Lecture 8: Emerging Parasitic Protozoa part 1 (Apicomplexans-1: Coccidia)

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Readings-Protozoa pt. 2 (Coccidia)

- Ch. 8 (p. 163 [table 8.2])
- Ch. 11 (p. 274, 276-78)
- Ch. 13 (p. 340)

Monsters Inside Me

- Cryptosporidiosis (Cryptosporidium spp., Coccidian/Apicomplexan):
  Background: http://animal.discovery.com/invertebrates/monsters-inside-me/cryptosporidium-outbreak.html
- Toxoplasmosis (Toxoplasma gondii, Coccidian/Apicomplexan)
  Background: http://animal.discovery.com/invertebrates/monsters-inside-me/toxoplasma-gondii-parasite.html

Learning objectives: Apicomplexan coccidia

- Define basic attributes of Apicomplexans- unique characteristics?
- Know basic life cycle and developmental stages of coccidian parasites
- Required hosts
  - Transmission strategy
  - Infective and diagnostic stages
  - Unique character of reproduction
- Know the common characteristics of each parasite
  - Be able to contrast and compare
- Define diseases, high-risk groups
- Determine diagnostic methods, treatment
- Know important parasite survival strategies
- Be familiar with outbreaks caused by coccidians and the conditions involved

Taxonomic Review

Table 7.1: Classification of Parasitic Protozoa and Associated Diseases
**Phylum Apicomplexa (the Sporozoa)**

- Obligate (intracellular) parasites
- Apical complex for attachment or penetration of cells
- >5,000 species – includes some of the most important human and animal pathogens

**Coccidia**

- Spore-forming, single-celled parasites belonging to the apicomplexan class Conoidasida, subclass Coccidiasina
- Infect the intestinal tracts, liver, kidneys, blood cells, other tissues of vertebrates and invertebrates
- Largest group of apicomplexan protozoa
- Characterized by a thick walled oocyst stage that is typically excreted with the feces

**Isospora belli**

- Only infects man
- Least common of the three intestinal coccidia that infect humans.
- Infects the epithelial cells of the small intestine
- Found worldwide, especially in tropical and subtropical areas.
- Infection occurs in immunosuppressed individuals, and outbreaks have been reported in institutionalized groups in the United States
- U.S.-Infections are more commonly observed in Hispanics, foreign-born patients, and HIV-positive homosexual men rather than those who acquired HIV by some other route such as intravenous drug use

**Isosporiasis**

- Note large oocyst with two sporocysts inside (these in turn contain sporozoites)
- This part of parasite development is similar for other coccidia

**Phylum Apicomplexa, Class Conoidasida**

- *Isospora belli*
- *Cryptosporidium* spp.
- *Cyclospora cayetanensis*
- *Toxoplasma gondii*
**Isosporiasis**

- **Disease**: Acute nonbloody diarrhea with crampy abdominal pain, which can last for weeks and result in malabsorption and weight loss.
  - In immunodepressed patients, and in infants and children, the diarrhea can be severe.
- **Diagnosis**: ID of large, typically shaped oocysts in stool, found in biopsies
  - Differential interference contrast (DIC), and epifluorescence.
- **Treatment**: Trimethoprim-sulfamethoxazol
- **Prevention**: Eliminate fecal-oral transmission, improved personal hygiene, sanitation

**An emerging threat?**

- Unlike crypto and cyclo, doesn’t cause large outbreaks
- Can be a chronic problem in AIDS patients
- Drug resistance?
  - HAART=highly active antiretroviral therapy

**Cyclospora cayetanensis**

- The first human cases were reported in 1979
- The species designation *Cyclospora cayetanensis* was given in 1994 to Peruvian isolates of human-associated *Cyclospora*.
- Not immediately infective
- 15,000 cases in U.S. annually
  - 90% food related

**Geographic Distribution**

- Cyclosporiasis has been reported worldwide, but is most common in Latin America, the Indian subcontinent, and southeast Asia
- Since 1990, at least 11 foodborne outbreaks of cyclosporiasis, affecting approximately 3600 persons, have been documented in the United States and Canada.

**Life Cycle**

When freshly passed in stools, the oocyst is not infective (thus, direct fecal-oral transmission cannot occur; this differentiates Cyclospora from another important coccidian parasite, Cryptosporidium).

