Protozoan Parasites

General Characteristics
- protozoa are a heterogeneous group of approximately 50,000 known species, many of which are parasitic
- protozoa are responsible for some of the most important diseases of animals & humans
- protozoan parasites kill, debilitate & mutilate more people in the world than any other group of disease organisms

Host range
- all animals are susceptible
- some protozoan parasites have highly specific host ranges (e.g. Eimeria). Others are less discriminate and will infect any host e.g. Giardia & Cryptosporidium (this may not be entirely true anymore...more later in the lecture to follow of course...).

Site of Infection
- most organs & tissues e.g. intestine, muscle, brain, liver & blood
- some live free within the intestine or blood while others are intracellular

Morphology
- single-celled eukaryotes & therefore most have a typical complement of organelles (nucleus, mitochondria, endoplasmic reticulum, golgi apparatus…) surrounded by a plasma membrane
- simple appearance but some have developed complexity through specialized organelles that aid in attachment, locomotion, feeding & cell entry
  - glycosomes - contain glycolytic enzymes
  - kinetoplasts - contain extrachromosomal DNA (modified mitochondria)
  - rhoptries - cell invasion

- Locomotion occurs by the use of flagella, cilia, pseudopodia or other specialized methods.

Life Cycle
- Reproduction can be asexual, sexual or a combination of both
  - Asexual e.g. budding, binary fission or schizogony (multiple fission)
  - Sexual reproduction involves fusion of identical gametes (isogametes) or gametes that differ in size (anisogametes)

- Some have a cyst stage (infective or resting stage) with a resistant covering that protects from environmental factors. Some protozoa also encyst within the host’s tissue (e.g. Toxoplasma).
- Life cycles may be simple occurring within a single host (e.g. Isospora) while others are complex and involve multiple hosts (intermediate and paratenic)
- Some infect hosts directly while others rely on a vector (e.g. insects) for successful transmission.
**Taxonomy, Systematics and Classification**

This is ever changing as new approaches (e.g. molecular biology) have increased our knowledge of the evolutionary relationships of these different groups.

We will use the following simplified taxonomy:

**Flagellates** - all possess flagella at some life stage  
   e.g. *Giardia, Hexamita, Histomonas, Trichomonas, Tritrichomonas, Trypanosoma & Leishmania*

**Ciliates** - all possess cilia at some life stage  
   e.g. *Balantidium*

**Amoebae** - all use pseudopodia for locomotion at some life stage  
   e.g. *Entamoeba, Naegleria & Acanthamoeba*

**Apicomplexa** - The coccidians & hemosporidians (the most important group of human & veterinary protozoan parasites)  
   e.g. *Eimeria, Isospora, Cryptosporidium, Sarcocystis, Toxoplasma, Neospora, Babesia, Theileria, Cytauxzoon, Leucocytozoon, Plasmodium*

**Microsporida** - Highly specialized fungi - dominant life stage is a spore  
   e.g. *Encephalitozoon*
# Gastrointestinal Protozoa Parasites by Host Species

<table>
<thead>
<tr>
<th>Host</th>
<th>Parasite Genera</th>
<th>Location</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td><em>Giardia</em></td>
<td>small intestine</td>
<td>zoonotic?</td>
</tr>
<tr>
<td></td>
<td><em>Cryptosporidium</em></td>
<td>small intestine</td>
<td></td>
</tr>
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<td></td>
<td><em>Isospora</em></td>
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<td></td>
<td><em>Sarcocystis</em></td>
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<td></td>
<td><em>Neospora</em></td>
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<td>definitive host</td>
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<tr>
<td></td>
<td><em>Entamoeba</em></td>
<td>colon</td>
<td>rare</td>
</tr>
<tr>
<td></td>
<td><em>Balantidium</em></td>
<td>colon</td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td><em>Giardia</em></td>
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<td>zoonotic?</td>
</tr>
<tr>
<td></td>
<td><em>Trichomonas</em></td>
<td>large intestine</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Cryptosporidium</em></td>
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</tr>
<tr>
<td></td>
<td><em>Isospora</em></td>
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<td></td>
</tr>
<tr>
<td></td>
<td><em>Sarcocystis</em></td>
<td>small intestine</td>
<td>definitive host</td>
</tr>
<tr>
<td></td>
<td><em>Toxoplasma</em></td>
<td>small intestine</td>
<td>definitive host</td>
</tr>
<tr>
<td></td>
<td><em>Besnoitia</em></td>
<td>small intestine</td>
<td>definitive host</td>
</tr>
<tr>
<td></td>
<td><em>Entamoeba</em></td>
<td>colon</td>
<td>rare</td>
</tr>
<tr>
<td>Cattle</td>
<td><em>Giardia</em></td>
<td>small intestine</td>
<td>zoonotic?</td>
</tr>
<tr>
<td></td>
<td><em>Cryptosporidium</em></td>
<td>small intestine &amp; abomasum</td>
<td>zoonotic?</td>
</tr>
<tr>
<td></td>
<td><em>Eimeria</em></td>
<td>small &amp; large intestine</td>
<td></td>
</tr>
<tr>
<td>Sheep &amp; Goats</td>
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<td>zoonotic?</td>
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<tr>
<td></td>
<td><em>Cryptosporidium</em></td>
<td>small intestine</td>
<td>zoonotic?</td>
</tr>
<tr>
<td></td>
<td><em>Eimeria</em></td>
<td>small &amp; large intestine</td>
<td></td>
</tr>
<tr>
<td>Swine</td>
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<tr>
<td></td>
<td><em>Cryptosporidium</em></td>
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<td>zoonotic?</td>
</tr>
<tr>
<td></td>
<td><em>Eimeria</em></td>
<td>small intestine</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Entamoeba</em></td>
<td>colon</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Balantidium</em></td>
<td>colon</td>
<td>pathogenic?/zoonotic?</td>
</tr>
<tr>
<td>Horses</td>
<td><em>Giardia</em></td>
<td>small intestine</td>
<td>rare/zoonotic?</td>
</tr>
<tr>
<td></td>
<td><em>Cryptosporidium</em></td>
<td>small intestine</td>
<td>rare/zoonotic?</td>
</tr>
<tr>
<td></td>
<td><em>Eimeria</em></td>
<td>small intestine</td>
<td>pathogenic?</td>
</tr>
<tr>
<td>Pet Birds</td>
<td><em>Giardia</em></td>
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<td>zoonotic?</td>
</tr>
<tr>
<td></td>
<td><em>Trichomonas</em></td>
<td>crop</td>
<td>commensal?</td>
</tr>
<tr>
<td></td>
<td><em>Cryptosporidium</em></td>
<td>small intestine/airways</td>
<td>zoonotic?</td>
</tr>
<tr>
<td></td>
<td><em>Eimeria</em></td>
<td>small intestine</td>
<td></td>
</tr>
<tr>
<td>Poultry</td>
<td><em>Giardia</em></td>
<td>small intestine</td>
<td>zoonotic?</td>
</tr>
<tr>
<td></td>
<td><em>Trichomonas</em></td>
<td>crop</td>
<td>commensal?</td>
</tr>
<tr>
<td></td>
<td><em>Hexamita</em></td>
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<td></td>
</tr>
<tr>
<td></td>
<td><em>Histomonas</em></td>
<td>cecum &amp; liver</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Cryptosporidium</em></td>
<td>small intestine</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Eimeria</em></td>
<td>small &amp; large intestine, cecum</td>
<td>many species/pathogenic &amp; non-pathogenic</td>
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<tr>
<td>Rodents &amp; Rabbits</td>
<td><em>Giardia</em></td>
<td>small intestine</td>
<td>zoonotic?</td>
</tr>
<tr>
<td></td>
<td><em>Cryptosporidium</em></td>
<td>small intestine</td>
<td>zoonotic?</td>
</tr>
<tr>
<td></td>
<td><em>Encephalitozoon</em></td>
<td>kidney, liver, brain...</td>
<td></td>
</tr>
<tr>
<td>Reptiles &amp; Amphibians</td>
<td><em>Giardia</em></td>
<td>small intestine</td>
<td>zoonotic?</td>
</tr>
<tr>
<td></td>
<td><em>Entamoeba</em></td>
<td>intestine</td>
<td>pathogenic in Snakes</td>
</tr>
<tr>
<td></td>
<td><em>Cryptosporidium</em></td>
<td>small intestine</td>
<td>zoonotic?</td>
</tr>
</tbody>
</table>
Giardiasis

**General Taxonomy - Flagellate**

**Agent and Host Range**
- Common intestinal disease of mammals & birds found worldwide (cosmopolitan), especially in warm climates, caused by various *Giardia* species. Giardiasis is a recognized zoonosis.
- Often called ‘Beaver fever’ in humans, but more likely that humans are source of infection.
- ~ 200 million people have symptomatic giardiasis in Asia, Africa & Latin America.
- Worldwide ~ 500,000 new cases reported each year.

Broad host range:
*Giardia duodenalis* (= *intestinalis* = lamblia)
- most important species of *Giardia* in veterinary medicine.
- infects a wide range of hosts including dogs, cats, cattle, sheep, birds & humans...
- animals act as ‘potential’ reservoir for human infections (zoonotic) but also vice versa.
- 7 genetic assemblages (genotypes) that vary from country to country.
  - Assemblages A & B are considered zoonotic, C to G are host specific.

Host-adapted species: not zoonotic?
*G. muris* - rodents
*G. microti* - muskrats and voles
*G. ardeae* - birds
*G. psittaci* - birds
*G. agilis* – amphibians

**Site of infection** - duodenum & upper small intestine. *Giardia* attaches to the brush border of epithelial cells by a ventral sucking/adhesive disk.

