Protozoan Parasites:

Lecture 18 - Amoebae, Ciliates & Coccidia

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Intestinal Amoebiasis

• Pathogenic obligate intestinal parasites
  – *Entamoeba histolytica*
    • Mammals (Zoonosis)
  – *Entamoeba invadens*
    • Captive reptiles

• Non-pathogenic
  – Many e.g. *Entamoeba coli*
    • Cattle, horses, pigs, humans...
• *Entamoeba histolytica*
  
  – Third leading cause of morbidity & mortality due to parasitic disease in humans
  – Estimated to be responsible for 50,000 & 100,000 deaths every year
  
  – Number of cases in animals?
Morphology

*Entamoeba histolytica/dispar*

- Two life stages: Trophozoite & Cyst

- **Trophozoite**
  - Amoeboid shape
  - 12-60 um (~20 um)
  - Nucleus
    - with central nucleolus
    - Chromatin ring
  - Ingested RBC’s uncommon but considered Dx for *E. histolytica*

[Image of Trichrome stain showing RBCs]
Morphology

*Entamoeba histolytica/dispar*

- **Cyst**
  - Round
  - 10-20 um
  - 1-4 nuclei
  - Blunt-ended chromatoid bodies (ribosomes)
Epidemiology

• Worldwide distribution
  – Tropical countries
  – Primarily a pathogen of primates
  – Humans
    • Reservoir for domestic animals
    • Zoonosis
  – Infections in animals reported but prevalence is unknown...
Epidemiology

- **Transmission:**
  - Fecal-oral & waterborne

- **Cysts:**
  - Viable for ~ 2 weeks
  - Killed at temperature >55°C
  - Super chlorination
Pathogenesis

• Trophozoites hydrolyze tissues of large intestine

• Intestinal lesions
  – Flask shaped ulcers - penetrate m.m. & s.m.
  – Enter general circulation & spread systemically

http://www.pathology.vcu.edu/education/microbiologyhtml
Pathogenesis

• Extra-intestinal lesions
  – Necrotic abscesses in lung, liver, brain & other organs
Clinical signs

• Variable
  – Asymptomatic to severe

• Common signs
  – Colitis, diarrhea, dysentery & vomiting
  – Additionally may see
    • Anorexia & weight loss
    • Hepatomegaly & fever with liver infections
Diagnosis

- **Fecal smears - Trophozoites & cysts**
  - Fresh (wet-mount) or stained
- **Centrifugal Fecal Flotation – cysts**
  - Cysts shed intermittently
  - Check multiple (3) samples in 7 days
- **Specialty lab required for Dx**
  - re: confounder *E. dispar*
    - Non pathogenic
  - **Enzyme Immunoassays (EIA)**
  - **PCR**
    - Fresh or frozen stool only
Control & Treatment

• Good hygiene & proper sanitation
• Metronidazole is recommended drug of choice for humans
• BUT little is known about treatment of amoebiasis in domestic animals
Intestinal Amoebiasis in Reptiles

- *Entamoeba invadens*
  - “Commensal” in turtles & crocodiles
  - Highly pathogenic in lizards, snakes & tortoises

- Don’t mix snakes, lizards or tortoises with turtles!
Intestinal Amoebiasis in Reptiles

*Entamoeba invadens*

- **Direct life cycle**
  - Fecal-oral & waterborne

- **Clinical signs**
  - Anorexia, weight loss, vomiting
  - Blood or mucus in the feces
  - Green coloured urates
  - Mid to caudal swellings of the body
  - Death

Pathogenesis

Gross Liver Necrosis

Congested Proximal GI (Colitis)

Ulcerative Colitis
Pathogenic Ameba
(Presumptive ID: Entamoeba invadens)
Intestinal Lumen

Pathogenic Ameba
(Presumptive ID: Entamoeba invadens)
Liver
Intestinal Amoebiasis in Reptiles

Entamoeba invadens

• **Diagnosis**
  – History, P.E. & overview of husbandry
  – Fecal exam for cysts - as before
  – PCR test being developed...

