Protozoa infections in immuno-compromised host

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Learning objectives

• After class, students will be able to:
  • Describe morphology, life cycle, signs and symptoms, epidemiology, prevention and control, laboratory diagnosis and treatment of opportunistic parasites of man
Opportunistic parasites

• Sporozoa/ Coccidia
  • *Toxoplasma gondii*
  • *Cystoisospora belli*
  • *Cryptosporidium*
  • *Etc.*

• Fungi
  • *Pneumocystis jirovecii*
  • Microspora
Recall

• Opportunistic parasites are those infecting host without signs and symptoms or with signs and symptoms but the host can recover without anti-parasitic drug.

• Opportunistic parasites become important as they produce severe disease and high mortality in immunocompromised hosts.
The Coccidia

• Collectively intracellular organisms in suborder Eimeriorina in Phylum Apicomplexa
• Eimeriorina has 10 families, at least 42 genera, and over 2,000 named species
• Life history has both asexual and sexual phase of development
Phylum Apicomplexa

• Obligate intracellular parasite
• Apical complex organelles for host cell invasion
  • Conoid
  • Polar ring
  • Rhoptry
  • Microneme
  • Subpellicular microtubules
• Sexual and asexual reproduction

http://www.nature.com/scitable/topicpage/the-apicoplast-an-organelle-with-a-green-14231555
Reproduction: terminology

• Asexual
  • Schizogony or merogony = multiple fission
  • 1 sporozite or 1 merozoite → many merozoites

• Sexual
  • Gamogony: 1 merozoite → 1 gamont
  • 1 microgamont → 1 microgamete
  • Sporulation: 1 zygote → 1 oocyst → many sporozoites
Molecular details of the moving junction (MJ) formed during host cell invasion by apicomplexan parasites.

Apicomplexan cell division flexibility.


Asexual reproduction

Taxonomy of coccidia

• Based on number of sporocysts and sporozoites in oocyst

Unsporulated oocyst

Sporont

Oocyst wall

Sporoblasts

Sporocyst wall

Sporocysts

Sporozoites

Sporulated oocyst
Taxonomy - examples

0+4  
*Cryptosporidium*
Naked sporozoites

2+8  
*Cystoisospora*
*Toxoplasma*
*Sarcocystis*
## Opportunistic parasites: Apicomplexa

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**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
    - with glycogen granules
    - Pepsin-resistant
  - Oocyst, 10 x 12 µm

*Toxon* = arc

Image from https://www2.bc.edu/~gubbelsj/Toxoplasma.html
In the small intestine

**Definitive host**

**Intermediate hosts**
Asexual reproduction in cells and tissues

**Accidental host**
Ingestion of meat containing tissue cysts

Feline Definitive host

See: scielo.br

Immature oocyst in feces

Oocyst sporulation in external environment
Oocyst sporulation in external environment

See: Toxophysics.wordpress.com
Intermediate hosts

Asexual reproduction in cells and tissues

Sporozites

Ingestion of sporulated oocysts

Acute phase of infection = tachyzoites
Chronic phase of infection → Bradyzoites in tissue cyst

In feces

Sexual reproduction

1-5 days

Unsporulated oocyst
In feces

Sporulated oocyst
In soil

Definitive host
The small intestine

Intermediate hosts
Cells, tissues and organs

Sporozoites
Tachyzoites
Bradyzoites
In Tissue cysts

Accidental host

See: Toxophysics.wordpress.com
See: iss.it
Watch video

• *Toxoplasma gondii* - Life cycle and Invasion
  
  • [https://www.youtube.com/watch?v=hlKOVvAeq88&list=PLoeuxutUxJnmwFiC4Yw8dgZzLjrPwumP](https://www.youtube.com/watch?v=hlKOVvAeq88&list=PLoeuxutUxJnmwFiC4Yw8dgZzLjrPwumP)

• Gliding
  
  • [https://www.youtube.com/watch?v=5qHNoTZMz6w](https://www.youtube.com/watch?v=5qHNoTZMz6w)
• **Cats**
  - Ingest tissue cysts (bradyzoites), PP = 3-10 days (main mode)
  - Ingest tachyzoites or sporozoites, PP longer

