
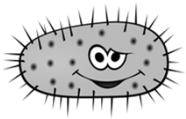

Updates & Unusual Cases In Parasitology/Mycology

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Spring 2016 CLPC

Objectives

- Discuss unusual case studies involving various parasites and fungi.
- Describe the epidemiology and lab diagnosis for each organism.

CASE STUDIES

Case #1

- 32 year old male in Missouri
- 3 month history of recurrent fevers (up to 103^{o}F)
- Additional symptoms:
  - Myalgia
  - Arthralgia
  - Generalized malaise
  - Cough producing minimal brownish sputum

Case #1

- Chest x-ray showed hazy infiltrate & small pleural effusion
- Treated with levofloxacin for 7 days (presumed community-acquired pneumonia)
- Patient's symptoms persisted……

Case #1

- Developed "sharp" & "splitting" headaches & blurred vision (with blind spots & floaters)
- Admitted to community hospital
  - CSF analysis performed - normal
  - CT scan of head – normal
  - Chemistry & liver function tests – normal
  - Urine culture - negative

Case #1

- CBC – Abnormal results:
  - WBC count: 12,000 cells/mm^3
  - Eosinophils: 30%
- Diagnosed with eosinophilic pneumonia
- Treated with prednisone (60 mg daily)
- Initially improved, but symptoms returned when dose was decreased

Case #1

- Referred to a large teaching hospital in Missouri
- Repeat CBC:
  - WBC count - 10, 100 cells/mm^3
  - 30% eosinophils
- Additional negative testing:
  - Rickettsia, Ehrlichia, Strongyloides, EBV, herpes simplex virus, CMV, HIV

Case #1

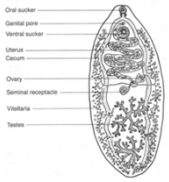
- Upon further questioning, the patient reported eating raw crawfish while intoxicated during a "float trip" on a Missouri river
  - 3 weeks prior to onset of symptoms
- CDC performed ELISA testing
  - Confirmed *Paragonimus* sp.
    - Lung trematode (fluke)

Case #1

- Patient treated with 3-day course of praziquantel
- Symptoms resolved within 3 days
- At 1 month follow-up, no symptoms
  - Leukocytosis & eosinophilia normal

Trematode Characteristics

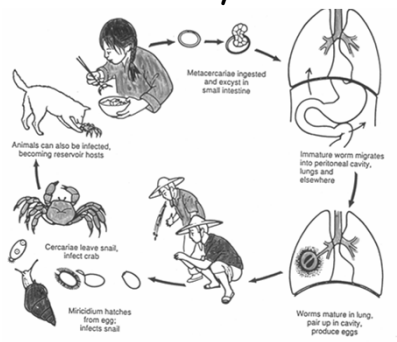
- Flukes
- Most are hermaphroditic
- Leaf shaped
- Eggs are usually operculated
- Require fresh water snail as intermediate host



Paragonimus westermani

- Common name: Oriental Lung Fluke
- Prevalent in Far East
- Intermediate hosts:
  - Snail
  - Crabs/Crawfish

Paragonimus westermani: Life Cycle

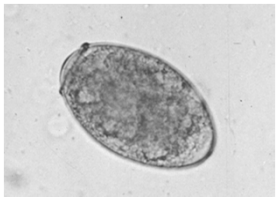


P. westermani: Clinical Disease

- Pronounced inflammation in lungs
- Formation of fibrotic capsule around adult in lungs
- Capsule filled with fluid conaining eggs
  - Capsule ruptures, causing cough with production of blood-tinged sputum

Paragonimus westermani: Diagnosis

- Patient history
- Recovery of eggs in sputum (or stool)
- Sputum is blood-tinged & may contain blown flecks (eggs)
- Serological testing



Why Unusual ???

- *Paragonimus* usually found in Asia
  - Estimated 300 million at risk for infection
    - With 195 million at risk in China
- Only 7 cases previously reported in North America
  - Without history of travel or consuming imported food items
  - Consumption of raw crawfish is uncommon

Why Unusual ???

