Lecture 7: Emerging Parasitic Protozoa part 1 (Intro, Intestinal Protozoa (non-Apicomplexan), FLA, Microsporidia)

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Readings-Protozoa pt. 1
• Ch. 8 (p. 163 [table 8.2])
• Ch. 11 (pp. 272-74, 275-76, 286)

Monsters Inside Me
• Primary amebic encephalitis (Naegleria fowleri, free living amoeba)

• Acanthamoeba keratitis (Acanthamoeba spp., free living amoeba)
  Background: http://animal.discovery.com/invertebrates/monsters-inside-me/acanthamoeba-keratitis.html

Learning objectives: Protozoans
• Describe basic characteristics of protozoa
• Know basic life cycle and developmental stages
• Required hosts
  — Transmission strategy
  — Infective and diagnostic stages
  — Unique character of reproduction
• Know the common characteristics of each group
  — Be able to contrast and compare
• Diseases, high-risk groups
• Diagnostic methods, treatment
• Know important parasite survival strategies

Protozoa (boring biology?)
• Unicellular Eukaryotes
• Motility
  — Cilia, Flagella, Amoeboid, Gliding
• Phylum Sarcomastigophora
  — Flagellates (Trypanosoma, Leishmania, Giardia, Trichomonas)
  — Ameobae (Entamoeba spp., Naegleria, Acanthamoeba)
• Phylum Ciliophora (Balantidium)
• Phylum Microsporidia (encompasses many genera)
• Phylum Apicomplexa-sporozoa (Cyclospora, Cryptosporidium, Toxoplasma, Plasmodium, Babesia)
• Usually range from 10–50μm

Taxonomic Overview
Parasitic Protozoa

- Infect a variety of hosts
- Multiplication within hosts, enabling huge numbers in short periods
- 50,000 species of protozoa, of which a fifth are parasitic
- Life cycles
  - Usually less complex than helminths
  - Many examples of direct and indirect
  - Cyst form offers protection against harsh conditions, allowing to survive extreme temperatures or harmful chemicals or lack of food, water, or oxygen

### Table 7.1: Major Protozoan Diseases

<table>
<thead>
<tr>
<th>Organism</th>
<th>Classification</th>
<th>Disease</th>
<th>Transmission</th>
<th>Principal Site(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giardia</td>
<td></td>
<td></td>
<td>Water, direct contact</td>
<td>Intestinal tract</td>
</tr>
<tr>
<td>Entamoeba</td>
<td></td>
<td>Amoebae</td>
<td>Water, food, direct contact</td>
<td>Intestinal tract</td>
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<tr>
<td>Intestinal</td>
<td></td>
<td></td>
<td>Water, food, direct contact</td>
<td>Intestinal tract</td>
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<tr>
<td>Sarcodinae</td>
<td></td>
<td></td>
<td>Food, contact with cat</td>
<td>Brain, heart, lungs, possible transfer to extra transmission</td>
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<tr>
<td>Crithidia</td>
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<tr>
<td>Trichomonas</td>
<td></td>
<td>Flagellates</td>
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<tr>
<td>Giardia lamblia</td>
<td></td>
<td>Flagellates</td>
<td></td>
<td></td>
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<tr>
<td>Trichomonas vaginalis</td>
<td></td>
<td>Flagellates</td>
<td></td>
<td></td>
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<tr>
<td>Balantidium</td>
<td></td>
<td>Ciliates</td>
<td></td>
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<tr>
<td>Heterolobosema</td>
<td></td>
<td>Free-living amoebae</td>
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<tr>
<td>Alveolata</td>
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<td>Free-living amoebae</td>
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<tr>
<td>Coelomobranchia</td>
<td></td>
<td>Free-living amoebae</td>
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<tr>
<td>Microsporidia</td>
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</table>

On the Menu: Lecture 7

**Intestinal Protozoa**
- Amoebae
  - *Entamoeba histolytica*
- Flagellates
  - *Giardia lamblia*
- *Trichomonas vaginalis*
- Ciliates
  - *Balantidium coli*

