany other syndromes, specially CNS disease. Some cats can become blind.
- **Other organs** which can also be affected include:
  - the bone (osteomyelitis),
  - mediastinum,
  - heart,
  - thyroid gland,
  - spleen,
  - liver
  - urinary tract.

11.5.2. Cryptococcosis in dogs

Compared to cats, dogs are more prone to develop disseminated Cryptococcosis Cryptococcus neoformans can affect the eyes and central nervous system (CNS) and cause optic neuritis, granulomatous chorioretinitis and meningoencephalitis. About 50 percent of dogs diagnosed to have lesions in their respiratory tract, which is often the lungs, and most dogs have a granuloma in more than one system. Lesions may develop in the nasal cavity. Cryptococcus neoformans is quite often attacks the kidneys, lymph nodes, spleen and liver of dogs, and general lack of almond, heart valves, thyroid, adrenal, muscle, pancreas, gastrointestinal tract, bone, myocardium, and prostate.
When Cryptococcosis cause lesions in dogs, they can be different from the kind of granuloma mass jelly made by many organisms (often with very little inflammation). Criptococosis lesions in dogs usually consists of an aggregate of encapsulated organisms covered by reticular connective tissue.

The average age of infected dogs is 3.5 years and, unlike cats, there is no gender predisposition. Overrepresented dog breeds include American Cocker Spaniels and Labrador Retrievers in North America, and Doberman Pinschers and Great Danes in Australia. Cryptococcosis affects the same four organ systems as with cats, but the CNS and eyes are more commonly involved in dogs than in cats. The clinical signs are similar to those found in cats except that fever (103-105° F) is seen more often in affected dogs (25% of cases).

Bowles et al. (2009) presented two young, large-breed female dogs with an acute onset of sneezing and nasal discharge.

- One patient had concurrent epistaxis and facial deformity. Decreased airflow was noted through the left nostril in Case 1, while Case 2 showed facial deformity.
- Nasal radiographs from Case 1 showed a soft tissue opacity in the left nasal cavity and frontal sinus.
- Rhinoscopy revealed roughened, erythematous nasal turbinates in both patients, and a mass in the left caudal nasal cavity of Case 1.
- Cryptococcus spp. were demonstrated histopathologically on a nasal biopsy.
- Tissue culture and serum antigen titres were positive for Cryptococcus spp. The diagnosis was chronic rhinitis secondary to Cryptococcus gattii infection in Case 1, and Cryptococcu neoformans infection in Case 2.

Byrnes et al. (2009) isolated Cryptococcus gattii from a 1.5-year-old dog with systemic cryptococcosis in Oregon. The dog had no link to Vancouver Island or British Columbia, Canada.

The 2 isolates were both the VGIIa Vancouver Island major genotype. Findings are consistent with expansion of the Vancouver Island outbreak onto the mainland Pacific Northwest region of the United States.

Trivedi et al. (2011) reviewed the medical records of cats and dogs with cryptococcosis. American Cocker Spaniels were overrepresented, compared with other dog breeds. Cryptococcus neoformans was more commonly detected in dogs (6/8). Six of 7 C. gattii isolates from cats were molecular type VGIII.

Bryan et al. (2011) collected blood and stool samples from 107 dogs in 5 remote communities in May and September 2007. Serology revealed that the dogs had been exposed to canine parvovirus, canine distemper virus, Bordetella bronchiseptica, canine respiratory coronavirus, and Leptospira interrogans.

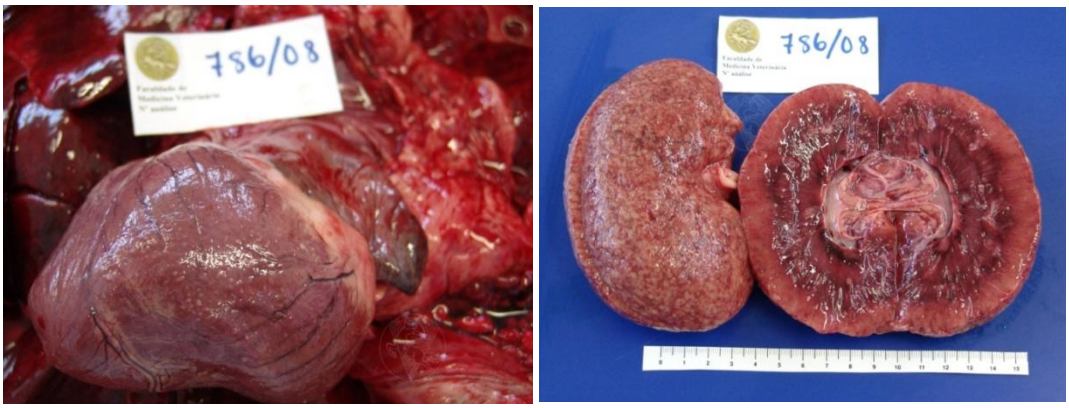
- Frequently affected sites in the dog include both the respiratory tract and CNS.
- Signs primarily of upper respiratory tract involvement have been documented in some dogs, especially those infected with C. gattii; however,
- Concurrent involvement of the lower respiratory tract or CNS is common in this species.

- **Disseminated cryptococcosis** is reported to be more common in dogs than cats.abnormal pupillary responses and blindness.
- **Meningitis** may appear as pain over the thoracolumbar spine or pelvic limbs, but hyperesthesia and nuchal rigidity are uncommon. Deficits of cranial nerves 5 to 12 are often found.
- **The CNS** is sometimes involved even if there are few or no obvious neurological signs..
- **Ocular lesions** reported cases are
  - chorioretinitis,
  - optic neuritis,
  - panophthalmitis,
  - retinal hemorrhages and
  - iridocyclitis
  - small transparent focal retinal detachments with a minimal inflammatory response. Ocular lesions often accompany other syndromes, especially CNS disease. Some dogs can become blind.

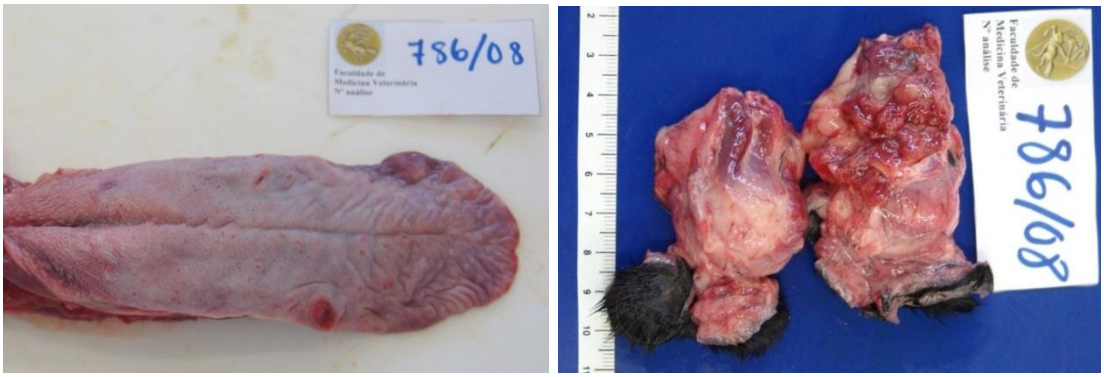
- **Cutaneous involvement**
  - Usually appears as fluctuant or firm papules and nodules.
  - Some skin lesions may ulcerate, but there is little or no pruritus.
  - Generalized skin disease suggests disseminated cryptococcosis.
  - Direct inoculation of organisms into the skin can occasionally cause solitary lesions.

Other organs which can also be affected.including

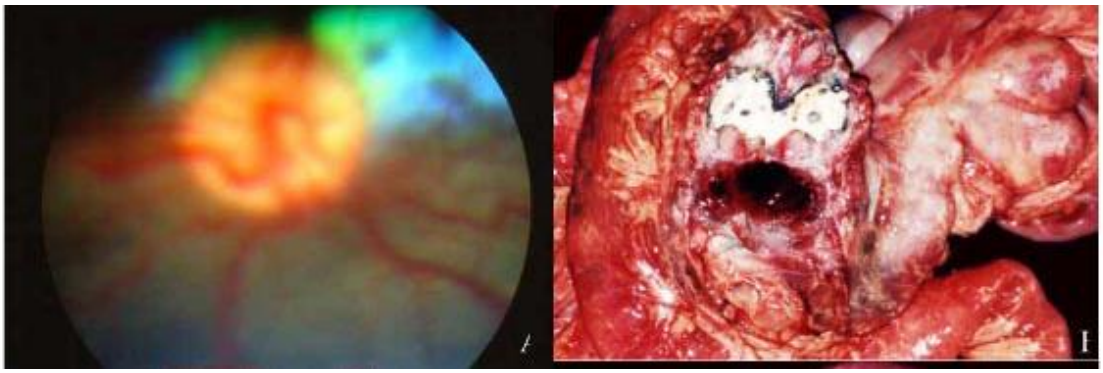
- the bone (osteomyelitis),
- mediastinum,
- heart,
- thyroid gland,
- spleen,
- liver and
- urinary tract



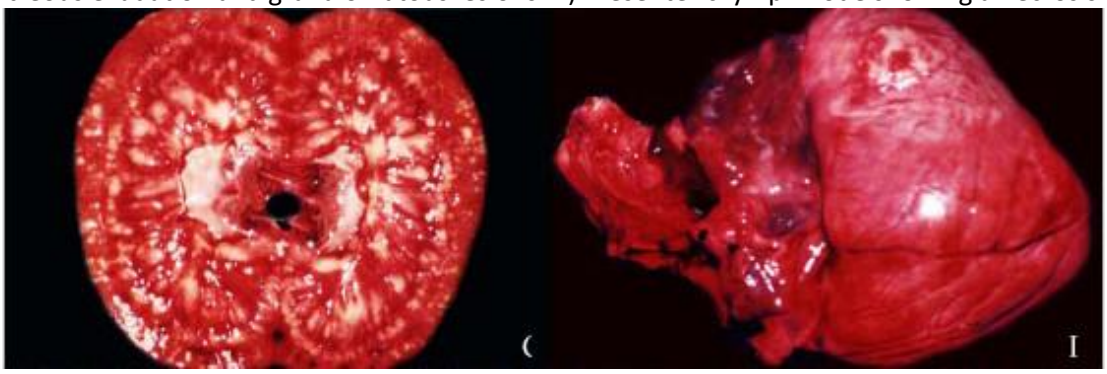
Cryptococcosis affecting several organs in a 5-year-old Labrador. Above, on the left, note the small pale nodules corresponding to cryptococcosis granulomas on the cardiac wall; on the right, similar nodules can be seen on both kidneys.,



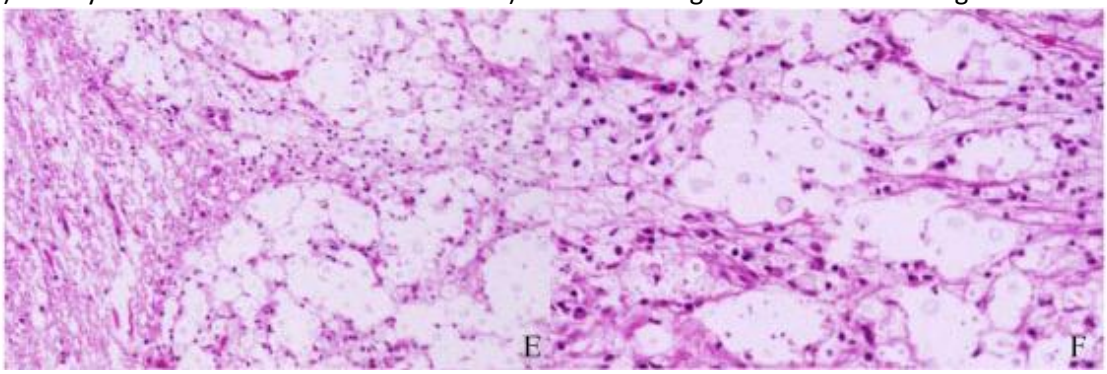
the lingual lesions and, on the right, the invasion of the right retrobulbar tissue by granulomatous inflammation caused by the *Cryptococcus*..www.fmv.utl.pt/atlas/cardiovascular pages_us cardio



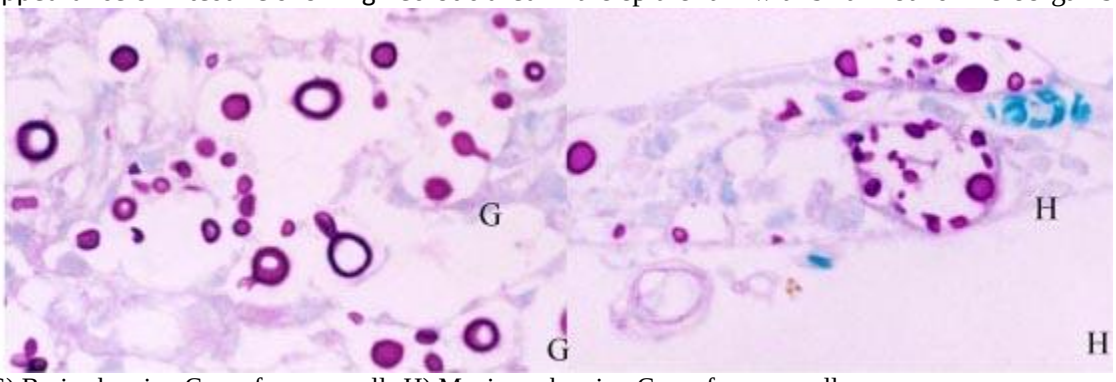
Organs of a dog with cryptococcosis. A) Fundus tapetal with discrete pigmented spots, papilloma, vitreous exudation and granulomatous lesions. B) Mesenteric lymph node showing a necrosis area.