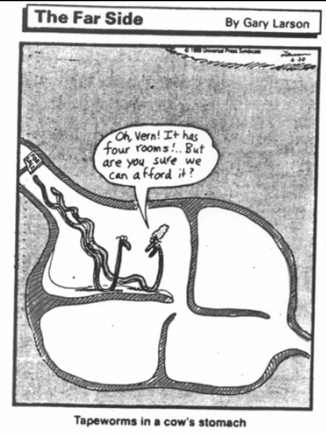
- Over 18 month period, this hospital diagnosed 2 more cases of *Paragonimus*
- All 3 cases were associated with intoxication, ingesting raw crawfish & "float trips" on Missouri rivers
  - Very likely that alcohol played role by relaxing normal dietary inhibitions
- Species determined to be *P. kellicotti*
  - Most common in midwest region of U.S.

Case #2

- January, 2013
- 41 year old male in Colombia
- Presented with fatigue, fever, cough, & weight loss for past several months
- Diagnosed with HIV in 2006
  - Non adherent to therapy
  - CD4 count dangerously low
  - Viral load very high

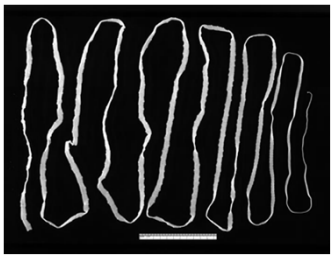
Case #2

- Stool exam revealed *Hymenolepis nana*
  - Intestinal cestode (tapeworm)



Cestode Characteristics

- Tapeworms
- Flat, segmented, ribbon-like
- Hermaphroditic (male & female reproductive organs)
- Usually require intermediate host
- Species determined by eggs, scolex, or gravid proglottid



Hymenolepis nana

- Common name: Dwarf tapeworm
- Most common cestode in U.S.
- No intermediate host required
- Eggs infective IMMEDIATELY upon passage
- Seen in daycares, institutions, & nursing homes

Hymenolepis nana : Life Cycle

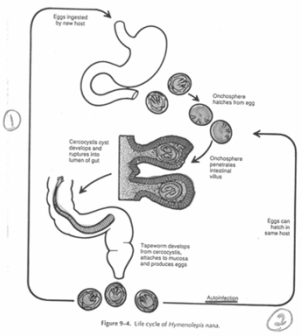


Figure 9–4. Life cycle of *Hymenolepis nana*.

Hymenolepis nana : Egg Morphology

Hymenolepis nana : Clinical Disease

- Usually asymptomatic
- Symptoms, if present, include:
  - Headache
  - Dizziness
  - Abdominal pain
  - Anorexia
  - Diarrhea

Hymenolepis nana : Diagnosis

- Eggs in stool specimen
- Adults &/or proglottids rarely seen

Case #2

- CT scan performed
  - Numerous tumors in lungs, liver & adrenal glands
- Treated with anti-HIV drugs & albendazole for tapeworm infection
- Conditions continued to worsen
- Additional samples submitted to CDC for analysis

Case #2

- Biopsy results clearly revealed cancer

BUT….

Things got a little bit weird!

Case #2

- Cancerous cells were tiny
  - 10x smaller than expected
  - Far too small to be human cells
- Scientists baffled; subjected cells to a bounty of tests
  - Revealed tumor cells contained *H. nana* DNA
- Unfortunately, the patient died 72 hours after the discovery was made

Case #2

- Scientists remain bewildered
  - *H. nana* is most common human tapeworm
  - Infects 75 million people worldwide
  - Yet nothing like this has been described before
  - Human disease caused by parasite-derived cancer cells
- While this case seems unique, most likely other cases that have gone unrecognized

Case Study #3

- African American female
- Born 3 months premature
- Birth weight: 1 pound, 9 ounces
- Placed on lipid emulsion therapy through a deep vein catheter due to low birth weight

Patient History

- 12 days old
  - Respiratory & metabolic complications
  - Diagnosed with necrotizing enterocolitis
- 14 days old
  - Positive blood culture for *Enterobacter cloacae*
  - Aggressive antibiotic therapy (Gentamicin & Amikacin)

HEMATOLOGY – CBC Results

		Reference Range
• **Day 1**		
– WBC count	54.7/uL	9.0 – 30.0/uL
– RBC count	3.12 x 10^6/uL	5.14 ± 0.7 x 10^6/uL
Hemoglobin	12.3 g/dL	19.3 ± 2.2 g/dL
Hematocrit	37.6 %	61 ± 7.4 %
• **DAY 14**		
– Platelet count	77x10^3/uL	150 – 375 x 10^3/uL