**Other Important Protozoa**
- Free-living amoebae
- *Microsporidia*

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**Entamoeba histolytica**

- Cosmopolitan distribution
- Infects 500 million people worldwide - causes Amebiasis
- 100,000 deaths per year have been attributed to complications of amebiasis, notably amoebic liver abscesses
  - Second in terms of deaths from parasitic protozoa
- More prevalent and more severe in tropical/subtropical locations
- Risk groups
  - Crowded conditions: orphanages, prisons, mental institutions may exacerbate transmission of disease.
- 1-5% infected in U.S.
- Highly pathogenic
- Humans=definitive host
- Fecal-oral transmission

**E. histolytica Life Cycle**
trophozoites and cysts

Symptoms and Pathology

- Asymptomatic infection ("luminal amebiasis")
- Invasive intestinal amebiasis (dysentery, colitis, appendicitis, toxic megacolon)
- Invasive extraintestinal amebiasis (liver abscess, peritonitis, pleuropulmonary abscess, cutaneous and genital amebic lesions).
- Death and illness due to dysentery and liver abscess.
- Typical flask-shaped lesions in the large intestines.

Amebiasis caused by Entamoeba histolytica

Diagnosis

- Microscopic identification of cysts and trophozoites in the stool
  - Characteristics: bulls-eye nucleus, ingested red blood cells, chromatin bar
- Differentiation from other amoebae based on morphologic characteristics of the cysts and trophozoites.
  - Entamoeba dispar (non-pathogenic) vs. E. histolytica, based on isoenzymatic, immunologic, or molecular analysis.

Treatment

- Asymptomatic - only carry cysts
  - Metronidazole (Flagyl)
  - Iodoquinol
  - Diloxanide furoate (Furamide) if passing cysts (not in U.S.)
  - Paromomycin
- Symptomatic
  - Acute amebic dysentery
    - Emetine HCL
  - Liver abscess
    - Flagyl
    - Tinidazole or Omidazole (not in US)
      - Followed by treatment with iodoquinol, paromomycin, or diloxanide furate

Prevention

- Safety of drinking water supplies
  - Cysts are fairly resistant to chlorination of drinking water
- Environmentally stable cysts
  - Heat water above 50°F
  - Freezing kills cysts
- Traveler precautions

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Outbreaks

- World's Fair, Chicago (1933)  
  >1400 cases, 100 deaths
- Los Angeles, CA. (1983)  
  38 patients over the course of 3 months were diagnosed, in comparison to a previous frequency of about 1 case per month.
- Tbilisi, republic of Georgia (1998)  
  A case-control study identified 177 cases, but outbreak was widespread may have affected 84,000–225,000 people.
- Emerging in Japan  
  Male homosexuals and mentally handicapped persons in institutions.
  - 500–600 cases of amebiasis reported annually, with 3-4 deaths

Giardia lamblia

- Obligate parasite that infects numerous mammalian hosts
- Fecal-oral transmission through food and water
- Distributed worldwide, more prevalent in warm climates, and in children.
  - Up to 2 million cases in U.S. per year
  - Prevalence 3-7% in developed countries
  - Average prevalence of 20% in undeveloped countries
- The most frequent cause of non-bacterial diarrhea in U.S.
- Risk groups in the US: travelers, children in day care, homosexual men

Giardia Life Cycle

- Acute disease develops after an incubation period of 1-14 days (average of 7 days) and usually lasts 1-3 weeks.
- Symptoms:
  - Diarrhea, abdominal pain, bloating, nausea, vomiting, steatorrhea (fatty diarrhea).
  - In chronic giardiasis, the symptoms are recurrent and malabsorption and debilitation may occur.
  - Suction force may damage microvilli.
  - Large number of parasites may interfere mechanically with digestion.
  - Symptoms may result from inflammation of the mucosal cells of the small intestine.
- Asymptomatic carriage
- Antigenic Switching occurs during differentiation to cyst stage when a parasite is transmitted from one host to another.
  - Enhances the probability of parasite being transmitted to reservoir animals that are partially immune to other variants.

