Coccidia
Toxoplasma gondii, Sarcocystis spp., Isospora belli, Cryptosporidium spp., Cyclospora cayetanensis

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This lecture handout is prepared for a course 317331, second semester, academic year 2018 of Department of Parasitology, Faculty of Medicine, CMU
Learning objectives

• After class, students will be able to:
  • Describe morphology, life cycle, signs and symptoms, prevention and control, laboratory diagnosis and treatment of coccidian of man
  • Differentiate oocysts of coccidian causing human infection
Coccidia

- Collectively, *intracellular* organisms in suborder Eimeriorina in Phylum Apicomplexa
  - Apical complex for host cell invasion
- 10 families, at least 42 genera, and over 2,000 named species
- Life history has both asexual and sexual phase of development

Reproduction

ASEXUAL: progeny=merozites

- Merogony or schizogony
  - Multiple fission- from one cell to many cells

- Endodyogeny
  - Internal budding from one mother cell to 2 daughter cells

- Endopolygeny
  - Internal budding to many daughter cells

SEXUAL: progeny=sporozoites

- Gamogony
  - Formation of gamont or gametocytes (sex cells: macro=female, micro=male)

- Gametogony
  - Formation of gametes

- Zygote
  - Following fertilization

- Oocyst
  - Zygote surrounded by cyst wall
Sexual reproduction

- Sporogony: production of sporozoites

  ![Diagram showing the process of sexual reproduction with stages: Zygote and Oocyst (immature or unsporulated).]
Taxonomy of coccidia

- Based on number of sporocysts and sporozoites in sporulated oocyst
Taxonomy- coccidia of medical importance

0+4 Cryptosporidium
Naked sporozoites

2+4 Cyclospora

2+8 Cystoisospora
Toxoplasma
Sarcocystis
# Coccidia of medical importance

<table>
<thead>
<tr>
<th></th>
<th>Life cycle</th>
<th>Opportunist</th>
<th>Affected organs/ Disease in man</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>Complex Cat=DH</td>
<td>+</td>
<td>Brain-Toxoplastic encephalitis Developing organs-Congenital toxoplasmosis</td>
</tr>
<tr>
<td><em>Sarcocystis bovihominis, S. hominis</em></td>
<td>Complex Cattle, pig=IH</td>
<td>-</td>
<td>Small intestine-Eosinophilic enteritis</td>
</tr>
<tr>
<td><em>S. nesbitti</em> or other spp.</td>
<td>Cobra, pthon=DH</td>
<td>-</td>
<td>Muscle</td>
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<tr>
<td><em>Cystoisospora belli</em></td>
<td>Simple</td>
<td>+</td>
<td>Small intestine-Diarrhea</td>
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<tr>
<td><em>Cryptosporidium</em></td>
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<td><em>Cyclospora cayetanensis</em></td>
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<td>+</td>
<td>Small intestine-Diarrhea</td>
</tr>
</tbody>
</table>

*Now classified as Gregarine*
Toxoplasma gondii

- Disease: toxoplasmosis
  - Congenital toxoplasmosis
  - Toxoplasmic encephalitis in AIDS
- Stages in life cycle
  - Tachyzoites, 6 x 2 µm: during *acute stage* of infection
  - Bradyzoites, during *chronic stage* of infection
  - Oocyst, 10 x 12 µm
Life cycle

- PP = 3 - 10 d

**Asexual phase**
- Bradyzoites
- In tissue cysts
- Tachyzoites
- Sporozoites

**Enteroepithelial cycle**
- Merogony
- Gametogony
- Fertilization

**Sporulation**
- Infective oocysts
- Containing sporozoites

rowdysites.msudenver.edu
Source of infections to human
(Clin Microbiol Rev 2012; 25(2):

1. hand-to-mouth (oocyst)
2. consumption of infected meat (tissue cysts), contaminated vegetable and water (oocyst)
3. Mother-to-child (tachyzoite)
4. Organ transplant (tissue cyst), especially heart
5. Blood transfusion (tachyzoite)
Pathogenesis, Signs, Symptoms

• Acute phase- tachyzoites invade and destroy host cells
  • Lymphadenopathy, flu-like symptoms in 10-20%
  • Incubation period 5-23 days

• Chronic phase
  • Immune pressure: Tachyzoites → bradyzoites (tissue cysts) in brain, muscle, liver
  • Symptomless

• Reactivate in AIDS → toxoplasmic encephalitis

• Congenital toxoplasmosis: chorioretinitis, hydrocephalus, intracerebral calcification
Epidemiology

- Worldwide distribution
- Organism isolated from free range chicken in some developing countries up to 100%.
- Farmed sheep up to 89% seroprevalence.
- Prevalence very low in farmed pigs and cattle in US.
- 25-30% of world human population are infected, but asymptomatic.
  - High prevalence in Latin America and Africa
- Congenital toxoplasmosis ranges from approximately 1 in 3000 births to 1 in 10,000 births.
- Risk of transmission from an infected mother is estimated to be 25% during the first trimester, and results in high fetal mortality rate.
Diagnosis & treatment

