FUNGAL AGENTS CAUSING INFECTION OF THE LUNG

Microbiology Lectures of the Respiratory Diseases

Prepared by: Rizalinda Sjahril

Microbiology Department
Faculty of Medicine
Hasanuddin University

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OVERVIEW OF CLINICAL MYCOLOGY

- Among 150.000 fungi species only 100-150 are human pathogens → 25 spp most common pathogens
- Majority are saprophytic Living on dead or decayed organic matter
- Transmission

  Person to person (rare)

  SPORE INHALATION OR ENTERS THE TISSUE FROM TRAUMA

  Animal to person (rare) – usually in dermatophytosis

OVERVIEW OF CLINICAL MYCOLOGY

- Human is usually resistant to infection, unless:
  - Immunoscompromised (HIV, DM)
  - Serious underlying disease
  - Corticosteroid/antimetabolite treatment
- Predisposing factors:
  - Long term intravenous cannulation
  - Complex surgical procedures
  - Prolonged/excessive antibacterial therapy

OVERVIEW OF CLINICAL MYCOLOGY

- Several fungi can cause a variety of infections: clinical manifestation and severity varies.
- **True pathogens** -- have the ability to cause infection in otherwise healthy individuals

Opportunistic/deep mycoses which affect the respiratory system are:

- **Cryptococcosis**
- **Aspergillosis**
- **Zygomycosis**

True pathogens are:

- **Blastomycosis**
- **Coccidioidomycosis**
- **Histoplasmosis**

} Seldom severe
Treatment not required unless extensive tissue destruction compromising respiratory status
Or extrapulmonary fungal dissemination

- **Paracoccidioidomycosis**

COMMON PATHOGENS OBTAINED FROM SPECIMENS OF PATIENTS WITH RESPIRATORY DISEASE

Fungi (Note: * dimorphic)	Common site of infection	Mode of transmission	Infectious form	Clinical form
BLASTOMYCES DERMATITIDIS*	Lungs, skin, long bones	(Usually) INHALATION	(probably) Conidia	YEAST
COCCIDIOIDES IMMITIS*	Lungs, skin, meninges	INHALATION	Arthro conidia	SPHERULES, ENDOSPORES
HISTOPLASMA*	Lungs, bone marrow, blood	INHALATION	Conidia	YEAST
PARACOCCIDIOIDES BRAZILIENSIS*	Lungs, skin, mucous membrane	INHALATION /TRAUMA	Conidia	YEAST
SPOROTHRIX SCHENKII*	Skin and lymphatics, lungs, meninges	TRAUMA, rarely inhalation	Conidia/ hyphae	YEAST
CRYPTOCOCCUS NEOFORMANS	Lungs, skin, meninges	INHALATION	Yeast [x]	YEAST
ASPERGILLUS	Lung, eye, skin, nail	INHALATION	Conidia	Hyphae

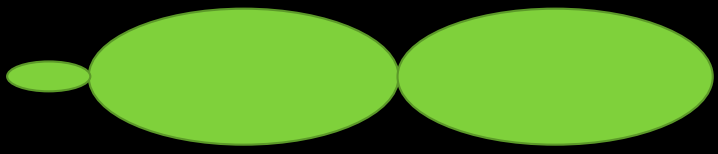
[x] conidia of telemorphic stage

OPPORTUNIST:

CRYPTOCOCCUS

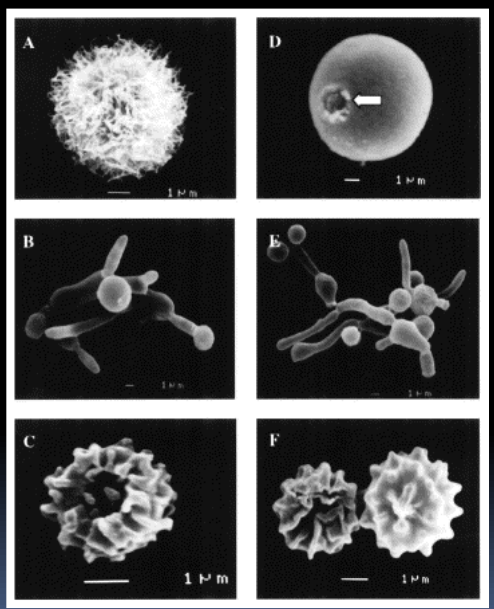
Cryptococcosis

- Etiology : *Cryptococcus neoformans.*
- Replicate by budding new yeast cells 4-6 µm, has large characteristic complex polysacharide capsule (>25 µm) --.Able to evade phagocytosis
- Culture appearance in Saboraud Dextrose agar containing no cycloheximide*: smooth, creamy, mucoid white colony in 2-3 days
- Phenoloxydase → produces melanine
- Produces urease in culture



C.neoformans
A-B-D-E

A.niger
C-F



- **Capsule of Cryptococci**:
  - protection against some stress conditions (dehydration)
  - strong immunomodulatory properties -- promotes immune evasion
  - capsular components are key virulence determinants
- Composed primarily of two polysaccharides:
  - glucuronoxylomannan (GXM) 90-95%
  - galactoxylomannan (GalXM) 5-8%
  - mannoproteins (MP) <1%
- acapsular strains (mutants) can be pathogenic for severely immunocompromised hosts

Host reaction to encapsulated cryptococci

- During the first hours of infection, a significant proportion of the yeast cells injected in the lungs are found inside phagocytic cells → this will overcome the antiphagocytic effect of the capsule.
- Mechanisms that allows phagocytosis:
  1) the presence of opsonins (antibodies and proteins from the complement system)
  2) direct interaction of the polysaccharide fibers with phagocytic receptors that occur after capsule structure rearrangements.