• **Treatment & Control**
  – Metronidazole
  – Don’t mix turtles with snakes, lizards & tortoises...
Amebiasis in Four Ball Pythons, *Python regius*

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**ABSTRACT.** Between September 13th and November 18th in 1999, four ball pythons, *Python regius* kept in the same display, showed anorexia and died one after another. At necropsy, all four snakes had severe hemorrhagic colitis. Microscopically, all snakes had severe necrotizing hemorrhagic colitis, in association with ameba-like protozoa. Some of the protozoa had macrophage-like morphology and others formed protozoal cysts with thickened walls. These protozoa were distributed throughout the wall in the large intestine. Based on the pathological findings, these snakes were infested with a member of *Entamoeba* sp., presumably with infection by *Entamoeba invadens*, the most prevalent type of reptilian amoebae.

**KEY WORDS:** ball python, colitis, reptilian amebiasis.

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Amoebic Meningoencephalitis

- Pathogenic Free-living
  - *Naegleria fowleri*
  - *Acanthamoeba* spp.
- Important causes of disease in humans & animals
- **Worldwide distribution**
- Infrequent cases in both humans & animals
Amoebic Meningoencephalitis

• *Naegleria fowleri*
  – Soil & thermally polluted water
  – Swimming pools, hot-tubs, tap water, sewage, aquariums...

• *Acanthamoeba spp.*
  – Soil, fresh-water...
  – Heating & air-conditioner units
  – Swimming pools, hot-tubs, tap water
Isolation and Molecular Typing of *Naegleria fowleri* from the Brain of a Cow That Died of Primary Amebic Meningoencephalitis

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*Naegleria fowleri* causes an acute and rapidly fatal central nervous system infection called primary amebic meningoencephalitis (PAM) in healthy children and young adults. We describe here the identification of *N. fowleri* isolated from the brain of one of several cows that died of PAM based on sequencing of the internal transcribed spacers, including the 5.8S rRNA genes.
Disseminated *Acanthamoeba* sp. infection in a dog

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Abstract

Several species of free-living amoebae can cause encephalomyelitis in animals and humans. Disseminated acanthamoebiasis was diagnosed in pyogranulomatous lesions in brain, thyroid, pancreas, heart, lymph nodes, and kidney of a one-year-old dog. *Acanthamoeba* sp. was identified in canine tissues by conventional histology, by immunofluorescence, by cultivation of the parasite from the brain of the dog that had been stored at \(-70^\circ\)C for two months, and by PCR. The sequence obtained from the PCR product from the amoeba from the dog was compared to other sequences in the *Acanthamoeba* sp. ribosomal DNA database and was determined to be genotype T1, associated with other isolates of *Acanthamoeba* obtained from granulomatous amebic encephalitis infections in humans.
Balantidiosis

*Balantidium coli*

- Normal ‘non-pathogenic’ flora of G.I. tract of pigs
  - *B. caviae* also in Guinea Pigs
- Pathogenic
  - Humans (Zoonosis) & other primates
  - Dogs (rare)
Morphology
Balantidium coli - 2 life stages

• **Trophozoite**
  – Variable sizes
    • 40-60 um & 90-120 um
  – Ovoid
  – **Ciliated**
    – Funnel shaped cytostome (gullet)
    – Macronucleus – genetic
    • Small sphere
    – Macronucleus – somatic
    • Central & bean shaped
Morphology

*Balantidium coli* - 2 life stages

- **Cyst**
  - Round to ellipsoidal
  - 50-75 um
  - Thick refractile wall

![Refractile cyst wall](image)
Life Cycle

Balantidium coli

- Direct
- Fecal-oral
- Waterborne or food
- Reproduction
  - Asexual
    - Transverse binary fission
  - Sexual
    - Conjugation
- Cyst = infective stage

Source: CDC
Epidemiology

Balantidium coli

• Prevalence in pigs
  – Warmer climates 20-100%
  – Temperate climates disease is rare

• Prevalence in other animals
  – Unknown?