C) Kidney with several small whitish areas D) Heart showing whitish area in the right ventricule



E) Microscopic appearance of kidney necrotic area with small round microorganisms. F) Microscopic appearance of intestine showing necrotic area in the epithelium with small round microorganisms.



G) Brain showing C. neoformans cells H) Meninge showing C. neoformans cells

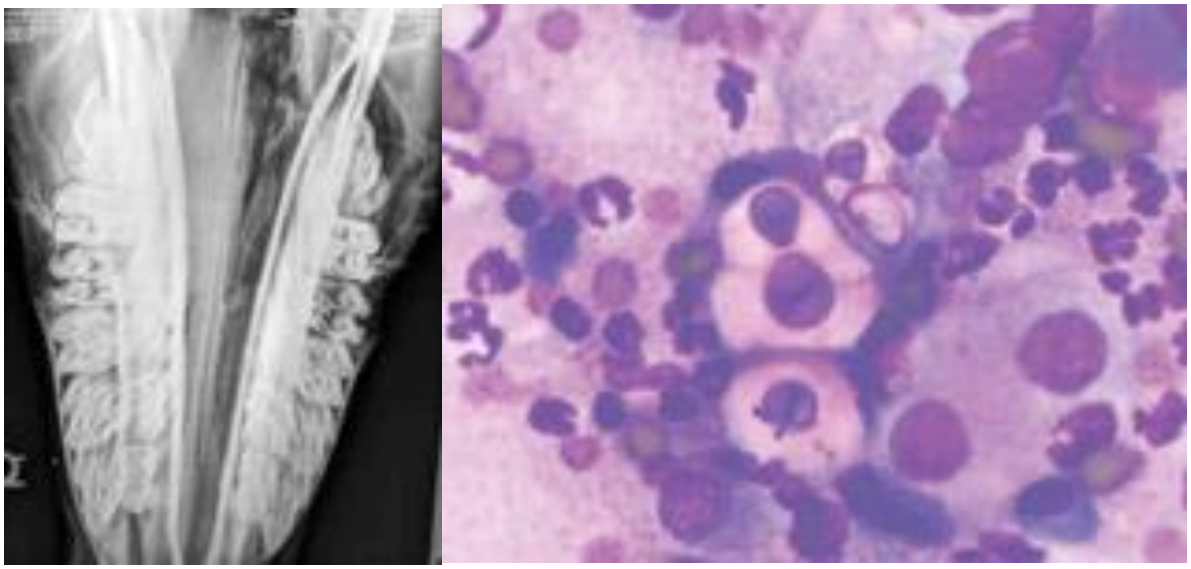
Arquivo Brasileiro de Medicina Veterinária e Zootecnia - Generalized systemic cryptococcosis in a dog after immunosuppressive corticotherapy

11.5.3. Cryptococcosis in horses

Published clinical cases in horses have described meningoencephalitis/ meningitis, lower respiratory disease or pneumonia, upper respiratory disease affecting the sinuses and/or nasal cavity, osteomyelitis, mass lesion in the intestinal tract, endometritis and abortions with mycotic placentitis, and disseminated disease.

- **Obstructive growths in the nasal cavities and sinuses**

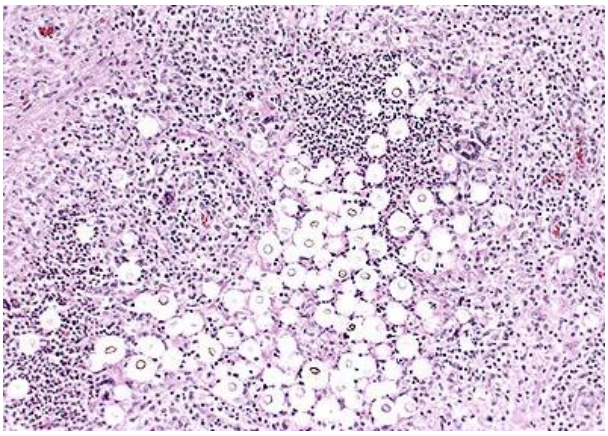
are a common presentation in some geographic areas. Lower respiratory disease was reported to be more frequent, where C. gattii is common. Cutaneous lesions were documented in a donkey

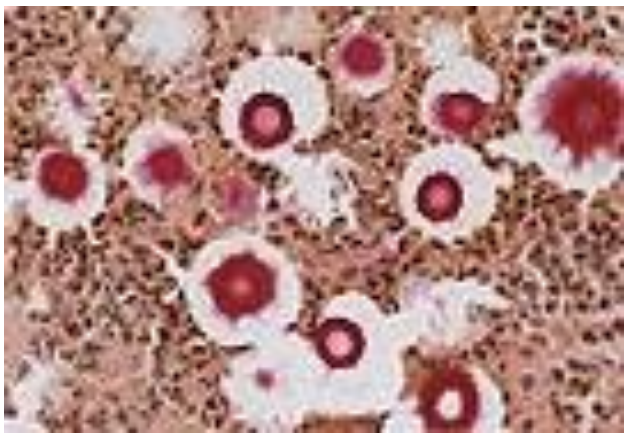


Radiograph of a horse with a history of chronic serosanguinous nasal discharge and dyspnea. Dorsoventral radiograph of the skull demonstrates significant increase in soft-tissue opacity within the nasal cavity and right maxillary sinus due to cryptococcal infection. An impression smear from a biopsy of a mass in the nasal passage of a horse with chronic serosanguinous nasal discharge and dyspnea. Allison J. Stewart DVM 360,2009

- **Cerebral cryptococcosis, due to infection with *Cryptococcus neoformans*.**

The brain contained numerous encapsulated yeast bodies compatible with *C. neoformans*. Similar organisms were present in the lung within the grossly visible nodules. Lesions in the lungs were those of pyogranulomatous pneumonia. This case is somewhat unusual, because in the CNS 1) C. neoformans is usually associated with meningitis and 2) the organism usually evokes minimal inflammatory response. In this case, lesions were confined to the midbrain; the meninges were spared.

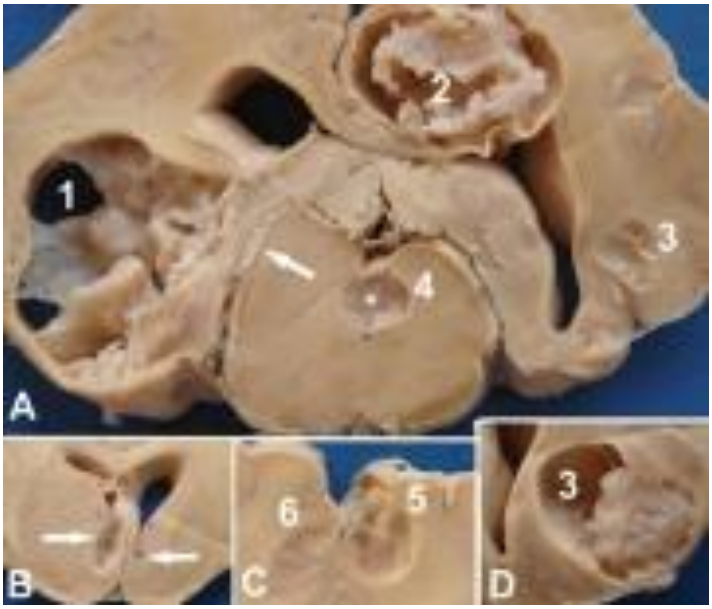




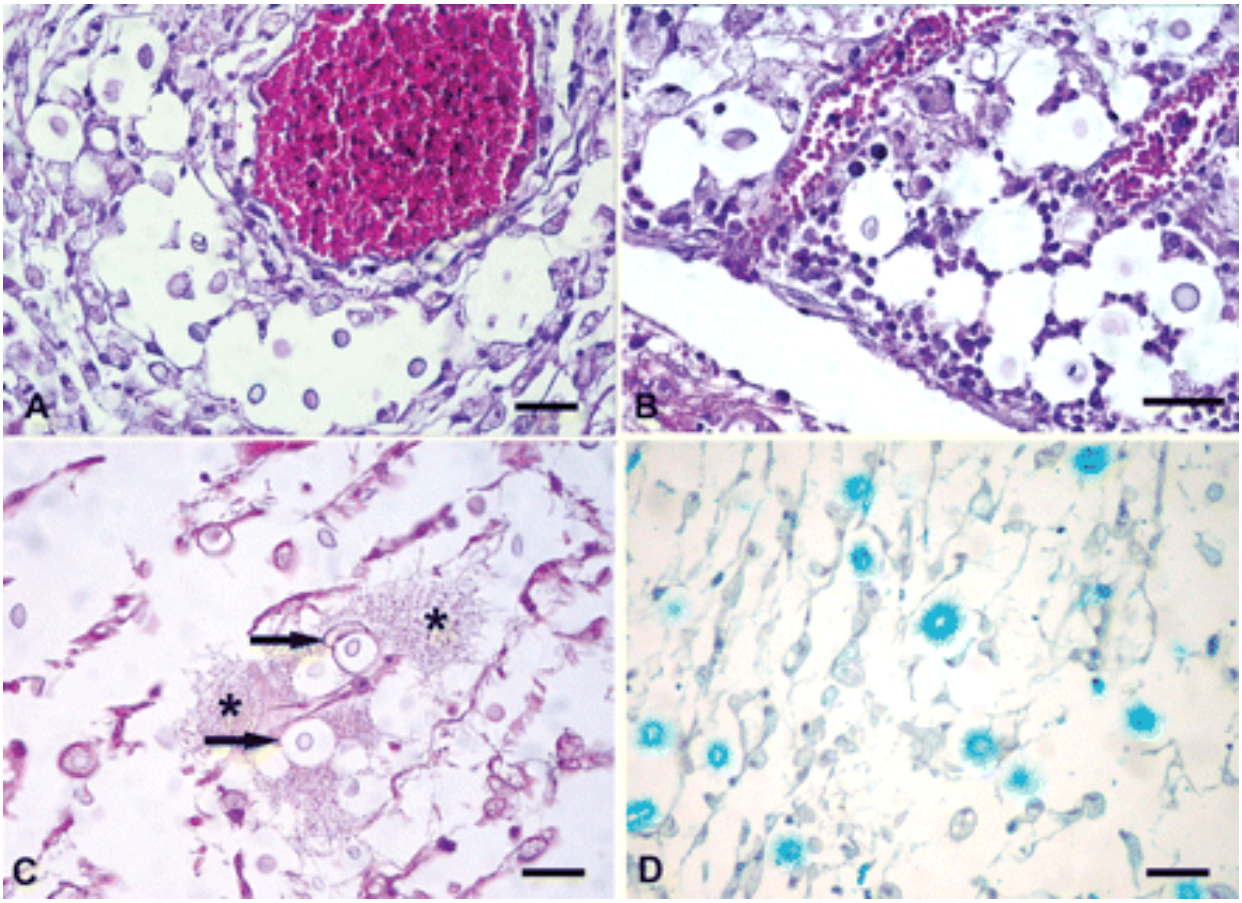
Left:Granulomatous encephalitis with the classic "soap-bubble" appearance associated with *Cryptococcus neoformans* in the cerebrum of a horse,H&E Right:Carmininophilic yeasts in the cerebrum of a horse. The clear "halo" around each yeast is due to shrinkage of the capsule during fixation. (Mucicarmine), Results AFIP Wednesday Slide Conference - No. 26 24 April 1996, www.askjpc.org

11.5.4. Cryptococcosis in cattle

Pulmonary infections, CNS involvement and cryptococcal mastitis have been reported in cattle. In outbreaks of mastitis, the clinical signs may include anorexia, decreased milk production and enlargement of the supramammary lymph nodes. The affected quarters are usually swollen and firm. The milk may be viscid, mucoid and grayish-white, or it may be watery with flakes. Neurological signs were the presenting complaint in a bull. The clinical signs included gait abnormalities and visual impairment in both animals, as well as various other signs such as depression, circling, head pressing, anorexia and abnormal reflexes. In the bull, the brain was the only affected organ at necropsy.