Ref: Feldmesser et al, 2000

Cryptococcosis - pathogenesis

- Major risk factors: HIV/AIDS, lymphoma, corticosteroid therapy, and idiopathic CD_4, T lymphocytopenia (T cell disfunction)
- Predisposing conditions: lymphoma, sarcoidosis or treatment of corticosteroid
- Aerosolized spores in soil reach the lung → in the tissue, spores start to produce capsule that enables to evade immune response.
- GXM binds to complement C_3 and interfere with antigen presentation.
- Lung lesions: intense granulomatous inflammation

Cryptococcosis – Clinical Manifestation

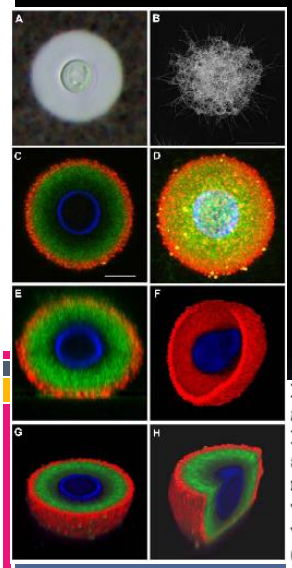
- Causes chronic meningitis and pneumonia.
- Silent hematogenous spread to the brain → clusters of cryptococci in the perivasc areas of the gray matter
- Symptoms:
  - Headache, nausea, staggering gait, dementia, irritability, confusion, blurred vision
  - Fever, nuchal rigidity often mild
- Cryptococcal pneumonia is mostly asymptomatic.
  - Chest pain (40%), cough (20%)

Cryptococcosis - diagnosis

- Cryptococcal meningitis: CSF → increased pressure, pleocytosis, glucose depression
- Isolation of fungi in CSF requires large volume specimen
- *C. neoformans* is thickly encapsulated when observed in mammalian tissues. However, upon culture in artificial media, capsule thickness is variable and strain dependent
  - Capsule in CSF can be stained by China Ink

- The capsule is not visible by regular microscopy because it is highly hydrophilic and due to its high water content it has the same refraction index as the medium.
- However, it can be easily made visible by several techniques
  - Ink →halo effect
  - scanning electron microscopy
  - Fluorescence microscope

Cryptococcus neoformans

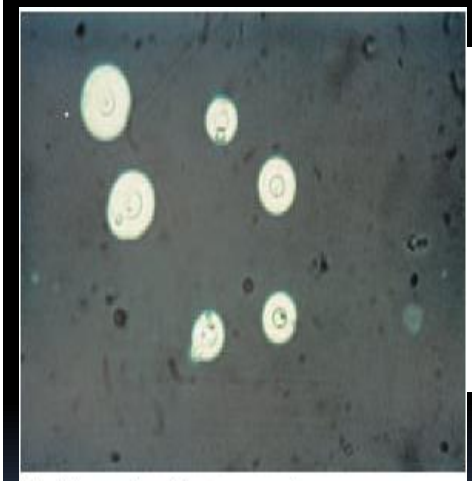


Different micrographs and compositions showing the polysaccharide capsule of *C. neoformans*. A) Suspension of the cells in India Ink; B) Scanning electron microscopy; C-H) Immunofluorescence using specific mAbs to the capsule (green and red fluorescence) showing also the cell wall localization (blue flurorescence). D) 3D image composition of a C. neoformans cell labeled with two different mAbs to the capsule. In blue, the cell wall. E) Side view of a section of cell shown in D. F-H) Sections showing the 3 dimension of the capsule, visualized after staining with mAbs (green and red). Pictures by Oscar Zaragoza, and from (Maxson et al, 2007b).

Opportunistic fungal infections

Cryptococcosis

Appearance of *Cryptococcus neoformans* on Niger seed agar.



Indian ink preparation of *Cryptococcus neoformans*.

Definition

Infection with the encapsulated yeast *Cryptococcus neoformans*.

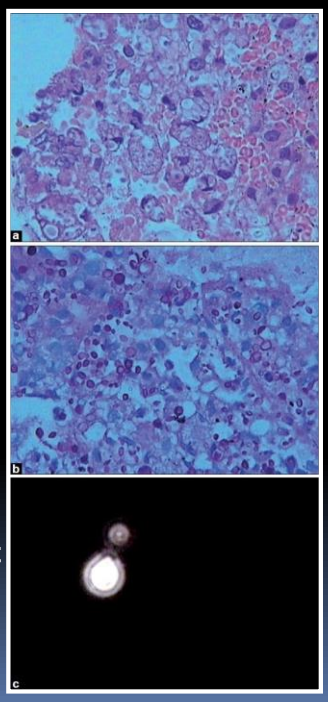
Most infections occur in immunocompromised patients, especially those with AIDS.

Meningitis is the most common clinical presentation.

(a)Section showing the presence of intracellular and extracellular organisms, H and E, ×40.

(b) PAS stained section showing the presence of capsulated organisms with morphology of Cryptococcus. ×40.