**Morphology** - 2 life stages

**Trophozoite**
- motile feeding stage, **non-infectious**
- bilaterally symmetrical, pyriform to ellipsoidal in shape, convex dorsal surface, a ventral sucking/adhesive disk, axostyle & 2 median bodies (specialized microtubules)
- approximately 12-20 µm long, by 7-10 µm wide
- 4 pairs of flagella, binucleate (2 diploid nuclei)
  - ‘Monkey face’
Cyst

- **infectious stage (immediately infectious to host)**
  - environmentally resistant, ~2 months under ideal conditions (temperature/humidity)
  - oval shaped, 9-12 µm long by 7-9 µm wide
  - internal structures >visible= with light microscopy,
    - contains 4 nuclei (i.e. 2 trophozoites/cyst),
    - axostyle,
    - median bodies
  - karyogamy (fusion of nuclei) - i.e. ‘Sex in the cyst’

**Life cycle**

- **simple direct**, cysts ingested by the host & excyst after exposure to both acid of stomach & alkaline of small intestine to release 2 trophozoites into duodenum
- trophozoites reproduce by **asexual** binary fission & feed & colonize small intestine
- severity of disease is dependent on the number of feeding trophozoites
- trophozoites encyst in response to increasing concentrations of bile within feces (i.e. reabsorption of water, dehydrates feces as it passes through intestine towards rectum, therefore bile concentration increases at the same time as free cholesterol decreases).
- both cysts & more rarely, trophozoites can be passed in feces
- cysts resistant & survive (infectious stage), where as trophozoites are fragile & die quickly (non-infectious)
- up to $10^6$ cysts per gram of feces
- **intermittent shedding of cysts**
- **prepatent period**: 7-10 days
Epidemiology

Transmission - via cysts (immediately infectious)
- direct: fecal-oral route is most important
- waterborne transmission common in human outbreaks
- cysts highly contagious & ingestion of as few as 10-100 cysts can establish an infection
- cysts are susceptible to desiccation but remain viable in cool moist areas for ~2 months
- cysts are resistant to ‘conventional’ water disinfectants (i.e. chlorination, filtration…)

Prevalence
- ubiquitous in the environment but varies among populations and geographic regions
- infections are most common in young animals (including humans) & exacerbated by stressful situations, poor sanitation/hygiene & crowded confinement conditions (e.g. barnyards, kennels, catteries, shelters, pet stores, puppy mills & for humans, daycare facilities…)

Dogs & cats
- up to 36% in puppies & 11% in kittens (8% for both for cases submitted to the AVC)
- US national prevalence was 4% based on > 1 million fecals (Little et al. 2009), 70% of dogs were > 1 year old

Livestock
- calves & lambs, up to 100% reported with infections most common in calves older than 30 days of age

Birds
- common in pet birds with over 60% reported in one study (cockatiels)
Pathogenesis
- highly variable & still controversial in some species as both parasite & host factors contribute to disease
- severity of disease dependent on dose of infection i.e. number of cysts ingested
- trophozoites do not invade tissue (normally) but instead attach to the brush border of the mucosal epithelium of the duodenum & upper small intestine.
- trophozoite colonies result in diffuse shortening of microvilli (sometimes villus atrophy) which reduces the absorptive surface area (malabsorption) of the small intestine & results in decreased intestinal enzyme activity (e.g. disaccharidases) & malabsorption of nutrients (glucose especially), electrolytes & water
  → results in increased intestinal motility of digesta (or ‘decreased transit time’)
- in some animals, enterocyte injury by the parasite disrupting tight junctions thereby increasing intestinal permeability & destruction of enterocytes
  - this may lead to more severe chronic intestinal disorders e.g. Inflammatory bowel disease, Crohn’s disease & food allergies by exposing immune system to novel antigens.

Clinical Signs
- most infections are asymptomatic
- when clinical signs occur, typically small bowel diarrhea (usually self-limiting) but can be acute or chronic & often will reoccur
- clinical signs range from slight abdominal discomfort to severe abdominal pain & cramping, explosive watery, pale, foul-smelling diarrhea with malabsorption
- steatorrhea (fat in stool) is common as is anorexia & occasionally vomiting
  - in people, acute giardiasis develops after 1-14 days (average ~ 7 days) & can last 1-3 weeks
  - in dogs, prepatent period usually 1-2 weeks & can last 1 day to months
- extra-intestinal signs of urticaria & pruritus (allergic diseases) have been reported in both dogs, humans & birds.
  - e.g. feather picking (allergic disease) in cockatiels (associated with giardiasis)

Diagnosis
- “most commonly mis -, under- & over- diagnosed parasite in vet practice”

Requires multiple fecal samples (3 consecutive or 3 over 5 days) as cyst shedding is intermittent

Fecal flotation
- Gold Standard: fecal flotation with centrifugation in ZnSO₄ (with or without Lugol’s iodine stain)
- cytoplasm within cysts collapses producing a crescent shaped refractile osmotic artifact

Direct smear
- saline smear of fresh diarrhea, trophozoites movement (via flagella) – ‘falling leaf’
- trophozoites die quickly so sample must be observed soon after collection (i.e. per rectum) or within ~ 20-30 minutes of ‘deposit’ & kept at body temperature in humid environment to avoid desiccation
- cysts **may** be observed in high numbers
- Lugol’s iodine can be used to stain both trophozoites & cysts

**Antigen detection**
- detect *Giardia*-specific antigen
  - ProSpec T Fecal ELISA (micro plate - multiple samples) - detect infections in cats & dogs (100 & 96%, sensitivity & specificity respectively)
  - IDEXX SNAP Giardia test (lateral flow ELISA - individual samples)
    - based on cyst wall protein released into the feces during encystation
    - approved for cats & dogs (> 90% sensitivity & specificity)

*** No current test is 100% reliable; therefore, best to use a combination of centrifugal fecal flotation & antigen testing. Accuracy increases with more than one fecal sample analyzed per animal re: intermittent shedding of cysts

**Treatment & Control**

**Treatment**
- **No licensed treatments**
  - anti-giardial therapy focuses on the trophozoite stage not the cyst.
  - fenbendazole (Panacur) & metronidazole (Flagyl) used **off-label** either alone or in combination.
  - Drontal Plus (a combination of praziquantel, febantel, & pyrantel pamoate) also effective.
  - most current treatment protocols recommend fenbendazole (bind α-tubulin in cytoskeleton of trophozoites & impacts energy metabolism by inhibiting glucose uptake)
  - one recent study in dogs used azithromycin (Zygner et al. 2008, Pol J Vet Sci. 11:231-4)
  - many human & veterinary cases of clinical resistance to metronidazole & albendazole-like compounds
  - many cases of treatment failure are most likely caused by re-infection

**Vaccination**
- GiardiaVax™ (Fort Dodge) to aid in the prevention of disease & cyst shedding by *Giardia* in dogs. Claim of 1 year protection in healthy animals 8 weeks of age & older. Contains killed *Giardia*. Dosage: 1 ml dose subcutaneously. A second dose is given 2 to 4 weeks after the first vaccination. Annual re-vaccination is recommended. Vaccinated dogs may still shed viable cysts & therefore owners should continue to use proper hygiene & sanitation practices.
  - a few studies showing variable efficacy (no significant differences between vaccinated animals & controls) in dogs, cats & even in cows!
  - Not recommended by American Animal Hospital Association 2006…
Control
- **good hygiene & proper sanitation** to limit exposure to infectious cysts.
- remove or reduce stressful situations if possible
- cysts can stick to the fur & be a source for re-infection, the positive animal should receive a bath at least once during treatment. Some authors recommend bathing animals on the last day of treatment.
- disinfectants on surfaces—bleach & Lysol (also VIM!) with high contact times (15-30 minutes) to ensure inactivation of cysts.
- hot, soapy water also works.

**Hexamitosis**

**General Taxonomy - Flagellate**

**Agent and host range**

*Hexamita meleagridis* - turkeys and game birds  
*Hexamita columbae* - pigeons  
- hexamitosis = infectious catarrhal enteritis of birds (turkeys, pigeons, quail, pheasants, partridge, ducks & peafowl)

**Morphology**

**Trophozoites**
- oval shaped, 6-12 um long  
- bilaterally symmetrical with 8 flagella  
- binucleate with prominent nucleoli

**Cyst**
- ‘rarely formed’

**Life Cycle & site of infection**
- **direct life cycle** — trophozoites is the infectious stage, reproduce by binary fission  
- **fecal-oral** route of transmission (feces containing trophozoites or cysts contaminate food or water)  
- trophozoites colonize crypts of duodenum & upper jejunum  
- disease of young birds (1-9 week old), recovered adults act as asymptomatic carriers  
- heavy losses in outbreaks in ring-necked pheasants  
- chickens not typically affected  
- hexamitosis is a problem in every commercial turkey-producing area  
- major problems occur in localized areas during a particular year, followed by one or more years in which incidence is low
Pathogenesis
- **catarrhal enteritis** (inflammation of the mucous membranes) and atony
  - results in distention of upper small intestine
  - swollen, bulbous, liquid filled small intestine

Clinical signs
- listlessness, inappetence, anorexia
- birds huddle together near heat source & "chirp" constantly (pain?)
- greenish-yellow, foamy or watery diarrhea
- rapid weight loss due to diarrhea (dehydration)
- convulsions due to lowered blood glucose levels shortly precede death
- affected birds that survive remain stunted

Diagnosis
- history, clinical signs & microscopic examination of intestinal contents
- trophozoites detected in fresh wet mounts of intestinal contents of the duodenum
- confounding flagellate organisms in the ceca are not disease producers

Treatment & Control
- remove carrier birds & disinfect buildings, feeders & waterers
- separate adult & young birds or use an all-in/all-out strategy
- biosecurity - prevent contact between turkey poult & captive or wild birds
- chlortetracycline, tetracyclines & oxytetracyline used in food animals...variable success
- Nitroimidazoles (carnidazole, metronidazole, ronidazole, & dimetridazole) are the most effective treatment options for non-food animals (i.e. pet birds).
- Treatment does not substitute for adequate sanitation & management programs.

Trichomonosis

General Taxonomy - Flagellate

Agent and Host Range
Pathogenic
*Trichomonas foetus* - Bovine Genital Trichomonosis = ‘Trich’
*Trichomonas foetus* - Feline Trichomonosis
*Trichomonas gallinae* - Avian Trichomonosis = ‘Canker’ or ‘Frounce’

Non-pathogenic?
*Trichomonas suis* = *Trichomonas foetus*
  - nasal passages, stomach, cecum, colon & occasionally the small intestine of swine

*Trichomonas* spp.
  - found in cecum and colon of horses, ‘accused’ of causing acute diarrhea
  - found in intestinal tracts of cats & dogs
Bovine Genital Trichomonosis

Agent and Host Range
*Tritrichomonas foetus* - infection of the reproductive tract of cattle (Trich)

Morphology
- trichomonads have only a single life stage the trophozoite (no cysts)
- pyriform shaped,
- 10-25 um long
- 3 anterior flagella
- an undulating membrane with a posterior free flagellum
- single nucleus
- axostyle

Life cycle & Site of infection
- **direct life cycle** - transmitted through ‘natural service’ (copulation)
- **trophozoites are the infectious stage**, reproduce by binary fission
  - Bulls: trophozoites reside in the prepuce, penis, epididymis & vas deferens
  - Cow/heifers: trophozoites reside in the vagina, cervix & uterus

Epidemiology

Transmission
Cows/heifers:
- trophozoites are transferred to cows & heifers from infected bull during copulation
- infections persist for weeks-months
- usually recover from infections but can be re-infected

Bulls:
- trophozoites transferred from infected cows/heifers to bulls during copulation
- infected bulls are often infected for life, but there is an association between age & infection
- younger bulls (< 3 years) may be refractory to infection while mature bulls remain infected due to markedly deeper epithelial crypts in the skin of the penis & prepuce (internal sheath), therefore providing a favourable environment for *T. foetus*.