Diagnosis and Treatment

- Identification of cysts or trophozoites (monkey-face morphology) in the feces
- Samples of duodenal fluid or duodenal biopsy may demonstrate trophozoites.
- Antigen detection tests by enzyme immunoassays, and detection of parasites by immunofluorescence.
- Treatment: Metronidazole, Tinidazole, Nitazoxanide in children.

Prevention

- Proper hygiene and disposal of waste
- Avoid drinking water from sources associated with outbreaks
- Avoid swallowing water while swimming in public pools, water parks
- Avoid livestock and exotic pets, zoonotic transmission
- In day care centers: wash hands and proper disposing of diapers
- Hikers and backpackers: boil, filter, chemically treat water
- Water filtration (moderate Chlorine resistance)

Major Outbreaks

- Europe
  - Sweden 1986: >1400 cases
  - Norway 1994: >1300 cases
- U.S.
  - Colorado (Vail-1978): 5,000 cases
  - Bradford, PA. (1979): 3500 cases
  - Berlin, N.H. (1977): 7000 cases
  - Florida (1996): 77 cases

Trichomoniasis: Diagnosis/Rx

- Clinical presentation: discharge, strawberry cervix
- In men, anterior urethral or prostatic secretions should be examined.
- Microscopic examination of vaginal and urethral secretions can establish the diagnosis by detecting actively motile organisms.
- Direct immunofluorescent antibody staining is more sensitive than wet mounts, but technically more complex.
- PCR assays

Treatment

- Metronidazole and tinidazole; therapy is usually highly successful.
- Strains of Trichomonas vaginalis resistant to both drugs have been reported.

Prevention

- Abstain from sexual contact, or to be in a long-term mutually monogamous relationship with a partner who has been tested and is known to be uninfected.
- Condom use for as long as both partners are infected.
- Any genital symptom such as discharge or burning during urination or an unusual sore or rash = consult a health care provider immediately.
- Possible transmission via contaminated towels, washcloths, clothing.
### Balantidium coli

- Largest protozoan and only ciliate infecting humans
  - Trophozoites: 50x35 µm and 100x70 µm, Cysts: 50-70 µm
- Tissue invasive in large intestines: causes dysentery
- Extraintestinal spread (e.g., lungs, liver)
- Pigs implicated as reservoir host
  - Other potential animal reservoirs include rodents and nonhuman primates.
- Worldwide distribution
- Periodic outbreaks in institutionalized populations

### Naegleria fowleri

- Usually found in warm bodies of water such as ponds, irrigation ditches, lakes, coastal waters, and hot springs, well water
  - Water temp. between 77–95°F
- Can cause primary amebic meningoencephalitis
- Other species are non-pathogenic to humans

### Naegleria fowleri in the USA

- In U.S., has caused infections in 15 southern tier states
  - 121 deaths in the U.S. from 1937 through 2007.
  - From 1998 to 2007, 33 infections were reported in the U.S., all but one died
  - 6 documented deaths in 2007, all in warmer regions (Arizona, Texas, Florida)
- Florida
  - Prior to 2008, primary amebic meningoencephalitis was not a reportable disease in Florida.
  - 30 cases have been documented from 1962 through 2007. Of the 30 cases, 19 were from Central Florida, (5 in Orange County in 2007)
  - Other counties: Baker, Brevard, Broward, Citrus, Lee, Manatee-Dade, Orange, Pinellas, Polk, Putnam, Seminole, and 1 unknown county. All cases resulted in death.