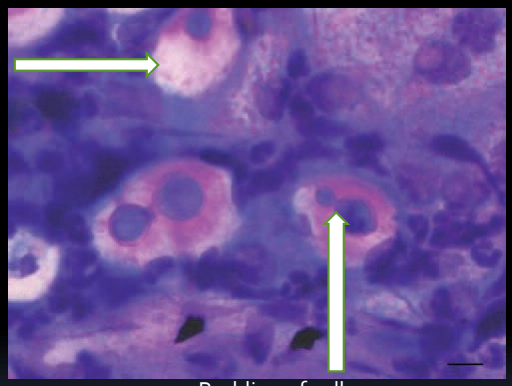
(c) India ink preparation showing capsulated yeast; there is mother yeast cell with the attached daughter cell that is budding off, ×40



Ref: Tarai B., et al, 2010

C. neoformans

Wide nonstaining capsule



Budding of cell

(modifiedWright's stain; bar = 4o µm)

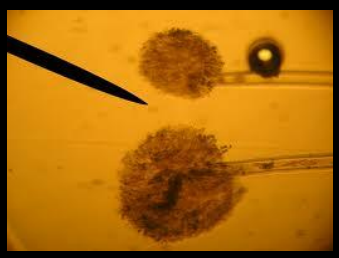
Opportunistic

ASPERGILLUS

Aspergillus

- Aspergillus spp. are molds with branching septate hyphae and characteristic conidia arrangement on the conidiophore.
- Fluffy colonies 1-2 days – 5 days full pigmented growth covering plate
- Most frequent spp:
  - *Aspergillus fumigatus*
  - *Aspergillus flavus*
  - *Aspergillus niger*

Aspergillus(KOH)



Growth on SAB Agar (grows in 48 hr)

Aspergillus (LCB)

Aspergillosis

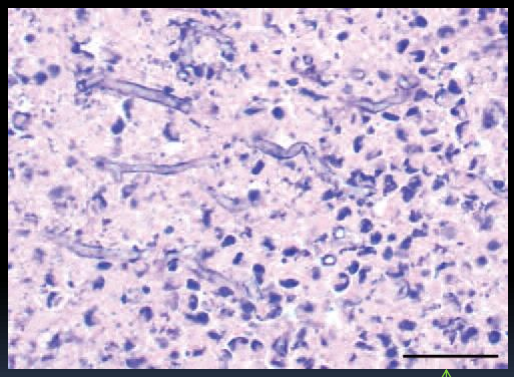
Forms of disease caused by Aspergillus

- Pulmonary aspergillosis
- Invasive aspergillosis
- Allergic bronchopulmonary aspergillosis

Aspergillosis

- Occurs in immunocompromised individuals, rapid progression to death.
- The only sign and symptom may be fever and dry cough.
- Conidia is small enough to enter the lung
- Adherence with fibrinogen and laminin.
- Extracellular elastase, proteinase, phospholipase → more virulent

Histologic microphotograph of Aspergillus spp in HE



Dichotomous Branching septate hyphae
I

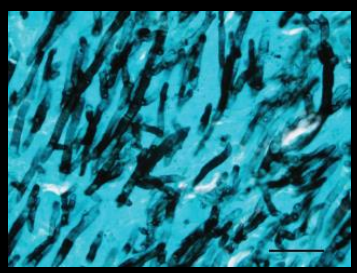
Bar = 30 um

Invasive aspergillosis

- Occurs in the presence of preexisting pulmonary disease(bronchiectasis, bronchitis, asthma, TB) or immunosuppression.
- Aspergillus invade tissues by forming branching septate hyphae → 'fungus ball' = aspergilloma within preexisting cavity.
- Invasion into blood vessels → hemopthysis
- Erosion to other organs→ fistula

Aspergillus - Diagnosis

- Isolation and identification
- Rapid growth, frequently as contamination
- Specimen: lung aspiration, biopsy and bronchoalveolar lavage



Grocott stain colours the hyphae of Aspergillus in lung tissue black

Aspergillus fumigatus bar represents 10 µm

Ref: Barton et al, 2013

Laboratory Diagnosis for Invasive aspergillosis

TABLE 2: Main approaches to laboratory diagnosis.

Test	Specimens	Advantages	Disadvantages
Direct microscopy	Respiratory	Low cost	Insensitive, labour intensive
Culture	Respiratory, tissue	Low cost, enables further analysis	Insensitive
Histopathology	Tissue	Enables proven diagnosis	Requires biopsy tissue
Galactomannan (GM)	Serum, BAL	Sensitive, specimens easy to obtain	Lacks sensitivity in patients on antifungals
β-D-glucan (BDG)	Serum	Sensitive, specimens easy to obtain	Lacks specificity
PCR (DNA detection)	Any	Sensitive, can be applied to any specimen	Labour intensive, expensive

Note: GM is carbohydrate molecule with mannose back bone and side chain galactofuranosil

Ref: Richard Barton, 2013

Characteristic microscopy and Available serologic tests

Fungi	Cytologic morphology	Serologic test
Cryptococcus neoformans	Round, thin walled yeast like cell (5-10 um), and large heteropolysacharide capsule (1-30 um) Capsule best stained in mucocarmine Narrow based budding No endospores	Caspular Ag ELISA (Ag) Latex agglutination (Ag)
Coccidioides immitis	Relatively large spherules (20-80 um; up to 200 mmwith double contoured cell wall The mature spherules are called sporangiospores (2-5 um)	Agar gel immunodiffusion (Ab) for TgM and IgG CF (Ab) may have some false positive results
Aspergillus spp	Broad (2-4 um) septate hyphae with parallel sides and acute, right angle branching	Aspergillus galactomannan EIA (sandwich immunoassay Ag test; some reactivity with penicillium, Alternaria and paecilomyces spp

Zygomycosis

- Zygomycosis(mucormycosis) is caused by any of zygomycetes (Absidia, Rhizopus, Mucor).
- Saprophyts
- Immunocompromised hosts with diabetes are infected
- Pulmonary disease is similar to other fungi
- Pathologic finding in tissue: ribbonlike non septate hyphae.

