Parasitic Protozoa

One cell menaces
**Protozoa**

- Single Cell
- Asexual multiplication provides the mechanism for the development of pathogenic protozoan populations.
- Pathology is generally seen as the dysfunction of host tissue
  - direct destruction of the host cells (*Coccidiosis, Malaria, Piroplasms*)
  - indirect destruction of host cells (*Entamoeba*)
  - barrier to tissue function (*Giardia*)
  - excessive activation of host immune system (*Trypanosomes*)
  - excretion of toxins
Coccidian Pathology

Pathology:
Cellular trauma
Organ dysfunction
Protozoa

- **Life Cycle Strategies**
  - Direct Life cycle -- uses only a single host species (e.g. *Eimeria*)
  - Indirect Life cycle -- require 2 or more hosts (e.g. *Sarcocystis, Trypanosoma*)

- Asexual stages only - thus “clonal” (e.g. *Giardia, Entamoeba*)
- Alternation of sexual and asexual stages (all of the apicomplexans)

- **Continuous life cycle**
  - Without host immunity; organism would continue multiplying (e.g. *Plasmodium, Trypanosoma*)

- **Single direction life cycle**
  - Once the life cycle is completed then all organisms are gone (except in the case of re-infection) “all in – all out” (e.g. *Eimeria*).
Protozoa

- Life Cycle Strategies - continued
  - High Host specificity (e.g., *Sarcocystis, Eimeria, Toxoplasma* - sexual stages only)
  - Low Host Specificity (*Cryptosporidium, Toxoplasma* - asexual stages only).

- Infectious when passed (*Giardia*)
- Requires time in environment to become infectious (*Eimeria*)
ANIMALIA

NEMATODA
- Nematodes
- Oxyurids
- Ascarids
- Strongylids
- Trichinellids

PLATYHELMINTHES
- Flatworms
- Digeneans
- Monogeneans
- Tapeworms

CHORDATA
- Fishes
- Ticks
- Mites

ARTHROPODA
- Flies
- Fleas
- Other insects
- Crustaceans

ANNELIDA
- Annelids

MOLLUSCA
- Mollusks

ECHINODERMATA
- Echinoderms

PROTOZOA

PROTISTA
- Sporozoa
- Ciliophora
- Adenophorea
- Sarcomastigophora
- Mastigophora
- Amoeba
- Ciliates
- Hemoflagellates
- Mucoflagellates
- Hemosporidians
- Coccidians
Protozoan Groups

Historically, protozoa have been grouped by mode of motility.

<table>
<thead>
<tr>
<th>Flagellates</th>
<th>Apicomplexans</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hemoflagellates</strong></td>
<td><strong>Intestinal Apicomplexans</strong></td>
</tr>
<tr>
<td><em>Trypanosoma cruzi</em></td>
<td><em>Cryptosporidium parvum</em></td>
</tr>
<tr>
<td><em>Leishmania infantum</em></td>
<td><em>Eimeria spp.</em></td>
</tr>
<tr>
<td></td>
<td><em>Cystoisospora spp.</em></td>
</tr>
<tr>
<td><strong>Mucoflagellates</strong></td>
<td><strong>Systemic Apicomplexans</strong></td>
</tr>
<tr>
<td><em>Tritrichomonas foetus</em></td>
<td><em>Toxoplasma gondii</em></td>
</tr>
<tr>
<td><em>Giardia spp.</em></td>
<td><em>Neospora caninum</em></td>
</tr>
<tr>
<td><strong>Ciliates</strong></td>
<td><em>Sarcocystis cruzi, S. neurona</em></td>
</tr>
<tr>
<td><em>Balantidium coli</em></td>
<td><strong>Blood Apicomplexans</strong></td>
</tr>
<tr>
<td><em>Entameoba histolytica</em></td>
<td><em>Babesia canis</em></td>
</tr>
<tr>
<td><strong>Amoeba</strong></td>
<td><em>Babesia gibsoni</em></td>
</tr>
<tr>
<td></td>
<td><em>Cytauxzoon felis</em></td>
</tr>
</tbody>
</table>
Hemoflagellates

Trypanosoma cruzi

Leishmania infantum
## Protozoan Groups

Historically, protozoa have been grouped by mode of motility.