Bovine; brain, coronal section. A, 4 cavities of different sizes are observed in lesions 1–4. The meninges of the mesencephalon are thickened (arrow), and a spongy granular tissue is observed in the mesencephalic aqueduct (asterisk). B, a spongy granular tissue is observed in the rostral ventral horn of left and right lateral ventricles (arrows). C, lesions 5 and 6 are observed in the frontal lobe. Lesion 5 is communicating with the meninges. D, lesion 3 (also showed in panel A) is observed in the occipital lobe. Riet-Correa et al. Journal of Veterinary Diagnostic Investigation **September 2011** vol. 23 no. 5 **1056-1060**

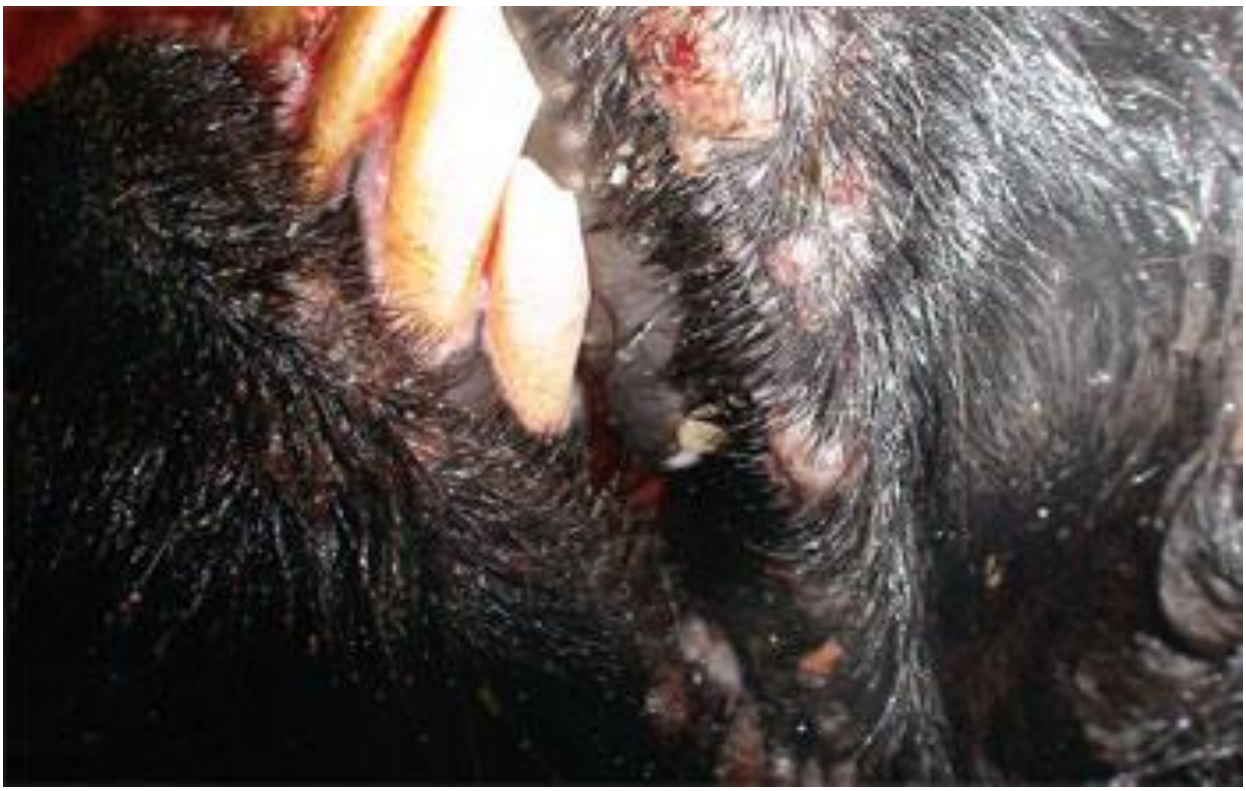


Bovine. Brain (**A**) and meninges (**B**) showing numerous yeasts with a nonstaining capsule giving an appearance of “soap bubbles” to the lesion. A few mononuclear cells are also observed, mainly in the meninges (panel B). **C**,**D**, numerous yeasts (arrows) are observed within the lesion. A fibrillar amorphous material (asterisks) is also present. Hematoxylin and eosin (panels A–C; bar = 50 µm) and alcian blue (panel D; bar = 50 µm).

11.5.5. Cryptococcosis in Camelids

Syndromes that have been reported in camelids include lower respiratory disease, CNS disease and disseminated infections.

The disease was reported in a six-year-old, black male alpaca, which started as skin eruptions on the lips. Papules soon ulcerated forming multifocal and coalescing ulcerative dermatitis that did not respond to antibiotic treatment for more than two months.

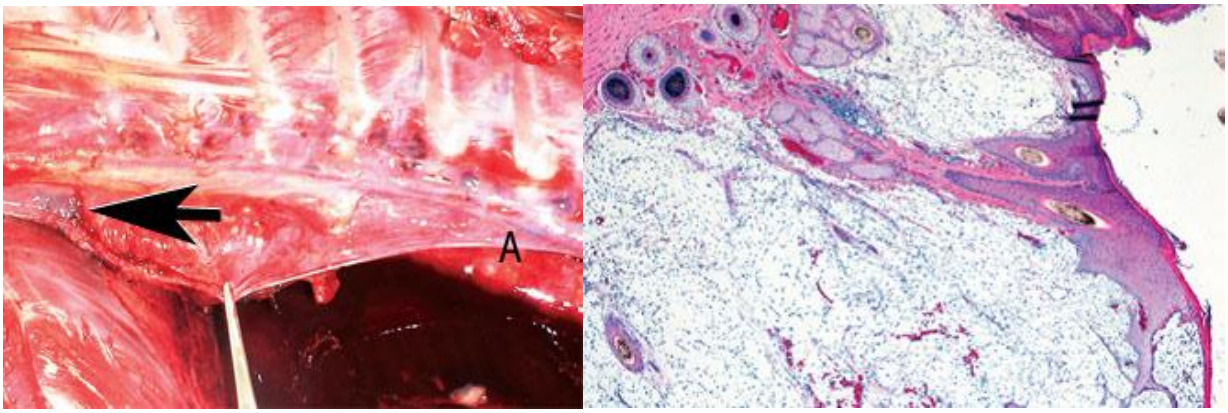


Papular to ulcerative chelitis evident on both lips of a six-year-old black alpaca infected with *Crypto gattii.*

Progressive respiratory disease ended up in complete obliteration of the left lung, which made the referring DVM suspect a neoplastic disease. Four months into the clinical disease, the animal succumbed to worsening respiratory distress with pericardial and pleural fluid associated with chronic weight loss. On necropsy, the left lung was diffusely consolidated with a large, blood-filled cystic cavity present in the middle of the cardiac lobe that was firmly adhered to the costal pleura.

Tracheobronchial and mediastinal lymph nodes were markedly enlarged and on cut surface were diffusely gelatinous with taut capsules. Also, discrete lymph nodes were visible along the ventral surface of the thoracic aorta.

Histological examination of the lungs, aforementioned lymph nodes and skin revealed mats of yeastlike organisms organized as soap bubble replacing most of the parenchyma in affected organs, including the skin. Little to no inflammation is associated with these fungal mats.

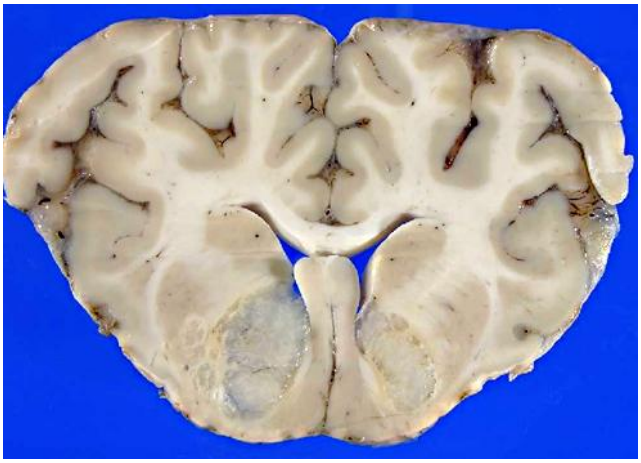


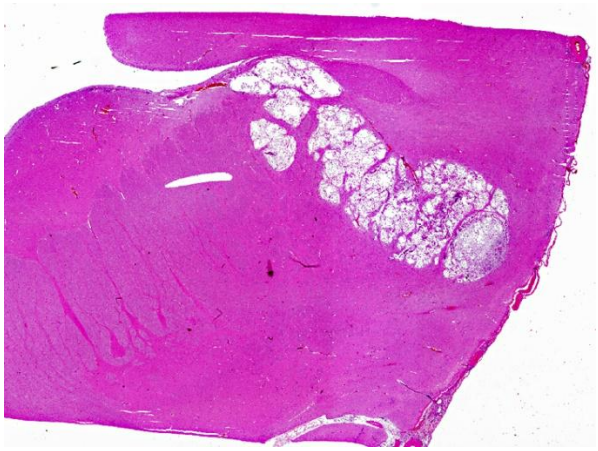
Left:Enlarged gelatinous lymph nodes (arrow) along the ventral surface of the aorta Right:Markedly enlarged skin by soap-bubble fungal mats separating the adnexa, characteristic of Cryptococcus gattii as

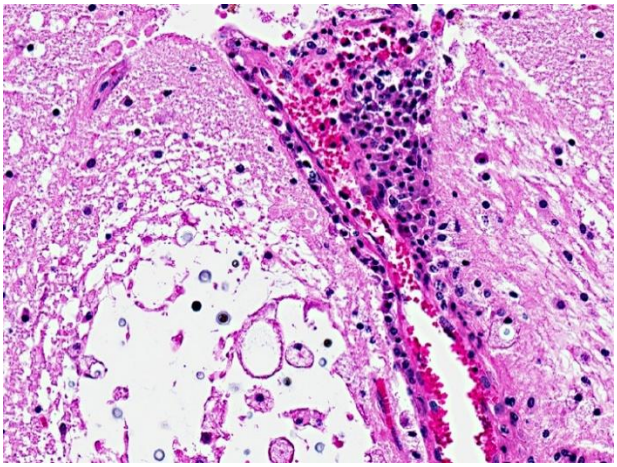
11.5.6. Cryptococcosis in elk (*Cervus Canadensis*)

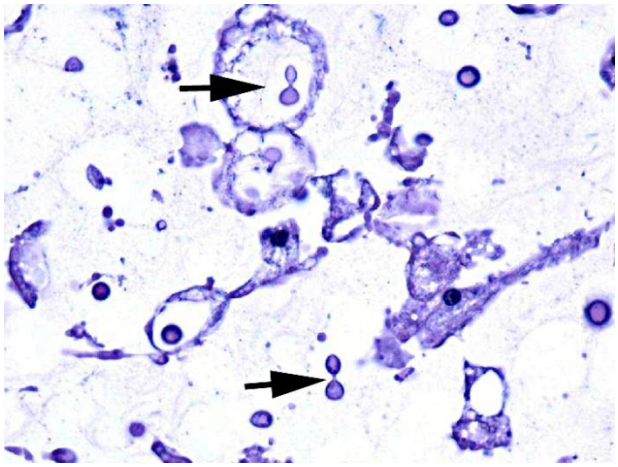
Cryptococcosis was recorded in an elk.

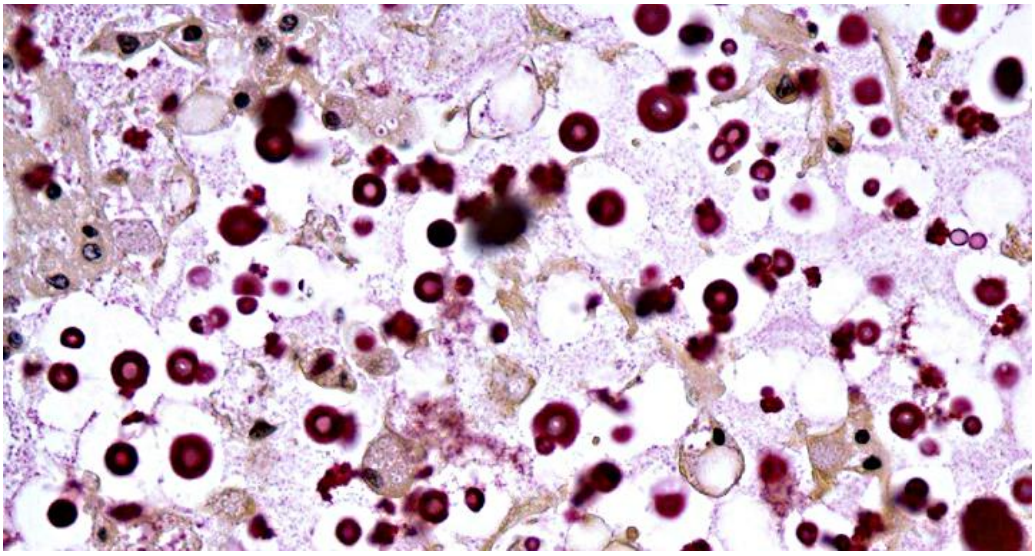
Brain: Disrupting the gray and white matter of the left aspect of the diencephalon, mesencephalon, and brain stem were multifocal to coalescing expansile inflammatory nodules composed of a central areas of necrosis surrounded by epithelioid macrophages, fewer lymphocytes and plasma cells, and scattered multinucleated giant cells. Within these foci there were numerous 5–18 μm diameter, round extracellular yeasts with a 1 μm thin wall, narrow-based budding, and a 2–8 μm thick, amphophilic, mucicarminepositive mucinous capsule. There was spongiosis of the adjacent neuropil with few infiltrating lymphocytes and plasma cells. Occasionally, there was expansion of the Virchow-Robin space by small numbers of lymphocytes, plasma cells and occasional macrophages (perivascular cuffing). The leptomeninges overlying the cerebrum and brainstem were expanded by moderate numbers of lymphocytes, plasma cells, and macrophages associated with the same yeast bodies.











