Systemic Mycoses

- Histoplasma capsulatum
- Blastomyces dermatitidis
- Coccidioides immitis
- Paracoccidioides brasiliensis
- Penicillium marneffei
- Sporothrix schenckii
- Aspergillus species
- Emmonsia species

Dimorphism

- Majority systemic mycoses are caused by dimorphic fungi
  - Molds in their normal soil environment, and on laboratory media when kept at 25-30°C.
  - Yeast when the temperature is raised to 37°C (as in the human host).
  - Nutritional factors such as certain amino acids can also enter into dimorphism, but are generally less important than temperature.

Histoplasmosis

- Occurs throughout the world
- Endemic areas
  - Mississippi and Ohio River Valleys in the US.
  - Mexico, Central and South America
- Once thought to be a highly lethal form of pneumonia with up to 90% mortality
- Now known to be a rather common infection in endemic areas.

Histoplasma habitat

- Soil saprobe
- Loves droppings from bats or birds.
  - Requires high levels of creatinine and nitrogen
  - Birds not susceptible to infection with Histoplasma
    - Likely related to their high body temperature
      - 40-42°C (104-108°F)
  - Occupational risk for people working with chickens.
  - Clearing Starling roosts has been associated with large outbreaks of fatal infections.
**Histoplasma capsulatum Disease**

- Usually self-limiting flu-like illness and does not require medical intervention.
  - 90% asymptomatic
  - 4:1 male predominance for clinical disease
  - Infants and young children more likely symptomatic
  - Chronic pulmonary disease
  - Disseminated disease in immunocompromised

**Pathobiology**

- In human infection---small yeast 2-5 µm in diameter.
  - Predominantly in macrophages.
- Nonactivated macrophages do not effectively kill H. capsulatum and can actually spread the disease.
  - Can multiply intracellularly, kill the phagocyte, and infect additional cells

**Histoplasmosis**

- Granulomatous lesions in the lungs
  - Very similar to tuberculosis lesions
  - Lymphocytes, macrophages, Langhans’ giant cells
- Severity of infection directly proportional to the number of conidiospores inhaled.
  - Miliary lesions when large numbers of the spores are inhaled.
- In most infections the cure is spontaneous and lasting immunity occurs.
- Histoplasma may remain viable and recurrence possible with decrease in CMI

**Pathobiology**

- Radiologic variations

**Calcification**

- Immunity and Treatment
  - Immunity dependent on CMI.
    - Antibody is of little importance
  - Healing of lesions leads to calcified granulomas similar to that seen in tuberculosis.
    - Old calcified nodules on chest X-ray not uncommon
  - Treatment reserved for life-threatening infections
    - Amphotericin B
    - Itraconazole
**Laboratory Diagnosis**

- **Histology**
  - GMS stain
  - Wright stain of blood or bone marrow
- **Direct Microscopic Exam**
  - KOH, Calcofluor—2-5um yeast
- **Culture**
  - Enriched media (BHI with Blood, Yeast Extract Phosphate, Inhibitory Mold Agar)
  - 2-4 weeks at 30°C
- **Antigen Detection**
  - Urine EIA (miravistalabs.com)
- **Serology**
  - Complement fixation
  - EIA
  - Immunodiffusion
- **Real-time PCR**

**Specimens for Fungal Infections**

- **Urine and other body fluids**
  - Centrifuge 2000g, 10 minutes
  - Plate pellet
- **Mince tissue, do not grind**
  - Place 3-4 pieces on plate and press into agar
  - Exception for Histoplasma—want to grind to release intracellular organisms

**Media for Primary Isolation of Systemic Fungi**

- **Non-inhibitory media**
  - Sabouraud’s dextrose agar
  - Potato Flake Agar
  - Potato Dextrose Agar
- **Selective Media**
  - Mycobiotic or Mycosel agar—cyclohexamide and chloramphenicol

**Media for Primary Isolation of Systemic Fungi (2)**

- **Enriched media w/ or w/o antibiotics**
  - Inhibitory mold agar—chloramphenicol and cyclohexamide
  - BHI with sheep blood w/wo antibiotics
  - Yeast extract phosphate agar with ammonia
- **Incubate plates or tubes at 30°C or 25°C**
  - Hold 4 weeks
- **For Blood Cultures**
  - Lysis Centrifugation
  - BACTEC MYCO/F or BacT ALERT MB

**Silver Stain**
Culture

Histoplasma capsulatum

H. capsulatum conidia 30C Incubation
Differentiation from other Fungi

- Must differentiate from *Sepedonium* and *Chrysosporium* species that produce tuberculate macroconidia
  - More rapid growing
  - Not dimorphic
  - Usually will not grow in the presence of cycloheximide
  - Distinguish using DNA probe

Nucleic Acid Probe Identification

- GenProbe® Assay
  - Rapid
  - Chemiluminescent assay using labeled probes specific for each agent
  - Labeled DNA probe hybridizes with rRNA of the fungus
  - Available for *H. capsulatum*, *Blastomyces dermatitidis*, and *C. immitis*

