Apicomplexans

Apicomplexa Intro

Cryptosporidium
Apicomplexan
Select Characteristics

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

- Zoite
  - Tear-shaped (cylindrical with pointed anterior and blunt posterior)
  - Sporozoite & merozoite stage
  - Extra-cellular and invasive stages

Apicomplexan Morphologic Stages

- Troph
  - amoeboid (various shapes)
  - trophozoite
  - Intra-cellular, feeding, metabolically active

Apicomplexan Morphologic Stages

“-ont”

- a “bag” of Zoites
- meront (= schizont), gamont
- intracellular

result of Endopolygeny = multi-nuclear division followed by cytoplasmic division
  - One mother cell produces multiple daughter cells
  - Aka: merogony (= schizogony), gametogony, sporogony
Apicomplexan Morphologic Stages

“-ont”

Apicomplexan Life Cycle Processes

- Asexual Processes
  - Sporogony (aka Sporulation) results in Sporozoites (infective stage)
  - Merogony (aka Schizogony) results in Merozoites

- Sexual Processes
  - Gametogony results in Gametes (microgametes & macrogametes)
  - Fertilization results in a Zygote & in some groups an oocyst
Apicomplexan Taxonomy

- **Conoidasida** - conoid apparatus, infect intestinal cells, oocyst stage
  - *Gregarinasina* (Primitive, mainly infects invertebrates)
    - *Cryptosporidium* spp. — direct life cycle
  - *Coccidiasina* (common coccidians)
    - *Eimeria* — direct life cycle
    - *Cystoisospora* & *Toxoplasma* — direct LC or facultative indirect LC (paratenic hosts)
    - *Neospora* & *Sarcocystis* — obligate indirect life cycle (requires intermediate host)
- **Aconoidasida** — no conoid apparatus, infects blood cells, indirect LC w/ blood-feeding arthropods
  - *Piroplasmidia* — transmitted by Ixodid ticks
    - *Babesia, Theileria, Cytauxzoon*
  - *Haemop sporida* — transmitted by biting flies
    - *Plasmodium, Haemoproteus, Leucocytozoon*
Protozoan Groups

Historically, protozoa have been grouped by mode of motility.

Flagellates

Hemoflagellates
Trypanosoma cruzi
Leishmania infantum

Mucoflagellates
Tritrichomonas foetus
Giardia spp.

Ciliates
Balantidium coli

Amoeba
Entameoba histolytica

Apicomplexans

Intestinal Apicomplexans
Cryptosporidium parvum
Eimeria spp.
Cystoisospora spp.

Systemic Apicomplexans
Toxoplasma gondii
Neospora caninum
Sarcocystis cruzi, S. neurona

Blood Apicomplexans
Babesia bigemina,
Babesia canis, B. gibsoni
Cytauxzoon felis
Cryptosporidium parvum

- Pathogenic Crypto of Cattle
- But very low host specificity

[C. parvum][C. hominis][C. meleagris]

[C. saurophilum][Opossum genotype I]

Pig genotype I

http://www.emedmd.com/content/cryptosporidium-and-cryptosporidiosis

Cryptosporidium sp. Morphology

- Microvilli: elongated, deformed
- Parasitophorous Vacuole: made of host and parasite components
- Meront: with developing merozoites
- Residual Body: germinative body from which merozoites bud
- Feeder Organelle: obtains nutrients from host cell

Uninfected Host Cell vs. Infected Host Cell
Life Cycle

- **Transmission**
  - Direct life cycle - fecal-oral, ingestion of oocyst

- **Invasion**
  - Sporocysts excyst from oocyst and invade microvillus border of enterocyte

- **Asexual reproduction** (Ileum, less in cecum & colon)
  - Merogony (schizogony)
    - [multi-nuclear division followed by cytoplasmic division]
  - Merozoites exit the enterocyte and infect the microvillus border of other enterocytes and goes through merogony again.
  - Number of asexual cycles: unknown, (probably variable depending on host response.)
**Life Cycle**