CHEMISTRY - Basic Metabolic Profile

		Interpretation
• **Day 1**		
– Potassium	6.3 mmol/L	Increased
– Chloride	110 mmol/L	Increased
– Glucose	193 mg/dL	Increased
– Calcium	6.2 mg/dL	Decreased
– BUN	38 mg/dL	Increased

- Laboratory results remained consistent through hospitalization (except platelet count)

Patient History

- Received multiple platelet transfusions
- Aggressive ventilator & cardiovascular therapy
- Condition continued to decline
- Life support was withdrawn
- Patient expired at 47 days old
- Autopsy performed at request of the infant's mother

Autopsy Results

- Confirmed the clinical diagnosis of *Enterobacter* sepsis
- Also revealed a disseminated budding yeast
  - Found in lungs, liver, small intestine, pancreas, spleen & kidneys
  - Identified as *Malassezia furfur*

Malassezia furfur

- Found worldwide
- Etiologic agent of pityriasis versicolor (tinea versicolor)
- Characterized by patchy lesions or scaling of varying pigmentation; ("fawn-colored liver spots" that fail to tan normally)

Malassezia furfur

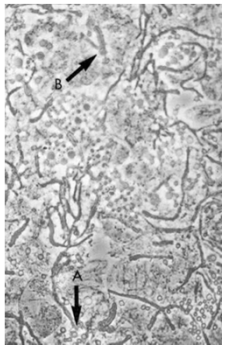
- Lipophilic yeast ("oil loving")
- Normal skin flora of humans & domestic animals & birds
- Normally colonizes infant's skin during first 1-3 months of life (routine handling)

Laboratory Diagnosis

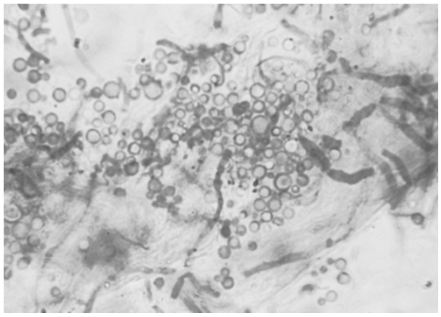
- Clinical specimen
  - Potassium hydroxide (KOH)
- Culture
  - Lactophenol cotton blue (LPCB) prep
  - Main problem: commercial culture broth & agar media do not contain essential long-chain fatty acids required for growth
  - Media must be overlaid with sterile olive oil to provide organism with required nutrients

Malassezia furfur

- Microscopic exam
  - Budding yeast
  - 4-8 um
  - Septate, often branched hyphae
  - Referred to as "spaghetti & meatballs" arrangement



Malassezia furfur - KOH



Treatment

- Temporary treatment
  - 1% selenium sulfide (usually found in shampoo: Selsun Blue)
  - Usually recurs when treatment is stopped

Predisposing Factors for Colonization of *M. furfur*

- Low birth weight
- Low gestational age
- Long duration of hospital stay
- Handling by multiple nursery personnel
- Lipid emulsion therapy
- Application of skin oils or lotions

Catheter Related Infections with *Malassezia furfur*

- Major problem in premature infants
- Catheter tip usually placed in right atrium or inferior vena cava
- Once catheter becomes colonized, immediate removal necessary
- Miconizole & Amphotericin B used to treat infections

Case Study #4

- 17 year old male in Texas
- Presented to medical center
- 3 week history of fever, malaise, shortness of breath & hacking cough productive of yellow sputum
- Stated he was diagnosed with bacterial pneumonia by another local medical facility 10 days previously

Patient History

- His symptoms persisted over past 10 days, despite taking antibiotics
- Laboratory tests were performed
- All results were normal except:
  - Increased WBC count (19.6 x 10^9L)
  - Increased neutrophils (89%)
  - Erythrocyte Sed. Rate (98 mm/hr)