### Free-living amoebae

- Species of Acanthamoeba, Balamuthia, Naegleria and Sappinia are important causes of disease in humans and animals.
  - 9 species of Acanthamoeba implicated in human disease.
- Naegleria fowleri and Acanthamoeba spp. commonly found in lakes, swimming pools, tap water, soil, and heating and air conditioning units.
- Balamuthia mandrillaris, is morphologically similar to Acanthamoeba and causes human disease.
- While infrequent, infections occur worldwide.
  - Can be isolated from all types of fresh water and soil habitats

### Comparing, Giardia, Trichomonas and E. Histolytica

<table>
<thead>
<tr>
<th>G. lamblia</th>
<th>E. histolytica</th>
<th>T. vaginalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>Flagellate</td>
<td>Amoeba</td>
</tr>
<tr>
<td>Life forms</td>
<td>Trophozoite, Resistant Cyst</td>
<td>Trophozoite, Resistant Cyst</td>
</tr>
<tr>
<td>Host</td>
<td>Many mammals, including humans</td>
<td>Humans, Only</td>
</tr>
<tr>
<td>Transmission</td>
<td>Ingestion of Cyst</td>
<td>Ingestion of Cyst</td>
</tr>
<tr>
<td>Disease Manifestations</td>
<td>Diarrhea/Dysentary</td>
<td>Diarrhea/Dysentery</td>
</tr>
</tbody>
</table>

Review, anyone?
3 deaths in Summer 2007 around Orlando area

Primary Amebic Meningoencephalitis

- Acute and usually lethal, central nervous system (CNS) disease
- The ameba enters the nasal passages, penetrates the nasopharyngeal mucosa, and migrates to the olfactory nerves, eventually invading the brain through the cribriform plate.
- The initial symptoms of PAM start 1-14 days after infection.
  - Headache, fever, nausea, vomiting, and stiff neck.
- As the ameba causes more extensive destruction of brain tissue, this leads to confusion, lack of attention to people and surroundings, loss of balance and bodily control, seizures, and hallucinations.
- Progresses rapidly (<10 days) and frequently to coma and death.

Diagnosis and Treatment

- In Naegleria infections, the diagnosis can be made by microscopic examination of cerebrospinal fluid (CSF).
- A wet mount may detect motile trophozoites, and a Giemsa-stained smear will show trophozoites with typical morphology.
- Often diagnosed post-mortem
- Can be found by MRI and treated with antibiotics if found early enough
- Amphotericin B is effective against N. fowleri in vitro
- After progression to PAM, less than 1% survival

Naegleria Prevention

- Avoid swimming or jumping into bodies of warm freshwater, hot springs, and thermally polluted water such as water around power plants.
  - Water above 80°F use caution
- Avoid swimming or jumping into freshwater during periods of high temperature and low water volume.
- Hold the nose shut or use nose clips when jumping or diving into bodies of warm fresh water such as lakes, rivers, or hot springs.

Acanthamoeba spp., Balamuthia

- Both are opportunistic protozoan pathogen that rarely causes disease in humans.
  - Acanthamoeba spp.
    - Approximately 400 cases have been reported worldwide with a survival rate of 2-3%.
    - No flagella and can’t tolerate water as hot as Naegleria can
    - Usually skin infections, but serious disease in immunocompromised
  - Balamuthia mandrillaris
    - Approximately 100 published and unpublished cases of Balamuthia amebic encephalitis (BAE) have been reported; most were fatal.
    - Extremely rare, mostly in immunocompromised

Life cycles

Acanthamoeba spp.

Balamuthia mandrillaris
Diseases

- Both can cause granulomatous amebic encephalitis (GAE) in individuals with compromised immune systems.
- *Acanthamoeba* crosses the blood brain barrier and invades connective tissue, induction of pro-inflammatory responses leads to neuronal damage which can be fatal within days.
  - Subacute symptoms including altered mental status, headaches, fever, neck stiffness, seizures, other neuropathies leading to coma and death
  - Also, granulomatous skin lesions and keratitis, corneal ulcers following corneal trauma or contaminated contact lens use.
- *Balamuthia*-induced GAE can cause focal paralysis, seizures, and brainstem symptoms such as facial paralysis, difficulty swallowing, and double vision.
  - Also causes a variety of non-neurological symptoms, and often causes skin lesions, through which the amoeba may enter the bloodstream and migrate to the brain.