- **Sexual reproduction**
  - Final generation of merozoites exit the enterocyte and infect the microvillus border of other enterocytes and go through gametogony (production of gametes)
  - **Macrogamete (egg)**
    - Some final merozoites remain a single cell and become a macrogamete (egg) within a macrogamont.
  - **Microgametes (sperm)**
    - Other final merozoites go through gametogony and develop 2 flagella (biflagellate) on each gamete; thus forming a microgamont
    - Exflagellation - when microgametes exit the microgamont in search of a macrogamete.
  - **Fertilization** - a microgamete fuses with a macrogamete forming a zygote
  - A cyst wall forms around the zygote and the immature oocyst exits the host cell into the lumen of the host’s gut.
Life Cycle

- **Sporogony (= Sporulation)**
  - The zygote, within the oocyst, goes through sporogony, forming 4 sporozoites.
  - Sporulation occurs within the lumen of the host gut, thus making the oocyst immediately infectious.

- **Dissemination**
  - **Thin-walled Oocysts**
    - Some oocysts have thin cyst walls and excyst within the same host
      - thus *autoinfection* causing low grade chronic pathology (diarrhea)
      - in the immunocompromised this may allow for hyperinfection and acute severe pathology / mortality.
  - **Thick-walled Oocysts**
    - Some oocysts have thick cyst walls and exit the host in the feces
      - thus contamination of the environment and transmission to the next host.
      - infectious when passed.
Cryptosporidium parvum

Ingestion

Sporulated Oocyst

Sporulated ooysts passed in feces
Cryptosporidium sp. on intestinal brush border

Higher Magnification
SEM’s of Crypto

Pathology

Pathology = Watery Diarrhea

Various processes contribute to Pathology

- Villus atrophy and dysfunction of absorptive enterocytes decrease absorption
- Crypt hyperplasia causes increased secretory activity
- Increased inflammatory cells (inflammation) increase permeability, with loss of fluids into the gut lumen.
Clinical Disease

- **Complaint** -- Mild to severe diarrhea
  - usually in neonatal calves, “Calf Scours”
  - most often reported in calves from 7-15 days of age.
- Most cases are self-limiting after several days.
- But persistent infection may cause marked dehydration, weight loss, and emaciation.
Calf Scours

http://calfcare.ca/calf-care-corner/feeding-to-fight-disease/

http://coloradodisasterhelp.colostate.edu/prefair/disease/dz/Cryptosporidiosis.html
Clinical Disease

Pathological findings
- large amounts of watery diarrhea
  - “cholera-like diarrhea”
- feces yellow or pale, watery, may contain mucus.
- subsequent severe dehydration, anorexia, debilitation.

- Usually self-limiting in immunocompetent hosts
- Severe & lethal in immunodeficient hosts
Diagnosis

- **Fecal Float Centrifugation**
  - very small oocysts
    - focus on the thin layer of fluid above bubbles
  - don’t confuse with yeast
- **Thin fecal smear with special staining**
  - acid fast stains
- **Molecular diagnostics**
  - Fluorescent antibodies, ELISA, PCR
Diagnostics

Acid-fast Stain

Fluorescent stain

Wet Mounts

http://www.imgrum.org/media/1000074980669288494_423165795

https://www.cdc.gov/dpdx/cryptosporidiosis/
Treatment

- Drugs are only suppressive (Paromomycin, Azithromycin, etc.)

- **Fluid-replacement therapy** for the dehydration caused by the diarrhea.
Control

- Sanitation, especially for young calves, and provide adequate amounts of colostrum
  - Hutch system for dairy calves
- Sanitation & hygiene for humans & others
- No Vaccines Available
Calf Hutches
Epidemiology

- *C. parvum* in Calves
  - Primarily in neonatal calves, but also in lambs, kids, foals, and piglets, as well as in humans (zoonotic)
  - Prevalence of 70% in 1-3 week old dairy calves
  - Calves 9-14 days old most likely source of oocysts.
  - A concurrent infection with rotavirus and coronavirus tends to make disease worse, than with *Crypto.* alone.