Artificial Insemination:
- semen is **not** typically infective unless contaminated with preputial fluid during collection
- contaminated semen will remain infectious through the addition of diluents, antibiotics, & the freezing process (i.e. *T. foetus* is cryopreserved)
Prevalence - surveys from the USA and Canada
- 11.9% of bulls in Florida, 15.8% of herds & 4% of bulls in California
- 6% of bulls in Saskatchewan

Pathogenesis
- invasion of the uterus leads to placentitis which results in detachment, death & abortion of fetus
- *T. foetus* may also invade the fetal tissues

Clinical Signs
Cows/heifers
- typically minimal - mild mucopurulent discharge
- **open cows** (infertility common) may see decreases in calf production by 50-80% with newly infected herd
- abortion (uncommon, < 5%) before 5 months gestation (due to small size of passed fetus, abortion may actually go unnoticed)
- vaginitis &/or pyometra (5%)
- retention of fetus and fetal membranes leading to endometritis & sterility

Bulls
- no clinical signs of infection

Diagnosis
- confirmation of infection by demonstration of trophozoites in preputial scrapings or washings (smegma), vaginal secretions, vaginal washings, or absorbed fetuses. Care must be taken to avoid contamination with gastrointestinal trichomonads (non-pathogenic but could be confounders)
- *T. foetus* has 3 anterior flagella & one trailing flagellum & displays a characteristic ‘rolling’ motion. If this is not observed in the washings they should be routinely cultured.
- commercially available >In Pouch TF= culture test kit
- **repeated sampling may be required to confirm infection status**
- confounding non-TF trichs (*Tetratrichomonas* spp. & *Pentatrichomonas hominis*) - pathogenic?
  - likely need PCR & immunofluorescent assay (IFA) to confirm

Treatment
Cows/heifers
- no treatment available
- 4 months sexual rest will **usually** clear reproductive tract of *T. foetus*
- **3% of cows remain carriers regardless of this ‘treatment’**

Bulls
- no approved drugs
- treatment not recommended, therefore **cull infected bulls**
Control
- cull infected bulls & use non-infected or virgin bulls
- test bulls prior to breeding (pre-breeding exam)
- use artificial insemination
- using younger bulls can reduce transmission (replace every two years)
- vaccine available for female herd (Trich Guard), annual vaccination

Avian Trichomonosis

Agent and Host Range
*Trichomonas gallinae* - infection of the crop of birds

Morphology
- trichomonads have only a single life stage - the trophozoite (no cysts)
  - pyriform shaped
  - 5-9 um long
  - 4 anterior flagella & an undulating membrane that incorporates a recurrent flagellum (i.e. embedded & does not extend beyond the undulating membrane)
  - single nucleus
  - axostyle

Life cycle & Site of infection
- direct oral-oral transmission
- trophozoites are the infectious stage and reproduce by binary fission

Pigeons
- ‘Canker’ (trichomonosis) most common condition of pigeons infecting about 80%
- *T. gallinae* considered normal oral flora in adult pigeons
- disease only occurring when birds are ‘stressed’
- trophozoites transferred from the crop of adults to squabs during feeding ‘pigeon milk’ (regurgitated food & fat laden crop lining cells)
- trophozoites from infected birds oral secretion may also be transmitted via contaminated surface water, waterers & feeders

Raptors (birds of prey)
- ‘Frounce’ in raptors, *T. gallinae* transferred to birds of prey when they feed on infected pigeons

Poultry - can contact the disease by consuming water or feed contaminated by infected pigeons
Pathogenesis
- trophozoites invade the mucosa of the upper intestinal tract (buccal cavity, sinuses, pharynx, esophagus, crop)
- liver and other organs are occasionally invaded
- raised yellow caseous lesions ‘cankers’ first appear in mouth & spread to the upper digestive tract. Birds often gape as the cankers make closing the mouth impossible
- lesions can enlarge, become confluent & caseous material may occlude the esophagus
- liver lesions appear on surface as solid, white or yellow circular masses

Clinical Signs
- birds will have difficulty closing their mouth. They will drool & make continuous swallowing motions
- greenish to yellow fluid in oral cavity or dripping from beak
- ruffled & emaciated appearance

Diagnosis
- clinical signs & gross lesions restricted to the upper portion of the digestive tract are suggestive of trichomonosis
- microscopic observation of large numbers of T. gallinae in direct smears from oral fluids or lesions in the mouth, crop or digestive tract is confirmatory
- commercially available In Pouch TF culture test kit works to culture T. gallinae
- repeated sampling may be required to confirm infection status
  - need PCR

Control & Treatment
- eliminate infected birds & suspected carriers from flock
- avoid feeding pigeons to raptors
- practice proper sanitation & ensure there is a source of fresh, clean water & food.
- prevent pigeons from contaminating water & food supply of poultry
- anti-flagellate drugs - dimetronidazole & ronidazole (Europe)

Feline Trichomonosis
- First cases in 1996 & now considered an emerging infectious diarrheal disease in cats worldwide
- problem mostly in young cats under stress (Observed more so in cat colonies e.g. purebred catteries & shelters BUT can be observed in single & multi-cat households)
- 31% infection in 117 cats from 89 catteries at International cat show
- first reports found that feral cats, in the same demographic had no trichs (see below).

Agent/Morphology
Trichomonas foetus (see above for bovine trichs re: morphology)

Epidemiology
- Trichomonads are usually commensal organisms & cause no clinical signs
- Transmission route unknown but since no cysts, the trich is likely transmitted directly
from cat to cat
- trichs can survive ~6-24 hours in feces (moist but firm). Therefore, mutual grooming may result in transmission between cats.
- a 2008 prevalence study (17 of 173 cats with and without clinical signs of diarrhea) found no correlation with breed or sex but the disease was more common in younger male & female cats of all breeds
  - Siamese & Bengal cats were overrepresented in the study
- infected cats (9 of 17) were more likely to also be infected with other enteric pathogens.
- cat trichs can infect heifers (2008) BUT now (2010) determined that regardless of this finding, they are distinctly different genotypes that can be differentiated by molecular assays.

Pathogenesis
- *T. foetus* colonizes the surface of the mucosa in the ileum, cecum & colon but less frequently, found within the lumen of the colonic crypts
- infiltration of lymphocytes & neutrophils, loss of goblet cells in intestinal mucosa
- mild to severe lymphoplasmacytic colitis
- mechanism unknown but hypothesized to involve disruption of normal flora, adherence to the epithelium & induction of host cytokines & enzymes

Clinical Signs
- range from asymptomatic infection to chronic large bowel diarrhea
- typically cats are in good body condition (BAR) & have good appetites
- diarrhea may be waxing & waning but often resolves ‘spontaneously’ after ~2 years
- cow-pie diarrhea with or without blood & mucus
- often with flatulence, tenesmus & fecal incontinence
- anus may appear edematous, painful, dribbling feces, rectal prolapse...

Diagnosis
- direct fecal smear of fresh feces (or feces kept at room temperature for ~6 hours)
  – observe erratic ‘rolling’ motion of trich (differs from *Giardia*)
- flotation methods useless as trophozoite is destroyed by flotation solutions
- cultivation of *T. foetus* using ‘In pouch TF-Feline Medium’ is the Gold Standard
- PCR – now commercially available for confirmation
- confounding organisms – e.g. *Pentatrichomonas* & *Giardia*

Treatment & Control
- No approved treatment
  - Ronidazole: 30 mg/kg twice daily for 2 weeks
  - resolves diarrhea & appears to eradicate infection
  - sanitation & hygiene, stress & density of cats all important in control
Histomonosis

**General taxonomy - Flagellate**

**Agent and host range**
- *Histomonas meleagridis* is a **cosmopolitan** parasite affecting gallinaceous fowl (turkeys, chickens, pheasant, quail, & grouse)

Turkeys - highly susceptible to infection & most infected turkeys die (**Blackhead disease**)  
Chickens - easily infected but usually a milder form of disease

**Site of infection**
- cecum & liver

**Morphology**
- various trophozoite stages  
  (flagellate & amoeba forms, i.e. pleomorphic) 5-30 um  
  - no cysts

**Life Cycle**
- divides by asexual, binary fission  
- direct transmission - **fecal-oral route** possible but considered **rare**  
  - **cloacal drinking** (turkeys & SPF chickens) transmission possible  
- **indirect transmission is the most common route by the cecal nematode, Heterakis gallinarum**  
  - trophozoites are ingested by the nematode & then trophs penetrate the tissues of the worm & finally become incorporated into developing nematode eggs  
  - birds become infected by ingesting *Heterakis* eggs in soil or by eating earthworms (paratenic host of the nematode)  
- by either route, *Histomonas* liberated into the intestine will penetrate the cecal wall & passes to the liver via the portal circulation.
Epidemiology
- commonly reported throughout Canada & USA
- *Histomonas* can remain infective within the *Heterakis* egg for 1-2 years
- affects all ages of turkeys with young birds being the most susceptible (3-12 weeks)
- chickens - only appears to affect young birds (4-6 weeks)
- higher incidence of histomonosis in turkeys concurrently infected with coccidia

Pathogenesis
- disease results when *Histomonas* penetrates the cecal wall & invades the liver via the blood stream
- cecal lesions - cecum becomes thickened (edematous) & lumen is filled with yellow caseous smelly exudate
- liver lesions - circular depressed (bulls eye) yellow-green to grey areas of necrosis (1-2 cm) which may coalesce to involve the entire liver
  - appear by approximately 10 days post infection

Clinical signs
- 2-3 weeks post infection
- hunched appearance, droopy wings & tail, ruffled feathers
- anorexia & emaciation, weakness & depression
- head may (or may not) turn black or cyanotic (due to deficient oxygenation of the blood)
- **foul smelling, brilliant yellow sulfur-coloured diarrhea**
- mortality high in young turkeys (50-100%)
- low mortality in chickens, but up to 30% has been reported in young birds

**Diagnosis**
- brilliant yellow (sulfur) feces combined with the cecal & liver lesions are pathognomonic
- *Histomonas* may also be demonstrated histologically.

**Treatment & Control**
- good sanitation must be practiced
- **turkeys & chickens must be raised separately**
- control *Heterakis gallinarum* in birds & limit access to nematode eggs & earthworms
- No current treatment approved - in the past, prophylactic & therapeutic treatment with nitroimidazoles (dimetronidazole, ipronidazole, and ronidazole)
- roxarsone a pentavalent arsenical has anecdotally been used in reducing mortality from blackhead, particularly in chickens
- herbal products have shown no efficacy.
Amoebiasis

General taxonomy - Amoeba

Agent and host range - (Zoonosis)
Numerous non-pathogenic species of amoebae that infect animals and humans
Pathogenic species include obligate parasites as well as free-living amoeba

- Pathogenic obligate parasites - Intestinal Amoebiasis
  Entamoeba histolytica - mammals
  Entamoeba invadens - severe disease and death in captive reptiles

- Pathogenic free-living amoeba - Primary Amoebic Meningoencephalitis
  Naegleria fowleri
  Acanthamoeba spp.