**Clinical Features**

- After an average incubation period of 1 week, symptoms occur: watery diarrhea, which can be severe.
- Other symptoms include anorexia, weight loss, abdominal pain, nausea and vomiting, myalgias, low-grade fever, and fatigue.
- Untreated infections typically last for 10-12 weeks and may follow a relapsing course.
Diagnosis and Treatment

• Identification of oocysts in stool specimens by light microscopy.
• Characteristic autofluorescence
• Trimethoprim-sulfamethoxazole
• Supportive measures include management of fluid and electrolyte balance, and rest.

Prevention/Control

• Wash fruits and vegetables
• Be cautious when traveling to developing countries.
• Wash hands before eating and preparing food.
• Oocysts not affected by normal chlorine concentrations
• Monitoring of water used for irrigation

Why we don’t look for it in U.S.
• No outbreaks associated with treated drinking water
• No good method for recovery and detection in drinking water

Outbreaks

• Linked to importation of fresh produce
  - Raspberries, blackberries, fresh basil, fresh baby lettuce leaves, snow peas
• Two major outbreaks linked to raspberries from Guatemala
• Before 1996, only three outbreaks of Cyclospora infection had been reported in the United States.
• Between May 1 and mid-July 1996 almost 1,000 laboratory-confirmed cases were reported to the CDC.
• These infections occurred in at least 15 states and Canadian provinces and the District of Columbia.

Cyclospora outbreaks in 1990s

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Species</th>
<th>Number of cases</th>
<th>Source of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>Palm Beach County</td>
<td>C. hominis</td>
<td>592</td>
<td>Fresh basil in chain restaurants</td>
</tr>
<tr>
<td>1997</td>
<td>Leon County</td>
<td>C. parvum</td>
<td>40</td>
<td>Lettuce</td>
</tr>
<tr>
<td>1997</td>
<td>Orange County</td>
<td>C. parvum</td>
<td>544</td>
<td>Lettuce</td>
</tr>
<tr>
<td>1999</td>
<td>Palm Beach County</td>
<td>Multiple fruits</td>
<td>Undetermined</td>
<td>Snow peas</td>
</tr>
</tbody>
</table>

Recent Florida Outbreaks

• 1996 –Palm Beach County, multiple clusters, raspberries, part of multi-state outbreak
• 1997 –Leon County –lettuce
• 1997 –Orange County –lettuce
• 1999 –Palm Beach County –undetermined (multiple fruits)
• 2005– 592 cases, fresh basil implicated in chain restaurants

Cryptosporidium spp.

• First recognized as disease agent in 1976
• Invade and grow intracellularly in mucosal epithelial cells of the stomach and intestine
• Human infections caused by C. hominis, C. parvum
  - C. hominis: only human-human transmission
  - C. parvum: Widespread in non-human hosts: found in >155 mammalian species
• Recognized as a cause of death in AIDS patients in 1980s
• Worldwide distribution
• Does not have mitochondria or apicoplast

Worldwide distribution

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Crypto Life cycle

Crypto Life cycle

Cryptosporidiosis

- A wide range of manifestations, from asymptomatic infections to severe, life-threatening illness
- Incubation period averages 7 days (but can range from 2-10 days).
- Watery diarrhea is the most frequent symptom, and can be accompanied by dehydration, weight loss, abdominal pain, fever, nausea and vomiting.
- In immunocompetent persons, symptoms are usually short lived (1-2 weeks); they can be chronic and more severe in immunocompromised patients-severe dehydration, death.
- While the small intestine is the site most commonly affected, symptomatic Cryptosporidium infections have also been found in other organs including other digestive tract organs, the lungs, and conjunctiva.
- Minimum infective dose –10 to 100 oocysts depending upon species.
- Estimated 3,000 cases per year in U.S. (under-estimated)

Laboratory

- Acid-fast staining methods for oocysts are most frequently used in clinical laboratories.
- For greatest sensitivity and specificity, immunofluorescence microscopy is the method of choice (followed closely by enzyme immunoassays).
- PCR
- Oocysts in stool specimens (fresh or in storage media) remain infective for extended periods, and must be rendered nonviable before processing

Treatment

- Rapid loss of fluids because of diarrhea can be managed by fluid and electrolyte replacement.
- Nitazoxanide has been approved for treatment of diarrhea caused by Cryptosporidium in immunocompetent patients.
- For persons with AIDS, anti-retroviral therapy, which improves immune status, will also reduce oocyst excretion and decrease diarrhea associated with cryptosporidiosis.