TRUE PATHOGEN

HISTOPLASMA

Histoplasma capsulatum (1)

- It is found worldwide, in soil and in bat's feces
- Endemic to the temperate zones: Americas, Asia, Africa
- Multiply by budding (blastoconidia)
- **Dimorphic**: yeast form 2-4 µm at 370C and Mold phase at 22-250C
- Grows in culture in weeks time
- Mycelial phase produces microconidia and macroconidia
- Able to survive in macrophage by modulating pH inside fagosome thus stops fusion with lysosome – virulence fc of Histoplasma
- Diagnostic structure: tuberculate macroconidium

Histoplasma Clinical Manifestation

- Most cases are asymptomatic
- Clinical symptoms of acute or epidemic histoplasmosis:high fever, non productive cough, asthenia and retrosternal pain, enlargement of the cervical lymph nodes, hepatosplenomegaly, erythema nodosum, erythema multiforme.
- X-ray : mediastinal lymphadenopathy, infiltrates
- Histoplasmin skin test positive in 3 weeks .
- Residual nodule may continue to enlarge over a year and mimic pulmonary neoplasma.
- Progressive pulmonary disease resembles pulmonary tuberculosis

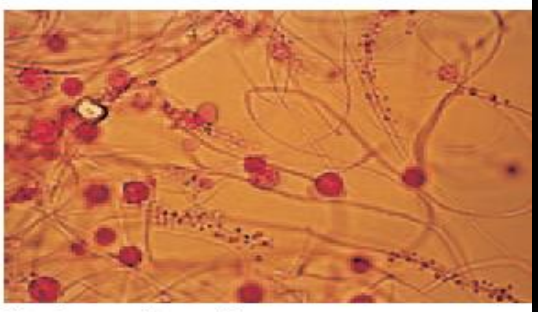
Histoplasma - Pathogenesis

- Reticuloendothelial system is the focus of infection.
- Inhaled microconidia/spores → changes to yeast form in the host body
- When phagocytosed (by macrophage and PMNs) it may grow inside macrophages by controlling lysosomal pH (increased to neutral) → remains able to multiply inside macrophage → to mediastinal lymph nodes → hematogenous spread
- Further lymphatic spread and development of primary lesion is similar to Mycobacteria

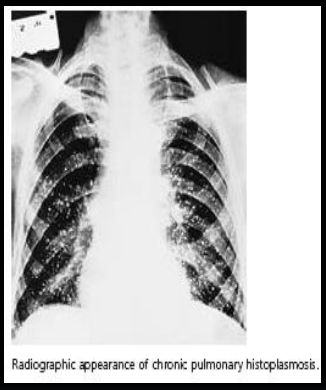
- 10-14 days in Macrophage– necrosis – caseation, fibrous encapsulation, calcium deposition, calcified granulomas
- → persist for years, dormant
- → reactivate if immunity decreases

Systemic mycoses

Histoplasmosis



Histoplasma capsulatum conidia.



Radiographic appearance of chronic pulmonary histoplasmosis.

Definition

A mild and transient pulmonary infection in normal individual caused by the dimorphic fungus *Histoplasma capsulatum*. Can proceed to a chronic infection of the lungs or more widespread infection in predisposed patients.

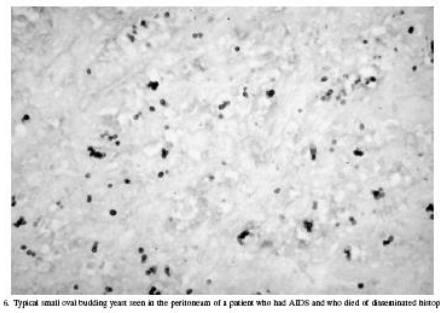


FIG. 6. Typical small oval budding yeast seen in the peritoneum of a patient who had AIDS and who died of disseminated histoplasmosis.

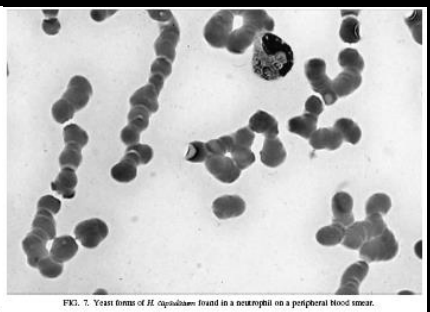


FIG. 7. Yeast forms of *H. capsulatum* found in a neutrophil on a peripheral blood smear.