Intestinal Amoebiasis

Morphology - 2 life stages
Trophozoite - ameboid shape, 12-60 um in size (average 20 um)
  - E. histolytica has a single nucleus & central nucleolus surrounded by chromatin ring
  - may contain ingested erythrocytes - which differentiates them from other amoeba
Cyst - round, 10-20 um in size and contain 4 nuclei with ‘rounded’ chromatid bar

Life Cycle
- simple & direct
- cysts are ingested & release trophozoites in lower ileum
- trophozoites colonize the large intestine & divide by binary fission
- trophozoites may invade the intestinal wall & spread systemically via the blood (especially to the liver)
- cysts are formed in lower colon & are passed in the feces

Epidemiology
- E. histolytica is primarily a parasite of humans & it is humans that act as a reservoir for domestic animals (Zoonosis)
- infections reported in primates, dogs, cats, pigs, rats & cattle BUT prevalence is unknown
- transmission is primarily fecal-oral route, but waterborne is another route
- cysts not as resistant as Giardia, killed by temperatures > 55°C & super chlorination.
Pathogenesis
- *E. histolytica* trophozoites hydrolyze the host large intestinal tissues via cysteine proteases
- intestinal lesions – ‘flask-shaped’ ulcers that penetrate the muscularis mucosa
- trophs penetrate through the submucosa to enter blood vessels and lymph
- extra-intestinal organ/tissue sites - liver and lung (other sites) where the parasite forms necrotic abscesses

Clinical signs
- infections may be asymptomatic (no mucosal invasion)
- vary dependent on severity of infection
- most common are colitis, diarrhea, dysentery (blood & mucus in stool) & vomiting
- anorexia, weight loss
- hepatomegaly & fever with liver infections

Diagnosis
- finding trophs or cysts on fresh or stained fixed fecal smears with heavy infections
- fecal flotation - cysts
- morphology is important - stained smears must be evaluated by specialized lab as many non-pathogenic amoeba are confused with pathogenic species

Control and 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 - Reptiles

*Entamoeba invadens*
- commensal in turtles & crocodiles
- highly pathogenic in lizards, tortoises & snakes
- Don’t mix snakes, lizards or tortoises with turtles!

Direct life cycle
- fecal-oral, waterborne

Pathogenesis
- intestinal lesions - ulcers that penetrate the muscularis mucosa
- trophs may penetrate through the submucosa to enter blood vessels & lymph
- extra-intestinal organ/tissue sites - primarily causes necrotic abscesses in liver

Clinical signs
- anorexia, weight loss, vomiting
- blood or mucus in the feces
- green coloured urates
- mid to caudal swellings of the body
- Death

Diagnosis
- History, P.E. & overview of husbandry
- Fecal exam for cysts - as before
- confounding non-pathogenic *Entamoeba* spp., therefore requires PCR for definitive Dx

Treatment & Control
- Metronidazole
- snakes & tortoises should never be housed with turtles or in areas where exposure to environment contaminated with turtle feces
- Don’t mix turtles with snakes, tortoises & lizards

**Amoebic Meningoencephalitis**

- This is considered an extremely rare disease in domestic animals BUT does happen (it also shows up on the NAVLE occasionally)
- more common in tropical (humid and high temperature) areas where water biosecurity is an issue.
- rapidly fatal caused by exposure to water heavily contaminated with mainly *Naegleria fowleri* or *Acanthamoeba* spp.
- amoeba are inhaled into the upper respiratory tract and migrate along the olfactory nerves into the cranium
- rapid destruction of brain tissue with death occurring in about 2 weeks
- treatment is rare as disease progresses so rapidly
- human have been treated with amphotericin B
- prognosis is poor and most diagnoses occur at post mortem
- Has been reported in North America (emerging disease? Unlikely but you should be aware of the opportunistic exposure to these primary amoebic pathogens)
  - rarely in dairy cattle in California and a dog in Illinois.
Balantidiosis

**General taxonomy** - Ciliate

**Agent and host range**
- *Balantidium coli* a common non-pathogenic resident of the G.I. tract of pigs
- primarily a disease of primates (**including humans**, therefore **zoonotic**) but has been reported in dogs

**Morphology** - 2 life stages

**Trophozoite**
- trophs are ovoid, covered with cilia & have a funnel shaped cytostome (mouth)
- variable sizes - small 40-60 um, large 90-120 um
- macronucleus - centrally located, bean shaped
- micronucleus - smaller and spherical, located near macronucleus

**Cyst**
- spherical to ellipsoid, 50-75 um long and have a thick refractile wall
- **cyst is the infectious stage**

**Life Cycle & site of infection**
- direct transmission of cysts (fecal oral route & waterborne)
- trophs released from cysts multiply by transverse binary fission (asexual) and colonize the large intestine
- trophs may reproduce sexually through conjugation
- trophs encyst in response to dehydration of feces as it moves through the large intestine and through desiccation of defecated fecal material

**Epidemiology**
- ubiquitous with a prevalence in pigs close to 100%
- prevalence in primates & other animals unknown
- transmission by a **fecal-oral or waterborne**
- human infections are rare but transmission from pigs is the likely source (**zoonotic**)

**Pathogenesis**
- most infections are asymptomatic and disease is rare (especially in pigs)
- when pathogenic, the parasite invades the tissues of the large intestine using enzymes
- secondary invasion often takes place following the induction of intestinal lesions
- rarely invades extra-intestinal tissues but peritonitis, appendix perforation and pulmonary involvement have been reported

**Clinical signs**
- in pigs, typically mild colitis & diarrhea (sloppy grey feces) with occasional severe ‘amoebic-type’ dysentery
**Diagnosis**
- trophs found in fresh fecal wet mounts
- cysts found in fresh fecal wet mounts, fixed smears or on fecal flotation

**Treatment and Control**
- hygiene and proper sanitation, especially in primate colonies and swine farms
- no treatment necessary for pigs
- tetracyclines used in the treatment of primates including humans.
Enteric Coccidiosis

General Taxonomy - Apicomplexa

Agent and host range
- enteric (intestinal) coccidiosis is a generic term used to describe disease caused by 2 genera:
  - *Eimeria*
  - *Isospora* (also called *Cystoisospora*)
- coccidia are all obligate intracellular parasites
- hundreds of recognized species of coccidia which infect all animals of veterinary importance
  - each specific species of *Eimeria* or *Isospora* tends to be host specific, infecting only a single host species or closely related species
  - BUT each host may be simultaneously infected with more than one coccidian species

Morphology
Zoite (sporozoite, merozoite)
- the functional unit of all Apicomplexans
- motile, banana-shaped, 2-8 um long
- zoites contain specialized structures at their apical end (apical complex, rhoptries, conoid) which are used for cell invasion and are only visible with an electron microscope
- *sporozoites* are the infective stage of the parasite and are found in sporulated oocysts
- *merozoites* are produced within the host cells through a form of asexual reproduction called **merogony** (synonym = **schizogony**)

Oocyst
- formed as a result of sexual reproduction
- round to ovoid and vary in size depending on species of coccidia
- oocysts are environmentally resistant stage of the parasite that require further development by **sporulation** to become infective
- unsporulated oocysts contain a diploid single cell called a **sporoblast** or **sporont** (equivalent to a zygote)
- sporulated oocysts (infectious stage) contain haploid **sporozoites** which may or may not be enclosed in a **sporocyst(s)**
- number of sporozoites / sporocysts depends on the Genus

*Eimeria*
- 4 sporocysts each containing 2 sporozoites = 8 sporozoites

*Isospora*
- 2 sporocysts each containing 4 sporozoites = 8 sporozoites
**Life Cycle** - generalized for all **monoxenous** (direct or one host life cycle) coccidia

Many stages, asexual reproduction (merogony & sporogony) & sexual reproduction (gametogony)

- **sporulated oocysts (infective stage)** are ingested by the host
- **sporozoites** emerge & invade an intestinal cell (epithelial or lamina propria)
- round up as **trophozoite** (feeding/growing stage) inside cell surrounded by a parasitophorous vacuole
- trophozoite grows to become **first generation meront** (also called schizont)
- **merogony** (asexual reproduction or schizogony) to produce first generation **merozoites**
- **merozoites** burst from the cell and invade new cells to become **second generation meronts** (the number of these asexual generations depends on the species of coccidia but 2 or 3 is usually the limit for many species)
- after a fixed number of asexual repetitions, **merozoites** produced by the **last merogony** enter a new host cell and develop into a **microgametocyte** or a **macrogametocyte** (*gametogony*)
- these mature into a single cell = macrogamete (haploid, female sex cell) or undergo repeated division to become biflagellate **microgametes** (haploid, male sex cells)
- **microgametes** are released and fertilize **macrogametes** to form a diploid zygote
- a resistant wall develops around the **zygote** to form an **oocyst**
- the host cell ruptures releasing the **unsporulated oocyst** (non-infectious) which passes out into the feces
- **oocysts** undergo **sporulation** (also called **sporogony**), a maturation process when conditions are correct (moisture & warm temperature ~20°C) to produce haploid **sporozoites** within the protective oocyst. Therefore, the **sporulated oocyst** is now infectious

**Infection is usually self-limiting in the absence of re-infection**

Oocysts can be very resistant to environmental conditions (e.g. resist freezing) & survive for months to a year
Generalized Coccidian Life Cycle
A useful introduction to the life cycle of *Isospora* coccidia
Generalized Coccidian Life Cycle
A useful introduction to the life cycle of *Eimeria* coccidia is available online at http://www.saxonet.de/coccidia/cycle.htm

**Epidemiology**

**Prevalence**
- high - especially in crowded conditions (poultry operations, feedlots, catteries etc.)
- all animals will have a mixed infection (some highly pathogenic, others not pathogenic)

**Transmission**
- fecal-oral route, contaminated food and water
Disease
- often asymptomatic
- **associated with raising animals in confinement**
- associated with **young animals** and **stress** (weaning, adverse weather, shipping etc.)
- adult animals develop immunity that protects from disease but not re-infection
- immunity is species specific (i.e. in cattle immunity to *E. bovis* does not offer any protection to *E. zuernii*)

Pathogenesis
- severity of disease is thought to be proportional to the number of infective oocysts ingested & location of infection (crypts and colon = more severe disease)
- parasite can destroy epithelial cells causing villus atrophy & intestinal lesions
- crypt hyperplasia can occur resulting in immature epithelial cells along the villi
- denudation of epithelium can result from infections of crypts
- hemorrhage is seen in severe disease
- **majority of pathology caused by asexually replicating stages vs. sexual stages**, therefore see clinical signs of disease prior to presence of oocysts

Clinical signs
- general signs of diarrheal enteritis & malabsorption
- blood may or may not be observed in feces (depends on species & severity of infection)
- systemic signs of blood loss if the infection has caused hemorrhage
- poor weight gain, emaciation & death

Diagnosis
- oocysts present on fecal flotation, but clinical signs usually appear before oocysts passed in feces
- history & clinical signs are important as just finding oocysts is not proof of coccidiosis
- gross intestinal lesions at necropsy and coccidia in observed lesions scrapings or histopathology

Infection with non-pathogenic species of coccidia is common
**Diagnosis is dependent on the age of susceptibility & finding oocysts in the feces of animals suffering clinical signs of coccidiosis**

Treatment & Control
- prophylactic drugs (most are coccidiostatic only e.g. Amprolium, Sulfa drugs) are used to control the infections (often mixed with feed or water). Some newer drugs have a cidal effect (e.g. Panzuril, Diclazuril, Toltrazuril) & should result in elimination of infection.
- treatment with drugs after clinical signs develop is rarely effective (or dramatic)
- supportive treatment & control of secondary infections is extremely important
- Environment - reducing exposure to oocysts through good management can prevent or reduce severity of disease
- oocysts resistant to most disinfectants but steam cleaning, immersion in boiling water or 10% ammonia solutions (e.g. food & water bowls)