Wednesday Slide Conference Online, DODVPR, Conference: 14 - 2011 Case: 02 brain - Elk

11.5.7. Cryptococcosis in goats

A case of a five year old buck showing severe neurological signs, including paraplegia and strong pain reaction to touch of the hindquarters region was described by **Stilwell and Pissarra (2014).**

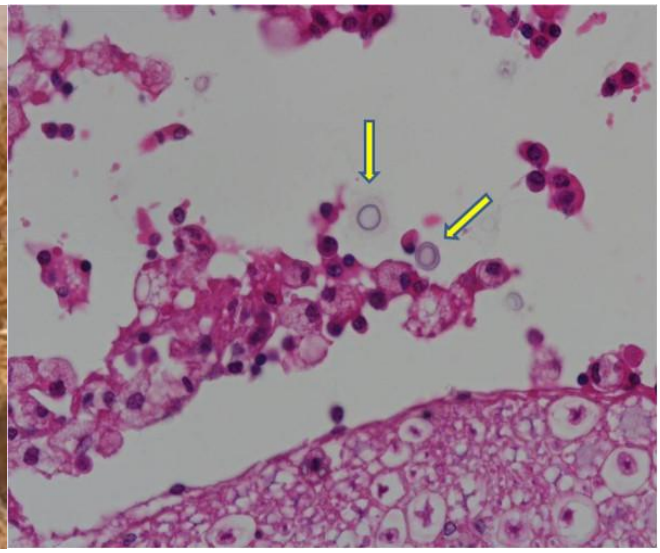
Postmortem examination revealed lumbar meningitis, lung nodules and caseous lymphadenitis lesions. Encapsulated Cryptococcus neoformans were identified from the lungs and meninges, showing that cryptococcal meningitis should be included in the differential diagnosis of goats showing paresis and hyperesthesia. The possibility of concurrent immunosuppression due to Corynebacterium pseudotuberculosis infection is raised.

Cryptococcal meningitis in a goat – a case report

Stilwell and Pissarra

BioMed Central

Stilwell and Pissarra *BMC Veterinary Research* 2014, **10**:84
http://www.biomedcentral.com/1746-6148/10/84



Neurologic signs - buck showing signs of hind limb paralysis and self mutilation (skin excoriation on abdominal flank) due to hyperaesthesia.

Histopathology exam - several round encapsulated microorganisms (yellow arrow) isolated from the lumbar meninges were identified as being *Cryptococcus neoformans* (HE, ×400).

11.5.7. Cryptococcosis in koala

Cryptococcosis is a common infectious diseases that is having an increasing impact on the health of threatened koala populations through high death rates and infertility

11.5.8. Cryptococcosis in Gorilla

Disseminated cryptococcosis was reported in a simian immunodeficiency virus-negative 27-year-old female Gorilla gorilla presenting with lethargy, progressive

weight loss and productive cough. The diagnosis was confirmed by positive lung biopsy culture, serum cryptococcal antigen, and cerebral histopathology demonstrating encapsulated yeasts. Molecular characterisation of lung culture isolate yielded Cryptococcus neoformans var. grubii. An immune-deficiency could not be demonstrated **(Mischnik *et al.*, 2014)**

11.5.9. Cryptococcosis in cheetah

Polo Leal *et al*. (2010) presented a case of a female cheetah (Acinonyx jubatus) kept in the Nacional Zoo of Havana. The animal came from South Africa. She began losing weight, and suffering asthenia, anorexia and breathing problems with abundant nasal secretion. Mycological testing of these secretions disclosed the presence of serotype B Cryptococcus gattii. Because of the origin and captive condition of the animal, it was believed that the infection had been latent for 16 months at least. They concluded that up to the present, in Cuba, all clinical Cryptococcus isolates were C. neoformans var. grubii, so it is considered that the infection was caught in the country of origin of the female cheetah. This is the first C. gattii isolate in Cuba from an animal coming from South Africa where this fungus is endemic.

11.5.10. Cryptococcosis in monkeys

Rare Monkey Succumbs to Cryptococcosis

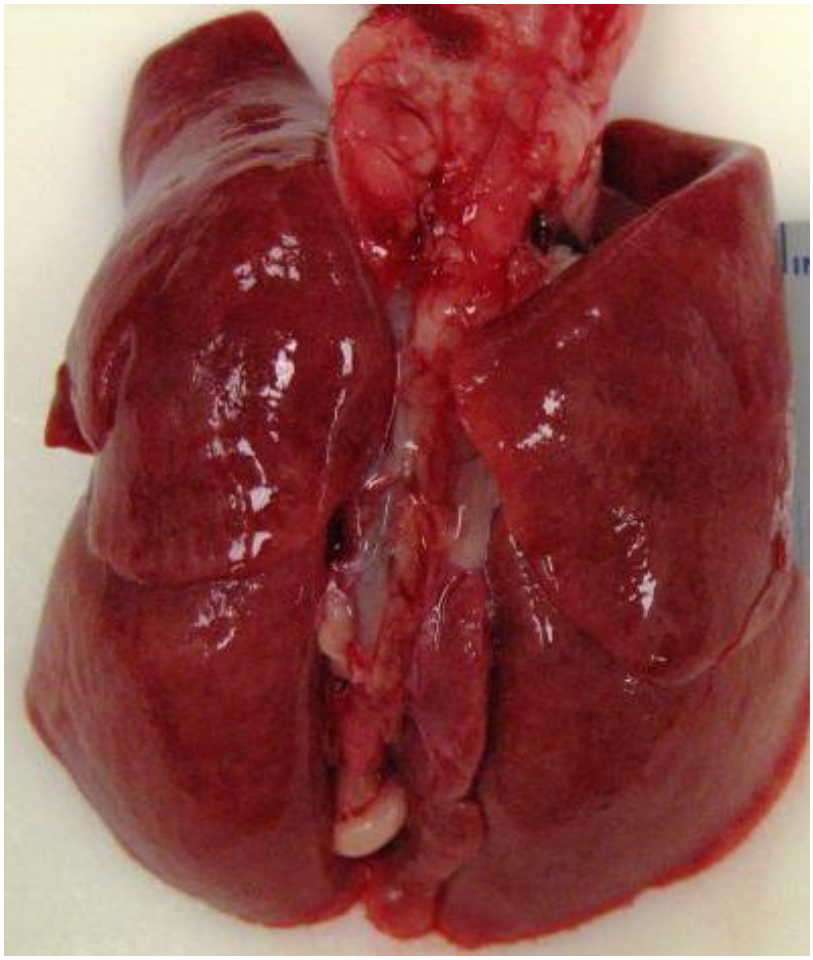
11.5.11. Cryptococcosis in ferrets

Various presentations, similar to those in other species, have been seen in ferrets. Some affected ferrets had disseminated disease, but localized masses (e.g., on the nose, spine or digit) were reported in others. Lymphadenopathy and respiratory signs are reported to be common in this species. Neurological signs, meningitis and ocular signs (chorioretinitis, blindness) have also been documented. Gastrointestinal signs were prominent in one ferret with disseminated disease; the presenting signs included lethargy, weight loss, diarrhea and retching, as well as dyspnea.

Morera et al. (2011) reported a domestic ferret (Mustela putorius furo) with lymphadenopathy and acute bilateral blindness. Cytologic evaluation and biopsy of an affected lymph node revealed pyogranulomatous lymphadenitis with intralesional yeast consistent with Cryptococcus sp. Subsequent studies demonstrated Cryptococcus gattii serotype B VGI/AFLP4 as the causative agent. Postmortem examination revealed disseminated cryptococcosis with prominent neurologic involvement. Nasal swabs of other ferrets and humans from the same household revealed that two ferrets and two humans to be asymptomatic carriers of the same strain of cryptococcus as the necropsied ferret.

Ropstad et al. (2011) described a case of bilateral exudative chorioretinitis in an 18-month-old male neutered ferret (Mustela putorius furo) with a generalized Cryptococcus gattii infection confirmed by PCR.. The postmortem histopathology confirmed the initial diagnosis of cryptococcosis and the presence of intraretinal Cryptococcus spp.

Morera *et al.* (2104) described two cases of cryptococcosis in ferrets in the Iberian Peninsula and Balearic Islands and documents a relationship of ferret cryptococcosis with environmental isolates in the same locations



Ferret lung cryptococcus pneumonia .ocw.tufts.edu

11.5.12. Cryptococcosis in reptiles

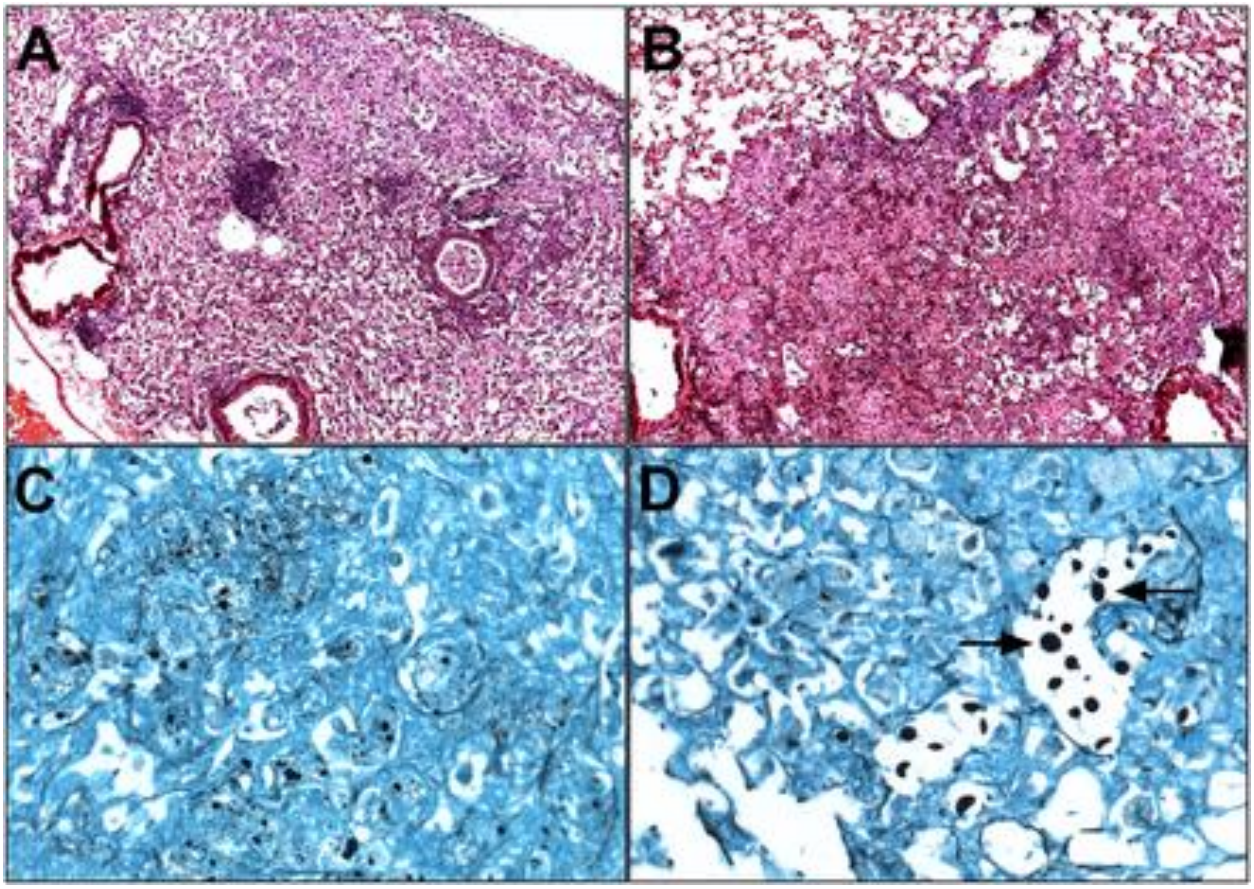
A few cases of cryptococcosis have been described in reptiles, including lizards and snakes.

11.5.13. Cryptococcosis in marine mammals

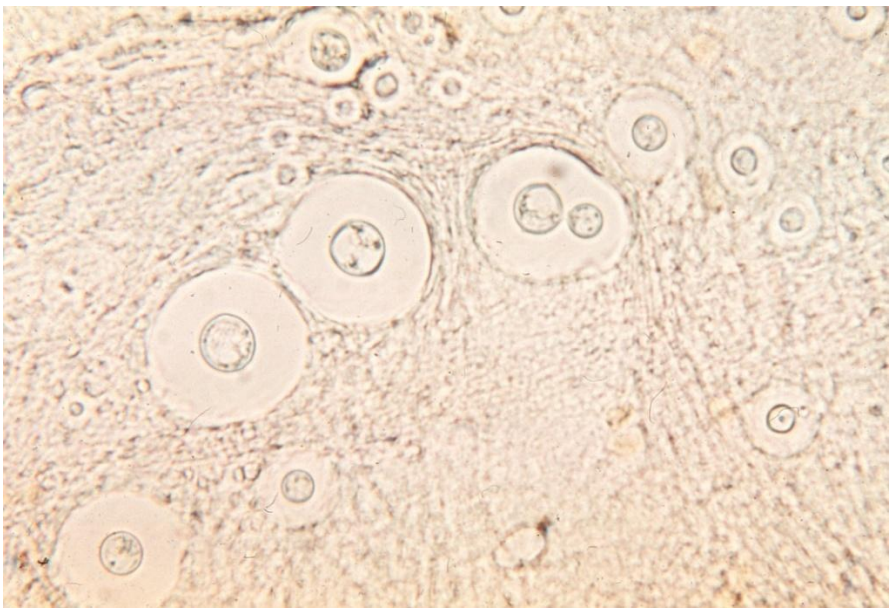
Mcleland *et al.* (2012) recovered *Cryptococcus albidus*, from a juvenile California sea lion (Zalophus californianus) rescued near San Francisco Bay, California. Yeast morphologically consistent with a Cryptococcus sp. was identified histologically in a lymph node and *C. albidus* was identified by an rDNA sequence from the lung. Infection with *C. albidus* was thought to have contributed to mortality in this sea lion, along with concurrent bacterial pneumonia.Cryptococcus albidus should be considered as a potential pathogen with a role in marine mammal morbidity and mortality.

