

## LECTURE #2: INTRO. TO PROTOZOA; HEMOFLAGELLATES: *TRYPANOSOMA*, *LEISHMANIA*

### I. Protozoa

- Single-celled organisms
- A single protozoan organism is too small to cause pathology, thus 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
- Various life cycle strategies
  - Direct life cycles using 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 (all of the apicomplexans)
  
  - Continuous life cycle
    - without host immunity; organism would continue multiplying (*Plasmodium*)
  - 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*).
  
  - High Host specificity (e.g., *Sarcocystis*, *Eimeria*)
  - Low Host Specificity (*Toxoplasma* – asexual stages only, *Cryptosporidium*).
  
  - Infectious when passed (*Giardia*)
  - Requires time in environment to become infectious (*Eimeria*)

Pathological manifestations may be limited to a single or few stages, may be dependent on host species, age, prior exposure, condition, and other factors. Infecting dose may or may not affect the clinical outcome, dependent upon parasite species, host, etc.

Control depends on knowledge of the complete life cycle of each parasite and its' host range. Diagnosis requires knowledge of diagnostic structures (e.g., oocysts, cysts) or lesions.

Effective therapy, when available, may not target all parasite stages. Consider how this might affect the response to therapy. Additional factors to consider are wildlife and/or non-target hosts, zoonotic potential and client psychology.

## II. Hemoflagellates

### General Morphology of Hemoflagellates

#### A. Cell body

- Special organelles
  - nucleus
  - kinetoplast (DNA & mitochondria associated with the flagellum)
  - flagellum (single; extends from the kinetoplast & emerges from anterior end)
  - undulating membrane (thin extension of cell membrane attached to the flagellum)

#### B. Forms

1. amastigote
  - spherical, kinetoplast close to nucleus, no flagellum or undulating membrane
  - mammalian intracellular form; divides
2. promastigote
  - Spindle-shape, kinetoplast at anterior end, long flagellum
  - Arthropod gut form; divides
3. epimastigote
  - Spindle-shape, kinetoplast near nucleus, long flagellum, short undulating membrane
  - Arthropod gut form; divides
4. trypomastigote
  - Spindle-shape, kinetoplast at posterior end, long flagellum, undulating membrane
  - Mammalian blood form; some species divide, some don't
5. metacyclic trypomastigote
  - Stumpy, kinetoplast at posterior end, short flagellum, undulating membrane
  - Mammalian and Arthropod infective form

### *Trypanosoma cruzi*

#### American Trypanosomiasis (Chagas Disease)

#### A. Morphology

- Trypomastigote in blood, doesn't divide.
- Amastigote in tissues, divides
- Epimastigote in bug gut, divides
- Metacyclic Trypomastigote – infective form.

#### B. Life Cycle

1. Mammalian hosts
  - Dogs (cardiac muscle) & humans
  - Opossums, armadillos, raccoons, wood rats, etc. (>100 mammal species)
    - Metacyclic Trypomastigote – infective form – rubbed into bug bite, skin scratch, oral or ocular mucosae. Or via ingestion of bug
    - Metacyclic Trypomastigotes invade local cells and macrophages, become amastigotes that multiply via binary division
    - amastigotes turn into trypomastigotes, which burst from host cells
    - trypomastigotes travel to other cells in the body via blood stream and invade host cells- usually cardiac muscles in dogs – turn into amastigotes and repeat multiplication and distribution cycle.
    - some trypomastigotes in the blood change into metacyclic trypomastigotes, which may be ingested by vector.
2. Arthropod hosts (vectors)
  - Triatomine (Reduviid) Bugs (*Triatoma*, *Rhodnius*, *Panstrongylus*) [kissing bug, assassin bug]
  - Stercorarian transmission (Infective metacyclic trypomastigotes in bug feces)
    - metacyclic trypomastigotes ingested by vector during blood meal
    - in midgut, trypomastigotes transform to epimastigotes, which multiply via binary division
    - in hindgut, epimastigotes transform to metacyclic trypomastigotes which are passed in the bug feces when the bug feeds on the mammalian host. (stercorarian transmission)
3. Transmission
  - Vector borne
  - Transplacental
  - Blood transfusion

C. Geographic Distribution

1. Central & South America, rarely in southern USA
2. Concern for imported and travel dogs.

D. Pathogenesis

1. Intracellular multiplication of parasite with eventual destruction of host cells
2. Especially cardiac muscle in dogs (some in smooth & skeletal muscles and CNS tissue)