**Avian Coccidiosis**
One of the most common & expensive diseases of poultry production

**Chickens**
- 9 species of *Eimeria* involved
  - *Eimeria acervulina* - most frequently encountered
  - *Eimeria tenella* - most pathogenic (high mortality)

- site of infection ranges from small intestine to cecum depending on species involved
- intestinal / cecal lesions range from minor to round, white/grey plaques to severe necrotic cores and hemorrhage with significant blood loss

**Clinical signs**
- bloody droppings and hemorrhagic diarrhea beginning 4 days post infection
- acute death (high mortality rates)
- emaciation, pallor & inappetence

**Control and Treatment**
- isolate sick birds, treat healthy birds with anti-coccidial medications (treating sick birds is futile)
- almost all poultry flocks receive preventative medication
  - ionophores (monensin, salinomycin, lasalocid)
  - amprolium
  - decoquinate
  - many others
- resistance is becoming widespread
- drugs must be rotated or use Ashuttle® program (one drug in starter feed, another in grower feed)
- vaccines have been developed but problems with administration
- raise young chickens separate from older birds (segregation of industry), All-in-All-out strategy
- Environment as in general section

**Turkeys**
- 7 species of *Eimeria* infect turkeys
  - *E. adenoeides, E. gallopavonis, E. meleagrimittis* are all pathogenic
- lesions are not as spectacular as in chickens, but mortality rates high in young birds

**Clinical signs**
- watery mucoid diarrhea
- anorexia
- ruffled feathers and general signs of illness

**Control and Treatment**
- preventative medication and control measures as with chickens
- sulfonamides, amprolium, lasalocid

**Ducks & Geese**
-In geese, **renal coccidiosis** caused by *Eimeria truncata* and **intestinal coccidiosis** caused by *E. anseris* are of pathogenic significance
-In ducks, many species of pathogenic coccidia have been described
-Ducks and geese have been treated for coccidiosis with various sulfonamides, but there is a lack of information in this area.

**Bovine Coccidiosis**
- 13 species infect cattle in North America and mixed infections are the rule
  - *Eimeria zuernii* & *Eimeria bovis* are most pathogenic
- coccidiosis occurs in calves 3 weeks to 6 months of age, but usually seen in calves from 2 - 6 months or in older calves housed in groups
- first generation meronts grow to giant size in endothelial cells of lacteals in the villi (*E. bovis*) or lamina propria (*E. zuernii*) of the ileum and produce thousands of merozoites
- prepatent period - 12 - 24 days

**Clinical signs**
- moderate infections produce diarrhea, listlessness, anorexia
- severe infections produce liquid bloody diarrhea (which may travel 2-3 m), emaciation and tenesmus
- hind quarters of calf can become soiled with feces
- secondary infections are common (especially pneumonia)
- animal that do not die in 7 - 10 days will usually recover

**Summer coccidiosis - Eimeria bovis**
- young calves 3 - 6 months that are on pasture for the first time
- warm temperatures & high humidity
- older cows carriers (asymptomatic) & infect the pasture
- calves have diarrhea to dysentery with tenesmus & projectile feces leading to dehydration & death if left untreated

**Winter coccidiosis - Eimeria zuernii**
- young calves that have diarrhea and tenesmus during severe cold weather
- other stress factors critical (weaning, shift from pasture to feedlot)

**Nervous coccidiosis - Eimeria zuernii**
- calves suffer muscular tremors, convulsions, blindness & 50% mortality in addition to acute diarrhea
- Pathogenesis is not understood but majority of cases occur during coldest months.

**Control & Treatment**
- Reduce stocking rates, minimize stress, clean housing & feed/water equipment.
- Drugs used for treatment & prevention and include:
  - Decoquinate, amprolium, monensin, sulfamethazine, sulfadiazine, lasalocid.
- During an outbreak, anticoccidial drugs should be given to calves that are not yet showing clinical signs.
- Supportive therapy & fluid replacement until gut epithelium is replaced is extremely important in calves with clinical coccidiosis.
- Prevention of successive transmission requires that housing be cleaned & sanitized between groups of calves. Do not mix calves of different ages.

**Ovine / Caprine Coccidiosis**
- 12 species of *Eimeria* infect sheep, 6 infect goats.
- Prepatent period of approximately 14 days.

**Sheep** - Primarily a disease of feedlot lambs or after shipping, usually occurring 12 - 21 days after arrival.
- Lambs have watery diarrhea for several days - 2 weeks (usually not bloody).
- Depression, inappetence followed by weight loss.
- Mortality is seldom more than 10%, but significant weight loss can occur.
- Soiled wool may attract flies = Fly strike (myiasis).

**Goats** - More susceptible than sheep.
- Clinical signs of diarrhea typically follow weaning.
- Heavily infected kids usually die and kids that recover may fail to grow normally.

**Control and Treatment**
- As in cattle.
- Raise lambs on slatted floor pens.
- Good sanitation.

**Equine Coccidiosis**
- *Eimeria leuckarti*.
- Coccidiosis is rare in horses.
- Few reports of diarrhea or weight loss in infected horses.

**Rabbit Coccidiosis**
- There are several pathogenic species in rabbits.

**Liver** - *Eimeria stiedai*.
- infects the bile duct epithelium
- causes biliary hyperplasia and cirrhosis resulting in diarrhea

**Intestine - Eimeria intestinalis, Eimeria flavescens**
- infect the crypts of small intestine (E. intestinalis) and cecum (E. flavescens)
- infection causes denudation of the epithelium & severe diarrhea

**Control & Treatment**
- prophylactic administration of sulfonamides or lasalocid
- prevent contamination of feeders & water supply
- proper management is important

**Swine Coccidiosis**
- coccidiosis is a severe disease of nursing piglets caused by *Isospora suis*
- ubiquitous where pigs are farrowed in confinement
- *I. suis* is responsible for 15 - 20% of piglet diarrhea
- infects enterocytes throughout the small intestine and occasionally cecum and colon
- prepatent period 4 - 5 days, patent period 2 weeks
- established *I. suis* infections interferes with *Salmonella typhimurium* infections

**Clinical signs**
- piglets 7 - 14 days of age having yellow-grey pasty diarrhea
- piglets covered with diarrhea smell like ‘soured milk’
- blood not present unless other disease agents involved
- depressed weight gains, high morbidity, moderate mortality

**Control & Treatment**
- current anti-coccidial agents are not considered effective
- nursing piglets do not eat or drink enough for drugs added to feed or water to be effective
- improved sanitation is required to reduce the number of infectious oocysts in environment

**Canine & Feline Coccidiosis**

**Dogs**
- *Isospora canis* - largest oocysts of canine *Isospora* species (38 x 30 µm)
  - develops in lamina propria of distal small intestine & may cause diarrhea associated with weaning stress (also shipping, change of ownership…)
- *Isospora ohiensis* complex
  - *Isospora ohiensis, Isospora burrowsi & Isospora neorivolta* cannot be separated based on oocysts size (20 x 17-20 µm)
  - *I. ohiensis* develops in the enterocytes in the small intestine, cecum & colon and can cause diarrhea in puppies & is associated with stress factors
Cats
- *Isospora felis* (40 x 30 µm) & *Isospora rivolta* (22 x 20 µm)
- develop in the enterocytes of small intestine & can cause diarrhea & enteritis in kittens (newborn - 4 weeks old)
  - stress factors are likely involved (e.g. weaning stress, shipping, change of ownership... & other disease agents)

Paratenic hosts
- *Isospora* infections in dogs & cats can be acquired through a paratenic host
- mice, cattle, sheep & other herbivores ... (other species) can act as paratenic hosts when they ingest the sporulated oocysts which then excyst releasing sporozoites that then invade the extra-intestinal tissue (mesenteric lymph nodes, brain, muscle)
- sporozoites become encysted within tissue and form enlarging monozoic or unizoic cysts which remain with the paratenic host for life
- when the paratenic host is ingested by the cat or dog, the parasite completes the life cycle similar to ingested sporulated oocysts from feces
- the significance of the paratenic host-monozoic cyst life cycle to dogs & cats is unknown since the typical fecal-oral route via oocysts is very efficient

Control & Treatment
- sanitation & hygiene to reduce exposure & re-infection to oocysts
- reduce stress (transport, weaning, change of companionship/ownership), remove animals from high density environments to prevent transmission
- controlled treatment data not available for most protocols & therefore anecdotally, administration of drugs (e.g. supha drugs) for clinical cases lessens morbidity & mortality & reduces the shedding of oocysts but response to treatment is seldom dramatic
- supportive care with fluid therapy for dehydrated cases as indicated

Problem
- clinical disease not well understood since there are no experimental models (i.e. disease not reproduced reliably)
- no information on virulence differences between strains
- clinical signs of diarrhea do not correlate with number of oocysts
Cryptosporidiosis

General Taxonomy - Apicomplexa

Agent and host range (Zoonosis)
- Cryptosporidiosis caused by small ‘coccidian-like’ (actually a gregarine) parasites of the Genus Cryptosporidium
- Many species of Cryptosporidium infect the microvillous region of epithelial cells lining the gastrointestinal and respiratory tracts of many different vertebrates
- Intracellular, extra-cytoplasmic parasites of epithelial cells

Mammals
- Cryptosporidium hominis - humans (host-adapted species)
- Cryptosporidium parvum and C. parvum-like species
  - infect the small intestine of cats, dogs, horses, cattle and humans (zoonotic)
  - very important cause of neonatal diarrhea in calves
- Cryptosporidium bovis – cattle (morphologically indistinguishable from C. parvum)
- Cryptosporidium andersoni
  - infects the abomasum of older calves (> 1 month of age) & mature cattle
  - may lead to sub-par performance in cattle (milk production and weight gain)
  - life-long infections
- Cryptosporidium suis - pigs
- Cryptosporidium muris - gastric infection of rodents

Birds
- Cryptosporidium baileyi
  - respiratory tract and cloaca of chickens & turkeys
- Cryptosporidium meleagridis
  - small intestine of chickens & turkeys

Reptiles - Cryptosporidium serpentis - intestinal infections

Fish
- Cryptosporidium molnari - intestinal infections

Morphology

Sporozoites, Merozoites, Meronts and Gamonts
- similar to coccidia

Oocysts
- C. parvum oocysts are spherical and very small 4 - 6 um
- C. andersoni oocysts are slightly larger 6 - 8 um
- all are sporulated (therefore immediately infective) when passed in feces
- Cryptosporidium oocysts contain 4 ‘naked’ sporozoites (no sporocysts)
Life Cycle
- direct (one host or monoxenous) life cycle, therefore similar to coccidia
- intracellular stages within parasitophorous vacuole located in the microvillous region of the hosts intestinal cells
- Type I (first generation) meronts release merozoites that go on to invade new cells and produce Type II (second generation) meronts or recycle back to produce Type I meronts
- Type II meronts release merozoites that invade new cells and differentiate into gamonts
- following sexual reproduction 80% of oocysts formed have thick walls and 20% have thin walls
- thick walled oocysts (sporulated) pass out of the host and infect new hosts
- thick walled oocysts are environmentally resistant
- thin walled oocysts (sporulated) rupture within the original host resulting in an autoinfection cycle
- autoinfection via thin walled oocysts-> persistent infections in immune deficient hosts

Cryptosporidium life cycle
Epidemiology

Prevalence
- ubiquitous
- infections are common in younger animals & neonates & in the immunocompromised

Transmission
- oocysts are highly resistant & remain viable for ~ 1 year (most standard concentrations of disinfectants fail to kill oocysts)
- direct fecal-oral transmission is common in confinement
- waterborne outbreaks are the most common source for human infections
  - Milwaukee - 1993
  - North Battleford - 2001

Risk Factors - *C. parvum* Zoonosis
- Positive
  - direct contact with cattle or indirect contact with water contaminated with cattle manure
  - person to person (e.g. swimming pools)
- Negative
  - pets (cats & dogs)
  - consumption of raw vegetables (tomatoes & carrots).
    Why? Possible protective effect of repeated low dose exposure leading to enhanced immunity.