<table>
<thead>
<tr>
<th>Flagellates</th>
<th>Apicomplexans</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hemoflagellates</strong></td>
<td><strong>Intestinal Apicomplexans</strong></td>
</tr>
<tr>
<td><em>Trypanosoma cruzi</em></td>
<td><em>Cryptosporidium parvum</em></td>
</tr>
<tr>
<td><em>Leishmania infantum</em></td>
<td><em>Eimeria</em> spp.</td>
</tr>
<tr>
<td><strong>Mucoflagellates</strong></td>
<td><em>Cystoisospora</em> spp.</td>
</tr>
<tr>
<td><em>Tritrichomonas foetus</em></td>
<td></td>
</tr>
<tr>
<td><em>Giardia</em> spp.</td>
<td><strong>Systemic Apicomplexans</strong></td>
</tr>
<tr>
<td></td>
<td><em>Toxoplasma gondii</em></td>
</tr>
<tr>
<td></td>
<td><em>Neospora caninum</em></td>
</tr>
<tr>
<td></td>
<td><em>Sarcocystis cruzi, S. neurona</em></td>
</tr>
<tr>
<td><strong>Ciliates</strong></td>
<td><strong>Blood Apicomplexans</strong></td>
</tr>
<tr>
<td><em>Balantidium coli</em></td>
<td><em>Babesia canis</em></td>
</tr>
<tr>
<td><strong>Amoeba</strong></td>
<td><em>Babesia gibsoni</em></td>
</tr>
<tr>
<td><em>Entameoba histolytica</em></td>
<td><em>Cytauxzoon felis</em></td>
</tr>
</tbody>
</table>
General Morphology of Hemoflagellates

Diagram:
- Flagellum
- Undulating Membrane
- Anterior
- Nucleus
- Posterior
- Cell Body (Elongate Shape)
- Kinetoplast
Forms of Hemoflagellates

Epimastigote

- Flagellum
- Undulating Membrane
- Kinetoplast
- Anterior
- Cell Body (Elongate Shape)
- Posterior
- Nucleus

Promastigote

- Flagellum
- Kinetoplast
- Anterior
- Cell Body (Elongate Shape)
- Posterior
- Nucleus

Amastigote

- Flagellum
- Anterior
- Kinetoplast
- Cell Body (Oval Shape)
- Posterior
- Nucleus

Trypomastigote

- Flagellum
- Undulating Membrane
- Kinetoplast
- Anterior
- Cell Body (Elongate Shape)
- Posterior
- Nucleus
Trypanosoma cruzi

- American Trypanosomiasis
- Chagas Disease
Trypanosoma cruzi

Life Cycle

Intermediate (vector) Host: Triatomine (Reduviid) bugs
- Become infected by taking a blood meal from infected mammalian hosts
- Trypanosome development / multiplication in the bug gut.
- Infects a 2nd mammalian host via stercorarian transmission - trypanosome passed in feces contaminates mammalian via bug bite, skin scratch, oral or ocular mucosae.

