Apicomplexa of Intestinal Pathology

Cryptosporidium, Eimeria, Cystoisospora

General Characteristics of Apicomplexa

A. Select Characteristics
- Intracellular with apical complex – organelle for invasion of host cell
- Gliding motility
- Life cycle alternates b/w sexual and asexual phases
- Many morphological stages

B. Morphology by stage
- Zoite
  - Tear-shaped (cylindrical with pointed anterior and blunt posterior)
  - Sporozoite (infective stage) & merozoite (reproduce in host cells and can infect new host cells)
    - Other “zoites” (not all apicomplexa will include these)
      - Bradyzoite = slow growing stage
      - Tachyzoite = fast growing stage
  - Extra-cellular and invasive stages
- Troph
  - amoeboid (various shapes)
  - trophozoite
  - Intra-cellular, feeding, metabolically active
- “-ont”
  - a “bag” of Zoites
  - meront (= schizont), gamont
  - intracellular
  - result of replication (endopolygeny)

C. Replication
- Asexual Reproduction
  - Endopolygeny = multiple cycles of nuclear division followed by cytoplasmic division
    - Sporogony (sporulation): replication within the oocyst resulting in sporozoites; usually only 1 round of replication
    - Merogony: (aka: schizogony) is replication of merozoites; usually many rounds of replication
  - Endodyogeny = single nuclear division followed by cytoplasmic division, forming 2 fully-formed daughter cells within the cytoplasm of the mother cell
- Sexual Cycle
  - Gametogony: merozoite develops into a gamete
    - Microgamete = male (replicates asexually)
    - Macrogamet = female
  - Fertilization = microgamete fertilizes a macrogamet and develops into a zygote or oocyst

C. Taxonomy
1. Conoidasida – conoid apparatus, infect intestinal cells, oocyst stage
   a. Gregarinasinina (Primitive, mainly infects invertebrates)
      i. Cryptosporidium spp. --- direct life cycle
   b. Coccidiasina (common coccidians)
      i. Eimeria --- direct life cycle
      ii. Cystoisospora & Toxoplasma --- direct LC or facultative indirect LC (paratenic hosts)
      iii. Sarcocystis -- obligate indirect life cycle (requires intermediate host)
2. Aconoidasida – no conoid apparatus, infects blood cells, indirect LC w/ blood feeding arthropods
   a. Piroplasmdia -- transmitted by Ixodid ticks
      i. Babesia, Theileria, Cytauxzoon
   b. Haemosporida – transmitted by biting flies
      i. Plasmodium, Heamoproteus, Leucocytozoon
Cryptosporidium parvum
Pathogenic Crypto of Cattle, but very low host specificity

A. Morphology
- Very small oocyst (5-8 um) with 4 sporozoites, already sporulated when passed
- "Superficial" parasite of the microvillus of gut cells (enterocytes)

B. Life Cycle
1. Transmission
   a. Direct life cycle – fecal-oral, ingestion of oocyst
2. Invasion
   a. Sporozoites excyst from oocyst and invade microvillus border of enterocyte
3. Asexual reproduction
   a. Merogony (schizogony) [multi-nuclear division followed by cytoplasmic division]
   b. Merozoites exit the enterocyte and infect the microvillus border of other enterocytes and goes through merogony again.
   c. Number of asexual cycle: unknown, (probably variable depending on host response.)
4. Sexual reproduction
   a. Final generation of merozoites exit the enterocyte and infect the microvillus border of other enterocytes and go through gametogony (production of gametes)
   b. Macrogamete (egg)
      i. Some final merozoites remain a single cell and become a macrogamete (egg) within a macrogamont.
   c. Microgametes (sperm)
      i. Other final merozoites go through multi-nuclear division, cytoplasmic division, and develop 2 flagella (bi-flagellate) on each gamete; thus forming a microgamont
      ii. Exflagellation – when microgametes exit the microgamont in search of a macrogamete.
   d. Fertilization – a microgamete fuses with a macrogamete forming a zygote
   e. A cyst wall forms around the zygote and the immature oocyst exits the macrogamont into the lumen of the host’s gut.
5. Sporogony (= Sporulation)
   a. The zygote, within the oocyst, goes through sporogony, forming 4 sporozoites.
   b. Sporulation occurs within the lumen of the host gut, thus making the oocyst immediately infectious.
6. Dissemination
   a. Thin-walled Oocysts
      i. Some oocysts have thin cyst walls and excyst within the gut of the same host
         1. thus autoinfection causing low grade chronic pathology (diarrhea)
         2. in the immunocompromised this may allow for hyperinfection and acute severe pathology / mortality.
   b. Thick-walled Oocysts
      i. Some oocysts have thick cyst walls and exit the host in the feces
         1. thus contamination of the environment and transmission to the next host.
         2. infectious when passed.