Prevention and Control

- Thick-walled cysts can persist in environment for a long time
- Resistant to chlorination, but filtration and ozonation OK
- Management of watersheds where filtration not possible
- Surveillance to keep public water supplies pathogen-free
  - PCR continual monitoring
- Avoid touching animals-petting zoos, pet stores
- Day care settings

Outbreaks

- 1987 Carrollton, Georgia: ~13,000 people
- 1993 Milwaukee, Wisconsin: ~400,000 people
  - Spring rains and runoff from surrounding farmland had drained into Lake Michigan and overburdened the water supply system. Dairy cattle were the most likely source of this outbreak
  - Contributed to the deaths of more than 100 AIDS and chemotherapy patients.
- Non-outbreak cryptosporidiosis cases reported nationally increased from 3,411 cases in 2004 to nearly 8,300 in 2007-this mirrors the increase in the number of nationally reported cryptosporidiosis outbreaks associated with treated recreational water venues (e.g., pools, water parks, and interactive fountains)
  - Seven reported treated recreational water-associated outbreaks in 2004, 19 in 2006, and reports of 26 in 2007
- Feb 2009-present: Sydney, Australia (over 600 cases)
Recent Florida Outbreaks

<table>
<thead>
<tr>
<th>Year</th>
<th>County</th>
<th>Cases</th>
<th>Site of Outbreak</th>
<th>Vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>Alachua</td>
<td>72</td>
<td>School</td>
<td>Hose</td>
</tr>
<tr>
<td>1996</td>
<td>Broward</td>
<td>22</td>
<td>Public Water</td>
<td>Pool</td>
</tr>
<tr>
<td>1998</td>
<td>Indian River</td>
<td>7</td>
<td>Pool</td>
<td>Surface Water</td>
</tr>
<tr>
<td>1999</td>
<td>Hillsborough</td>
<td>6</td>
<td>Pool</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>Volusia</td>
<td>38</td>
<td>Public Water</td>
<td>Interactive Water Fountain</td>
</tr>
<tr>
<td>2000</td>
<td>Nassau</td>
<td>19</td>
<td>Pool</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>St. Johns</td>
<td>5</td>
<td>Pool</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>Volusia</td>
<td>38</td>
<td>Public Water</td>
<td>Drinking Water</td>
</tr>
<tr>
<td>2002</td>
<td>Brevard</td>
<td>37</td>
<td>Other</td>
<td>Other</td>
</tr>
</tbody>
</table>

http://www.doh.state.fl.us/ENVIRONMENT/COMMUNITY/foodsurveillance/pdfs/3_panel_crypto.pdf

Toxoplasma gondii

- Cats are definitive hosts, act as reservoirs
- Serologic prevalence data indicate that toxoplasmosis is one of the most common of human infections throughout the world.
  - >500 million infected worldwide
- More than 60 million people in the U.S. carry the parasite, but very few have symptoms
  - Studies in 1988-2004 and 1999-2000 found 22.5% and 15.8% people (12-49 age) had antibodies, respectively
  - Third leading cause of death attributed to foodborne illness in the U.S.

Toxoplasma parasite stages

- T. gondii primarily exists in three forms: oocysts, tachyzoites, and bradyzoites.
- Oocysts are only produced in the definitive host (cats)
- When passed in feces and then ingested, the oocysts can infect humans and other intermediate hosts. They develop into tachyzoites, which are the rapidly multiplying trophozoite form
- They divide rapidly in cells, causing tissue destruction and spreading the infection.
  - Tachyzoites in pregnant women are capable of infecting the fetus.
- Eventually tachyzoites localize to muscle tissues and the CNS where they convert to tissue cysts, or bradyzoites.
- Bradyzoites can cause infection if ingested in contaminated food

Toxoplasma Life Cycle

- Human infection through ingestion of sporulated Toxoplasma oocysts or bradyzoites (tissue cysts)
- Tachyzoites may be found in various sites throughout the body of the host.
- Tachyzoites in macrophage cytoplasm (carried and disseminated throughout the body by infected macrophages)
- Oocysts can survive in the environment for several months and are remarkably resistant to disinfectants, freezing, and drying, but are killed by heating to 70°C for 10 minutes.
- Generally an asymptomatic infection
- 10% to 20% of patients with acute infection may develop cervical lymphadenopathy and/or a flu-like illness.
- The clinical course is usually benign and self-limited; symptoms usually resolve within a few months to a year.
- Immunodeficient patients often have central nervous system (CNS) disease but may have retinocchoroiditis, or pneumonitis.
Cerebral toxoplasmosis (AIDS)

- In people whose immune defenses are weakened because of AIDS, cancer or immunosuppressant medication, a new toxoplasmosis infection may spread out of control and become deadly, or dormant *Toxoplasma* parasites from infection may suddenly become active again and cause severe illness.
- In patients with AIDS, toxoplasmic encephalitis is the most common cause of intracerebral mass lesions.