Dogs & Cats
- reported prevalence of 5 - 10% but this is likely an underestimate

Livestock
- prevalence of up to 100% in calves
- infections usually occur in calves less than 3 weeks of age and may occur in conjunction with other agents of neonatal diarrhea (rotavirus, coronavirus and *E. coli*)
- in calves the prepatent period is 4 - 6 days, patent period 7 - 14 days
- reported prevalence in Canadian survey found crypto in 17% of horses, 11% of pigs and 23% of sheep

Poultry
- common wherever birds are raised commercially i.e. disease of confinement
- 27% reported in broilers

Pathogenesis

Intestinal cryptosporidiosis
- infections occur in the enterocytes of the distal small intestine - ileum
- *C. baileyi* infects the cloaca and bursa of Fabricius in chickens and turkeys
- *C. parvum* infections are associated with villous atrophy, villous fusion, crypt hyperplasia and enterocyte sloughing
- increased epithelial turnover has been reported which results in an immature population of absorptive cells along the villus
- results in malabsorptive diarrhea
Abomasal cryptosporidiosis (C. andersoni)
- infects gastric peptic glands
- causes dilation of gland and may impact performance (?)

Respiratory cryptosporidiosis (C. baileyi)
- results from inhalation or aspiration of oocysts
- impairs mucociliary elevator in trachea and bronchi (few to no cilia found in heavy infections)
- accumulation of mucus and sloughed epithelial cells
- results in air sacculitis and pneumonia

Clinical signs
Intestinal cryptosporidiosis
- diarrhea (typically pasty-yellow in calves, occasionally with blood or mucus)
- diarrhea may be mild and intermittent or severe profuse and watery (may last up to two weeks)
- general signs of enteritis and dehydration (anorexia, abdominal cramping)
- low mortality unless other pathogens or stress factors (sever cold & poor management) are involved

Respiratory cryptosporidiosis
- sneezing, coughing and other signs of respiratory distress

Diagnosis
Histopathology
- various stages of Cryptosporidium can be seen in sections of ileum as round ‘blebs’ (parasitophorous vacuoles) on the microvilli border

Fecal exam
Sugar flotation
- C. parvum oocysts are spherical and very small 4 - 6 um
- C. andersoni oocysts are slightly larger 6 - 8 um
- both float just under the cover slip
- very difficult to see oocyst, requires experience as they are easily confused with the abundant yeast that are found in feces
- oocysts may take on a pink refractile appearance

Staining
- acid fast stains of fixed smears - oocysts are stain red, yeast stain green
- methylene blue and iodine also used with fecal wet mounts
- immunofluorescence antibody stain - specific and sensitive but limited to diagnostic and research labs
Treatment & Control
- no treatment currently available, although halofuginone & azithromycin have recently (2008) shown some efficacy in calves
- sanitation and good management practices are necessary to reduce exposure to oocysts
- supportive care (fluids, electrolytes, reduce stress)
- adequate nutrition is important but should be administered in small volumes so as not to overload the gut (i.e. absorptive capacity is reduced during cryptosporidiosis)
- cryptosporidiosis may be a chronic disease of the immunocompromised and can be life threatening (AIDS patients, immunosuppressive therapy)
- calves & lambs must receive adequate colostrum
Tissue-cyst forming Coccidia

**General Taxonomy - Apicomplexa**

all tissue-cyst forming coccidia have **heteroxenous** (two host) life cycles

asexual reproduction (with the exception of *Sarcocystis* spp.), sexual reproduction and oocyst formation take place in intestinal epithelium or sub-epithelium of a **carnivore definitive host**

asexual development also takes place in **extra-intestinal tissues of the intermediate host**

tissue cysts are formed in the extra-intestinal tissues of the **intermediate host**

**Causative agents and host ranges**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Definitive host</th>
<th>Intermediate host</th>
<th>Veterinary importance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>felids - domestic &amp; wild</td>
<td>all warm blooded vertebrates</td>
<td>very important</td>
</tr>
<tr>
<td><em>Neospora caninum</em></td>
<td>dogs, coyotes &amp; other canids?</td>
<td>cattle, sheep, dogs (all warm blooded vertebrates)</td>
<td>very important</td>
</tr>
<tr>
<td><em>Sarcocystis</em> spp.</td>
<td>dogs, cats, humans (species specific)</td>
<td>cattle, sheep, pigs, horses (species specific)</td>
<td>important</td>
</tr>
<tr>
<td><em>Frenkelia</em> spp.</td>
<td>raptors</td>
<td>small mammals</td>
<td>minimal</td>
</tr>
<tr>
<td><em>Besnoitia</em> spp.</td>
<td>cat</td>
<td>cattle</td>
<td>minimal</td>
</tr>
<tr>
<td><em>Hammondia</em> spp.</td>
<td>cat, dog</td>
<td>mice, herbivores</td>
<td>minimal</td>
</tr>
</tbody>
</table>
General Morphology

Intestinal stages
- are the same as for other coccidia (merozoites, microgamonts, macrogamonts)

Extra-Intestinal stages

Tachyzoites
- crescent shaped cells, approximately 6 um long
- proliferate rapidly by endodyogeny forming colonies (pseudocysts)

Bradyzoites
- approximately 7 um long
- multiply slowly within tissue cysts (or sarcocysts) by endodyogeny

Merozoites (Sarcocystis spp. ONLY)
- are extra-intestinal stage
- reproduce by endoploygeny (develop internally and later bud simultaneously at the surface)
- merozoites of Sarcocystis do not have rhoptries

Metrocytes (Sarcocystis spp. ONLY)
- merozoites transform into metrozoites which are precursors to bradyzoites
- metrozoites are ovoid and reproduce rapidly by endodyogeny

All extra-intestinal stages have a similar general coccidian zoite morphology

Tissue cysts
- contain bradyzoites which divide slowly resulting in increasing sizes of tissue cysts
- young tissue cysts may be 5 um long but can grow to 50 um
- Toxoplasma tissue cysts occur in many organs (liver, kidney, lung) but are most common in nervous tissue (brain, spinal cord, eye) and muscular tissue (skeletal and cardiac muscle)
- Neospora tissue cysts found in the CNS including the retina
- Toxoplasma tissue cysts have thin walls (< 1 um)
- Neospora tissue cysts have thick walls (>1 um and up to 4 um)

Sarcocysts (Sarcocystis spp. ONLY)
- commonly form in skeletal and cardiac musculature and in nervous tissue
- contain metrocytes and bradyzoites, often compartmentalized by septa
- sarcocyst walls contain minute undulations which become folded into villar protrusions as sarcocysts age
- sarcocysts may be microscopic or grossly visible (500 um - 1 cm, depending on species)
Toxoplasmosis

General Taxonomy - Apicomplexan parasite, Heteroxenous coccidia

Causative agent and Host Range (Zoonosis)

Toxoplasma gondii has a worldwide distribution

- **Definitive host** - cats & other felids.
- **Intermediate host** - indiscriminate, any warm blooded animal (mammal or bird)

Site of infection

- **Intestinal phase**, invades epithelial cells of the small intestine of the definitive host only (cats & other felids).
- **Extra-intestinal phase** within any cell or tissue (muscle, nervous tissue, liver, eye) of both intermediate and definitive hosts.

Morphology & Life cycle

**Infective stages** (transmission) - sporulated oocyst, tissue cyst (bradyzoites), tachyzoites.

**Definitive host** (cats & other felids)

- Ingests **tissue cysts** (bradyzoites) or sporulated oocysts which excyst within gut and release infective stages (bradyzoites and sporozoites) which invade intestinal epithelial cells of small intestine and undergo merogony (asexual reproduction to produce merozoites). Merozoites then penetrate other intestinal cells and develop into gametocytes (male or female). Fusion of male gametocyte with female gametocyte produces unsporulated oocyst which is extruded into lumen of intestine and evacuated with fecal material.

Sporogony

- maturation of the oocyst in the **environment** (1-5 days) to infective stage (**sporulated oocyst**). Viable in environment for 12-18 months.

Prepatent & patent period

- Tissue cysts (bradyzoites)
  - prepatent period 3-10 days, patent period 7-21 days - millions of oocysts
- Tachyzoites
  - prepatent period > 13 days, patent period 1-2 days - few oocysts
- Oocysts
  - prepatent period > 18 days, patent period 1-2 days - few oocysts
  - only 20% of cats fed oocysts will develop patent infection – therefore fecal-oral transmission in cats is not efficient
Intermediate hosts (& felids)

- Ingests **tissue cysts** *(bradyzoite)* or **sporulated oocysts** which excyst within gut & release (bradyzoites or sporozoites) that penetrate many cell types within lymph nodes, muscle, liver, lung etc.) & undergo rapid asexual replication to produce tachyzoites. Tachyzoites then re-infect more cells which results in a multiplication of the infection prior to disseminating systemically to invade all tissues. Tachyzoite multiplication continues until the host immune system responds which alters development towards the formation of tissue cysts containing slowly dividing bradyzoites which remain dormant for the life of the host.

- **Tachyzoites** cross placenta to fetus during primary maternal infections during pregnancy.

Transport hosts or vector— invertebrates & vertebrates eat feces with oocysts or carry on body (fur, hairs, scales) to new environment in which new intermediate host is exposed (e.g. people petting dog that rolled in or ate cat feces).