C. Pathogenesis = intestinal epithelial injury
1. Villus atrophy and dysfunction of absorptive enterocytes decrease absorption and surface area
2. Crypt hyperplasia causes increased secretory activity
3. Increased inflammatory cells (inflammation) increase permeability, with loss of fluids in to the gut lumen.

D. Clinical Disease (“Calf Scours”)
1. Complaint -- Mild to severe diarrhea, usually in neonatal claves - first 3 weeks of life
   a. most often reported in calves from 5-15 days of age.
   b. persistent infection may cause marked weight loss and emaciation.
2. Pathological findings
   a. large amounts of watery diarrhea (cholera-like diarrhea)
   b. feces yellow or pale, watery, and may contain mucus.
   c. subsequent severe dehydration, anorexia, debilitation.
3. Usually self-limiting in immunocompetent hosts
4. Severe & lethal in immunodeficient hosts
5. Differential Diagnoses for “Calf Scours”
   a. Bovine viral diarrhea virus (BVDV)
   b. Coccidia
   c. Cryptosporidium
   d. Salmonella
   e. Clostridium
   f. E. coli
   g. Nutritional causes

E. Diagnosis
1. Centrifugation with Sheather’s solution (sugar solution - higher specific gravity than cysts so they float to the top and debris sink)
   a. very small oocysts -- focus on the thin layer of fluid above bubbles
   b. don’t confuse with yeast
2. Thin fecal smear with special staining (acid fast stains)
3. Serology and molecular diagnostics: Fluorescent antibodies, ELISA, PCR

F. Treatment
1. Some drugs are suppressive against Cryptosporidium (Paromomycin, Azithromycin, etc.)
2. Fluid-replacement therapy for the dehydration that is caused by the diarrhea.

G. Control
1. Sanitation, especially for young calves, and provide adequate amounts of colostrum
   a. Hutch system for dairy calves
2. Sanitation & hygiene for humans and others
3. Oocysts are viable for months unless exposed to extreme temperatures, drying, or disinfectants
4. No Vaccines

H. Epidemiology
1. C. parvum in Calves
   a. Primarily in neonatal calves, but also in lambs, kids, foals, and piglets, as well as in humans (zoonotic)
   b. Prevalence of 70% in 1-3 week old dairy calves
   c. Calves 9-14 days old most likely to excrete oocysts.
   d. A concurrent infection with rotavirus and coronavirus tends to make disease worse, than with Crypto. alone.
2. Other Crypto. species are less pathogenic and may be more host-specific
   a. (ex. C. felis, C. canis, C. hominis)

I. Zoonosis
1. Highly zoonotic
2. Transmitted to humans
   a. predominantly human to human
   b. direct contact with animals
   c. water-borne infection from contamination of water sources with animal feces.
   d. Farm workers at high risk.
3. Waterborne municipal out-breaks, as well as food-borne outbreaks
4. Highly dangerous for immunocompromised patients.

====================================================================

**Eimeria spp**

*Common Coccidians of Hoof stock and Poultry*

*Many Eimeria spp. with high host specificity*

A. Morphology
   • Oocyst
     o Species-specific size, shape, outer coat, presence or absence of polar cap, etc.
     o Single-cell embryo when passed
     o Sporulated oocyst contains 4 sporocysts with 2 sporozoites each = 8 sporozoites total
   • Intracellular parasites of enterocytes.