Rotstein et al. (2010) described cutaneous nodules and enlarged lymph nodes in a spinner dolphin (Stenella longirostris). Numerous Cryptococcus gattii VGI yeast were observed in multiple organs with minimal inflammation. This case represents the first reported infection of C. gattii in a dolphin from Hawaii.

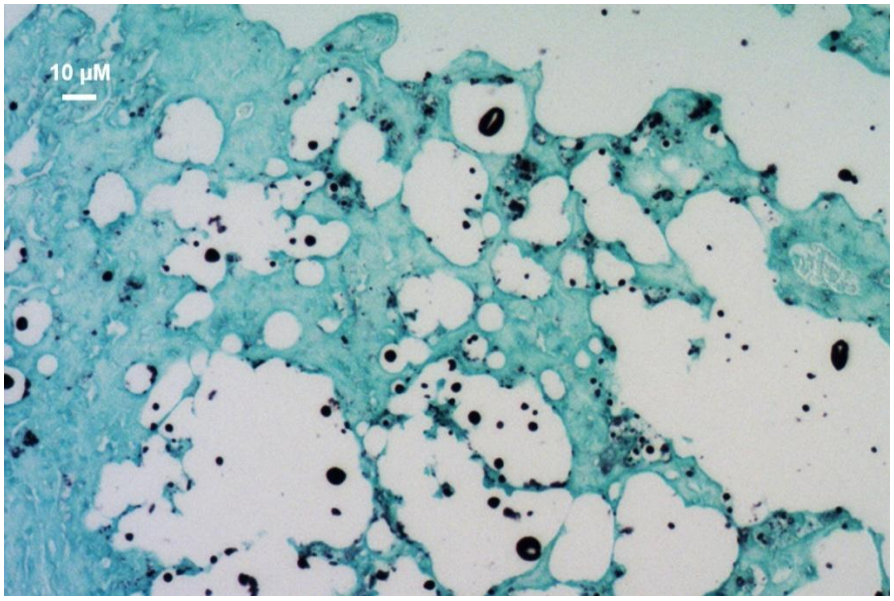
11.5.14. Cryptococcosis in mice



mbio.asm.org
X-Linked Immunodeficient Mice Exhibit Enhanced Susceptibility to Cryptococcus neoformans Infection



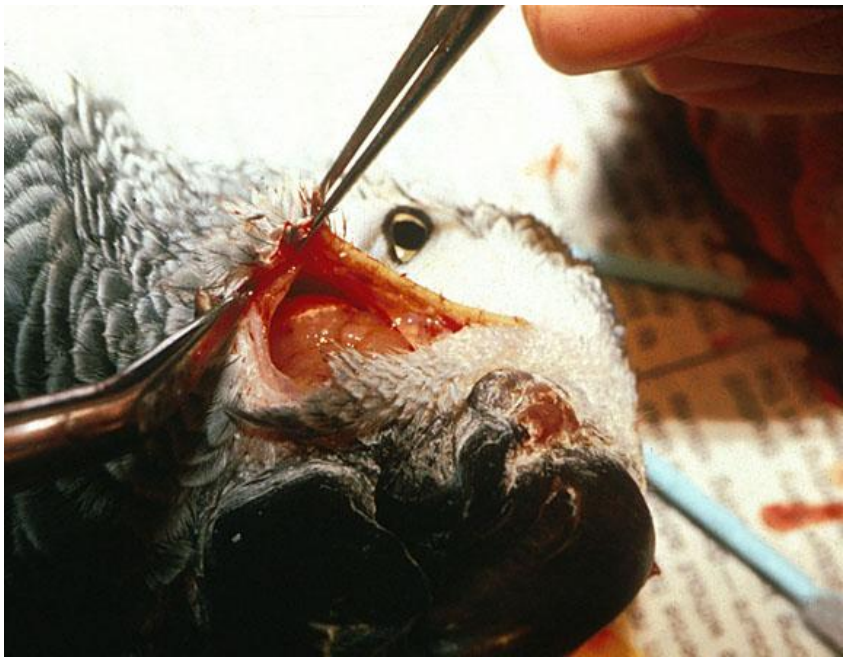
Cryptococcus neoformans in mash preparation of mouse brain. Note large capsule and narrow budding isthmus. www.microbeworld.org



Images of Cryptococcus neoformans taken in the course of the Texas A&M team's research show silver-stainedCryptococcus cells -- indicated in black -- in mouse lung tissue (above) and a scanning electron microscope (SEM) picture of the yeast cells (below).
(Credit: Xiaorong Lin, Texas A&M University.)

11.5.15. Cryptococcosis in Birds

Some psittacine birds with cryptococcosis have signs of an upper respiratory tract obstruction. These birds often have proliferative lesions, which may resemble neoplasia, around the beak or nares. The infection can progress to involve structures close to the nasal cavity, such as the rhamphotheca, nasopharynx, palate and sinuses. No internal organs, including the lower respiratory tract and CNS, were affected in most of these cases. However, severe invasive or disseminated disease affecting the lung, air sacs, CNS or other internal organs has been reported in a few psittacines. Cryptococcosis seems to be very rare in pigeons. One racing pigeon developed a localized subcutaneous swelling below the eye. Localized disease was also reported in another pigeon, while a third bird had widely disseminated lesions. Fatal C. gattii infections have been reported in captive kiwis. Extensive granulomatous pneumonia was found in two of these birds at necropsy, while the third had disseminated disease involving the heart, kidneys and proventriculus.



Cryptococcal rhinitis caused by *Cryptococcus neoformans* var. *gattii* in an African grey parrot from the Adelaide Zoo.

11.5. 16. Cryptococcosis in amphibians

Amphibian populations are declining worldwide and a major cause is a deadly fungus thought to be spread by bullfrogs, but a two-year study shows they can also die from this pathogen, contrary to suggestions that bullfrogs are a tolerant carrier host that just spreads the disease.

. Bullfrogs may help spread deadly amphibian fungus, but also die from it.
Credit: Image courtesy of Oregon State University

12. Diagnosis of cryptococcosis

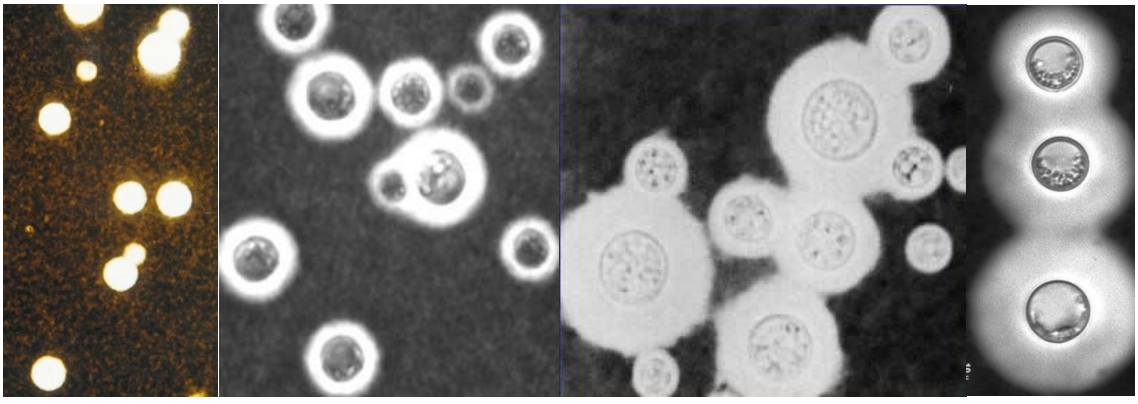
The workup in patients with suspected cryptococcosis includes the following:

- **Cutaneous lesions**: biopsy with fungal stains and cultures
- **Blood**: fungal culture, cryptococcal serology, and cryptococcal antigen testing
- **Cerebrospinal fluid**: India ink smear, fungal culture, and cryptococcal antigen testing
- **Urine and sputum:** cultures, even if renal or pulmonary disease is not clinically evident
- **Cryptococcal pneumonia**: culture of bronchoalveolar lavage washings

12.1. Direct microscopic examination of India ink preparation

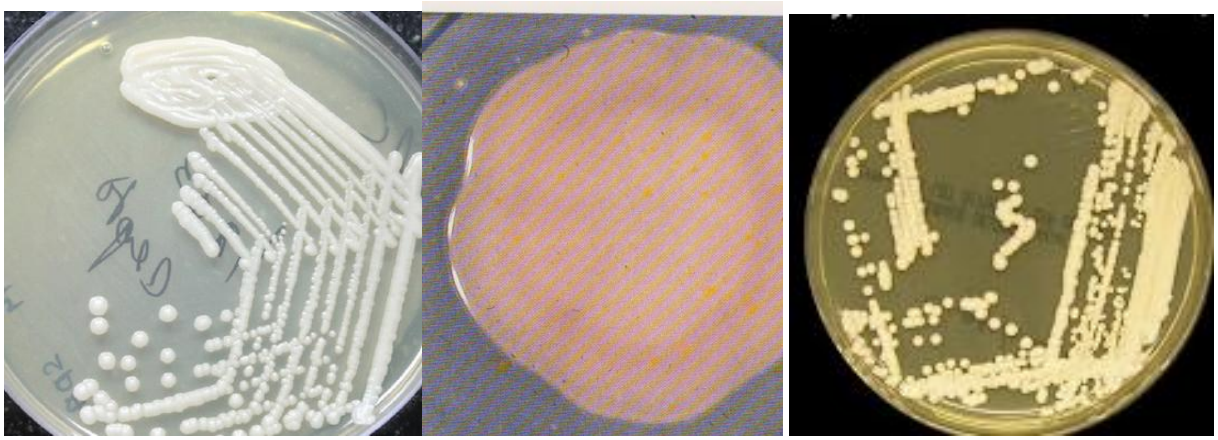
Preliminary diagnosis of cryptococcal infection is made by direct microscopic examination of india ink preparations of samples.

Method: Mix the specimen with a small drop of India ink on a clean glass slide. Place a cover slip over the smear and press gently. The preparation should be brownish, not black. Examine the smear microscopically (100X) for the presence of encapsulated cells as indicated by clear zones surrounding the cells.



12.2. Isolation and identification

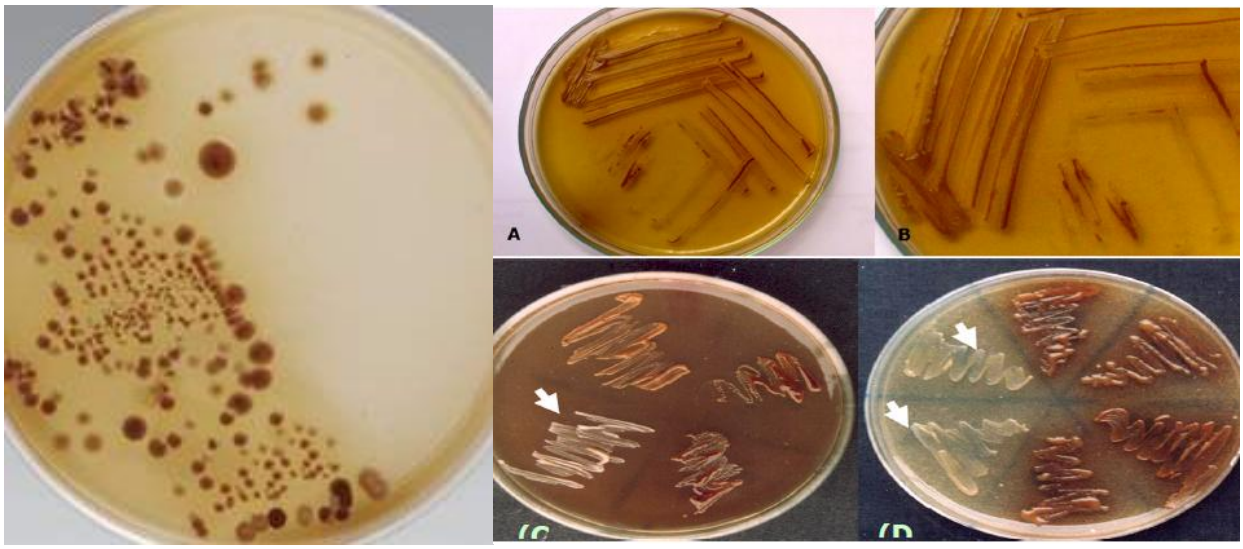
12.2.1. Definitive diagnosis is confirmed by the culture of specimens, often the cerebrospinal fluid (CSF) or blood, and sometimes in respiratory secretions. *Cryptococcus neoformans* and *C. gattii* grow well at 37°C. On Sabouraud dextrose agar colonies appear soft, creamy, opaque in 3-5 days, then colonies become mucoid and creamy to tan.