• Isolation of parasite impractical
• Primarily diagnosed by serology (antibody detection)
• Treatment: pyrimethamine and sulfadiazine, plus folinic acid.
• Infected pregnant women: spiramycin to reduce risk of fetal infection
Prevention and control

- Sporulated oocysts survive in:
  - moist environment for a year,
  - cold water for several years
  - -10 C for 4 months
  - 35 C for 32 days

- Not killed by water treatment, e.g., chlorination, ozone treatment

- Die in arid, cool environment

- Tincture of iodine (2%) - 3 hours

- Clean litter box daily and rinse with boiling water

- Thorough hand washing after exposure to soil or cat litter box

- Boil or filter natural water source

- Cook meat

- Freeze meat at -12 C for at least 3 days

- Fruits and vegetables should be peeled or washed well.
What are the chances of toxoplasmosis causing problems during pregnancy?

If you get toxoplasmosis for the first time during pregnancy, the risk to your child largely depends on when you were infected:

- Infection in early pregnancy is less likely to spread to your baby, but if problems do develop they are likely to be more serious.
- Infection later in pregnancy is more likely to spread to your baby, but any problems that develop are likely to be less severe.

It's estimated that only 1 in 10,000 babies is born with toxoplasmosis in the UK.


How can I prevent toxoplasmosis?

- Avoid exposures to cat feces; get someone else to change the kitty litter.
- Keep cats off counters.
- Do not give your cat raw meat.
- Wash your hands thoroughly after contact with your cat or contact with raw meat.
- Keep counters clean and cook meat thoroughly.
- When eating out, order meat well done.
- Good hygienic measures prevent transmission.

http://americanpregnancy.org/pregnancy-complications/toxoplasmosis/
Further readings


**Sarcocystis spp.**

**Eating meat**

- Intestinal sarcocystosis
  - Usually no signs and symptoms, a few cases of segmental eosinophilic enteritis
- Human as definitive host
  - *S. hominis*: bovine = IH
  - *S. suis* *ominis*: porcine = IH
- Not opportunistic parasite

**Drinking contaminated water or food**

- Muscular sarcocystosis
  - Fever, and/or myalgia, arthralgia, headache, fatigue
  - Later myositis: pain and swelling. Eosinophilia, high CPK
- Human as accidental host
  - *S. nesbitti* of snake (cobra)
- Not opportunistic parasite
Morphology

- Oocysts: thin-walled, sporulated when come out in faeces; “8”-like
- Sporocyst size:
  - 15 x 9 μm (S. hominis); 13 x 9 μm (S. suihominis)
- Sarcocysts with septa
  - Bradyzoites (banana shape)
Humans as definitive (final) hosts for Sarcocystis species.

Intestinal sarcocystosis

Muscular sarcocystosis

Intermediate host ingested by snake
Snake (Definitive host) species reticulated python/Cobra
Sporocyst in snake faeces
Cysts in the muscle
Monkey/rodent? (Intermediate hosts)
Contaminated food or water
Man (incidental intermediate host)

Incubation period about 2 weeks
Muscle biopsy

Food and waterborne parasitology
October 2015; 1: 2-11.

Asian Pac J Trop Biomed
Diagnosis

• Intestinal sarcocystosis
  • Microscopic observation of oocysts or sporocysts in fecal smear
    • Wet fecal smear- sporulated oocysts
    • Acid fast stain- irregular staining (some stained, some don’t)

• Muscular sarcocystosis
  • Tissue biopsy, section and stained.

From: Parasite.czu.cz

From: jcm.asm.org
Epidemiology

- Worldwide, affect livestock rather than human
- Muscular sarcocystosis outbreaks in Malaysia
  - 1999 (7 cases),
  - 2011 (32 cases),
  - 2012 (100 cases),
  - 2014 (6 cases)

Treatment, prevention and control

• Human sarcocystosis requires no treatment (self recovered)

• Supportive treatment of muscular sarcocystosis
  • Albendazole, ivermectin (suppress symptoms),
  • Metronidazole and cotrimoxazole (prolong symptoms)
  • Oral steroid (reduce inflammation)

• Cook meat (intestinal sarcocystosis)

• Filtered drinking water, avoid fresh fruit or salad (muscular sarcocystosis)

32 year old student from China with facial swelling from swollen temporalis and masseter muscles (Italiano CM, et al. PLOS neglected trop dis 2014)
Cystoisospora belli

- Only in human and primate
- Asexual and sexual reproduction in epithelial cell of the small intestine
- Oocyst sporulation outside host: 1-5 days
- Acute infection: diarrhea, self-limited  
  - Incubation period 1-2 weeks
- Immunodeficient person: severe diarrhea
- Diagnosis: fecal exam for unsporulated oocyst, 20-23 x 10-19 μm
- Treatment: trimethoprim and sulfamethoxazole
Cryptosporidium

- Parasite of mammals, birds, reptiles, fish, amphibians: quite host-nonspecific
- Over 17 species infect human
- Main species = *C. parvum, C. hominis*
  - *C. parvum* - human and calves
  - *C. hominis* - human and primate
- Oocyst 5 µm in diameter, smallest of all human coccidian
  - 4 naked sporozoites
• New classification of *Cryptosporidium* as Gregarine and a facultative parasite

• “*Cryptosporidium* is a ubiquitous, pleiomorphic, facultatively epicellular gregarine protozoan, capable of extended existence in the environment, that is elusive, opportunistic and zoonotic with the potential to cause disease and death in humans and domestic animals.”