• **Man acquires infection from**
  - Consumption of improperly cooked meat (tissue cysts)
  - Drinking unfiltered water (oocysts)
  - Contaminated hand (oocysts)
  - Organ transplant, blood transfusion (tachyzoites)
Pathogenesis, Signs, Symptoms

- **Acute phase** - tachyzoites invade and destroy host cells
  - Lymphadenopathy, flu-like symptoms
- **Chronic phase**
  - Immune pressure: Tachyzoites → bradyzoites (tissue cysts) in brain, muscle
  - Become asymptomatic
- Reactivate in AIDS → toxoplasmic encephalitis
- Congenital toxoplasmosis: chorioretinitis, hydrocephalus, intracerebral calcification
Diagnosis, prevention & treatment

- Isolation of parasite impractical
- Primarily diagnosed by serology (antibody detection)
- Serological survey shows worldwide distribution including Thailand
- Prevention by cook meat, proper handling of cat’s feces
- Treatment: pyrimethamine and sulfadiazine, plus folinic acid.
Toxoplasmosis: An Important Message for Cat Owners

What role do cats play in the spread of toxoplasmosis?
Cats get *Toxoplasma* infection by eating infected rodents, birds or other small animals, or anything contaminated with feces from another cat shedding the microscopic parasite in its feces. After a cat has been infected, it can shed the parasite for up to two weeks. The parasite becomes infective one to five days after it is passed in the feces of the cat. The parasite can remain infectious in the environment for many months and contaminate soil, water, food, vegetables, sandboxes, grass where animals graze for food, litter box, or a place where an infected cat may have defecated.

What is toxoplasmosis?
Toxoplasmosis is an infection caused by a microscopic parasite called *Toxoplasma gondii*. More than 60 million people in the United States are infected with this parasite.

What are the symptoms of toxoplasmosis?
*Individuals with healthy systems:*
Most people who become infected are asymptomatic or have mild signs of illness, which are often caused by an illness associated with the common cold. The symptoms include:
- Fever
- Headache
- Myalgia
- Muscle aches
- Fatigue

*Individuals with immunocompromised systems:*
- Severe disease affecting the nervous system, eye, heart, and other organs
- Pulmonary toxoplasmosis
- Encephalitis
- Retinitis
- Chorioretinitis

*Cystoisospora belli*

- Only in human and primate
- Merogony, gametogony, oocyst formation in epithelial cell of the small intestine
- Oocyst sporulation outside host: 1-5 days
- Acute infection: diarrhea, self-limited
- Immunodeficient person: severe diarrhea
• Diagnosis:
  • Fecal exam: simple smear or acid fast stain
  • Unsporulated oocyst, 20-23 x 10-19 μm
  • Contains 1 or 2 sporoblasts
• Treatment: trimethoprim and sulfamethoxazole
Cryptosporidium

- Parasite of mammals, birds, reptiles, fish, amphibians: rather host-nonspecific
  - Man & Mammals: *C. hominis*, *C. parvum*, *C. ubiquitum*, *C. suis*, etc.
  - Birds: *C. meleagridis*, *C. baileyi*, *C. galli*
- Human: mostly *C. parvum*, *C. hominis*
- Oocyst 5 µm in diameter, smallest of all human coccidian
  - 4 naked sporozoites

Unstained faecal smear (marvistavet.com)

Acid-fast stained smear (getfor.4t.com)
• Life cycle similar to *Cystoisospora belli* (development occurs in GI epithelium) except:
  • Organism is intracellular, extracytoplasmic
  • Sporulation in host cells-
    • thick-wall oocysts
    • thin-wall oocysts (autoinfective)
• PP app. 2 days, IP app. 7-10 days
Emerging infectious disease
Impaired intestinal absorption + enhanced secretion
Watery diarrhea, abdominal cramp, fever
The median maximum number of stool per day is 12
Self-limited in 2 wk.
Infected persons secrete large amount of oocysts in feces
Infectious dose 10-100 oocysts
• 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
• Infection worldwide including Thailand
  • Account for 5% of patient diarrhea
• Contaminated drinking water, food, direct contact
  • Clam
  • Outbreak involving 403,000 persons in Milwaukee associated with drinking water
• No effective, specific chemotherapy
• Diagnosis by stool exam for oocysts
  • Acid fast stain fecal smear
Pneumocystis jirovecii

• First identified in 1909 in rat lung, named *Pneumocystis carinii* of uncertain taxonomy
• Cause infantile pneumonia
• Recently classified as atypical fungus in Phylum Ascomycota, Family Pneumocystidaceae
• Renamed *Pneumocystis jirovecii* to be distinctive from rat’s species
Morphology

• Extracellular, obligate, host-specific, yeast-like parasitic fungi
• Live in alveoli
• Trophic forms (1-4 µm) adhere to alveolar epithelium
• Precyst stages (early, intermediate, late)
• Mature cyst or ascus (8-10 µm) with 8 intracystic bodies (spores)
Putative life cycle of Pneumocystis.