Cryptococcus colonies on Sabroud's dextrose agar

12.2.2. Demonstration of brown colour effect

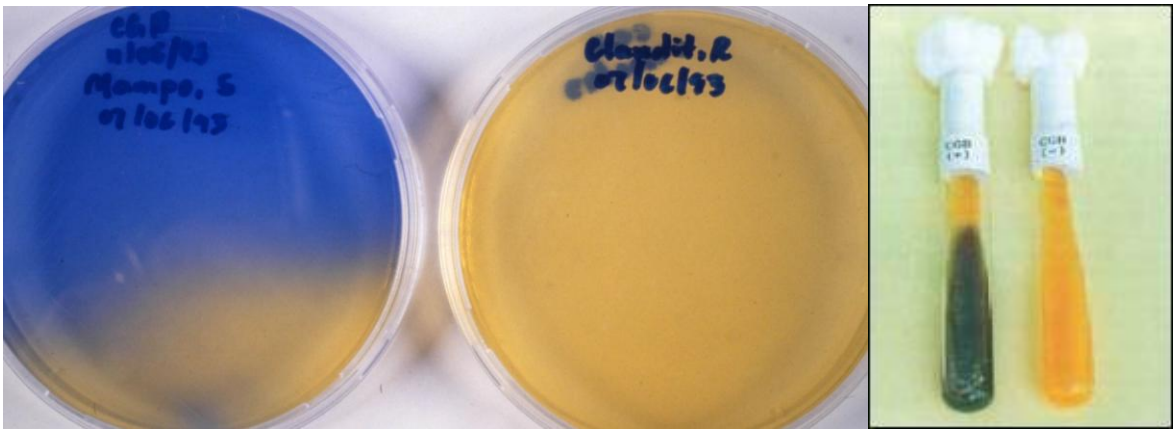
Cryptococcus colonies are brown on bird seed agar, modified tobacco and Eucalyptus leave extract agar as well as on Pal′s medium. Other yeasts develop white to creamy colonies.



Colonies on bird seed agar Colonies on mod.Tobacco (A), Eucalyptus (B-C) and Pal′s media showing BCE

12.2.3. Differentiation of *C. gattii* and *C. neoformans* on canavanine glycine bromothymol blue (CGB)

On canavanine gjycin bromthymol blue (CGB) medium, *Cryptococcus neoformans* develop non-coloured coloniesand , while *C. gattii* develops blue colonies.



Blue colonies of *C. gattii* (left) and non-coloured colonies of *C. neoformans* (right)

12.2.4. Biochemical identification

Cryptococcus neoformans and *C. gattii* do not ferment sugars, but assimilate several sugars such as glucose, galactose, sucrose, maltose and inositol, but not lactose or nitrate and hydrolyses urea.

	Assimilation									Color of culture	St prod	37°C
	Su	Ma	La	Ce	Me	St	Rh	Er	Mz			
Nitrate-positive species:												
Cr. albidus var. *albidus*	+	+/w	v	+	v	v	v	–		hyaline	+/w	–(+)
Cr. albidus var. *aerius*	+	+	+	+	+	v	v	–		hyaline	–	–
Cr. elinovii	–	+	+	+	–	+	+	–	+	hyaline	+	–
Cr. infirmo-miniatus	+	+	+ s/w	+	–	+	v	–		red	+	–
Cr. kuetzingii	+	–	v	+	–	–	–	–		hyaline	+	–
Cr. macerans	+	+/w	+/w	+	+w/–	+s	–(+)	+		red	+	–
Cr. terreus	–	+/w	+	+	–	+/w	+	–	–	hyaline	+	–
Nitrate-negative species:												
Cr. ater	+	+	+	+	–	+/w	+	–		hyaline[1]	+	–
Cr. dimennae	+	–	+	+	–	–	+	–		hyaline	+	–
Cr. flavus	+	+	+w	+	+	+w	+w	+		sl. yellow	–	–
Cr. gastricus	–	+	+	+	–	+	+	–		hyaline	+	–
Cr. heveanensis	+	+	+	+	–	+w	+	+		hyaline	+	–
Cr. hungaricus	+	+	v	+	v	+w	+/w	–(+)		red	+	–
Cr. laurentii	+	+	+	+	+	+/w	+(w)	+		hyaline	+	v
Cr. luteolus	+	+	–/w	+	+	+w	+/w	+		hyaline	+	–
Cr. magnus	+	+	+	+	–	+w	–	–		hyaline	+w	–
Cr. melibiosum	–	–	+	+	+	–	–	–		hyaline	+w	+w
Cr. neoformans var. *neoformans*	+	+	–	+/w	–	v	+	v		hyaline	+/w	+
Cr. neoformans var. *gattii*	+	+	–	+/w	–	v	+	v		hyaline	+/w	+
Cr. skinneri	–	–	–	+	–	–	+	–/w		hyaline	+w	–
Cr. uniguttulatus	+	+	–	–	–	+w	+/w	–		hyaline	+/w	–

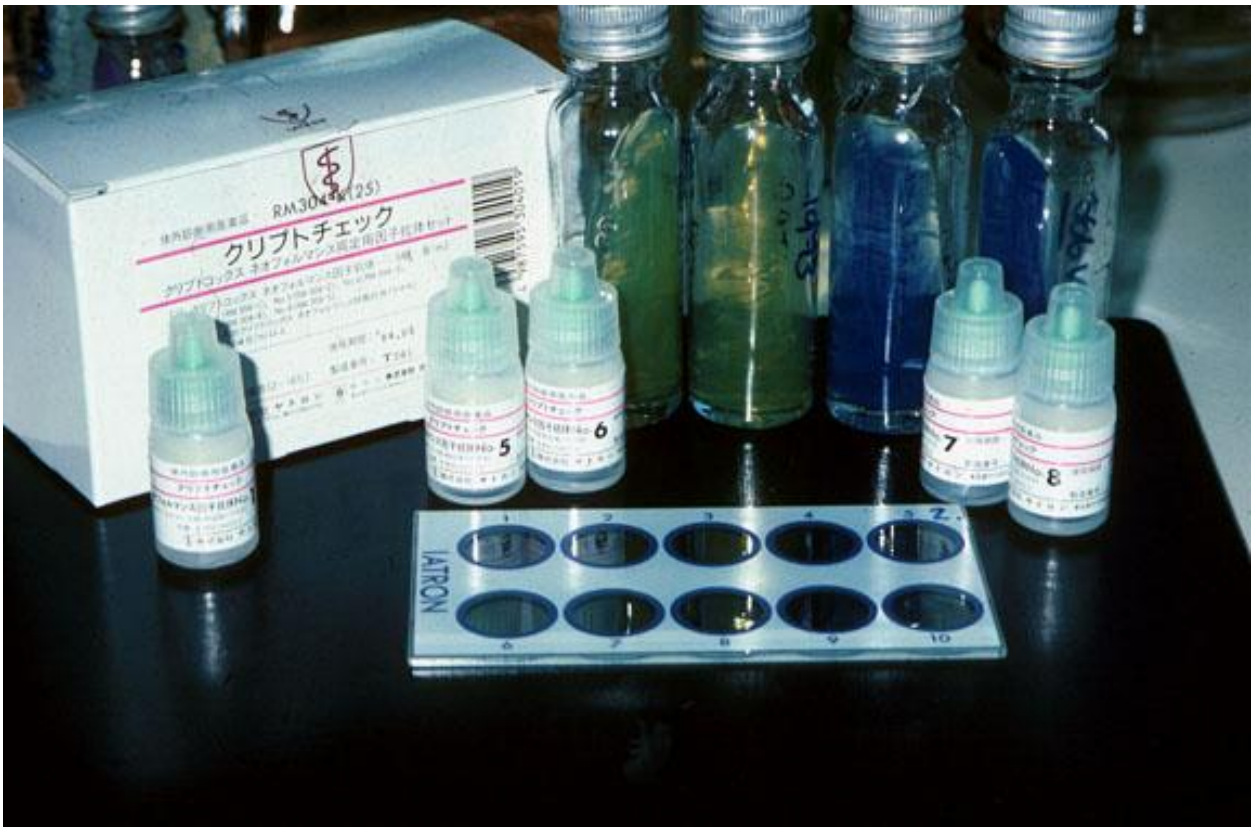
Su = sucrose, Ma = maltose, La = lactose, Ce = cellobiose, Me = melibiose, St = soluble starch, Rh = L-rhamnose
Er = erythritol, Mz = melezitose, St prod = starch formation, CGB = CGB agar turns blue.
[1] After several weeks growth dark brown or black on certain media.

12.2.5. Serotyping of *Cryptococcus neoformans* and *C. gattii*

Method: To determine the antigenic formulas of *Cryptococcus* species, equal volumes of factor serum and heat-killed cell suspension are mixed on a glass slide and rotated for 5 min, and then the results of agglutination are observed. The formation of aggregates within 5 min is considered positive. Smaller clumps are recorded as weakly positive. PSS is used for a negative control.

Group and species	Strain	Slide agglutination with factor sera[a]								Serotype
		1	2	3	4	5	6	7	8	
None										
C. neoformans										
Serotype A	CDC551	+	+	+	–	–	–	+	–	A
Serotype D	NIH52	+	+	+	–	–	–	–	+	D
Serotype A-D	CBS132	+	+	+	–	–	–	+	+	A-D
Serotype B	NIH112	+	+	–	+	+	–	–	–	B
Serotype C	NIH18	+	–	–	+	–	+	–	–	C

[a]+, positive; +w, weakly positive; –, negative.



Iatron serotyping kit can be used to serotype isolates of *Cryptococcus neoformans*.

12.2.6. Molecular typing

Numerous molecular techniques have been applied to subtype *C. neoformans* and *C. gattii* strains, only three methods were proved to produce comparable results: PCR Fingerprinting, AFLP, and MLST. M13 PCR Fingerprinting and *URA5* RFLP:

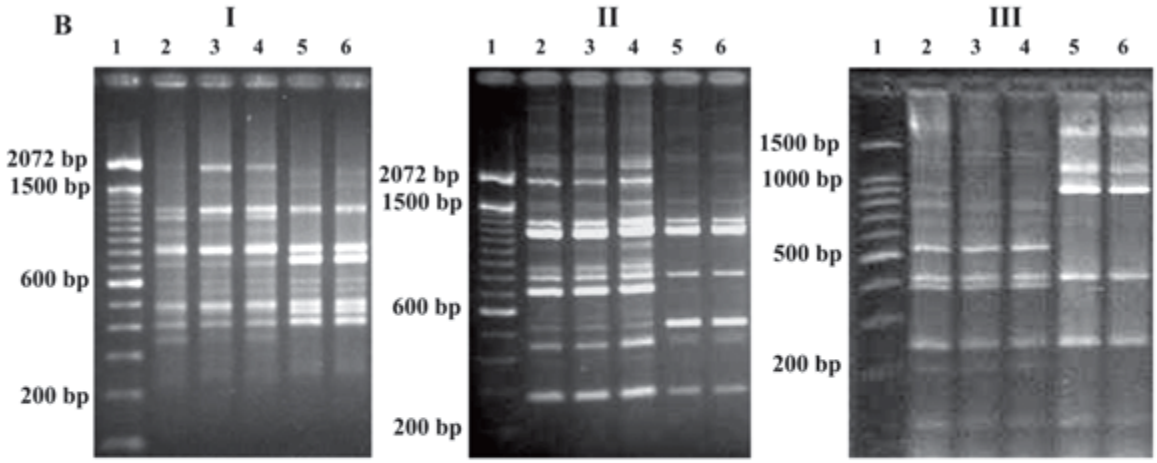


Fig. 2 - A. PCR fingerprinting with primer $(GACA)_4$ (AI) and PLB1-RFLP with *AvaI* (AII) patterns of *C. neoformans*. Lane 1*, molecular weight marker; 2-5, molecular types VNI, VNII, VNIII, VNIV; 6-9, molecular types VGI, VGII, VGIII, VGIV – **B.** Representative gel of $(GACA)_4$-PCR fingerprinting (BI), Primer 6-RAPD (BII) and PLB1-RFLP (BIII) from clinical isolates of *C. neoformans*. Lane 1*, molecular weight marker; 2-4, clinical isolates 377, 379 and 387 (molecular type VNI, serotype A); 5-6, clinical isolates 382 and 384 (molecular type VNII, serotype A). * Molecular weight marker (Gibco 100bp) to the $(GACA)_4$-PCR fingerprinting (AI and BI) and Primer 6-RAPD (BII). Molecular weight marker (Promega 100bp) to the PLB1-RFLP (AII and BIII).

