Lecture #2: Intro. to Protozoa; Hemoflagellates: Leishmania, Trypanosoma

I. Protozoa

- Eukaryote, single-celled organisms
- Most protozoa are not pathogenic nor require a host
- A single protozoan organism is too small to cause pathology, thus asexual multiplication provides the mechanism for pathogenic protozoan populations.
- Taxonomy is ever changing
  - Eukaryotic tree converging on a model composed of a few large, hypothetical supergroup
  - Supergroups with pathogenic protozoa we will discuss include Alveolates and Excavates
- 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)
  - 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, sexual stages of Toxoplasma)
  - Low Host Specificity (Cryptosporidium, asexual stages of Toxoplasma).
  - Infectious when passed (Giardia)
  - Requires time in environment to become infectious (Eimeria)
- Pathology = how the pathogen causes disease in the host; it is generally seen as the dysfunction of host tissue
  - direct destruction of the host cells (Coccidia, Piroplasms (Babesia, Cyttauxzoon, Malaria),)
  - indirect destruction of host cells (T. foetus, Giardia)
  - changes in host immune system (Babesia, Leishmania, Trypanosoma)
  - excretion of toxins (most all parasitic protozoa)

Pathological manifestations may be limited to a single or few stages in the parasite life cycle. It may be dependent on host factors (species, age, prior exposure, physical condition, and others). 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. Serological and molecular methods available for some protozoa pathogens.

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 (also called Trypanomastiads or Kinetoplastida)

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 (depend on the hemoflagellate, host and life stage)
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

Leishmania infantum / Leishmania chagasi
Viscerocutaneous Leishmaniasis

A. Morphology
- Amastigote in mammalian host, macrophages & tissues, divides
- Promastigote in sandfly gut, divides, infective form

B. Life Cycle
1. 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 infective promastigotes, which multiply via binary division
     - promastigotes migrate to mouthparts of sandfly and are injected when the sandfly feeds on the mammalian host (salivarian transmission)
2. Mammalian hosts (incidental or reservoir)
   - 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.
3. Transmission
   - Vector-borne, Transplacental, Blood Transfusion
   - ? Direct Transmission ? (contact, bites, fighting) (Foxhounds?)
   - Foxhounds in United States
     - a. 1980-90 infected foxhound from Europe brought into US for breeding
     - b. Many generations of vertical transmission; ~10% of all American Foxhounds infected with L. infantum. Many are asymptomatic
4. Incubation – maybe 3 months to 7 years

C. Geographic Distribution
- Many different species of Leishmania, world-wide. L. infantum/chagasi and L. mexicana most common sp. identified in US (L. infantum from import and L. mexicana in Southwest US)
- >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
• Tissue damage by sandfly recruits phagocytic immune cells
• Parasite is phagocytosed by macrophages and other immune cells (neutrophils, monocytes, dendritic)
• If a strong cell-mediated immune response (activation of T-helper 1 cells) occurs, this leads to enhanced killing by macrophages and mild disease or immune clearance
• Symptoms of immune-mediated pathology (asymptomatic immune-control of infection to autoimmunity to inflammatory cutaneous lesions, to immune-complex deposition in tissues)
• If not cleared, illness usually multisystemic
• Death ultimately caused by renal failure (Immuno-complex glomerulonephritis)

E. Clinical Disease
• A variety of factors dictate the wide range of clinical disease
  o Immune response (cell-mediated vs. humoral)
  o Presence of co-infections
  o Pathogen load of infection
  o Species of Leishmania
• Client complaint
  o Skin lesions, ocular abnormalities, epistaxis (nose bleed), weight loss, lethargy
• Clinical findings
  o 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) ask about travel history
     • Serology, Immunofluorescence, ELISA (may cross-react with T. cruzi)
     • PCR (blood, bone marrow, spleen, conjunctiva, cutaneous lesions, lymph node)
     • Amastigotes in cytology specimens (Lymph nodes, skin, spleen, etc.)
       o unreliable due to low numbers of amastigotes