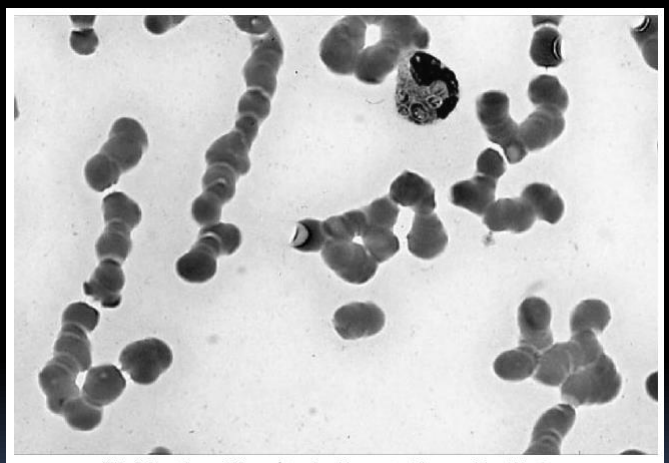
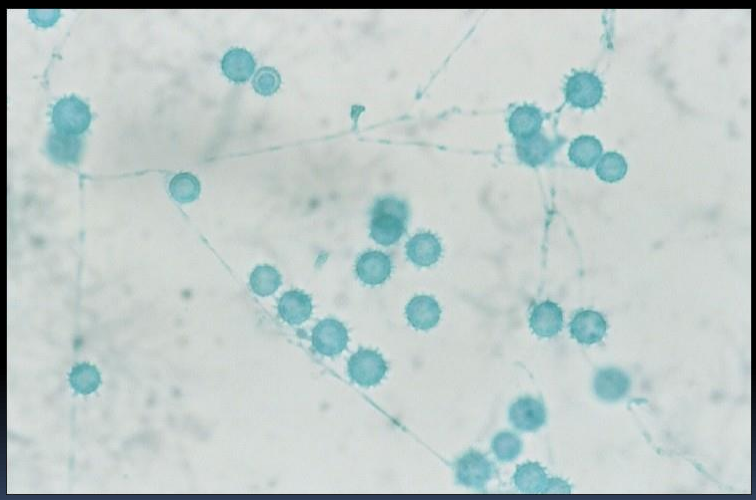


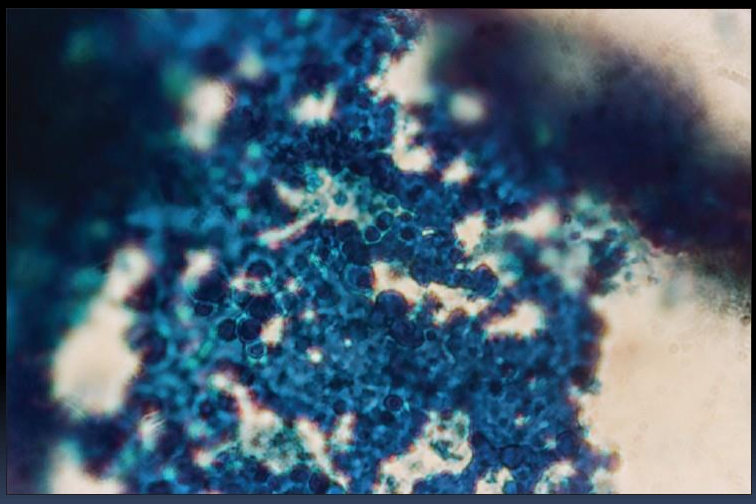
FIG. 7. Yeast forms of *H. capsulatum* found in a neutrophil on a peripheral blood smear.

Ref: Kauffman , 2007



Numerous tuberculate macroconidia of Histoplasma capsulatum on culture on SDA. ×400. LPCB mount.

Baradkar. 2011



Yeast forms of *H. capsulatum* observed on BHIA.
×400. LPCB mount

Histoplasma diagnosis

- **Culture** → GOLD STANDARD :Grows up to 4 weeks
  - INFECTIOUS → Work in Biosafety cabinet !!
- **Histopathology** → rapid but less sensitive than culture or antigen detection
  - Disseminated histoplasmosis → use blood and bone marrow, Wright or Hematoxylin Eosin staining shows intracellular histoplasma, tuberculate macroconidium and dimorphism

Histoplasmosis diagnosis

- **Antigen Detection → EIA** (immunodifusion)
- **PCR Assays → Real Time PCR**
- **Antibody Tests → false neg in the initial phase of disease and in immunocompromised patients**
- **Skin Tests → high background positivity in endemic area → rarely useful**

Histoplasmosis- treatment

- Mild cases: symptomatic
- Severe/prolonged acute pulmonary infection and disseminated disease : antifungal therapy
  - Amphotericin B → agent of choice
  - Itraconazole

OPPORTUNIST:

BLASTOMYCES

Blastomyces dermatitidis

- Caused by the dimorphic fungus that changes to mycelial at 25oC. Produces microconidia, but no macroconidia
- Blastomyces is similar to histoplasma, but larger yeast cells (8-15 µm), has broad base buds and thick wall.

Blastomyces – clinical manifestation

- Most clinical features are similar to histoplasmosis (asymptomatic or cough or mild fever).
- Infection typically presents as an acute or self-limited pneumonia, but chronic pulmonary, cutaneous, and disseminated forms of blastomycosis
- Disseminated infection: skin lesions

Blastomyces - pathogenesis

- Has surface glucan and glycoprotein adhesin (BAD1) for binding to host cells.
- Yeast are large cells, thick double walls, extracellular

Blastomyces – Clinical manifestation

- Pulmonary infection: cough, sputum production, chest pain, fever.
- Hilar lymphadenopathy, nodular pulmonary infiltrates with alveolar consolidation → resembles pulmonary tumor, tuberculosis, other mycosis.
- Skin lesions: occur on exposed skin

Blastomyces - Diagnosis

- The presence of large yeast cells with broad-based buds (blastoconidia) in KOH preparation
- Biopsy → H & E staining
- Culture: grow in weeks, but conidia not distinctive
- Immunodiffusion test
- Serologic tests mostly negative

TRUE PATHOGEN:

COCCIDIOIDES

Coccidioides

- History
- Clinical symptoms
- Fungal Morphology
- Diagnosis and Treatment

Coccidioides – History

- Named after a medical student: Alejandro Posadas
- Skin lesion → cultured → observed microscopical hyphae and spherule in the culture

Coccidioides – disease

- The disease Coccidioidomycosis is also known as
  - = Posadas-Wernicke disease
  - = Desert rheumatism
  - = San Joaquin Valley Fever
  - = Coccidioidal Granuloma
- **Endemic** in the southwestern US and Central America (Mexico); but recently has been reported in India, Turkey, Japan, (and possibly will be in other countries) as a disease obtained after travelling in the endemic areas.