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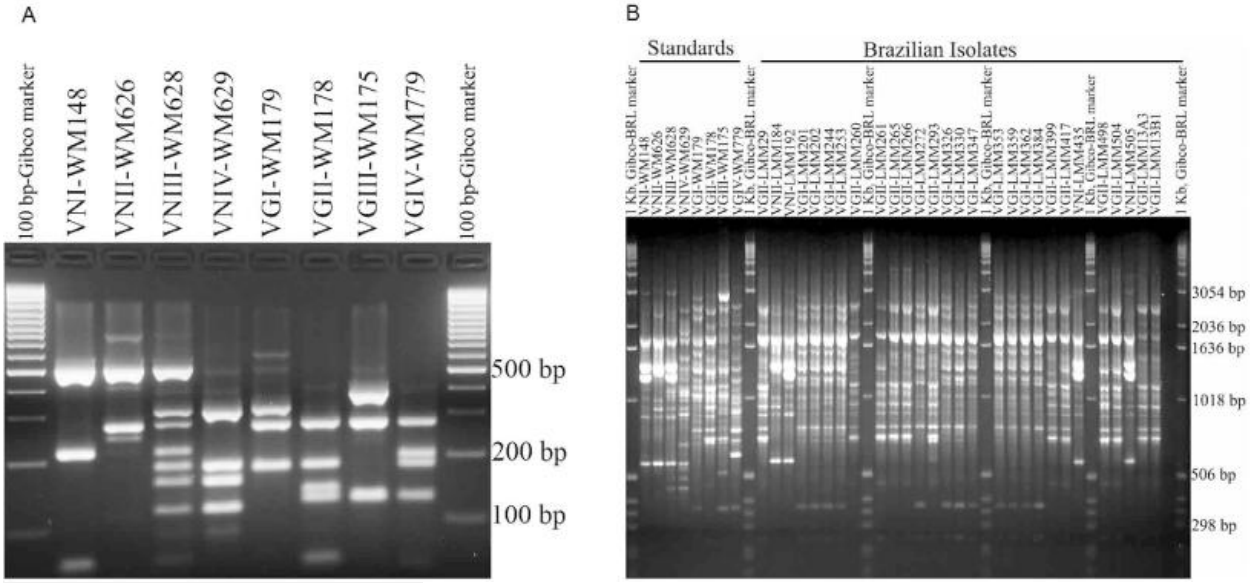


Memórias do Instituto Oswaldo Cruz -.www.scielo.br 2A demonstrates the RFLP profiles of the eight molecular types (VNI-VNIV, VGI-VGIV) resulting from the URA5-RFLP technique using the standards isolates, ..

12.3. Serological diagnosis of cryptococcosis

Cryptococcal antigen from cerebrospinal fluid is the best test for diagnosis of cryptococcal meningitis in terms of sensitivity. Rapid diagnostic methods to detect cryptococcal antigen by latex agglutination test, lateral flow immunochromatographic assay (LFA), or enzyme immunoassay (EIA).

12.3.1. Cryptococcal antigen (CRAG) detection

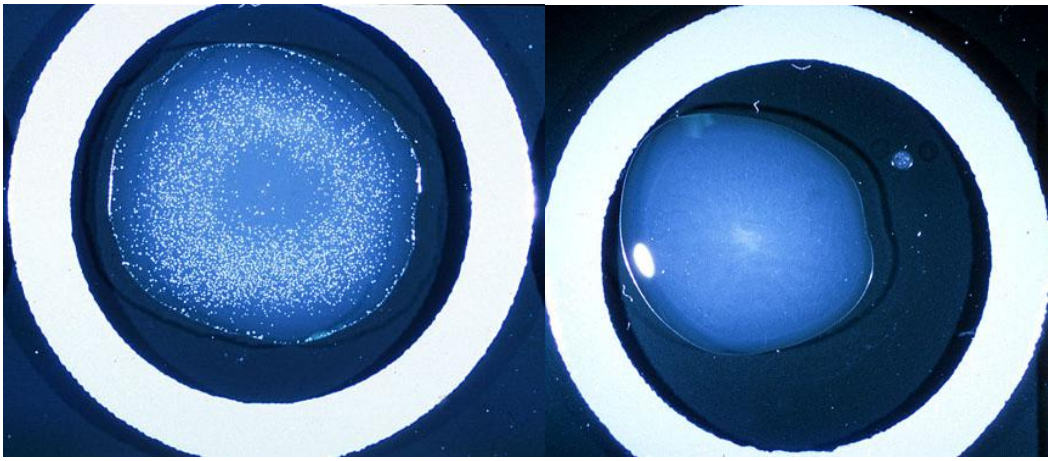
i. Rapid latex agglutination

(latex particles coated with polyclonal cryptococcal capsular antibodies), a direct antigen detection assay, is performed on blood, cerebrospinal fluid (CSF), or body fluids such as pleural fluid and bronchoalveolar lavage samples.

Latex agglutination kits :

- Crypto-La (International Biologicals NJ) polyclonal Ab
- Myco-immune (American Microscan NJ) polyclonal Ab
- IMMY (Immuno-Mycologics, Okla) polyclonal Ab
- CALAS (Meridian diagnostics, Ohio) polyclonal Ab
- Eiken tet (Eiken Co, Tokyo)
- Pastorex Cryptococcus (Sanofi Diagnostic Pasteur, France) monoclonal Ab
- Murex Cryptococcus (Murex Diagnostics, Ga) IgM based monoclonal

This qualitative and semiquantitative test detects capsular polysaccharide antigens of *Cryptococcus neoformans* in serum and cerebrospinal fluid. It utilizes latex particles coated with anticryptococcal globulin. This latex reacts with the cryptococcal polysaccharide antigen, causing a visible agglutination. The system contains sufficient reagents to perform 50 to 100 patient tests and includes sample diluent, detection latex, control latex, antibody control, negative and positive controls, pronase, test slide, reaction reference photograph and package insert.



Positive latex test Negative latex test

Interpretation of the test

- Sensitivity and specificity are 93% to 100% and 93% to 98%, respectively.
- False-positive results occur at a rate of up to 0.4% due to the presence of rheumatoid factor or infections with *Trichosporon beigelii*, *Stomatococcus mucilaginosus*, *Capnocytophaga canimorsus*, or *Klebsiella pneumoniae*.
- False-negative results are caused by a low fungal burden.

Serum CRAG (sCRAG)

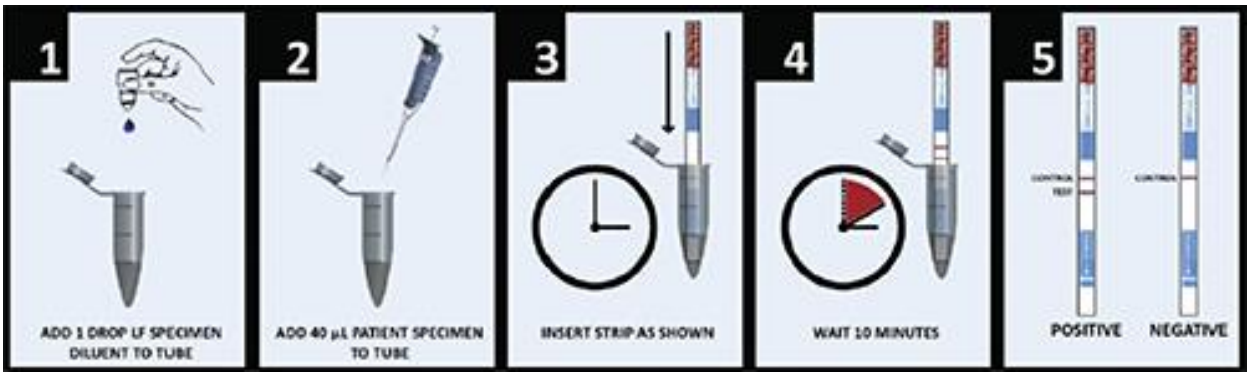
- is positive in >99% of HIV-positive patients with cryptococcal meningitis, usually at titres >1:2048, although significant disease can be present below this.
- In pulmonary cryptococcosis, sCRAG is usually negative if the infection is confined to the lung.
- A positive result may indicate disseminated disease.
- The presence of CRAG in the pleural fluid can be useful when cultures are negative.

ii. Cryptococcal antigen lateral flow assay.

- The LFA is a point-of-care dipstick test that uses gold-conjugated, monoclonal antibodies impregnated onto an immunochromatographic test strip to detect cryptococcal capsular polysaccharide glucuronoxylomannan antigen (CRAG) for all 4 *C. neoformans* serotypes (A–D)
- If cryptococcal antigen is present in a specimen, suspended, gold-conjugated antibodies bind to the antigen.
- The gold–antibody–CRAG complex migrates by capillary action up the test strip, interacts with immobilized monoclonal antibodies against CRAG, and forms a red line.
- The LFA kit contains immunochromatographic test strips, positive controls, and assay diluent that can be stored at room temperature for ≤2 years.

Method

- One drop of diluent (≈40 µL) is added to a container of 40 µL of patient specimen.
- The dipstick is inserted into the container and incubated at room temperature for 10 min.



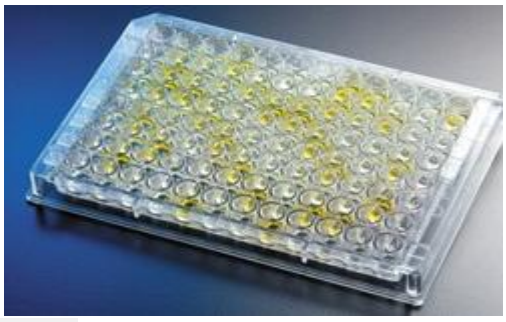
lateral flow assay (Immuno-Mycologics, Norman, OK) (source: Immy CrAg LFA package insert; ...

iii. Cryptococcal Antigen enzyme immunoassay (CrAg EIA)

The detection of *Cryptosporidium* antigen by enzyme immunoassay is a highly sensitive and specific test. It is extremely useful in cases where microscopic techniques are inconclusive.

ELISA kits

- **Merodoan Premier ELSA kits (Polyclonal capture, monoclonal detector)**
- **Monoclonal Ab of different isotypes for both capture and detection**
- **Biotin amplified sandwich ELISA**



Cryptococcus neoformans Antigen Test Kit (ELISA) The Premier® Cryptococcal Antigen enzyme immunoassay (EIA) is a screening or a semi-quantitative test system for the detection of capsular polysaccharide antigens of *Cryptococcus neoformans* in serum and cerebrospinal fluid (CSF).

The Cryptococcal Antigen enzyme immunoassay (CrAg EIA) is a qualitative or semi-quantitative (titration) test system for the detection of capsular polysaccharide antigens of Cryptococcus species complex (Cryptococcus neoformans and Cryptococcus gattii) in serum and cerebrospinal fluid (CSF). It is a direct

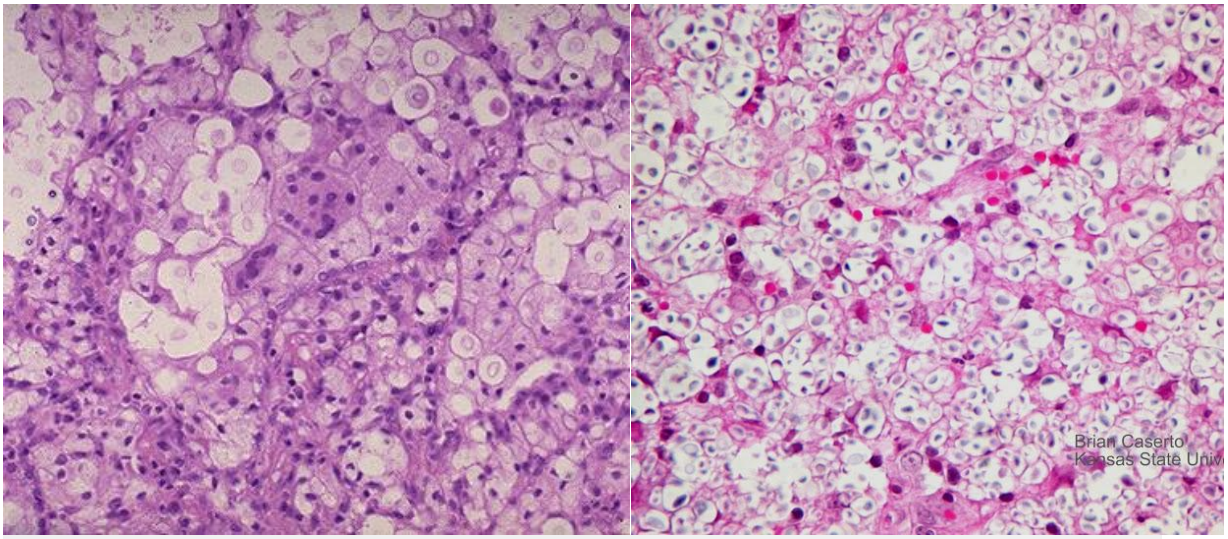
immunoenzymatic sandwich microplate assay, and has been considered the most sensitive and specific EIA for the detection of cryptococcal antigen.