---

**Toxoplasma gondii** - Feline Life Cycle
Toxoplasma gondii - Complete Life Cycle

**Prevalence**
- varies based on location, behavioural & cultural practices. Typically higher prevalence in warm moist climates & low altitudes (i.e. support longer environmental survival times of oocysts) and where raw meat consumption is high (tissue cyst transmission).
Cats
- North America - 30% (4 - 100%)
- Spain - 45%
- Japan - 6%

Humans
- North America - 15 - 23%
- Veterinary staff (Ontario 2002) - 14.2%
- U.K. - 30%
- France - 80%
- New Zealand - 60%

Other animals - seroprevalences vary considerably by region
- wild rodents - 20 - 60%
- wild birds - 13.4 - 66.7%

Pathogenesis/Clinical Signs

Definitive host (cats & other felids)

Intestinal phase
- more common in young cats (infected transplacentally or via suckling) typically in kittens (< 3 months) or just weaned & now hunting/scavenging
- no pathology, no clinical signs (i.e. no diarrhea)
- some newborn kittens shed oocysts (typically < 3 weeks)

Extra-intestinal phase
- clinical or acquired toxoplasmosis (infected transplacentally or via suckling) typically only in kittens (< 3 months)
- onset of clinical signs ranges from slow to rapid
- clinical illness is common (varies with stage of gestation at time of infection & dependent on the degree & localization of the tissue injury e.g. affecting most commonly the liver, lungs, heart, pancreas, eyes & CNS)
- common clinical signs are anorexia, depression, lethargy, dyspnea due to pneumonia, hepatomegaly, encephalitis & chorioretinitis
- also intermittent fever, icterus due to hepatitis, vomiting, diarrhea, abdominal effusion, hyperesthesia on muscle palpation, stiffness of gait, shifting leg lameness, dermatitis, blindness & neurologic deficits...
- Death in cases with severe respiratory & CNS signs (rare)
- Abortion in queens is rare
Intermediate hosts (extra-intestinal phase only)
Two recognized types of disease:

1) Acquired Toxoplasmosis:
   Domestic animals
   Sheep & Goats - mild febrile response
   Cattle & Horses - clinical signs rare
   Swine - clinical signs rare
   Dogs < 1 year old (considered rare) – typically presents with respiratory, neuromuscular
   or gastrointestinal system involvement with fever, tonsillitis, dyspnea, diarrhea &
   vomiting, myositis & muscle atrophy, CNS signs (ataxia, tremors, chorioretinitis) being
   typical clinical signs. Clinical signs in all dogs is dependent on the degree & localization
   of the tissue injury

   Humans (Zoonosis)
   Healthy people:
   Most have no clinical signs
   Flu-like symptoms - transient headache, muscle & joint pain, fatigue, lymphadenopathy

   Immunodeficient people: (AIDS, Organ transplant recipients, Cancer / Chemotherapy)
   Severe disease
   Pneumonia, myocarditis, encephalitis, intracranial calcifications, chorioretinitis

   Reactivated latent infection: with onset of immunosuppression

2) Congenital Toxoplasmosis:
   - Primary maternal infection acquired during pregnancy for transmission to
     placenta/fetus
     Major concern in humans & sheep > goats
     Not recognized in cattle

   Sheep > Goats
   - major cause of abortion (74 % New Zealand, 54% Norway)
   - early pregnancy infection - fetal death & resorption, no abortion, no visible signs
   - mid pregnancy infection - fetal death & abortion about 3 weeks before term
   - late pregnancy infection - still born lambs or weak lambs which fail to thrive
   - primary lesions - focal inflammation & necrosis of the placental cotyledons
     (small white chalky - 2 mm diameter)
   - fetal lesions in heart, brain & liver (tachyzoites & tissue cysts)
   - subsequent pregnancy abortions are rare - Ewe immune

   Humans (Zoonosis)
   - abortion rare, premature birth common
- at birth – ‘classic triad’ of disease:
  - chorioretinitis, hydrocephalus, intracranial calcification
- later in life - Mental retardation, blindness, epilepsy
- reactivated latent infections

**Diagnosis:**

- **centrifugal fecal flotation** with Sheather Sugar (preferred?) or ZnSO₄
  - oocysts are found in cat feces rarely & prevalence of *T. gondii* oocyst in feces is low (< 1% of cats shed oocysts on any given day). Shedding is typically for 1-2 weeks therefore, fecal flotation is considered of little value? That being said, we find ‘oocysts’ in cases submitted to AVC...but confounding coccidian organisms *Hammondia & Besnoitia* spp. (not pathogenic) are morphologically indistinguishable on flotation. (can be distinguished via PCR, sporulation, infection experiments only).
  - best to consider all oocysts (10-12 µm) to be *T. gondii* until proven otherwise...

- **serology** of value in Humans > Sheep but less so in cats
  - generally, acute / recent infection - elevated IgM titres with low/normal IgG or fourfold or greater, increasing or decreasing IgG or other antibody titres (after treatment or recovery)
  - chronic / Past infection - low IgM titres & higher IgG

In general for assessing human health risk, serologic tests from healthy cats
(1) seronegative cat is not likely currently shedding but will shed if exposed. This cat is the greatest risk to human health.
(2) seropositive cat is probably not shedding oocysts & is less likely to shed oocysts if re-exposed or immunosuppressed. Still recommended that potential exposure to oocysts (infected intermediate hosts) be minimized.

- ELISA, PCR- *Toxoplasma* Reference Lab (human)

**Treatment:**

- **No evidence that any drug can totally clear the body of infection, so clinical illness can recur in all animals infected with *T. gondii***
  - Drug therapy has been used to reduce shedding oocysts in acutely infected cats e.g. clindamycin, toltrazuril (usually short course 1-12 days).
  - Also, cats & dogs with clinical toxoplasmosis e.g. clindamycin, trimethoprim-sulfonamide, azithromycin (minimum 4 weeks)

**Prevention & Control:**

**Cats**
- raise kittens indoors as once weaned, hunting begins and exposure risk is high
- all cats should be prevented from hunting & scavenging of both intermediate hosts (e.g. mice) as well as mechanical vectors (insects...sow bugs, earthworms, flies, cockroaches...)
- countries where pets are fed more raw meat products have higher % of cases of feline & canine toxoplasmosis
- do not feed raw or undercooked meat & unpasteurized dairy
- freezing or irradiation prior to cooking can kill tissue cysts without affecting meat quality
- feed cats only ‘processed’ commercially available food

**Domestic Animals - Livestock**
- prevent cats defecating in feed & water
- bio-security on swine farms to prevent access to rodents/birds (tissue cysts)
- control cat population on farm
- Toxovax® in sheep / goats - one dose = lifetime protection (New Zealand & UK)

**Humans**
- do not eat meat (uncooked or rare)
- eat or drink only pasteurized dairy products
- empty litter box daily (gloves, spouse)
- pregnant women or those considering starting a family follow the above recommendations but contact your health professional
Neosporosis

**General Taxonomy** - Apicomplexan, Heteroxenous coccidian

**Agent and host range**
*Neospora caninum* is an important cause of **fatal neuromuscular disease of dogs** & a **major cause of reproductive failure & abortion in cattle**.

*N. caninum* is the most commonly identified etiologic agent of **abortion in cattle**

- structural & biologic similarities with *Toxoplasma*

**Morphology**
- all stages are morphologically identical to *Toxoplasma gondii* with the exception of the tissue cysts
- *T. gondii* have thin walled tissue cysts and *N. caninum* have thick walled tissue cysts (?)

**Life Cycle & site of infection**
- Canids (dog & coyote) have been confirmed as **definitive hosts** but nothing is known regarding the frequency of oocyst shedding (likely similar to cats with *Toxoplasma*)
- oocyst transmission has been confirmed experimentally in mice, cattle and sheep
- dogs become infected after ingesting tissue cysts, but infectivity of tissue cysts to other canids (foxes, wolves) is unknown & their significance as definitive hosts has yet to be determined
- tachyzoites are found in many cells but **tissue cysts** (up to 100 µm) are found only in the CNS, peripheral nerves & retina
Neospora caninum life cycle
http://www.cvm.uiuc.edu/faculty/attach/mmcalli/neocycle.pdf

Epidemiology
Transmission
- percentage of dogs/canids shedding oocysts is unknown
- **congenital transmission** (predominant route) occurs in dogs, cattle, sheep, goats, pigs, horses (may be *N. hughesi* see later in notes) cats, mice, monkeys...
- **Persistent infection:** repeated congenital transmission may occur & can occur over several generations in dogs, cows...
- dogs may also acquire infections by ingestion of tissue cysts (carnivorousness)

Prevalence
- *Neospora* is a common cause of abortion in both beef & dairy cattle worldwide
- serologic surveys report up to 100% of cattle have been exposed in some herds
- In Maritime dairies - 73% of herds and 19% of cows found to be positive
- seroprevalence in dogs varies (~20% in North America) but some studies have found higher prevalence rates in dogs on dairy farms vs. dogs from urban areas
- most clinical cases of neosporosis occur in congenitally infected young purebred dogs
- In 2008 birds (chickens & pigeons) were also shown to be able to be infected. Another reservoir? Prevalence in birds is currently unknown.
- A 2008 retrospective study using immunofluorescence antibody and ELISA methods evaluated 3,232 serum samples from the general population and 518 serum samples from a high-risk group (exposure based on occupation) that showed no evidence of human exposure to *Neospora caninum* in England.

**Pathogenesis**
- presumed to be similar to toxoplasmosis
- destruction of host cells by tachyzoites resulting in focal areas of necrosis
- often focal encephalitis, encephalomyelitis, myositis & myocarditis characterized by necrosis & non-suppurative inflammation

**Clinical signs**

**Cattle**
- abortion is major clinical sign
  - cows of any age can abort between 3 months gestation and term
  - most abortions occur around 5 - 6 months
  - fetus may die *in utero*, be resorbed, mummified, autolysed or stillborn
- infected calves born alive may have neurological signs, be underweight, unable to rise, hind limbs and/or forelimbs may be flexed or hyperextended
- calves may also be born infected without clinical signs
- calves may also be born free of infection (cycle is broken)
- ataxia, decreased patellar reflexes and asymmetrical appearance of the eyes (exophthalmia) may also be observed

**Dogs**
- subclinical infection is common
- clinical cases predominantly have muscular abnormalities & neurologic deficits
  - gradual muscle atrophy & stiffness producing a rigid hyper-extended limbs & limb paresis which develops into full paralysis (with pelvic limbs > thoracic limbs)
  - paralysis of the jaw with difficulty swallowing
  - muscle flaccidity, muscle atrophy, heart failure
  - dermatitis has been reported & is usually severe
  - worst cases show cervical weakness, dysphagia, megaesophagus leading to death
- most severe infections are in dogs < 6 months with youngest pups showing clinical signs at 3-9 weeks of age
- littermates are often infected
- older dogs are less commonly affected but have multifocal CNS signs & polymyositis & less commonly myocarditis, dermatitis, pneumonia
- death can occur in any age dog
- Experimental infections have shown that early fetal death, mummification, resorption & weak born pups but not abortion (as seen in cattle)
- Purebred dogs overrepresented in studies...German shorthaired pointer, Labrador retriever, Boxer, Golden retriever, Basset hounds & Greyhounds...