G. Treatment
  1. Antimonial drugs and Purine analogues
  2. In the US, some drugs not available (meglumine antimoniate) or are expensive (miltefosine)
  3. Clinical improvement, but none can eradicate infection

H. Control
• Insect repellants (vector control) – collars with imidacloprid/flumethrin (Seresto), spot-ons, etc.
• Vaccines have been developed in Brazil & Europe (Leishmune, Leish-Tec, CaniLeish)
• Domperidone approved for use in Europe; immune-stimulatory drug (stimulates cell-mediated immune response); high doses may cause cardiotoxicity

I. Zoonosis --- Dogs are very important reservoir for human infections.
• Humans
  o Visceral Leishmaniasis - L. donovani, L. infantum = L. chagasi
  o Mucocutaneous Leishmaniasis – L. braziliensis
  o Cutaneous Leishmaniasis – L. tropica, L. mexicana, etc
• Cats
  o Mucocutaneous Leishmaniasis – L. infantum
  o Cutaneous – L. mexicana, etc.
  o Few feline Leishmaniasis cases, even in endemic regions

Trypanosoma cruzi
American Trypanosomiasis (Chagas Disease)
A. Morphology
• Epimastigote in bug (vector) gut, divides, transforms into metacycle trypomastigote
• Metacyclic Trypomastigote – infective form, excreted from vector
• Amastigote intracellular, in tissue, divides by binary fission, transforms back into trypomastigote
• Trypomastigote in blood stream, inside erythrocytes, doesn’t divide.

B. Life Cycle

1. Mammalian hosts
   • Dogs (cardiac muscle) & humans
   • Opossums, armadillos, raccoons, wood rats, etc. (>100 mammal species) - reservoirs
     o Metacyclic Trypomastigote – infective form – stercorarian transmission, rubbed into bug bite, skin scratch, oral or ocular mucosae (via ingestion of bug)
     o Metacyclic Trypomastigotes invade local cells and macrophages, become amastigotes that multiply via binary division
     o Amastigotes turn into trypomastigotes, which burst from host cells
     o 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.
     o Trypomastigotes in the blood may be ingested by vector.

2. Arthropod hosts (vectors)
   • Triatomin (Reduviid) Bugs (*Triatoma, Rhodnius, Panstrongylus*) [kissing bug, assassin bug]
   • Stercorarian transmission (Infective metacyclic trypomastigotes in bug feces)
     o trypomastigotes ingested by vector during blood meal
     o in midgut, trypomastigotes transform to epimastigotes, which multiply via binary division
     o 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
   • Mucous membranes
     o Ingestion of infected bugs (probably how most dogs and small mammals become infected)

C. Geographic Distribution

1. Central & South America, increasingly detected southern USA
2. FYI: in TX seroprevalence ranges from 6-18% in shelter dogs and working dogs
3. Concern for imported and travel dogs

D. Pathogenesis

1. Intracellular multiplication of parasite and autoimmune destruction of host tissue 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 (1st month)
   • Inflammation at site of transmission
   • lymphadenopathy and non-specific febrile disease
     o 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.
   • Infected dogs more likely to have ventricular arrhythmias and ECG abnormalities
   • 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 and autoimmune destruction of host tissue

F. Diagnosis

1. Parasite detection
   • Blood smear -- trypomastigotes during acute phase
- Cardiac biopsy / histology -- amastigotes in pseudocysts
- Xenodiagnosis
- PCR detect parasite DNA

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

3. Molecular
   - PCR

G. Treatment
   1. Mainly symptomatic treatments to manage arrhythmias and heart failure.
   2. Human-approved drugs = Benznidazole, Nifurtimox – require CDC permission?

H. Control
   - Vector control
   - Breeding Control
   - Screen Blood donors

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

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