Blastomycosis

- *Blastomyces dermatitidis*
- Agent of North American Blastomycosis,
- Geographical distribution is similar to *H. capsulatum*
- More common in Wisconsin than *H. capsulatum*.
**Clinical Manifestations**

- Two clinical presentations
  - A primary cutaneous infection which usually remains localized to one area of the body
  - May indicate systemic disease
  - Primary pulmonary infection with possible secondary dissemination
    - 30-45 day incubation
    - Mimics flu progressing to cough, weight loss, chest pain, low grade fever
    - 75% with isolated pulmonary disease
  - Infection may involve any organ
  - Secondary cutaneous infection
  - Asymptomatic in >50% of those infected

**Systemic Disease**

- Common sites of infection in systemic disease
  - Bones—long bones, ribs, vertebrae
  - Joints
  - Genitourinary tract—prostate, epididymis
  - CNS—common in AIDS (40%), uncommon in immunocompetent (<5%)

**Cutaneous Form**

- A chronic suppurative granulomatous lesion.
- The presence of epithelial microabscesses and characteristic yeasts in the tissues is considered diagnostic.
- It is important to obtain urine and sputum samples from a patient with cutaneous blastomycosis since systemic spread may occur.

**Blastomycosis**

- The epidemiology is poorly understood
  - Lack of a good skin test reagent
  - Ecologic niche not well established
- Difficult to recover from the soil in endemic areas.
- Eagle River, Wisconsin outbreak 1985
  - First time Blastomyces isolated from the environment at the site of an outbreak
  - Isolated from soil containing decayed vegetative matter and from decomposed wood.
**Histology**

- **Body fluids or tissue specimens**
  - Look for the characteristic yeast form.
  - Large (8-15 µm) and thick walled.
  - The wall is prominent; "doubly refractile" on bright field microscopy.
  - A single daughter cell (bud) is present with a broad connection between the two cells (**BROAD-BASED BUDDING**).
Culture Characteristics

- On culture:
  - Slow growing gray/white mold
  - Delicate, septate hyphae
  - Conidia usually absent on blood-containing media. May be sparse on PDA and SAB
  - “Lollypop” conidiation

Blastomyces dermatitidis

- This form of conidia is also found in such fungi as Chrysosporium sp., Pseudallescheria boydii (Scedosporium), and various Trichophyton sp.
- Differentiation from these other species can be made by the following characteristics:
  - Slower growth
  - Growth in the presence of cycloheximide
  - Dimorphism
  - Nucleic acid probes

Blastomyces Mold Phase
• Coccidiomycosis is sometimes known as "San Joaquin Valley fever". Up to 95% of the residents of the endemic area are skin test positive (coccidioidin test positive)
• Lower Sonoran Life Zone
  - Arid climate, hot summers, few winter freezes, low altitude, alkaline soil, sparse flora
  - Drought followed by heavy rains—Increased infections
  - 100,000 infected annually in U.S.
• Variety of animals infected
  - Positive cultures around rodent burrows
• Archaeology students discover new "infected" sites
Clinical Manifestations

- The primary disease is pulmonary, secondary to inhalation of small numbers of arthrospores
- Usually resolves spontaneously as an influenza-like infection.
  - 60% asymptomatic
  - 40% influenza-like illness, LRI or systemic illness
  - Cough, sputum, chest pain, malaise, fever, chills, night sweats, arthralgias, anorexia

Clinical Manifestations

- In a minority of cases a more chronic pulmonary infection occurs
  - Granulomatous lesions of the lung
  - Can lead to cavitation
- In rare cases (0.5%) dissemination occurs which can lead to rapidly fatal results.
- Reactivation infection occurs

Coccidiomycosis

Lesions variable:
- Papules
- Pustules
- Plaques
- Nodules
- Ulcers
- Abscesses

Histology

- Histological examination useful in confirming a diagnosis.
  - Spherules: 10-60 µm in diameter, but they may be as big as 200 µm.
  - Immature spherules can be similar in size to the large yeast cells of *B. dermatitidis*
  - The spherules contain endospores 2-5 µm in diameter
    - Similar in size to *Histoplasma capsulatum*
    - Will not see budding

Culture

- Culture:
  - The organism grows fairly rapidly.
    - Visible growth on Sabouraud's agar within a few days.
Microscopic Features

- **Arthrospores:**
  - Formed by fragmentation of hyphae
  - Very thick walled. Provides them with resistance to drying.
  - “Barrel-Shaped”
  - As a culture ages on media the entire hyphal mass may fragment and form arthrospores.
- **Spores are extremely infectious - Handle with extreme care.**
- **Remember:** Arthrospores can be made by other fungi.
  - Malbranchea sp., Gymnoascus uncinatus, Auxarthron sp.
  - Geotrichum and Trichosporon can also form arthrospores
- Confirm the identification nucleic acid probe test

Select Agent Regulations

- Report to CDC within 7 days of ID
  - Responsibility of lab confirming ID
  - Select Agent APHIS/CDC Form 4
- Secure against loss, theft, or release
- Destroy all subcultures and specimens
- Good News
  - Proposed to remove Coccidioides from SA list

Work Safely!