• Transmission
  – Fecal-oral, waterborne
  – Infections more common in areas of high swine: human ratio
  – BUT disease is still considered uncommon
Pathogenesis

- Most infections- asymptomatic
- *B. coli invades tissue of large intestine*
  - Hyaluronidase enzyme
  - Rare
    - But may invade other tissue
    - Similar to intestinal amoebiasis
Clinical signs

• Pigs
  – Most are asymptomatic
  – But may be mild colitis & diarrhea (rare)
    • Sloppy grey feces
• Humans & other primates
  – Diarrhea
  – Occasionally severe
    • “Amoebic dysentery-like symptoms”
Diagnosis

• **Fecal smears**
  – Trophozoites & cysts
  – Fresh - wet-mount
  – Fixed / stained

• **Centrifugal Fecal Flotation**
  – Cysts

• **Cysts shed intermittently**
  – Check multiple samples
    • 3 fecals in 7 days...

Unstained trophozoite & cyst

Stained cyst
Treatment & Control

• Hygiene & proper sanitation
  – Especially on swine farms & primate colonies

• Pigs
  – No treatment necessary (asymptomatic)
  – If clinical cases (rare)
    • Tetracyclines & metronidazole

• Dogs, cats, humans & other primates
  – Tetracyclines & metronidazole
Severe peritonitis due to *Balantidium coli* acquired in France

**Abstract** The case reported here concerns an alcoholic pork-butcher who presented with severe colitis with peritonitis, caused by the only ciliate protozoan capable of infecting humans, *Balantidium coli*. This parasite is common in a variety of domestic and wild mammals, mainly pigs; however, its prevalence rate in humans is very low—particularly in industrialised, northern countries, including France. The infection is most frequently acquired by ingesting food or water contaminated by pig faeces, and it may be asymptomatic or may cause acute or immunocompromised individuals; thus, it is important to consider the risk of this parasitic disease in this particular patient population.

**Case report**

A 54-year-old male patient was admitted to hospital for diarrhoea associated with fever. This patient was a pork-butcher, and had suffered for many years from hyperten-
Characterization of a New Species of the Ciliate *Tetrahymena* (Ciliophora: Oligohymenophorea) Isolated from the Urine of a Dog: First Report of *Tetrahymena* from a Mammal

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Summary. An approximately 10 yr-old male Dalmation was admitted to the Exeter Animal Hospital presenting symptoms of continuous urination, polyuria/polydipsia, and regurgitation. Urinalysis showed glucosuria, pyuria, proteinuria, casts, and white blood cells. Microscopic examination of urine revealed considerable numbers of a ciliated protozoan. The ciliate was isolated and aseptically cultured in proteose peptone medium. Cytological staining of cells with the Chatton-Lwoff silver nitrate and silver proteinate procedures demonstrated that the ciliate was a species of the genus *Tetrahymena*, measuring about 50 x 25 \( \mu \text{m} \), placing it within the *pyriformis* species complex. Polymerase chain reaction amplification of the small subunit rRNA (SSrRNA) gene followed by DNA sequence analysis confirmed this identification. Analysis of the complete SSrRNA gene demonstrated significant differences in primary sequence from all other members of the *pyriformis* species complex and justified the designation of a new species, *Tetrahymena farleyi* sp. n.
Enteric Coccidiosis
General Taxonomy - Apicomplexa

- 2 Genera - *Eimeria* & *Isospora*
- Obligate intracellular parasites
- Hundreds of species infect all animals
  - Host specific
    - Infecting only a single host species or closely related species
  - Mixed infections
    - Hosts simultaneously infected with more than one coccidial species
Morphology

- **Zoite** = functional unit of all Apicomplexans
  - Motile, banana-shaped
  - 2-8 um long
  - Specialized structures
  - Apical complex, rhoptries & conoid
    - Function in cell invasion
    - Visible only by EM
Morphology

Zoite = sporozoite & merozoite

• Sporozoites
  – Infective stage is the **sporulated oocysts**

• Merozoites
  – Produced in host cells
  – Asexual reproduction called merogony
    • synonym = schizogony
Morphology
Oocyst

- Oocyst
  - Result of sexual reproduction
  - Round - ovoid
  - Size variable
  - Species dependent
  - Environmentally resistant stage
    - May survive up to 1 year
    - Oocysts don’t survive
      - < -30°C or >40°C
    - Require sporulation to become infective (24-48 hours)
Morphology

Oocyst

- **Unsporulated oocysts**
  - Contain a diploid single cell called a sporoblast or sporoont = zygote
  - Not infectious