12.3.2. Detection of Cryptococcus antibodies

Cryptococcal antibodies are not helpful in diagnosis because of their poor sensitivity and specificity

12. 4. Histopathology

i) Hematoxylin and eosin stain shows lightly basophilic cell wall surrounded by a clear zone.
ii) Cryptococcus neoformans will stain with Periodic acid–Schiff or silver methenamine
iii) Mucicarmine stains the capsule - shows clear zone containing carminophilic material. Alcian blue also stains the capsule
iv) A combined Periodic acid–Schiff - alcian blue stain contrasts the cell wall and capsule.
v) Capsule-deficient variety is demonstrated by:
(a) Immunofluorescent antibody.
(b) Fontana-Masson silver staining



Lung - nontumor - Cryptococcus neoformans Nasal cryptococcosis in a cat | veterinary pathology forum
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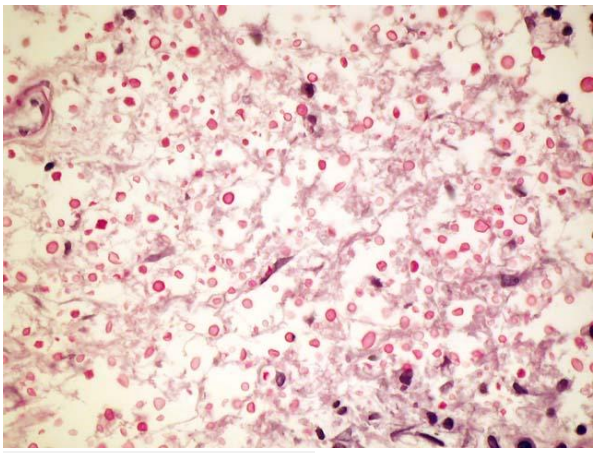
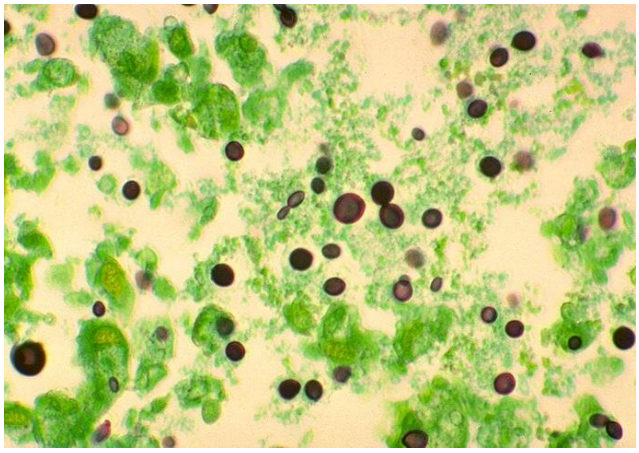
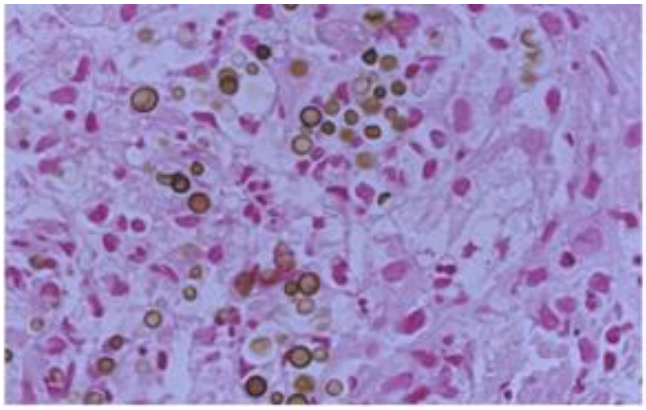
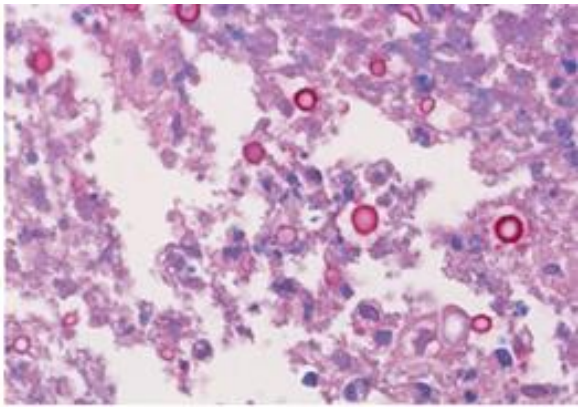




ww.pathologyoutlines.com Cryptococcal meningitis Cryptococcus in the lung. Methenamine silver stain





Cryptococcal prostatitis. Left:Fontana-Masson stain demonstrates melanin in the cell wall. Right: The mucoid capsule of the pathogen is stained with mucicarmine.

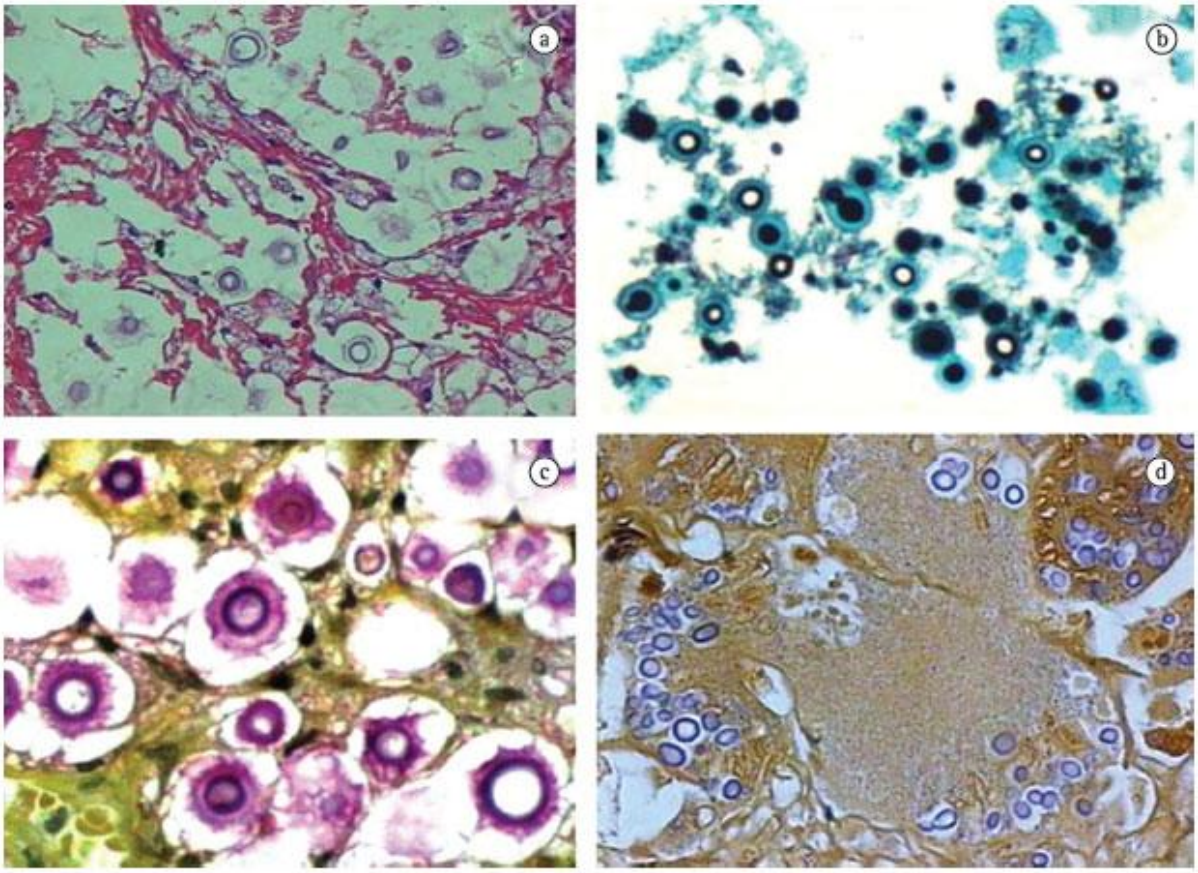


Figure 5 – Structures arranged extracellularly. Absence of inflammatory response. Note complete destruction of tissue architecture—minimally reactive histological pattern. (a) Round or ovoid fungal elements presenting budding on narrow base. H&E; magnification, ×40. (b) Microorganisms exhibiting evident mucicarminophilic capsular structure stained magenta. Grocott-Gomori; magnification, ×40. (c) Capsule-deficient C*ryptococcus* spp. Reactivity of the cryptococcal cell wall containing pigments of melanin. Mayer's mucicarmine; magnification, ×40. (d). Fontana-Masson; magnification, ×40.

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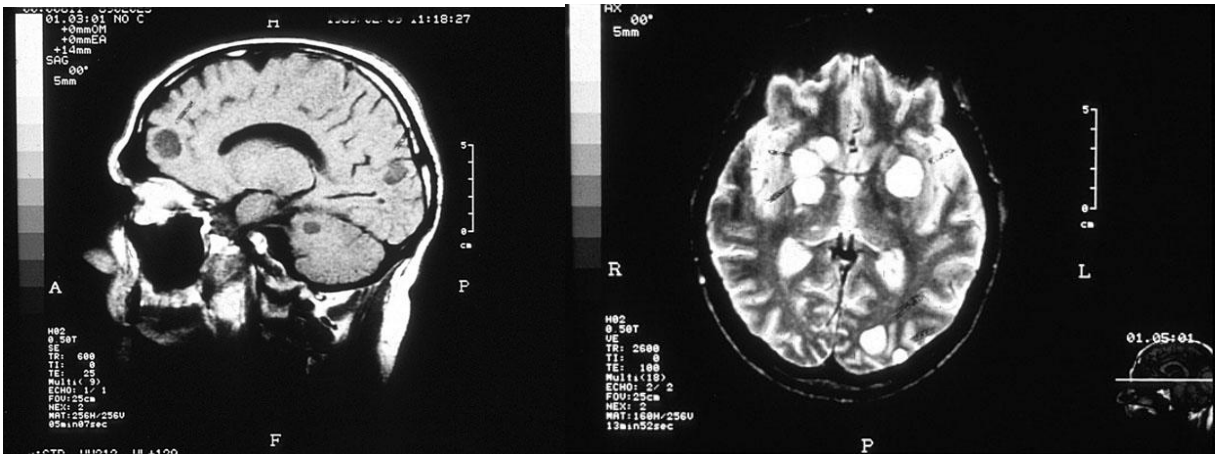
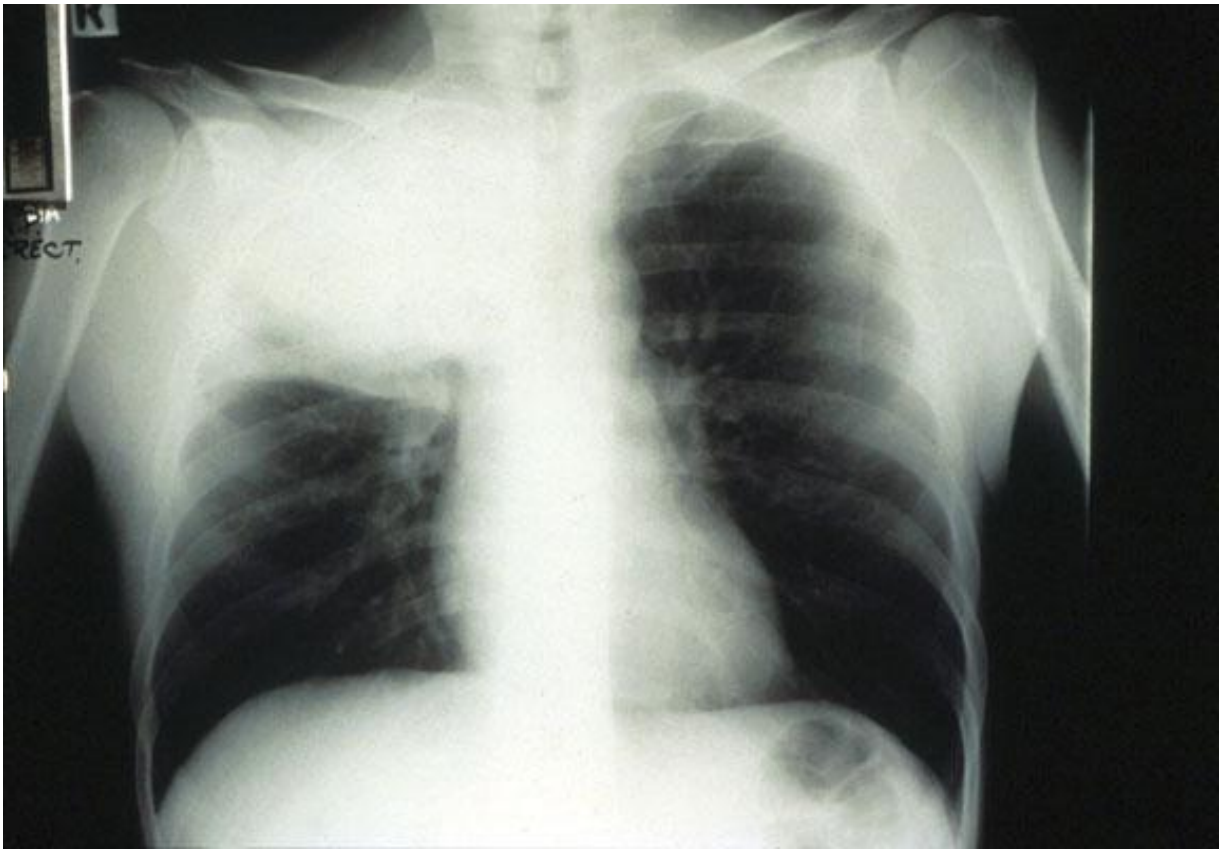
12.5. Radiology



MRI scans showing multiple cryptococcomas [white masses] in the brain. (Courtesy Prof. Tania Sorrell, Sydney NSW).


X-ray showing pulmonary cryptococcal infection [right upper lobe]. (Courtesy Prof. Tania Sorrell, Sydney NSW).

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