Laboratory Results

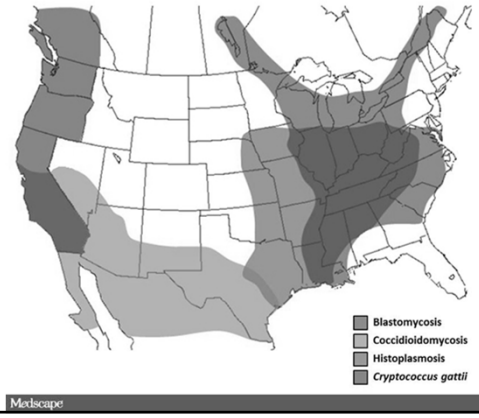
- Two sputum collections failed to produce acceptable specimens for culture
- Bronchial lavage performed
- Culture yielded
  - normal flora
  - no acid fast bacilli, *Mycoplasma* sp., *Legionella* sp., or viruses

Laboratory Results

- Fluorescent potassium hydroxide (KOH) prep of bronchial lavage
  - Revealed many broad-based budding yeast
- After 3 days of proper incubation, fungal culture revealed heavy growth of broad-based budding yeast
- Identified as *Blastomyces dermatitidis*
  - Pulmonary blastomycosis

Blastomyces dermatitidis

- Causative agent of blastomycosis
- Occurs primarily in N. America & Africa
- Highest incidence in AR, KE, LA, MS, NC, & TN
- Lives in soil & wood products (moist environment)
- Men are more commonly infected (9:1)



Blastomyces dermatitidis

- Dimorphic organism (ability to grow in mold & yeast form)
- Classified as one of the systemic mycoses
- Disease contracted by inhalation of infectious conidia (no person to person transmission)
- All laboratory procedures to identify these organisms must be performed under a biological safety cabinet
- Fatal unless treated

Clinical Disease

- Initially asymptomatic, or exhibit flu-like symptoms
- Progresses to pulmonary disease (cough, weight loss, chest pain & fever)
- Invasive disease may follow (ulcerative lesions of skin & bone)
- In immunocompromised persons, multiple organs may be involved, & rapidly fatal

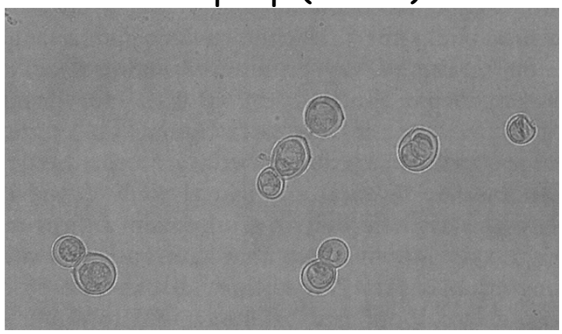
Laboratory Diagnosis

- Diagnosis requires identification of the organism in tissue or isolation in culture
- First morning sputum, bronchial washings & other pulmonary secretions, exudative material from lesions & tissue may be examined & cultured
- Serology not always reliable

Direct Examination

- Typical findings on KOH is diagnostic:
  - Large, spherical, refractile yeast cells
  - 8-15 um in diameter
  - Double-contoured wall & buds connected by a broad base
  - Stains gram negative

Blastomyces dermatitidis: KOH prep (Yeast)



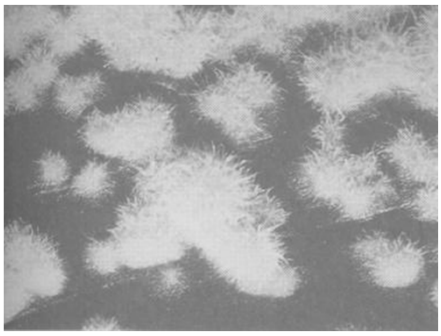
Culture

- Even though direct smear is diagnostic, cultures should also be performed
- Two sets of cultures should be set up, one incubated at 25°C & the other at 37°C
- Slow growing (2-6 weeks)

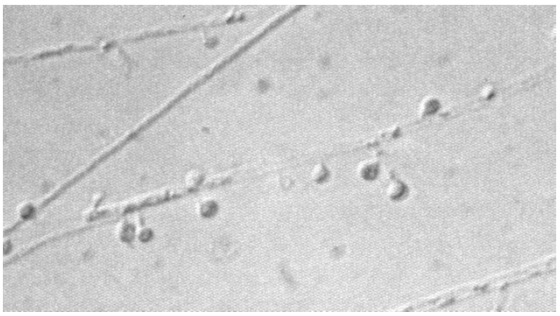
Culture

- Colonies appear waxy; develops a white, fluffy aerial mycelium; may eventually turn brown or grey
- Microscopically: delicate septate hyphae with single, round conidia attached to conidiophore; resembling lollipops
- Converts to a yeast form when incubated at 37°C in as little as 3 days

