LECTURES #4, #5 & #6: APICOMPLEXA 1

Apicomplexa of Intestinal Pathology

Cryptosporidium, Eimeria, Cystoisospora

General Characteristics of Apicomplexa

A. Morphology by stage

- Zoite
  - Tear-shaped (cylindrical with pointed anterior and blunt posterior)
  - Sporozoite & merozoite 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 endopolyogeny = multi-nuclear division followed by cytoplasmic division
    - 1 mother cells produces multiple daughter cells
    - also called merogony (= schizogony), gametogony, sporogony

B. Other Characteristics

- Gliding motility
- Apical Complex – organelle for invasion of host cell
- Life cycle alternates b/w sexual and asexual phases

C. Taxonomy

1. Conoidasida – conoid apparatus, infect intestinal cells, oocyst stage
   a. Gregarinasina (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. Piroplasmidia -- transmitted by Ixodid ticks
      i. *Babesia, Theileria, Cytauxzoon*
   b. Haemosporida – transmitted by biting flies
      i. *Plasmodium, Heamoproteus, Leucocytozoon*

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Cryptosporidium parvum

Pathogenic Crypto of Cattle, but very low host specificity

A. Morphology

- Very small oocyst (5-8 um) with 4 sporocysts, 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. Sporocysts 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 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
1. Villus atrophy and dysfunction of absorptive enterocytes decrease absorption
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
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

E. Diagnosis
1. Fecal Float
    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. 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. 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.

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Eimeria spp
Common Coccidians of Hoof stock and Poultry

A. Morphology
- Oocyst
    - Species-specific size, shape, outer coat, presence or absence of polar cap, etc.
    - Single-cell embryo when passed
    - 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 (sporulated) 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..

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 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
3. Diarrhea may occur prior to oocyst excretion.

F. Treatment
1. Ionophores (Monensin, Lasalocid, etc.) although often used, are <u>not</u> 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. 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
    a. Coccidiostats act to limit the number of successful coccidial organisms, especially in young hosts.
        i. Kills 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”: Sulfaquinoxaline, Sulfamethazine, Sulfamethoxine
    e. Concern for the development of resistance – rotate coccidiostats.
    f. WARNING -- IONOPHORES (MONENSIN, LASALOCID, ETC.) ARE HIGLY 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 environment al 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*
    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 & <u>prophylaxis</u> with coccidiostats
        i. resistance a problem , rotate through a variety of coccidiostats
    c. Chickens -- *E. tenella, E. necatrix, E. acervulina, E. maxima, E. mivati,* and *E. brunetti*.
    d. Turkeys -- *E. adenoides, E. meleagrimitis*

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Cystoisospora spp
Common Coccidians of Carnivores

A. Morphology
- Oocyst
  - Species-specific size, shape, oval to spherical, single-cell embryo when passed
  - 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 (homoxenous) OR
   b. Facultative Indirect life cycle -- Rodent or bird paratenic host (heteroxenous)
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. <u>Number of asexual cycles and number of merozoites per merogony is species-specific.</u>
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, 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.

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 Stress)

E. Diagnosis
1. Clinical Signs
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
   a. *Cystoisospora canis* – oval oocyst, non-pathogenic
   b. *C. ohioensis* -- spherical, may cause diarrhea
2. Feline
   a. *Cystoisospora felis* – oval oocyst, non-pathogenic
   b. *C. rivolta* -- spherical, diarrhea in new born kittens
3. Swine
   a. *Cystoisospora suis*
      i. Neonatal DZ – 1-2 week old piglets
      ii. Diarrhea, dehydration, weight loss, (High morbidity, Low mortality)
      iii. must distinguish b/w coccidiosis v/s viral or bacterial piglet diseases
      iv. Increased piglet age; then decreased susceptibility and decrease pathology
      v. Immunity complete against reinfection.
      vi. Rigorous sanitation w/ steam cleaning and detergents
      vii. Also 8 *Eimeria spp*. – non-pathogenic, but need to sporulate oocysts for identification.

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