Ref: De Deus Filho, 2009

Coccidioides – Clinical Form

1. Primary Pulmonary → THIS FORM IS THE MOST FOUND
2. Progressive pulmonary
3. Disseminated diseases: skin, bone, endocarditis, meningitis, bowel, genito-urinary infection

Coccidoides - infection of the lungs

- After inhalation of spores flowing in the dust 1-15 days
  - 60% benign and resolves spontaneously
  - 40% progressive disease with pulmonary and other organs symptoms
- Symptoms: malaise, cough, chest pain, fever, dyspnea, hemoptysis, fever, arthralgia 2-6 weeks (Valley Fever), diverse skin reactions : maculopapular rash, erythema multiforme, erythema nodosum (common in women)
- Chronic: pulmonary cavity

Coccidioides immitis

- Disseminated disease is more common in men, and related with racial orientation and immune status
- Differential Diagnosis:
  - nonspecific pneumoniae
  - Tuberculosis
  - Pneumoconiosis
  - Silicosis

Coccidioides immitis

- Symptomatic infection typically presents as **pneumonitis** with hilar adenopathy and cutaneous rashes → subacute and self limiting : Valley Fever
- Hematogenous spread: extensive granulomatous reactions and tissue damage in the skin, bones and joints, meninges, and genitourinary tract.

Coccidioidomycosis – Diagnosis

- X ray: Cavity and fungus ball formation
- Fibre optic bronchoscopy
- Laboratory:
  - Serology
  - **Culture on Saboraud Agar**
  - **Microscopy on Histology preparations**
  - **Nucleic Acid Amplification Techniques (NAAT)**

Coccidioides – culture

- Grow at most media, at room temperature
- Day 3-4: White cotton-like colonies
  - Micr: hyalin hyphae, septate, ramified, Ø 2-4 µm
- Day 5: forms (multinucleated) arthroconidia
  - Arthroconidia in lab culture is highly infectious
  - Arthroconidia detaches: barrel shape Ø 2-4 µm, containing endospores
  - Arthroconidia if inhaled from the air → in the lungs → converts into spherules and progeny endospores

Coccidioides immitis

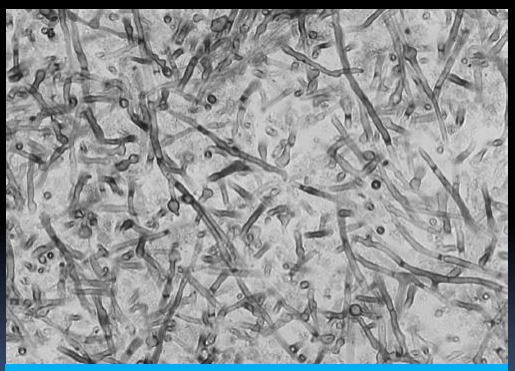
- Culture:
  - Grows on most media
  - Grows in 5 days
  - Colonial structure of the mycelial phase is not diagnostic → MYCELIAL PHASE IS HIGHLY INFECTIOUS → perform lab work in biological containment cabinet !!

Coccidioides - Microscopy

- Tissue biopsy
  - KOH
  - Hematoxylin eosin
  - Periacid Schif (PAS)
  - Grocott-Gomori Methenamin Silver Stain
- Microscopy of tissue: spherules containing endospores

Case Report:

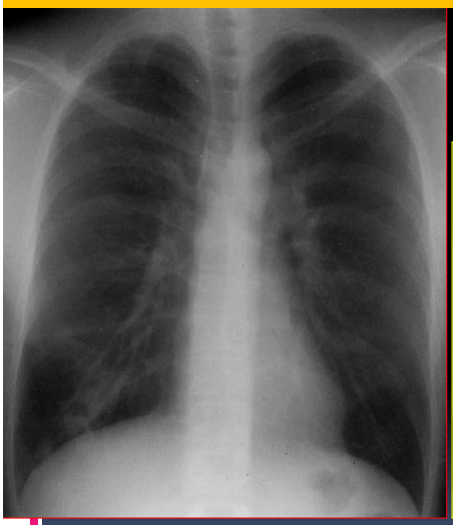
Fungus Ball detected in a Japanese man's lung after a short stay in an endemic area



Histological examination of the fungus ball showed numerous septate hyphae with terminal expansion (Grocott stain ×400).

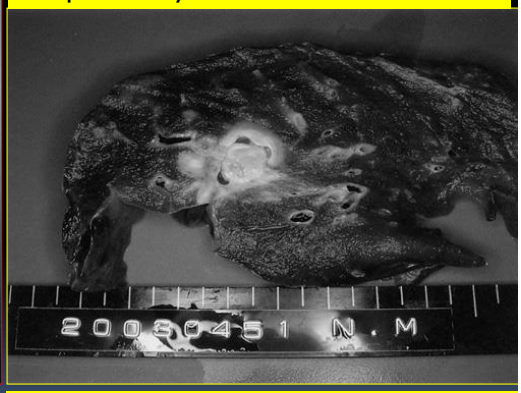
Osaki T et al, 2005

Chest radiograph on admission showing a thin-wall cavity in the right lower lung field.