Blastomyces dermatitidis – Culture (5% sheep blood agar)



Blastomyces dermatitidis - Mold



Treatment

- Patient was placed on intravenous antifungal therapy (Amphotericin B)
- Condition improved dramatically

- Advances in antifungal therapy have lowered the mortality rate from 90% to 10%
- Amphotericin B remains the drug of choice
- Ketoconazole & Itraconazole are used in non-life-threatening cases

Further Patient History

- Possible origin of illness
  - Patient stated he spent several weeks at his grandfather's house in Tennessee, which had recently been flooded
  - He used a wheelbarrow to transport mud, rotting leaves & other debris from the house
  - Most likely the source of the infection

Case Study #5

- Previously healthy 31 year old male
- Special Forces unit of the U.S. Army
- Deployed to Afghanistan in March, 2014
- Returned home to U.S. in September, 2014
- Febrile illness (spikes to 104°F)
  - Began 3 months after leaving Afghanistan (December, 2014)

Patient History

- Reported using personal protective measures & malarial prophylaxis
- No history of blood transfusions
- No other travel history
- Further testing performed to rule out HIV, CMV, hepatitis & malaria
  - All testing was negative

Laboratory Testing

- CT scan revealed enlarged liver
- Bone marrow & liver biopsies were negative for microscopic exam and culture

Further history and testing

- Patient remembered being bit by several sandflies
- Liver biopsy re-examined by light microscopy
  - Probable leishmanial parasites were noted

Laboratory Testing

- Serological testing performed to confirm diagnosis:
  - Indirect fluorescent antibody test
  - Marked reactivity
- Final Diagnosis: Visceral Leishmaniasis (February, 2014)
- Patient became afebrile 1 week after therapy with Amphotericin B

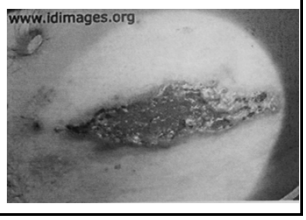
Leishmania species

- Etiologic agent of leishmaniasis
- Transmitted by bite of sand fly
- Endemic in more than 70 countries worldwide
- World Health Organization estimates approx. 1.5 million new cases each year
  - 90% of cases in Afghanistan, Brazil, Pakistan, Peru, Saudi Arabia, & Syria

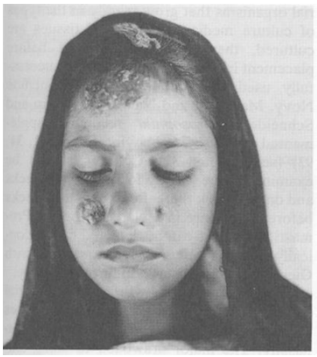
Clinical Disease

- Cutaneous
  - Firm, painless lesion develops at site of bite
  - Incubation period may be as short as 2 wks. or as long as 3 years
  - Usually remains localized at insect bite site
  - Secondary infections can complicate healing process

Cutaneous Leishmaniasis



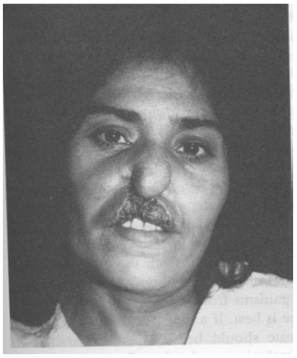
Cutaneous Leishmaniasis



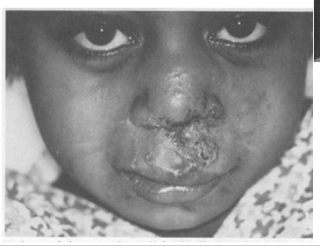
Clinical Disease

- Mucocutaneous
  - Lesion similar to cutaneous leishmaniasis
  - Metastatically spreads to nasal or oral mucosa, if untreated
  - Results in progressive ulceration & erosion of mucosal linings
  - Death usually due to secondary infections &/or malnutrition