Mammalian Host: Dog (Cardiac Muscle) (many others including humans)
- Trypomastigote invades host cells transforms to amastigote
- Amastigote multiplies and transforms to trypomastigote stage which burst out of host cell (= pathology)
- Trypomastigote in blood dispersal of trypanosome in host body and transfer to Triatomine bug.
Trypanosoma cruzi

Amastigotes in cardiac muscle

Trypomastigotes in the Blood

Stercorarian transmission:
Organisms in bug's feces enter wound or mucous membrane

Bug ingests organism with blood meal

Triatomid Intermediate Host
Trypanosoma cruzi

- Amasitgote
- Intracellular form
Trypanosoma cruzi

- Trypomastigote
- Blood form
*T. cruzi* - intermediate hosts -- Triatomid bugs

Stercorarian transmission
Transmission

- Vector-borne -- Triatomid bugs
- Transplacental
- Blood Transfusion
Geographic Distribution

- **Central & South America**
- **Canine cases rare in southern USA**
  - Texas, Arizona, New Mexico, California, Oklahoma
- **Various sylvatic hosts - seropositive**
  - raccoons, opossums, armadillos, etc.
  - Maryland, Virginia, South Carolina, Georgia
- **Concern for imported and travel dogs.**
Pathology

- **Cardiac Muscle Disease in Dogs**
- Repeat cycles of intracellular multiplication of parasites with destruction of host cells.
Clinical Disease – Acute Phase

- **Acute Phase (1st month)**
  - Inflammation at site of transmission
  - Lymphadenopathy and non-specific febrile disease
    - diarrhea, vomitus, anorexia, lethargy
  - Parasitemia (Trypomastigotes in blood)
Clinical Disease – Latent Phase

- Latent Phase (months to years post-infection)
  - usually asymptomatic
  - immunosuppression (disease, therapy, age) may cause relapse to acute phase
  - quiescent in tissues
Clinical Disease – Chronic Phase

- Chronic Phase (maybe years post-infection)
  - Gradual decline to death, usually about 2 years after diagnosis.
  - Chronic general weakness with progressive heart failure
  - Active multiplication & destruction of host tissues
  - Also important is the autoimmune destruction of host tissues
Pathology
Pathology

Chicken - cardiac muscle

Infected

Normal

Fig. 11: Pathology resembling human Chagas heart disease in a 6 month old F1 progeny of a kDNA-positive hen. A: cardiomegaly and dilation of the heart chambers; B: negative control heart of a 6 month-old chicken twice smaller than the sick (A) chicken heart; C: severe, destructive myocarditis and extensive target cell lysis carried out by the immune effector's mononuclear cells. H-E 200X; D: control section of a healthy chicken heart. H-E 200X.

Diagnosis

1. Parasite detection
   - Blood smear -- trypomastigotes
     - High numbers in acute phase, fewer in chronic phase
   - Cardiac biopsy / histology -- amastigotes in pseudocysts
   - Xenodiagnosis

2. Immunodiagnostics
   - Immunofluorescence, ELISA (may cross-react with *Leishmania*)

3. Molecular tests
   - PCR
Trypanosoma cruzi

Trypomastigotes in Blood smear
Trypanosoma cruzi

Amastigotes in Cardiac Biopsy or Post-mortem

2. Benznidazole, Nifurtimox require CDC permission
Control

- Vector control
  - Dx Triatomids & their habitat
- Breeding control
  - v/s Transplacental Transmission
- Screen Blood donors
  - v/s Transfusion Transmission
Trypanosoma cruzi

Thatched huts provide diurnal hiding habitat for Triatomid vectors for human Chagas disease in Brazil.
Human Chagas Disease

- **Endemic areas:**
  - Mexico, Central America, South America
- **Triatomids thrive in poor housing conditions**
  - Mud Walls, Thatched Roofs
- **Dogs are important reservoirs for human infections**
Take Home

- Rare Canine disease
  - Destruction of cardiac muscle
  - Acute Phase - non-specific febrile dz
  - Latent phase - asymptomatic / quiescent
  - Chronic phase - heart failure with myocarditis & arrhythmias
Leishmania infantum

- = *Leishmania chagasi*
- Viscerocutaneous Leishmaniasis
**Leishmania infantum**

**Life Cycle**

**Intermediate (vector) Host: Sandfly**

*(Phlebotomus spp. [old world]; Lutzomyia spp. [new world]*)

- Become infected by taking a blood meal from infected mammalian hosts
- Leishmania development / multiplication in the fly gut.
- Infects a 2\textsuperscript{nd} mammalian host via salivarian transmission - leishmania injected into host when fly takes blood meal.