Ref: Osaki T et al, 2005

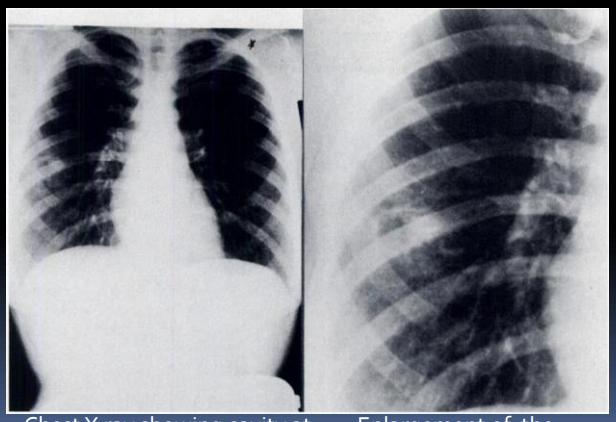
Macroscopic view of the resected lung demonstrates a cavity of 1.5×1.0 cm diameter encapsulated by a thick and fibrous wall.



The cavity contains a gray fungus ball and indents the pleura.

Case report:

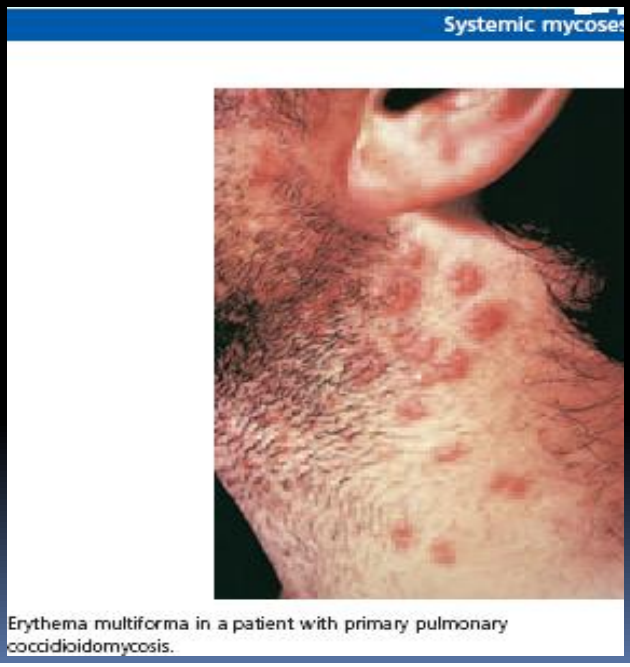
Fungus Ball in the lung of a 20 y.o. women 15 years living in an endemic area



Chest X ray showing cavity at the right lower lung

Enlargement of the radiography:

Ref: Winn et al, 1994



Erythema multiforma in a patient with primary pulmonary coccidioidomycosis.

Coccidioides – Etiology

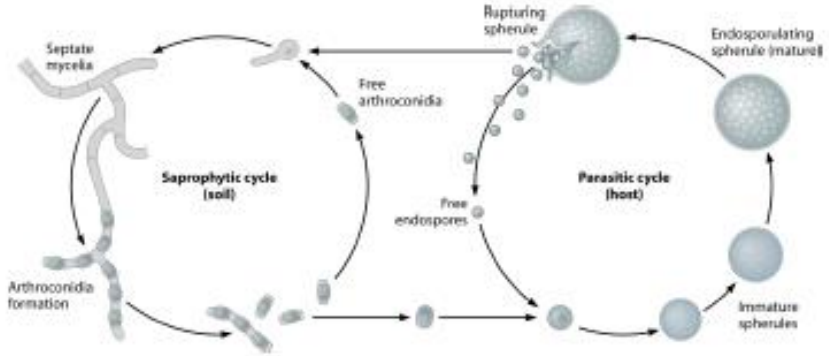
- *Coccioides immitis*
- *Coccidioides posadasii*

Dimorphic life cycle

- Arthroconidia (2-3 x 4-6 µm) if inhaled, enters the bronchioles and convert into invasive-spherules.
- The spherules enlarge (20 to 100 mm) and segment internally into hundreds of endospores.
- Endospore is capable to become another spherule
- Spherules are coated with an extracellular matrix which restricts PMN access.
- Arthroconidia has antiphagocytic action due to the outer portion of the cell wall

Coccidioides Dimorphic life cycle

Ref: Nguyen et al, 2013



Coccidioides tests

- Skin Test (coccidioidin or spherulin) will show delayed hypersensitivity – proof of past infection
- Serology:
  - IgM – Acute disease
  - IgG antibody -- a decrease indicates effective R/ but an increase indicates non effective R/ → intensify R/ or change R/
  - If negative result -- does not rule out an infection

Coccidioidomycosis Treatment

- Localized acute pulmonary infection and no risk factors for complications → assess the self limiting process and R/ azole antifungals
- Extensive spread or immunosuppression → R/ azole or polyenes antifungals and/or surgical debridement.
  - 1st class = polyenes (amphotericin B desoxycholate)
  - 2nd class = fluconazole, itraconazole, voriconazole, posaconazole

True Pathogens

PARACOCCIDIODOMYCOSIS

Paracoccidioidomycosis

- A soil saprophyte
- Inhalation of propagules → lung → disseminate mucous membrane, lymph node, skin, adrenal gland, oral,nasal, GI mucous membranes.
- Subacute infection (in children) becomes chronic systemic mycoses (in adults)
- Among rural men workers restricted to Latin America
- Is the most frequent endemic systemic mycosis in many countries of South America.