Mucocutaneous Leishmaniasis



Mucocutaneous Leishmaniasis



Clinical Disease

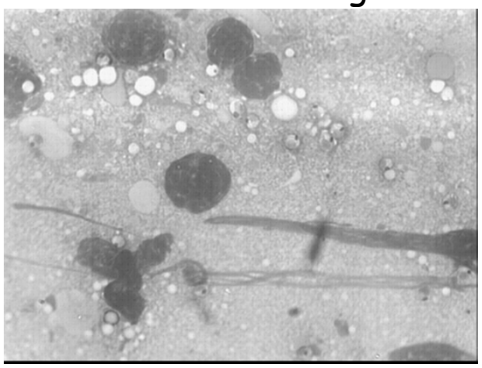
- Visceral Leishmaniasis
  - Fever, anorexia, weight loss, diarrhea
  - Marked enlargement of liver & spleen
  - Death results from complications such as septicemia, pneumonia or dysentery
  - Incubation period usually 2-4 months
  - Onset of disease may be insidious or acute

Visceral Leishmaniasis

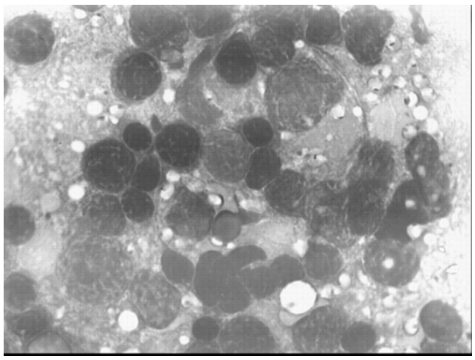
Laboratory Diagnosis

- Definitive diagnosis
  - Amastigotes in patient specimen
  - Specimen of choice: aspirate or biopsy from lesion, liver, spleen, bone marrow
- Additional testing
  - Marked increase in gamma globulins (IgG & IgM)

Leishmania Amastigotes



Leishmania Amastigotes



Prevention & Treatment

- Prevention
  - Personal protection against sand fly bites
  - No vaccine available
- Treatment
  - Pentostam is drug of choice
  - Amphotericin B also used
  - Follow-up smears should be examined 1-2 weeks post-therapy

UPDATES & ADDITIONAL INFORMATION

Guinea Worm: 2nd Human Disease to be Eradicated

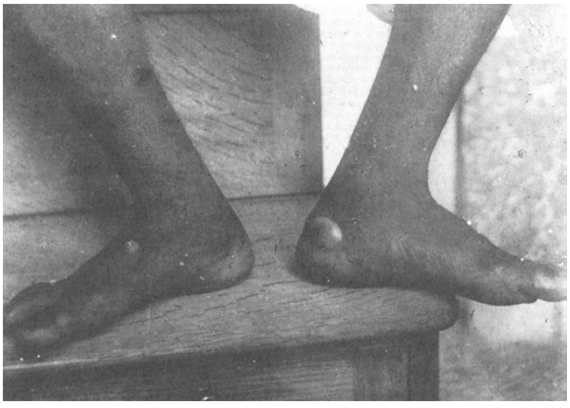
- Set to become the 2nd human disease in history (after smallpox) to be eradicated
- Will be the 1st parasitic disease to be eradicated
- Will be the 1st disease to be eradicated without the use of a vaccine or medicine

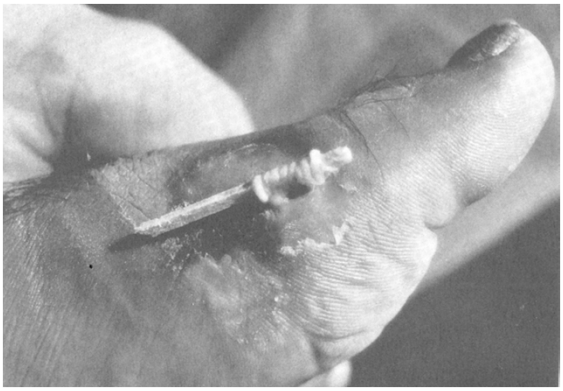
Guinea Worm: 2nd Human Disease to be Eradicated