**Mammalian Host: Dog** *(reticuloendothelial system = macrophage system)*

- Leishmania phagocytized by macrophages
- Amastigote form multiplies and burst out of host macrophage
- Other macrophages phagocytize amastogotes and transport parasite throughout body. Repeat
- Infected macrophages ingested by Sandfly
Leishmania infantum

Amastigotes in Macrophages in the Spleen

Salivarian transmission: Organisms injected into host when Sandfly feeds

Sandfly Intermediate Host

Sandfly ingests organism with blood meal

Amastigotes in Macrophages in the Blood
Sandfly Vectors
Transmission

- Vector-borne (Sandflies)
- Transplacental
- Blood Transfusion
- ? Other ? – American Foxhounds
  (Autochthonous - disease acquired in same place (ex. same colony, kennel))
  - Direct transmission (contact, licking, bites, fighting)
  - Perinatal (gestation, birth, and/or nursing)

(autochthonous = aw-tok-tha-nus)
Classical *Leishmania infantum* life cycle

4. Amastigotes transform to promastigotes in the sand fly midgut and migrate to proboscis

1. Promastigotes injected into skin of naive mammal and taken up by macrophages

Sand fly stage

3. Sand fly takes a bloodmeal from infected mammalian host and ingests infected macrophages

Mammalian stage

2. Promastigotes transform into amastigotes inside macrophages, multiply and spread to various tissues

Proposed *Leishmania infantum* life cycle in foxhounds in the United States

1. Cells infected with *Leishmania infantum* passed to pups during gestation, birth, and/or nursing
   *Vertical transmission*

2. Direct contact (such as fighting) with blood from infected foxhound to uninfected foxhound
   *Horizontal transmission*
Pedigree of American foxhounds with *Leishmania infantum* infection as presented in this report. Females are denoted by circles, males by squares. Black indicates CDC confirmed seropositive for *L. infantum*. Half black indicates *L. infantum* q-PCR positive but to date seronegative. Grey indicates status unknown. Dogs 3 and 4 are presented in this report. Dog 8 was euthanized due to poor body condition prior to q-PCR testing. Dog 9 was lost to follow-up.

Geographic Distribution

- >70 countries: Southern Europe, Africa, Asia, Caribbean, Central & South America
  - Concern for imported and travel dogs.

- Sporadic in US Foxhound colonies.
  - (Oklahoma, Kansas, NY, Ohio, NC)
Multi-system Disease
- In dogs, the disease most often manifests as skin and ocular pathology

Immune-mediated pathology
- From immune-control of infection w/o symptoms to autoimmune pathology

Death ultimately caused by Renal Failure
(Immuno-complex glomerulonephritis)
Clinical Disease in Canines

- Various issues - vary by case
  - Incubation period from 3 months to several years

- Client complaint
  - Skin lesions, ocular abnormalities, epistaxis (nose bleed), weight loss, lethargy

- Clinical findings
  - Dermal lesions, lymphadenopathy, fever, ocular dz (uveitis), splenomegaly, signs of liver dz (hyperglobulinemia, hypoalbuminemia), signs of anemia (non-regenerative anemia), signs of kidney dz (proteinuria)
Figure 1. Major clinical signs associated with Canine Leishmaniasis.

A: alopecia on the muzzle
B: periocular dermatitis with keratoconjunctivitis and hyperkeratosis;
C: hyperkeratosis of the nasal mucosa;
D: generalized non-pruritic exfoliative dermatitis;
E: ulcerated lesion in the ear;
F: crust with vascular injury on the tip of the ear;
G: lymphadenomegaly of the popliteal lymph node;
H: cachexia (wasting syndrome); (ka-kex-ea)
I: onychogryphosis (hypertrophy of claws). (on-i-ko-gri-fo-sis)

Photos of animals infected by *L. infantum* belong to archives from Laboratory of Pathology and Biointervention (LPBI - CPqGM).