- Other *Crypto.* species are less pathogenic and may be more host-specific.
  - (ex. *C. felis, C. canis, C. hominis*)
**Causes Of Calf Scours Based On Age Of Onset**

<table>
<thead>
<tr>
<th>Causes</th>
<th>Age Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVDV</td>
<td>Can impact animals at any age</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>4-14 days</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>7-30 days</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>7-16 days</td>
</tr>
<tr>
<td>E. Coli</td>
<td>0-3 days</td>
</tr>
<tr>
<td>Coccidia</td>
<td>21+ days</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Can impact animals at any age</td>
</tr>
<tr>
<td>Clostridium</td>
<td>0-28 days</td>
</tr>
</tbody>
</table>

**NUTRITIONAL CAUSES** *(Milk or milk replacer fed at inconsistent temperatures or improper solid concentrations.)*

Can impact animals at any age

**DAYS IN AGE**

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40+ |

**GOALS WHEN TREATING SCOURS**

1. Maintain caloric intake. Keep the calf on its normal feeding schedule.
2. Restore hydration status.
3. Stabilize the intestinal tract.

Causes of Calf Scours

- E.coli: 38%
- Rotavirus: 34%
- Coronavirus: 4%
- Coccidiosis: 18%
- Cryptosporidiosis: 6%

http://timmontgomery-farmsupplies.com/product/calf-clear/
Dehydration Decisions

CALF DEHYDRATION ASSESSMENT CHART

<table>
<thead>
<tr>
<th>% of dehydration</th>
<th>Demeanor</th>
<th>Sunken eye</th>
<th>Skin tenting</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 6%</td>
<td>Normal</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>6-8%</td>
<td>Depressed</td>
<td>2-4 mm</td>
<td>1-3 seconds</td>
</tr>
<tr>
<td>8-10%</td>
<td>Depressed</td>
<td>4-6 mm</td>
<td>2-5 seconds</td>
</tr>
<tr>
<td>10-12%</td>
<td>Comatose</td>
<td>6-8 mm</td>
<td>5-10 seconds</td>
</tr>
<tr>
<td>&gt; 12%</td>
<td>Dead</td>
<td>8-12 mm</td>
<td>&gt; 10 seconds</td>
</tr>
</tbody>
</table>

DECISION TREE FOR DEHYDRATED CALF TREATMENT

Calf with dehydration and diarrhea

- Suckle reflex present
  - Calf standing
    - ≤ 4 mm eye recession
      - ≤ 6-second neck skin tent
    - Hypertonic saline IV

- Suckle reflex absent
  - Calf not standing
    - Hypertonic saline IV

ORAL ALKalinizing ELECTROlyte SOLUTIONS

Geof Smith, DVM, PhD, Dipl. ACVIM, North Carolina State University, offers this algorithm for practical fluid therapy of dehydrated calves with diarrhea.

http://www.bovinevetonline.com/bv-magazine/Saving-winter-calfes-139313623.html
Zoonosis

- Highly zoonotic
- Transmitted to humans
  - predominantly human to human
  - direct contact with animals
  - water-borne infection from contamination of water sources with animal feces.
  - Farm workers at high risk.
- Water-borne municipal out-breaks, as well as food-borne outbreaks
- Highly dangerous for immunocompromised patients.
CDC – Crypto outbreaks reports

- 17 Water-borne -- 1990 to 2015 -- Municipal water, drinking fountains, public recreational swimming pools, interactive recreational water fountains, etc.
  - 1993 Milwaukee outbreak most famous

- 6 Food-borne outbreaks - 1993-2005 -- Fresh apple cider, unpasteurized milk, chicken salad, raw vegetables, etc.

- 9 Human to Human or animal to human outbreaks -- 1984 to 2015 Children day care, Summer camps, Zoos, wildlife center, State fairs, VETERINARY STUDENTS.

Abstract: Cryptosporidiosis was diagnosed in 10 veterinary students. Exposure to the pathogen was associated with direct contact with infected calves and contact with contaminated materials. Affected students had fever (50%), headache (50%), nausea (70%), diarrhea (80%), and vomiting (40%). Clinical signs persisted for 30 hours to 16 days after the onset of clinical signs of disease. Although one student required hospitalization, the remaining students recovered without treatment.
In-Class Discussion

A 12-day old calf is showing severe scours. What is your diagnosis?

Treatment plan?

Zoonotic concerns?
In-Class Discussion

Contrast the life cycles, ecology, and diagnosis of the protozoa that cause diarrhea in Large Animals.