- **Sporulated oocysts**
  - Contain haploid sporozoites which may or may not be enclosed in a sporocyst(s)
  - Infectious stage

- # sporozoites / sporocyst depends on the Genus
Morphology
Sporulated Oocyst

• **Eimeria**
  – 4 sporocysts each containing 2 sporozoites
  – = 8 sporozoites

• **Isospora**
  – 2 sporocysts each containing 4 sporozoites
  – = 8 sporozoites
General Coccidian Life Cycle
Monoxenous - direct or one host
General Coccidian Life Cycle

Monoxenous - direct or one host

Asexual replication (merogony) produces merozoites

Sporozoite infects intestinal cells

Sporulated oocyst (mature & infective)

Unsporulated oocyst (immature & not infective)

Sexual replication (gamogony) produces micro & macrogametes (fertilization produces unsporulated oocysts)
Epidemiology

• **Prevalence**
  – High - especially in crowded conditions
  – Poultry operations, feedlots, catteries...
  – All animals will have a mixed infection
  – Highly pathogenic & non-pathogenic

• **Transmission**
  – Fecal-oral route
  – contaminated food & water
Epidemiology

• Often asymptomatic
• Associated with raising young animals in confinement
• Associated with young animals & stress
  – Weaning, adverse weather, shipping...poor husbandry
Epidemiology

• Adult animals
  – Developed immunity
  – Can be re-infected
    • Typically asymptomatic
  – Carriers
  – Immunity is species specific
    • e.g. cattle immunity to *Eimeria bovis* does not offer any protection to *Eimeria zuernii*
**Pathogenesis**

- **Severity of disease**
  - Proportional to the **number of infective oocysts ingested** & **location of infection**
  - Crypts & colon = more severe disease
Pathogenesis

- Destruction of epithelial cells
  - Villous atrophy & intestinal lesions
  - Crypt hyperplasia
    - Results in immature epithelial cells along the villi
- Denuding of epithelium
  - Results from infections of crypts
- Hemorrhage in severe disease
Pathogenesis

- Majority of pathology caused by the asexual replicating stages versus the sexual stages.
- Therefore, you see the clinical signs of disease prior to the presence of oocysts in the feces.
Clinical signs

- Diarrheal enteritis & malabsorption
  - Blood may or may not be observed in feces
    - Depends on species & severity of infection
    - Usually self-limiting in the absence of re-infection
- Systemic signs of blood loss if the infection has caused hemorrhage
- Poor weight gain, emaciation & death
Diagnosis

• Oocysts present on **fecal flotation**
  – Clinical signs usually appear before oocysts passed in feces

• **History & clinical signs are important**
  – Finding oocysts is **not proof of coccidiosis**
    • Infection with non-pathogenic species of coccidia is common
  – **Gross intestinal lesions at necropsy & coccidia observed in lesion scrapings or histopathology**
Diagnosis

- Dependent on the **age of susceptibility & finding oocysts in the feces of animals suffering clinical signs of coccidiosis**
Treatment & Control

- Prophylactic drugs are used to control the infections (often mixed with feed or water)
- Treatment with drugs after clinical signs develop is rarely effective
- Supportive treatment & control of secondary infections
- Good management - reduce exposure to oocysts & reduce stress in young animals can prevent or decrease severity of disease
Coccidia:
Life cycle & treatment effectiveness?

Sanitation: within 24-48 hours i.e. before oocysts are mature & become infective (sporulated oocyst)

Asexual stages
Prophylactic drugs
Current drugs only work on asexual stages outside of host cells

Sexual stages
Supportive therapy
- No current drugs kill/target sexual stages in cells

Life cycle of coccidia (Eimeria sp.)
1. Infection
2. Merogony
3. Merogony II
4. Merogony III
5. Gamogony
6. Sporogony
7. Moist and aerial litter
8. Moist and aerial litter
9. A
10. B
11. Asexual stages
12. Sexual stages

Host cell nucleus

No current drugs kill/target sexual stages in cells

Disease model

- Asexual stages
- Sexual stages
Protozoan Parasites: Coccidia Part II & Cryptosporidium