Congenital Toxoplasmosis

- Results from an acute primary infection acquired by the mother during pregnancy.
- Abortions and stillbirths after primary infection
- 60% of infected newborns are asymptomatic (later - retinochoroiditis)
- Hydrocephaly or microcephaly, intracranial calcifications, deafness, seizures, hepatosplenomegaly, jaundice, fever, anemia, pneumoniacerbral palsy, damage to the retina, and mental retardation.
- Some sequelae of congenital toxoplasmosis are not apparent at birth and may become apparent years later
- The most dangerous period to acquire toxoplasmosis is at 24 to 30 weeks of pregnancy (10% chance of the baby being severely affected)

Retinochoroiditis

- Inflammation of the retina and choroid (thick vascular area at back of eye), can result from congenital infections or from acute or reactivated infections acquired postnatally.
- With congenital infection, retinochoroiditis can develop weeks to years after birth.
- The lesions are focal in nature and generally self-limiting.
- Symptoms can include blurred vision or other visual defects.
- The disease is rarely progressive in immunocompetent individuals, but can scar the retina.
- The disease can be quite severe in AIDS patients and continue to progress.

Laboratory Diagnosis

- Serologic testing is the routine method of diagnosis.
- Observation of parasites in patient specimens, such as bronchoalveolar lavage material from immunocompromised patients, or lymph node biopsy.
- Isolation of parasites from blood or other body fluids, by intraperitoneal inoculation into mice or tissue culture.
- Detection of parasite genetic material by PCR, especially in detecting congenital infections in utero.

Prevention of Toxoplasmosis

**Environmental Exposures**
- Avoid drinking untreated drinking water, particularly when traveling in less developed countries.
- Avoid contact with soil or sand that is contaminated with cat feces.
- Feed cats only canned or dried commercial food or well-cooked table food, not raw or undercooked meats.
- Cats infested with *Toxoplasma* typically shed infective eggs for one period of 3 - 21 days during their lives.
- If you are pregnant or immunocompromised:
  - Avoid changing cat litter if possible.
  - Keep cats indoors.
  - No new kittens

**Prevention-Foodborne**
- Cook food to safe temperatures.
  - Beef, lamb, and veal roasts and steaks should be cooked to at least 145°F throughout.
  - Pork, ground meat, and wild game should be cooked to 160°F.
  - Whole poultry should be cooked to 180°F in the thigh.
- Peel or wash fruits and vegetables thoroughly before eating.
- Freeze meat for several days before cooking to greatly reduce chance of infection.
**Tx**

- Treatment is not needed for a healthy person who is not pregnant.
- Treatment may be recommended for pregnant women or persons who have weakened immune systems.
- Sulfadiazine + pyrimethamine or clindamycin + pyrimethamine

**Toxoplasma and Human Behavior**

- Chronic infection causes subtle behavior changes in people?
  - Reaction time is affected
  - Women seem to become more intelligent, outgoing, conscientious, sexually promiscuous, and kind; changes in men seem to cause opposite trends.
- *Toxoplasma gondii* tend to encyst is in the amygdala of the brain (controls emotions-responses to social situations, sexual responses, aggression, fear, anxiety)
- Infected mice have increased activity levels and show increased aggression, and rats show a marked decrease in their natural fear of cat odors (more likely to be eaten by a cat?)

**Cause for Concern?**

- Mental disorders
  - Studies show that people with schizophrenia and other mental health problems have a higher incidence of exposure to *T. gondii* than healthy controls.
  - Antipsychotic drugs commonly used for people show activity against *T. gondii* parasites and infected rodents show reduced behavioral changes when treated with these drugs.
  - Since 1993, a total of 19 studies of *T. gondii* antibodies in persons with schizophrenia and other severe psychiatric disorders and in controls have been reported
  - UK (2009) toxoplasmosis parasite is statistically linked to the development of schizophrenia and other psychoses - by producing the enzyme tyrosine hydroxylase, which increases the production of L-DOPA, the precursor of dopamine in the brain.
- Organ, bone marrow transplantations
- Blood transfusions

**Outbreaks**

- Outbreaks associated with drinking water
  - First documented outbreak associated with municipal water supply: British Columbia (1995)-100 cases
  - Brazil (2001-2002)-155 cases
  - India (2001-2004)-178 ocular toxoplasma cases
- Unknown source
  - Turkey, 2002: 171 cases