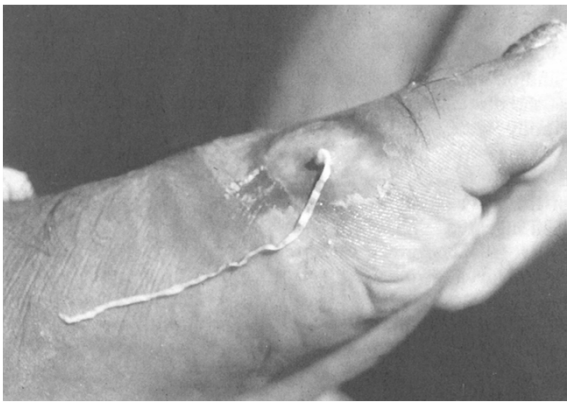
- *Dracunculus medinensis* – nematode worm causing parasitic infection
- Contracted by drinking contaminated water
- Since 1986, The Carter Center, founded by former U.S. President Jimmy Carter, has led international campaign to eradicate the disease

Drucunculus medinensis

- Intermediate host: copepod (water flea)
- Copepod containing infective larvae ingested in contaminated drinking water
- Larvae mature to adults & migrate to lower extremities and forms painful blister
- When blister touches water, larvae released into water

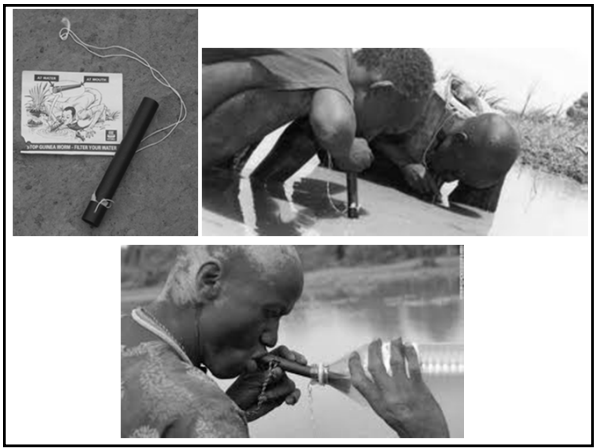






Guinea Worm Prevention

- The Carter Center
  - Strategy is to work with ministries of health to educate people to stop the spread of disease
    - CDC, WHO, & UNICEF
  - Community-based interventions to educate & change behavior
    - Filter or boil all drinking water
    - Preventing infected persons from entering water sources



Guinea Worm Prevalence

- 1986
  - Estimated 3.5 million people in 21 countries in Africa & Asia affected
- 2014
  - 126 cases (most in South Sudan, Africa)
    - Reduced by more than 99.9%
    - Prevented at least 80 million new cases

Guinea Worm Prevalence UPDATE….

- 2016
  - Last cases will be seen
  - Certification of disease-free status takes 3 years from final case reported
- Estimated cost
  - $350 million
  - Compare to $9.5 billion to eradicate polio
  - No drugs or vaccinations for guinea worm

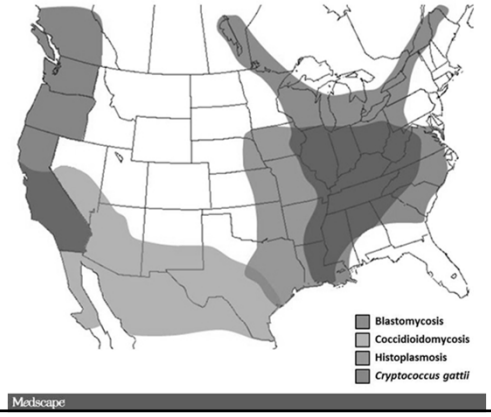
Recent Article in MMWR
June 19, 2015

"Coccidioidomycosis
in a State Where It is Not Known to Be Endemic"

Missouri, 2004 - 2013

Coccidioides immitis

- Causative agent of coccidioidomycosis
- Dimorphic organism
- Lives in the soil
- Endemic in some areas of U.S.
  - semiarid areas with hot summers, wet winters & infrequent frosts - deserts
- Seen worldwide due to travel to endemic areas



Clinical Disease

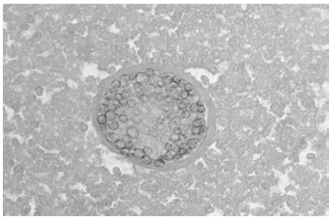
- Most virulent of all human mycotic agents (inhalation of only a few arthroconidia produces disease)
- Incubation is 7-28 days
- Symptoms include fever, malaise, dry cough, chest pain, night sweats & anorexia
- Most cases resolve in 3 weeks to 3 months
- Can become disseminated

Laboratory Diagnosis

- Variety of specimens
  - Sputum
  - Trachial aspirate
  - Lung biopsy
  - CSF
  - Blood culture