B. Life Cycle
   1. Transmission
a. Direct life cycle – fecal-oral, ingestion of oocyst

2. Invasion
   a. Sporozoites excyst from oocyst and invade enterocyte

3. Asexual reproduction
   a. Merogony (schizogony) [multi-nuclear division followed by cytoplasmic division]
   b. Merozoites exit the enterocyte and infect other enterocytes and goes through merogony again.
   c. Number of asexual cycles and number of merozoites per merogony is species-specific.

4. Sexual reproduction
   a. Final generation of merozoites exit the enterocyte, infect other enterocytes, and go through gametogony (production of gametes)
   b. Macrogamete (egg)
      i. Some final merozoites remain a single cell and become a macrogamete (egg) within a macrogamont.
   c. Microgametes (sperm)
      i. Other final merozoites go through multi-nuclear division, cytoplasmic division, and develop 2 flagella (bi-flagellate); thus forming a microgamont
      ii. Exflagellation – when microgametes exit the microgamont in search of a macrogamete.
   d. Fertilization – a microgamete fuses with a macrogamete forming a zygote
   e. A cyst wall forms around the zygote and the immature oocyst exits the macrogamont into the lumen of the host’s gut and is passed in the feces.

5. Dissemination
   a. Oocysts (unsporulated) exit the host in the feces and contaminate the environment.

6. Sporogony (= Sporulation)
   a. Sporogony occurs in the environment.
      i. Appropriate temperature, moisture, and oxygen are required for sporogony.
      ii. Some species can take as little as 1 day to sporulate in optimal conditions
   b. After sporulation, the oocyst is ready for transmission to the next host.

C. Pathogenesis
1. Exponential destruction of enterocytes with each merogonic cycle, thus causing malabsorption, destruction of epithelial lining and hemorrhagic ulcers.
2. Traumatic permeability, with loss of fluids and blood in to the gut lumen
3. Hypersecretion due to immune response.
4. Pathogenicity depends upon dose, host health status, immunological competence

D. Clinical Disease
1. Complaint -- Mild to severe diarrhea (bloody, mucoid, or watery)
   a. most often reported in young or naïve animals.

2. Range of Pathology
   a. Hemorrhagic diarrhea / dysentery, tenesmus, fever, anemia, weakness, weight loss, death.
   b. Location of pathologic lesions is coccidian-species specific.

3. Manifestation of Coccidiosis varies
   a. Individual animal
      i. Non-clinical, but large numbers of oocysts in feces
      ii. Acute, severe, fatal, bloody diarrhea, but no oocyst in feces – prior to prepatency
      iii. Disease caused by 1) An overwhelming dose of oocyst OR 2) a moderate dose + stress
   b. Herd or flock
      i. Regularly recurring diarrhea issues with each successive cohort of young animals.

E. Diagnosis
1. Clinical Signs
2. Fecal Float Centrifugation or McMaster Slide
3. Diarrhea may occur prior to oocyst excretion

F. Treatment
1. Ionophores (Monensin, Lasalocid, etc.) although often used, are not effective for the treatment of acute disease.
2. Treatment is mainly to eliminate incoming coccidial organisms; not to eliminate the ones already causing pathology. i.e. treatment does not stop occurring pathology.
3. Give supportive fluid-therapy for symptoms
4. Treatment difficult as feed & water consumption is depressed.
5. Treat prophylactically for control – medicated feed or water with coccidiostats.
G. Control

1. Sanitation, especially for young and naïve animals
   a. Keep susceptible animals out of moist area, where oocysts will sporulate.
   b. Hutch system for dairy calves
   c. Direct sunlight and dryness best disinfectants

2. Good nutrition important

3. Coccidiostats (won’t cure an infected animal, rather the goal is to limit infection of newly exposed animals so they don’t get sick but develop natural immunity)
   a. Coccidiostats act to limit the number of successful coccidial organisms, especially in young hosts.
      i. Kills or inhibits growth of most entering organisms, but not all.
      1. Allows for the development of immunity without disease. A “natural vaccine”.
   b. Extremely important in systems of intense and / or confinement rearing of poultry, ruminants.
   c. Prophylaxis -- Decoquinate (Deccox), Monensin (Rumensin), Lasalocid (Bovatec)
   d. Treatment -- Amprolium (Corid), “Sulfa Drugs”: Sulfamonomoxaline, Sulfamethazine, Sulfamethoxine
   e. Concern for the development of resistance – rotate coccidiostats.
   f. WARNING -- IONOPHORES (MONENSIN, LASALOCID, ETC.) ARE HIGHLY TOXIC TO HORSES