E. Clinical Disease

1. Acute Phase (1<sup>st</sup> month)
  - Inflammation at site of transmission
  - lymphadenopathy and non-specific febrile disease
    - diarrhea, vomitus, anorexia, lethargy
  - rare cases quickly develop to hepatosplenomegaly and acute myocarditis
  - Parasitemia (Trypomastigotes in blood)
2. Latent Phase (months to years post-infection)
  - usually asymptomatic
  - immunosuppression (disease, therapy, age) may cause relapse to acute phase
  - quiescent in tissues
3. Chronic Phase (maybe years post-infection)
  - Gradual decline to death, usually about 2 years after diagnosis.
  - Chronic general weakness with progressive heart failure
  - Right side congestive heart failure w/ myocarditis and arrhythmias leading to bilateral dilation and eventual death.
  - Active multiplication in the tissues

F. Diagnosis

1. Parasite detection
  - Blood smear -- trypomastigotes during acute phase
  - Cardiac biopsy / histology -- amastigotes in pseudocysts
  - Xenodiagnosis
2. Immunodiagnosics
  - Immunofluorescence, ELISA (may cross-react with *Leishmania*)
3. Molecular
  - PCR

G. Treatment

1. Mainly symptomatic treatments to manage arrhythmias and heart failure.
2. Benznidazole, Nifurtimox – require CDC permission

H. Control

- Vector control
- Breeding Control
- Screen Blood donors

I. Zoonosis --- Dogs are important reservoirs for human infections

=====

## *Leishmania infantum* / *Leishmania chagasi*

### Viscerocutaneous Leishmaniasis

#### A. Morphology

- Amastigote in macrophages & tissues, divides
- Promastigote in sandfly gut, divides, infective form

#### B. Life Cycle

##### 1. Mammalian hosts

- Dogs (spleen, liver, bone marrow, lymph nodes, skin) & humans
  - Promastigote – infective form – injected when sandfly takes a meal. Phagocytized by macrophages & transforms into amastigote.
  - Amastigotes multiply via binary division, bursts from cell & is phagocytized by macrophages and is disseminated throughout the body via macrophages.
  - Some amastigotes within macrophages are ingested by another sandfly.

##### 2. Arthropod hosts (vectors)

- Sandfly (*Phlebotomus spp.* [old world]; *Lutzomyia spp.* [new world])
- Salivarian transmission (Infective promastigotes from fly mouthparts)
  - amastigotes ingested by vector during blood meal
  - in midgut, amastigotes transform to promastigotes, which multiply via binary division
  - promastigotes migrate to mouthparts of sandfly and are injected in new when the sandfly feeds on the mammalian host. (salivarian transmission)

##### 3. Transmission

- Vector-borne, Transplacental, Blood Transfusion
- ? Direct Transmission ? (contact, bites, fighting) (Foxhounds?)

##### 4. Incubation – maybe 3 months to 7 years

#### C. Geographic Distribution

- >70 countries Southern Europe, Africa, Asia, Central & South America
- Sporadic in US (Oklahoma, Kansas, NY, Ohio, NC)
- Concern for imported and travel dogs.

#### D. Pathogenesis

- Multisystemic Disease
- Immune-mediated pathology (asymptomatic immune-control of infection to autoimmunity)
- Death ultimately caused by Renal Failure (**Immuno-complex glomerulonephritis**)

#### E. Clinical Disease

- Various issues - vary by case
- 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)

#### F. Diagnosis

##### 1. 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

#### G. Treatment

1. Antimonial drugs and Purine analogues
2. Some only available through the CDC
3. Temporary clinical improvement, but none can eradicate infection

H. Control

- Insect repellants – collars, spot-on's, etc.
- Vaccines have been developed in Brazil & Europe (Leishmune, Leish-Tec, CaniLeish)

I. Zoonosis --- Dogs are very important reservoir for human infections

J. Other:

- Visceral Leishmaniasis (Kala-azar)
  - Human - *L. donovani*, *L. infantum* = *L. chagasi*
- Viscerocutaneous Leishmaniasis
  - Dog - *L. infantum* = *L. chagasi*
- Mucocutaneous Leishmaniasis
  - Human – *L. brasiliensis*
- Cutaneous Leishmaniasis
  - Human – *L. tropica*, *L. mexicana*
  - Cat – *L. Mexicana*

=====