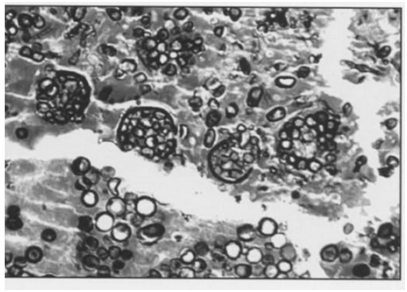
Laboratory Diagnosis

- Direct Examination (KOH)
  - Reveals non-budding, thick-walled spherules containing endospores – unique to this organism



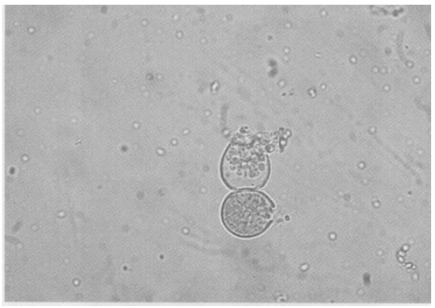
Patient Specimen – Lung Tissue

- Gomori-methenamine silver stain



Coccidioides immitis

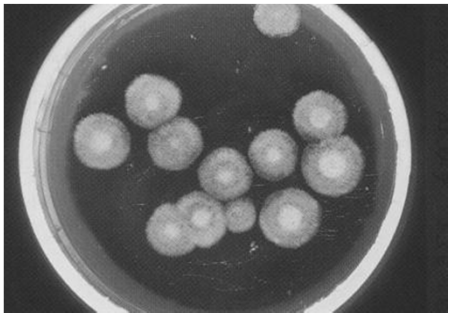
Resembles *Blastomyces dermatitidis*



Laboratory Diagnosis

- Culture
  - Rapid growing (3-7 days)
  - Young culture is cobweb-like; colony enlarges in a circular "bloom"
  - Microscopic: characteristic alternating arthroconidia (septation of hyphae that exhibits empty spaces between barrel-shaped arthroconidia)

Coccidioides immitis Culture



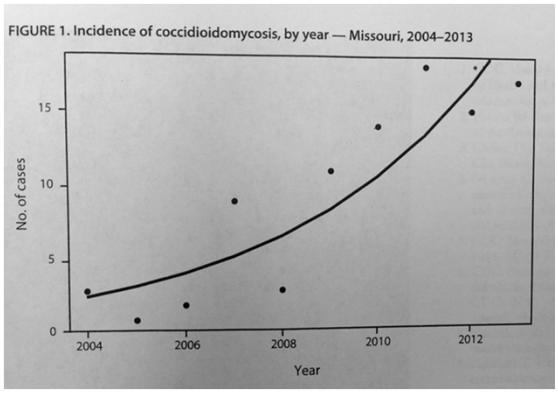
Coccidioides immitis : Mold

Treatment & Prevention

- Two forms of treatment
  - Surgical: Removal of infected tissue
  - Medical: Antifungal therapy with Amphotericin B
- Prevention
  - Reducing exposure to soil dust (wet down soil to lessen potential for aerosols)
  - Vaccine effective in animals, but ineffective in humans

Statistics

- During 1998 – 2011
  - 97% of all cases reported to CDC were from Arizona & California
  - Recently seen cases increase nationally nearly eightfold
    - Many of these cases are in areas that are not endemic
    - Could be due to increased awareness among health care providers & the public
    - Also better availability of diagnostic tests



Rapid Tests for Fungal Infections

- Demand for fast, easy to use, & sensitive testing is on the rise
- Labs desperately need accelerated detection methods to identify fungi quickly
- The sooner an infection is identified, the sooner the patient will receive a potentially life-saving treatment

Rapid Tests for Fungal Infections

- NanoLogix, Inc.
  - An Ohio-based biotechnology company
  - Developing testing that incorporates speed & specificity in diagnosing viruses, bacterial infections, & fungal infections
  - Results in a few hours to overnight

Rapid Tests for Fungal Infections

- BioNanoFilter (BNF)
  - Antibody/antigen test for fungal infections
  - Recently granted a patent
- N-Assay
  - Unique ELISA multiwell machine-readable assay for bacteria & fungi
  - Results in a few hours
  - High sensitivity & specificity
  - Projected to provide point-of-care rapid diagnostics

Any Questions?