Paracoccidioidomycosis - etiology

- *P.brasiliensis*
- *P.lutzii*

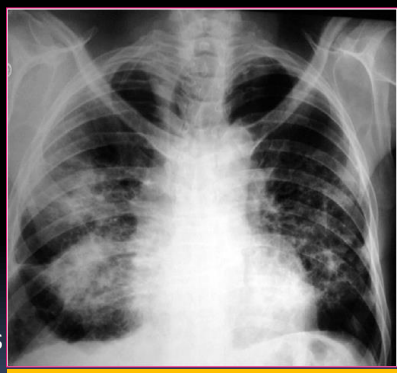
Paracoccidioides brasiliensis

- Paracoccidioidomycosis = South American Blastomycosis
- Occurs mainly in men → estrogen receptors block the change of hyphae to an invasive yeast form
- Causes primary pulmonary infection even in immunocompetent person
- Dimorphic fungi: slow growing (20-30 days)
- Thermodimorphic: growth is induced by temperature

Paracoccidioidomycosis - Differential Diagnosis

1. Pulmonary tuberculosis and atypical mycobacterioses
1. Sarcoidosis
2. Histoplasmosis
3. Idiopathic diffuse interstitial pneumonitis
1. Chronic silicosis
2. Coccidioidomycosis
3. Chromoblastomycosis
4. Cutaneous and visceral leishmaniasis
5. Leprosy
6. Cutaneous and laryngeal neoplasia

Ref: Hahn et al, 2014

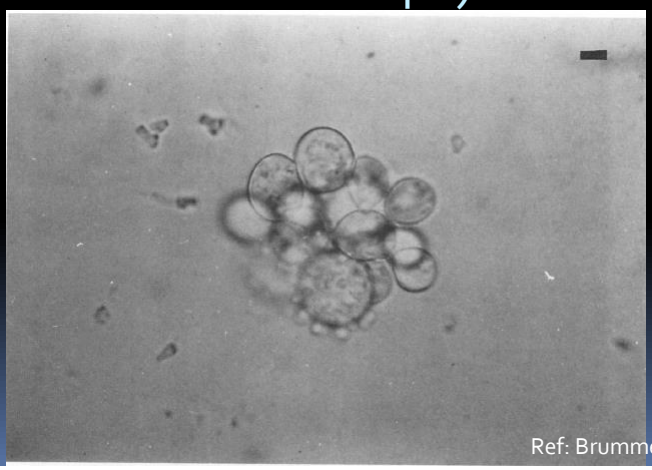


X ray showing Bilateral diffuse interstitial infiltrate

Paracoccidioides brasiliensis

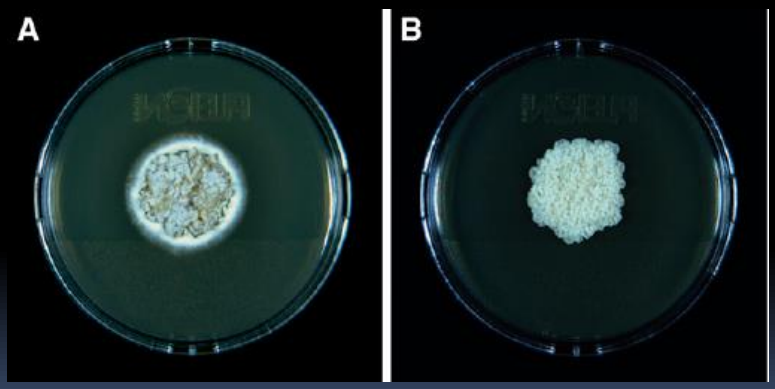
- Diagnosis:
  - Culture → Saboraud Agar
  - Direct microscopy on tracheal aspirate: multiple budding on a large yeast cell (20-60 um) → pilot's or **steering wheels** or Mickey mouse appearance
  - Serology: Antifungal antibody
    - Immunodiffusion (ID)
    - Counter immunoelectrophoresis (CIE)
    - ELISA

Culture of a conidia at 36oC converts it into **multiple budding** yeast cell (as viewed under the microscope)



Ref: Brummer et al, 1993

Thermo-dimorphism of *Paracoccidioides lutzii*



Hyohae at 25oC on Potato Dextrose Agar Yeast at 37oC on Fava –Netto Medium

Organism	Growth		Tissue	Source	Primary Disease	Disseminated Disease
	Culture 25oC	Culture 37oC				
C. neoformans	Encaps. Yeast	Encaps. Yeast	Encaps. Yeast	Environm, worldwide	Pnie	Chronic meningitis
H. capsulatum	Mold, Tuberculate Macroconidia	Small yeast	Small intracell yeast	Environm, US midwest	Pnie, hilar adenopath	RES enlargemnt
B. dermatitidis	Mold	Yeast		Environm, Us midwest	Pnie	Skin and bone lesion
C. immitis	Mold, arthrocon.	(spherule)	Spherules	Environm, Sonoran desert	Valley fever	Pnie, meningitis, skin, bone
P. brasiliensis	Mold	Yeast, mltiple blastokon.		Environm, latin america	Pnie	Mukokutan, RES