CYST or ASCUS
Disease and treatment

- Pneumocystis pneumonia, interstitial plasma cell pneumonia
  - Premature infants
  - AIDS
  - Organ transplant subjects
  - Malignancies
  - Steroid treatment, long term
- Fever, cough, dyspnea
- Radiograph: bilateral, diffuse, reticular, or granular opacities
- Trimethoprim-sulfamethoxazole (TMP-SMX)
  - Chemoprophylaxis when CD4 < 200 cells/microliter

Histological picture: Alveolar spaces filled with eosinophilic, vacuolated or foamy exudate.
Epidemiology

• Niches uncertain, airborne transmission
• Human acquires infection during infancy, asymptomatic, latent infection
• Reactivated when immunocompromised
Diagnosis

• Specimen from lungs
  • Induced sputum
  • Bronchoalveolar lavage (BAL)

• Stain for cysts
  • Toluidine Blue O

• Stain for trophics forms and intracystic bodies
  • Giemsa stain
P. carinii asci/cyst forms stained with methenamine silver.

Beck J M, and Cushion M T Eukaryotic Cell 2009;8:446-460
Pneumocystis carinii trophic and cyst forms.

Beck J M, and Cushion M T Eukaryotic Cell 2009;8:446-460
Watch Video

- Pneumocystis Pneumonia & Pneumocystis Jirovecii
- https://www.youtube.com/watch?v=cuZb539SaaY
Microspora

• A group of organisms: small single-celled, obligate intracellular parasites belonging to Phylum Microspora
• Early- eukaryotic protozoa
• Recently- classified as fungi
• >2,000 species infecting vertebrates and invertebrates
• 14 species infecting human- truly opportunistic
• *Enterocytozoon bieneusi, Encephalitozoon intestinalis*= most common
Some microsporidia infecting human

- Encephalitozoon cuniculi
- Encephalitozoon hellem
- Encephalitozoon (Septata) intestinalis
- Enterocytozoon bieneusi
- Trachipleistophora hominis
- Trachipleistophora anthropopthera
- Pleistophora ronneafiei
- Vittaforma (Nosema) corneae
- Nosema ocularum
- Brachiola vesicularum
- Brachiola (Nosema) algerae
- Brachiola (Nosema) connori
- Microsporidium (Nosema) africanum
- Microsporidium (Nosema) ceylonensis
- Trachipleistophora anthropopthera
- Trachipleistophora hominis
Morphology

• Spores are relatively small, 1.0–3.0 µM × 1.5–4.0 µM

• Special organelles- coiled polar tube or filament- for infecting host cell
  – 4-30 coils, used for species differentiation

• Merogony and sporogony in cytoplasm, mostly without parasitophorous vacuole
Life cycle: 2 alternatives
Disease

- Truly opportunistic, patient groups similar to *P. jirovecii*
- Small intestine, eyes, muscle, respiratory tract, urinary tract, biliary tract, skin
- *E. bieneusi* - the small intestine
  - Persistent diarrhea, abdominal pain, weight loss
- *E. intestinalis*-
  - Persistent diarrhea
  - Extraintestinal dissemination: sinusitis, keratoconjunctivitis, encephalitis, tracheobronchitis, interstitial nephritis, hepatitis, or myositis
• Transmission
  – Fecal-oral route (contaminated water and food)
  – Reservoir hosts
    • *E. bienneusi*: in pigs, monkeys
    • *E. intestinalis*: farm animals
• Diagnosis
  - Modified trichrome- red spore with belt
  - EM- for species diagnosis

• Treatment:
  - albendazole
Further readings

• Chapter 84. *Toxoplasma gondii* [http://www.ncbi.nlm.nih.gov/books/NBK7752/]