Thompson RCA et al, 2016

• Life cycle similar to *Cystoisospora belli* (development occurs in GI epithelium) except:
  • Organism is epicellular
  • Sporulation in host cells-
    • thick-wall oocysts
    • thin-wall oocysts (autoinfective)
• PP app. 2 days, IP app. 7-10 days
• New classification of *Cryptosporidium* as Gregarine and a facultative parasite

Signs and symptoms

• Emerging infectious disease
• Impaired intestinal absorption + enhanced secretion
• Watery diarrhea, abdominal cramp, fever
• In immunocompetent persons, self-limited in 2 wk.
• In immunocompromised persons, severe diarrhea and malabsorption syndrome
• An important opportunist in AIDS patients

• 4 Clinical patterns:
  • < 4 stool/day
  • diarrhea < 2 mo.
  • Diarrhea > 2 mo.
  • Fulminant infection, 2L watery stool daily (CD4 < 50/microliter)

• Extraintestinal dissemination can occur,
  • most common= biliary tract
Epidemiology

- *Cryptosporidium* was ranked fifth out of 24 potentially foodborne parasites in terms of importance as a foodborne pathogen (FAO/WHO, 2014)
- Much higher prevalence than other intestinal coccidiosis
- Infection worldwide including Thailand (i.e., account for 5% of patient diarrhea)
- Oocysts survive in water and soil at 4 C or -4 C more than 12 weeks; resist chlorination
- Transmission- Contaminated drinking water, food, direct contact
Outbreak

• 1993- Outbreak involving 403,000 persons in Milwaukee associated with drinking water with an estimated illness-associated cost of US $96.2 million and 100 deaths.

• 2010, the second largest waterborne outbreak occurred in the Swedish city of Östersund with an estimated 27,000 individuals infected.

• Food reported to source of infection: salad, “Yukke” (Korean-style beef tartare) and/or raw liver, unpasturised milk, Béarnaise sauce containing chopped fresh parsley
Parasite factors contributing to high prevalence

1. Oocysts are shed infective and ready to infect upon ingestion.
2. Oocysts are resistant to disinfection including chlorine.
3. Neonatal calves (*C. parvum*) can excrete up to 30 billion oocysts, thus spreading in environment.
4. Oocysts may continue to be shed after GI symptoms resolve.
5. Infectious dose is low (10–100 oocysts).
6. Oocysts on fruit and vegetables are still infectious for several days to weeks in a household refrigerator.
Diagnosis, treatment, prevention and control

• Diagnosis by stool exam for oocysts
• No effective, specific chemotherapy
• Prevention by drink filtered or boiled water, cook food, wash hands

• Further readings

Oocyst, stool smear, acid fast stain
**Cyclospora cayetanensis**

- Man is the only host
- Infect the small intestine, similar life cycle with *C. belli* → diarrhea
  - Unsporulated when come out with faeces
  - Sporulation requires 7-15 days in external environment
  - 2 sporocysts, each with 2 sporozoites
- IP app. 7 days

Signs and symptoms

• Diarrhea (5-15 times/day)
• Self-limited (19-57 days), recurrent
• More severe in AIDS patients
• Traveler's diarrhea
  • Implicated in Europeans returning from Asian/South American countries
  • Indonesia, Nepal, Thailand, Guatemala, Mexico
• Imported vegetables, salad & fruits = most frequent source of infection
  • Oocysts survive a week at 4 C
• As of September 11, 2018, CDC was notified of 511 laboratory-confirmed cases of *Cyclospora* infections in people from 15 states and New York City who reported consuming a variety of salads from McDonald’s restaurants in the Midwest.

• Twenty-four (24) people were hospitalized. No deaths were reported.
• Diagnosis
  • Stool exam for oocysts
  • Acid fast stain- must differentiate from Cryptosporidium
    • Oocyst slightly bigger than Cryptosporidium oocyst, i.e., 8.6 µm
    • Staining not uniform: dark red, unstained
• Prevention & control: avoid exotic salad & vegetable or wash well
• Treatment: Trimethoprim, sulfamethoxazole
Conclusion

• Coccidia are members of the protozoa in phylum Apicomplexa
  • They are obligate intracellular parasites, except Cryptosporidium which are closer to gregarine and are epicellular, facultative protozoan parasite.
• Toxoplasmic encephalitis is fatal in AIDS due to reactivation of tissue cysts.
• Congenital toxoplasmosis leads to abnormality of child.
• Intestinal coccidiosis causes diarrhea, and coccidia are opportunistic parasite, except *Sarcocystis*.
• Cryptosporidiosis in spread in much wider area than other intestinal coccidia and has no specific therapeutic agent.
End of lecture