Prevention

- Encephalitis diseases: similar to *Naegleria*
- IC careful in environment-soil, water
- *Acanthamoeba* keratitis
  - Wear and replace contact lenses according to the schedule prescribed by your eye care provider.
  - Remove contact lenses before any activity involving contact with water, including showering, using a hot tub, or swimming.
  - Wash hands with soap and water and dry before handling contact lenses.
- MRSA can infect and replicate inside of *Acanthamoeba polyphaga*; Since *A. polyphaga* can form cysts, cysts infected with MRSA can act as a mode of airborne dispersal for MRSA.
  - Pathogens that emerge from amoeba are more resistant to antibiotics and more virulent?

Microsporidia

- One general phylum
- Obligate, spore-forming, intracellular parasites that invade vertebrates and invertebrates.
- Polar tube or polar filament found in the spore used to infiltrate host cells.
- More than 1,200 species belonging to 143 genera have been described as parasites infecting a wide range of vertebrate and invertebrate host.
- First human case was described in 1959 in a Japanese child.
- Transmission route is unclear
- Since 1985, microsporidia have been identified as a cause of opportunistic infections associated with persistent diarrhea and weight loss in persons with AIDS

Diagnosis and Tx

- In *Acanthamoeba* infections, the dx from microscopic examination of stained smears of biopsy specimens (brain tissue, skin, cornea) or of corneal scrapings
- Confocal microscopy or cultivation of the causal organism, and its identification by direct immunofluorescent antibody, may also prove useful.
- Post-mortem biopsy reveals severe oedema and hemorrhagic necrosis.

Treatment

- The misdiagnosis of bacterial encephalitis often leads to erroneous treatment that is ineffective.
- In the case that *Acanthamoeba* is diagnosed correctly, amphotericin-B, rifampicin, trimethoprim-sulfamethoxazole, ketoconazole, fluconazole, sulfadiazine, albendazole are only tentatively successful.

Microsporidia species

- Encephalitozoon cuniculi
- *Enterocytozoon bieneusi* (inflammation of the small intestine, with chronic diarrhea)
- *Trachipleistophora anthropophthera*
- *Trachipleistophora hominis*
- *Pleistophora intestinalis*
- *Nosema corneum*
- *Vittaforma corneae*
- *Brachiola algerae*
- *Enterocytozoon bisphagnosis*
- *Encephalitozoon intestinalis*
- *Naegleria fowleri*
- *Balamuthia mandrillaris*
- *Septata inferens*
- *Trachipleistophora communis*
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Wide range of disease

- Occurs mainly, but not exclusively, in severely immunocompromised patients with AIDS.
- Chronic diarrhea and wasting are the most common symptoms of microsporidiosis.
- Disseminated infection is characterized by symptoms of cholecystitis (inflammation of the gallbladder), renal failure, respiratory infection, headache, nasal congestion, ocular pain and sinus involvement.
- Respiratory infection may cause cough, dyspnea (labored breathing) and wheezing.
- With ocular infection, symptoms range from foreign body sensations, eye pain, light sensitivity, redness, excessive tearing or blurred vision.
- Finally, infections of the brain or other nervous tissue cause seizures, headache and other symptoms depending the precise area of infection.

Diagnosis and Treatment

- DX: Microscopic ID-light and TEM, IFA, PCR
- Ocular microsporidiosis:
  - Oral Albendazole plus topical fumagillin.
  - Corneal infections with V. corneae often do not respond to chemotherapy and may require keratoplasty.
- Oral fumagillin has been effective to treat Enterocytozoon bieneusi infections, but it has been associated with thrombocytopenia.
- Albendazole for gastroenteritis caused by Encephalitozoon intestinalis and to treat disseminated microsporidiosis (various species) and skin and deep muscle infection (Brachiola algerae).

Prevention

- Transmission is still unclear, but possibly by inhalation, ingestion.
- Contaminated food and water sources?
- Highly resistant spores can survive outside host for up to several years.
- Proper disinfection, sterilization in health care settings.