**Diagnosis**

**Cattle**
- examination of the fetal tissues (brain, heart, liver, lungs) for presence of organisms or characteristic lesions (focal areas of necrosis and non-suppurative inflammation)
- histological examination of tissues requires immunohistochemical staining to differentiate *Neospora* tachyzoites from *Toxoplasma* tachyzoites
- detection of antibodies in fetal fluids and serum of cows

**Dogs**
- detection of specific antibodies in the serum or CSF
- suspect congenital neosporosis if several litter mates are showing clinical signs
- gross lesions include ulcerative dermatitis, necrosis of CNS and yellowish-white streaks in muscles
- immunohistochemistry
- fecal flotation – sporadic & inconsistent shedding, therefore finding oocysts in canine feces is considered rare. Oocysts are similar to confounding organisms e.g. *Hammondia* spp. & *Besnoitia* spp. (both not pathogenic) & also *T. gondii* (coprophagy)

**Treatment and Control**

**Cattle**
- protect feed and water sources from contamination
- do not let dogs eat fetal membranes, fetuses or dead calves
- cow that aborts once may abort again and therefore may have to be culled
- no treatment for neosporosis in livestock
- a conditional license has been granted for a vaccine but no efficacy data has been published

**Dogs/Canids**
- prevent canids from eating raw meat, fetal membranes, fetuses or dead calves by proper disposal
- Effective treatment for disease is limited but may be attempted with sulfonamides, pyremethamine and clindamycin (as used in *T. gondii*)
- clinical improvement in the presence of muscle contracture & rapidly advancing paralysis is not likely
- treat all littermates as soon as Dx is made in one pup
- older dogs and pups > 16 weeks respond better to treatment
- no therapy to treat bitch from transmitting to pups & may transmit to successive litters.
Sarcocystosis

**General Taxonomy** - Apicomplexan, Heteroxenous coccidian

- there are over 90 species recognized in various definitive (carnivore) and intermediate (herbivore) hosts

*Sarcocystis* require predator-prey or scavenger-carrion relationships to complete the life cycle

**Causative agent and Host Range**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Definitive host</th>
<th>Intermediate host</th>
<th>Pathogenicity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. cruzi</em></td>
<td>dog &amp; other canids</td>
<td>cattle &amp; bison</td>
<td>pathogenic</td>
<td>most pathogenic species in cattle</td>
</tr>
<tr>
<td><em>S. hirsuta</em></td>
<td>cat</td>
<td>cattle</td>
<td>mildly pathogenic</td>
<td></td>
</tr>
<tr>
<td><em>S. hominis</em></td>
<td>humans</td>
<td>cattle</td>
<td>mildly pathogenic</td>
<td>pathogenic for humans (found in Europe)</td>
</tr>
<tr>
<td><em>S. tenella</em></td>
<td>dog &amp; other canids</td>
<td>sheep</td>
<td>pathogenic</td>
<td>most pathogenic in sheep</td>
</tr>
<tr>
<td><em>S. arieticanis</em></td>
<td>dog</td>
<td>sheep</td>
<td>mildly pathogenic</td>
<td></td>
</tr>
<tr>
<td><em>S. gigantea</em></td>
<td>cat</td>
<td>sheep</td>
<td>mildly pathogenic</td>
<td>macroscopic sarcocysts can result in carcass condemnation</td>
</tr>
<tr>
<td><em>S. capacanis</em></td>
<td>dog &amp; other canids</td>
<td>goat</td>
<td>pathogenic</td>
<td>most pathogenic species in goats</td>
</tr>
<tr>
<td><em>S. miescheriana</em></td>
<td>dog &amp; other canids, raccoons</td>
<td>pigs</td>
<td>pathogenic</td>
<td></td>
</tr>
<tr>
<td><em>S. suihominis</em></td>
<td>humans</td>
<td>pigs</td>
<td>pathogenic</td>
<td>pathogenic to pigs &amp; humans</td>
</tr>
<tr>
<td><em>S. fayeri</em></td>
<td>dog</td>
<td>horse</td>
<td>mildly pathogenic</td>
<td></td>
</tr>
</tbody>
</table>
Morphology

Oocyst
- contain 2 sporocysts
- oocyst wall is thin and ruptures before passing in feces
- **sporocysts are passed in feces of definitive host**
  - sporocysts each contain 4 sporozoites
  - sporocysts are small ellipsoid and vary in size dependent on species (7 - 22 um) in length

Sarcocyst
- found primarily within the striated muscle cells of heart, tongue, esophagus, diaphragm and skeletal muscle
- microscopic to grossly visible (up to 1 cm in length) depending on species
- structure and thickness of sarcocyst wall varies with each species but is often striated and may possess villar protrusions
- internally, groups of metacytes or bradyzoites may be divided into compartments by septa originating from the sarcocyst wall

Life Cycle
- obligatory predator-prey or scavenger-carrion life cycle (heteroxenous = two host)
- asexual stages develop only in intermediate host
- sexual stages only in definitive host
  - bradyzoites are liberated from sarcocysts following ingestion by a carnivore and penetrate the mucosa of the small intestine
  - immediately transform into microgamonts and macrogamonts are found in goblet cells near tip of villi within 6 hours of infection
  - after fertilization of macrogamonts by microgamonts, a wall develops around the zygote forming an oocyst
- oocysts sporulate within the lamina propria and often rupture (due to their thin fragile wall) releasing sporocysts into the intestinal lumen which are then passed in the feces
- sporocysts ingested by the intermediate host release their sporozoites within the small intestine
- sporozoites enter vascular endothelial cells (primarily arteries of mesenteric lymph nodes) and undergo first-generation merogony (schizogony)
- merozoites are released and enter the endothelial cells in capillaries, small arteries or in other organs
- the number of generations of schizogony and type of host cells invaded vary with each species
- merozoites liberated from the terminal generation of schizogony enter bloodstream and invade myocytes (or occasionally neural cells)
- within the host cell, merozoites are found within a parasitophorous vacuole (the membrane of which becomes the sarcocyst wall)
- merozoites then become round to ovoid and transform into metrocytes
- metrocytes divide rapidly and after repeated divisions give rise to bradyzoites
- sarcocysts become infectious for the definitive host at approximately 75 days after infection (depending on species)
- immature sarcocysts (containing only metrocytes are not infectious)

\[ Sarcocystis \text{ cruzi} \]

Life cycle

Dubey, Fayer, Speer (1989) Sarcocystis of Animals & Man

**Epidemiology**
- ubiquitous in distribution
- nearly 100% of cattle and sheep are infected with Sarcocystis
- up to 90% of equids have been found infected
- prevalence of Sarcocystis is relatively low in pigs (usually less than 20%)
- in humans, sporocysts observed in feces of 2 - 10% of people in France, Germany and Poland
Pathogenesis
- most species are non-pathogenic
- pathogenic species are generally pathogenic to intermediate hosts only (humans are an obvious exception) and have a dog as the definitive host
- pathogenicity is dependent on the number of sporocysts ingested (>10,000 sporocysts needed to produce disease in cattle)
  - pathogenesis of disease primarily result of necrosis and hemorrhaging associated with meronts (schizonts) in the tissues
  - intense inflammation also associated with rupture of second generation meronts
  - gliosis and placental necrosis of the fetus occurs if infection during pregnancy

Clinical signs
Acute infection
- fever, anorexia, swollen lymph nodes, weight loss, muscle twitching
- decreased lactation
- mid to late term abortion or stillbirth (if infected during pregnancy and showing clinical signs)
- CNS signs and nasal discharge
- death if infected with high dose of sporocysts

Chronic infection
- inappetence, muscle atrophy and emaciation
- cessation of lactation
- icteric mucous membranes, hypersalivation
- hair loss on neck, rump and tip of tail (rat tail)
- lateral recumbency and CNS signs which eventually lead to death

Diagnosis
- serology may not be useful (due to high prevalence and cross reactivity)
- presumptive diagnosis based on clinical signs (anorexia, fever, excessive salivation, abortion, rat tail)
- observation of vascular meronts or sarcocysts at biopsy or in histological sections
- examination of fetal tissues following abortion (brain & placenta) for organisms, but parasite not consistently found
- must be differentiated from Toxoplasma and Neospora
- definitive host - observation of sporocysts in fecal flotations

Treatment and Control
- prevent carnivore fecal contamination of feed and water
- prevent dogs/cats from eating uncooked meat
- treatment with anticoccidial drugs (amprolium, salinomycin, halofuginone) may be attempted in the intermediate host
- treatment is not necessary for the definitive host
Equine Protozoal Myeloencephalitis (EPM)

General Taxonomy - Apicomplexa, Heteroxenous coccidian

Agent and host range

*Sarcocystis neurona* & *Neospora hughesi* are the causative agents for EPM
- most cases due to *S. neurona*

- EPM is considered one of the most important neurologic diseases in horses in some areas of North America
- the disease may be frustrating to owners and veterinarians to diagnose and treat

Life Cycle

*S. neurona*

- **definitive host** - the opossum which are nocturnal and a recognized scavenger that eats anything, including carrion
- large number of road-killed animals in some areas may contribute to the spread of EPM
- **intermediate hosts** - cat, skunk, raccoon and armadillo...?
- the **horse** is the aberrant intermediate host infected when it accidentally ingests feed contaminated with opossum feces containing sporocysts
- the horse tissue only contains merozoites and not sarcocysts (which are found in ‘true’ intermediate hosts)

*N. hughesi*

- little is known about the life cycle

Epidemiology

- serologic testing has revealed that between 22 - 65% of horses throughout the USA have been exposed to *S. neurona*
- less than 25% have been exposed to *N. hughesi*
- less than 1% of horses develop EPM
- risk factors
  - horses 1 -6 years old and > 13 years old
  - farms with previous cases of EPM
  - show and racing horses
  - close association with woods on premises, exposure to opossums
  - fall season and inadequate food security
- recently the disease was produced experimentally in horses following shipping
- indicates that stress plays an important role in expression of clinical signs
**Pathogenesis**
- invasion of white and grey matter at random sites along the entire CNS results in massive lesions that can destroy large portions of the brain and multiple segments along the CNS
- parasite may produce a non-suppurative meningitis

**Clinical signs**
- focal or multifocal signs of neurologic disease (dependent on where the parasite is located within the CNS)
- disease may progress gradually with slow changes in clinical signs or progress rapidly to severe signs. Individual horses therefore range in severity of disease presentation and progression
- signs include mild incoordination of either thoracic or pelvic limbs, muscle weakness, focal muscle atrophy and asymmetric ataxia
- cranial nerve deficits may indicate depression, head tilt and facial paralysis
- horses may exhibit abnormal airway function or seizures (rarely)
- rule outs: EPM can look like other equine neurologic diseases, including; Wobbler syndrome, the neurological form of herpes virus infection, rabies, West Nile virus or Eastern and Western equine encephalitis

**Diagnosis**
- antemortem Dx of is difficult, observation of clinical signs characteristic of neurological disease (therefore a thorough neurological exam must be performed)
- immunoblot analysis is used to detect specific antibodies to *S. neurona* in the serum and CSF
- postmortem diagnosis based on demonstration of protozoa in CNS lesions or presumptive diagnosis from characteristic lesions if no organisms are present

**Treatment and Control**
- combination of sulfadiazine and pyrimethamine administered for at least 6 months
- two approved treatments
  - ponazuril, an anticoccidial compound (oral paste - 5mg/kg daily for 28 days)
  - nitazoxanide, a benzamide active against protozoa, bacteria and helminths
- diclazuril and toltrazuril have also been used to treat EPM
- EPM vaccine - efficacy studies ‘still underway’…
- remove opossums humanely from pastures and woodlands
- remove carrion from property
- prevent exposure of horses to opossum feces (easier for stabled horses)
- prevent opossums from entering barns, hay and grain storage areas
- A 2008 paper suggested that protocols involving intermittent administration of ponazuril (every 7 days) may have application in prevention of EPM.
- high-pressure wash or scrub all infected areas
  - disinfectants alone are ineffective for cleaning contaminated areas