FYI only

Figure 5: Different patterns of cutaneous lesions in Canine Leishmaniasis.

A) Exfoliative periocular alopecia and blepharitis;
B) Ulcerative nasal mucocutaneous lesions;
C) Papular dermatitis in the inguinal region;
D) Nodular crateriform lesions bordering the muzzle;
E) Ulcerative erythematous lesions on the plantar surface of the paw and between pads;
F) Onychogryphosis. (on-i-ko-gri-fo-sis)

https://parasitesandvectors.biomedcentral.com/articles/10.1186/1756-3305-4-86
A) Epistaxis (nose bleed);
B) Bilateral uveitis and corneal opacity;
C) Purulent conjunctivitis and blepharitis;
D) Exfoliative alopecia in the rear leg and popliteal lymphadenomegaly;
E) Marked cachexia (wasting) and generalized exfoliative alopecia.

https://parasitesandvectors.biomedcentral.com/articles/10.1186/1756-3305-4-86
Diagnosis

- Combination of findings
  - Clinical Findings (physical exam, CBC, biochemical profile, urinalysis)
  - Serology, Immunofluorescence, ELISA
    - (may cross-react with *T. cruzi*)
  - PCR
  - Amastigotes in cytology specimens
    - Lymph nodes, skin, spleen, etc.
    - unreliable due to low numbers of amastigotes
Interpretation of cytology requires time and expertise for the detection of Leishmania amastigotes when parasites are in low numbers and freed from the cells. Note the nucleus (N) and the kinetoplast (K) of extracellular amastigotes (arrows) in a fine needle aspirate of a reactive lymph node from a dog with clinical leishmaniosis (x100, Diff-quick stain);
B) High numbers of intracellular and extracellular Leishmania amastigotes in a fine needle aspirate of a reactive lymph node from a dog with clinical leishmaniosis (x100, modified Giemsa stain).

https://parasitesandvectors.biomedcentral.com/articles/10.1186/1756-3305-4-86
Treatment

Antimonial drugs and Purine analogues
- Some only available through the CDC
- Temporary clinical improvement, but none can eradicate infection
Control

- **Vector Control**
  - Insect repellants (sandflies)
  - collars, spot-on’s, etc.

- **Breeding control**
  - v/s Transplacental Transmission

- **Screen Blood donors**
  - v/s Transfusion Transmission

- **Vaccines have been developed in Brazil & Europe**
  - (Leishmune, Leish-Tec, CaniLeish)
Zoonosis

Dogs are very important reservoir for human infections

Visceral Leishmaniasis (Kala-azar) (FYI)
- Human - *L. donovani, L. infantum = L. chagasi*

Viscerocutaneous Leishmaniasis
- Dog - *L. infantum = L. chagasi*

Mucocutaneous Leishmaniasis (Espundia) (FYI)
- Human - *L. brasiliensis*

Cutaneous Leishmaniasis (Oriental Sore) (FYI)
- Human - *L. tropica, L. mexicana*
- Cat - *L. mexicana*
A dog presents with generalized exfoliative dermatitis, alopecia.

Physical exam: popliteal lymphadenomegaly

History: 8 months ago had been on a trip to the Mediterranean with its owner.

OR A Foxhound from Kentucky
In-Class Discussion

A dog from Michigan presents with increasing exercise intolerance.

History: Had moved from southern Texas a year ago.

Labs:
  • HW Antigen test - negative
  • HW MF Knott’s test - negative
What you're expected to know

See Review Table:
Systemic Protozoa
Posted on-line at Parasitology Website:
https://parasitology.cvm.ncsu.edu/vmp930/lecture.html

The information in the review tables is basic information that you should know. You should also be able to use that information via critical thinking to answer more complex case-based questions.