4. Requires a coordinated control strategy (must have all these components)
   a. Coccidiostats
   b. Sanitation
   c. Good Nutrition
   d. Low Stress
   e. Don’t mix age groups
      i. Adults source of environmental contamination and source of infection for young animals.
   f. At first sign of disease
      i. Separate sick animals for supportive care
      ii. Begin treatment of whole herd / flock.

5. Vaccines – used in Poultry coccidiosis
   a. Oocyst cocktails, irradiated, mutated – ex. Inovocox vaccine

H. Epidemiology

1. Eimeria spp.
   a. Ubiquitous
   b. Very, very host specific (thus no cross-species infection or zoonosis)
   c. Each host species may have many Eimeria species, but few are pathogenic.

2. Host risk factors
   a. Immunodeficient: young, stressed, poor nutrition
   b. Immunologically naïve
   c. Immunity is coccidian-species specific.
      i. Ex. Eimeria bovis infection does not confer protection against Eimeria zurneii
   d. Immunological experience provides incomplete or complete protection.
      i. Incomplete = Reinfection usually leads to asymptomatic shedding of oocyst.

3. Environmental risk factors
   a. Primary infective dose – pathology proportional to infecting dose.
   b. Moist, cool habitats promote sporulation of oocysts
      i. Spring, Fall higher risks
   c. Crowded conditions
      i. Can quickly become highly contaminated with oocysts
         1. Pathogenesis proportional to infecting dose.
         ii. Stresses hosts thus decrease immune-competence

4. Consider Immunity and Environmental factors: Which is more likely to have a serious coccidian outbreak? Confinement chickens or Free-range chickens.

I. Host & pathogenic Eimeria species.

1. Bovine -- Eimeria bovis, Eimeria zurneii (differentiated by oocyst size)
   a. Once oocysts appear in feces it is too late
   b. Supportive therapy against dehydration is most important

2. Sheep -- Eimeria ovinoidalis
3. Goats -- *E. ninakohlyakimovae, Eimeria arloingi*
4. Swine -- *8 Eimeria spp.* but low pathogenicity
5. Horse -- *Eimeria leuckarti* -- non-pathogenic
6. Poultry
   a. Massive destruction of epithelial cells - hemorrhage, malabsorption.
      i. often prior to patency
      ii. young birds at greatest risk
   b. Sanitation & prophylaxis with coccidiostats
      i. resistance a problem, rotate through a variety of coccidiostats
   d. Turkeys -- *E. adenoides, E. meleagrititis*
   e. Clinical signs: bloody feces, pale combs, ruffle feathers, low appetite, high mortality, necropsy
      shows coagulated blood in ceca

=================================

**Cystoisospora spp (formally Isospora)**

**Common Coccidians of Carnivores**

*Cystoisospora* spp. are very host-specific

A. Morphology
   • Oocyst
     o Species-specific size, shape, oval to spherical, single-cell embryo when passed
     o Sporulated oocyst contains 2 sporocysts with 4 sporozoites each = 8 sporozoites total
   • Intracellular parasites of enterocytes.

B. Life Cycle
   1. Transmission
      a. Direct life cycle — fecal-oral, ingestion of oocyst
         i. homoxenous = a life cycle in which only one host is parasitized
         OR
      b. Facultative Indirect life cycle -- Rodent or bird paratenic host (not needed for the parasite’s development but helps maintain parasite)
         i. heteroxenous = a life cycle in which a parasite has more than one host
   2. Invasion
      a. Sporozoites excyst from oocyst and invade enterocyte
      OR
      b. Sporozoite excyst from prey tissue and invade enterocyte
   3. Asexual reproduction
      a. Merogony (schizogony) [multi-nuclear division followed by cytoplasmic division]
      b. Merozoites exit the enterocyte and infect other enterocytes and goes through merogony again.
      c. Number of asexual cycles and number of merozoites per merogony is species-specific.
   4. Sexual reproduction
      a. Final generation of merozoites exit the enterocyte, infect other enterocytes, and go through gametogony (production of gametes)
      b. Macrogamete (egg)
         i. Some final merozoites remain a single cell and become a macrogamete (egg) within a macrogamont.
      c. Microgametes (sperm)
         i. Others final merozoites go through multi-nuclear division, cytoplasmic division, and develop 2 flagella (bi-flagellate) on each gamete; thus forming a microgamont
         ii. Exflagellation – when microgametes exit the microgamont in search of a macrogamete.
      d. Fertilization – a microgamete fuses with a macrogamete forming a zygote
      e. A cyst wall forms around the zygote and the immature oocyst exits the macrogamont into the lumen of the host’s gut and is passed in the feces.
   5. Dissemination
      a. Oocysts (unsporulated) exit the host in the feces and contaminate the environment.
   6. Sporogony (= Sporulation)
      a. Sporogony occurs in the environment.
         i. Appropriate temperature, moisture, and oxygen are required for sporogony.
ii. Some species can take as little as 1 day to sporulate in optimal conditions
b. After sporulation, the oocyst is ready for transmission to the next host.
i. Ingestion of sporulated oocyst by definitive host
   OR
ii. Ingestion of sporulated oocyst by paratenic host & sporozoites encyst in tissue of paratenic host = “cystozoite”.

C. Pathogenesis
1. Destruction of enterocytes, causing malabsorption, epithelial lining destruction, hemorrhagic ulcers.
2. Traumatic permeability, with loss of fluids and blood into the gut lumen.
3. Hypersecretion due to immune response.

D. Clinical Disease
1. Complaint -- Mild to moderate diarrhea (bloody, mucoid, or watery)
   a. most often reported in nursing or recently weaned pets
   b. Immunocompromised or stressed animals may break with coccidiosis (shipping, shelter, kennel)

E. Diagnosis
1. Clinical Signs and animal age/history
2. Fecal Float Centrifugation
3. Diarrhea may occur prior to oocyst excretion.

F. Treatment
1. Sulfadimethoxine (Albon) although often used, are not effective for the treatment of acute disease.
2. Other sulfa drugs may also be used.
3. Give supportive therapy for symptoms

G. Control
1. Sanitation
   a. Especially for young and naïve animals
   b. Important in kennels & catteries
2. Prevent access to Paratenic hosts (rodents)
3. Good Nutrition Important
4. Keep Stress Low

H. Epidemiology
1. Cystoisospora spp.
   a. Ubiquitous
   b. Very, very host specific (thus no cross-species transmission or zoonosis)
2. Host risk factors
   a. Immunodeficient: young, stressed, poor nutrition
3. Environmental risk factors
   a. Moist, unsanitary conditions promote sporulation of oocysts within 3-4 days
   b. Access to paratenic hosts (rodents, birds)

I. Host & pathogenic Cystoisospora species.
1. Canine (puppy diarrhea)
   a. Cystoisospora canis – oval oocyst, non-pathogenic
   b. C. ohioensis -- spherical, may cause diarrhea
2. Feline (kitten diarrhea)
   a. Cystoisospora felis – oval oocyst, non-pathogenic
   b. C. rivolta -- spherical, diarrhea in new born kittens
3. Swine (piglet diarrhea)
   a. Cystoisospora suis
      i. Neonatal DZ – 1-2 week old piglets
      ii. Non-hemorrhagic diarrhea, dehydration, weight loss, (High morbidity, Low mortality)
      1. must distinguish b/w coccidiosis v/s viral or bacterial piglet diseases
      iii. As piglet age increases, the susceptibility and pathology decrease
      iv. Immunity complete against reinfection.
      v. Rigorous sanitation w/ steam cleaning and detergents
      vi. Coccidiostats are ineffective
      vii. Diagnose: McMaster method, stained fecal smears, autofluorescence microscopy (sensitive)
      viii. Multiple sampling days due to sporadic shedding of oocysts
      ix. Also 8 Eimeria spp. size and number of sporocysts to differentiate from C. suis

======================================
======================================