Blood and Tissue Protozoa
Blood and Tissue Protozoa

• Flagelates

*Trypanosoma brucei gambiense* – sleeping sickness (chr, Af)
*Trypanosoma brucei rhodesiense* – sleeping sickness (ac, Af)

*Trypanosoma cruzi* Chagas – Chagas’ disease (South Am)

*Leishmania* spp. – leishmaniasis (world wide)

• Apikomplexa (Sporozoa)

*Plasmodium* spp. – malaria (tropical and subtropical)
*Babesia* spp. – babesiosis (world wide)
*Toxoplasma gondii* – toxoplasmosis (world wide)
Morphological Forms

- Amastigote
- Promastigote
- Epimastigote
- Trypomastigote
T. brucei gambiense
T. brucei rhodesiense
African trypanosomiasis - epidemiology

- They are present in a variety of mammals, both wild and domestic.
- West African disease is caused by *T. brucei gambiense*. It is present in tropical rain forests and rural regions in Central and West Africa, and disease is greatest in the dry seasons.
- East African disease is caused mostly by *T. brucei rhodesiense*. Because the reservoir of disease is mostly in wild game such as antelope, infection in humans is limited.
African tripanosomiasis

- They are transmitted by the blood-sucking tsetse fly
- Trypomastigotes are transmitted from the salivary glands of the fly to the human during a blood meal
- The organism can multiply within the bloodstream and evade the host defenses by undergoing antigenic variation
- Congenital and blood transfusion infections are rare
African tripanosomiasis

- The number of parasites in the circulation dramatically rises and falls.
- Generally, fever and other clinical symptoms are associated with the peaks in parasitemia.
- Further examination of parasites obtained from successive peaks reveals that they are antigenically distinct, or exhibit variant antigenic types (VAT).
Progression of African Trypanosomiasis

Blood → Lymphatics → CNS → DEATH

± chancre
- intermittent fever
- headache

- continued febrile episodes
- lymphadenopathy
- deteriorating health

- anorexia
- minor neurological symptoms
- apathy, lassitude

- convulsions
- coma
- concurrent infections

- severe sleep disturbances
- severe neurological symptoms

months to years

months to years

weeks to months

$T_g$

$T_r$
Clinical Manifestations

Trypanosomal chancre

Winterbottom’s sign

Invasion of the CNS by crossing the blood-brain barrier

If untreated, the CNS stage of the disease will almost always progress to include convulsions or coma followed by death in both *T. gambiense* and *T. rhodesiense* infections.
African trypanosomiasis

Glossina sp. (tsetse fly)

Catcher
Trypanosoma gambiense and rhodensiense

• In African trypanosomiasis, only trypomastigotes are found in infected humans

Specimens of choice:
• Serous fluid aspirated from the chancre (early infection)
• Blood (best during febrile episodes)
• Aspirates of lymph glands
• CSF
African trypanosomiasis – diagnosis

Winterbottoms’ sign

Lumbal puncture
**Trypanosoma gambiense and rhodensiense**

Methods:

- Direct microscopic examination – wet and Giemsa stained preparations (thin and tick blood smears)
- Microhaematocrit tube method – examination of the plasma just above the buffy coat
- Culture – usually unsatisfactory
- Animal inoculation – intraperitoneal in mice and rats
- Immunological testing – detection of specific Ab in blood and CSF (IFAT and ELISA)
- Rapid tests – CATT (card direct agglutination trypanosomiasis test)
Trypanosoma gambiense and rhodensiense

Blood smear – Giemsa stain

Blood smear – EM
Trypanosoma cruzi Chagas
Epidemiology

*T. cruzi* is present in South America, Central America, and Mexico.

- It is present in many species of wild and domestic mammals.
- Most cases in humans occur during childhood, and the disease is much more common in areas of poverty and in rural areas.
- An estimated 16 to 18 million people are currently infected with *T. cruzi* in Latin America.
Trypanosoma cruzi Chagas Life cycle

- transmitted via blood-sucking insects (kissing bugs), in the insects' feces. From there, the infectious trypomastigotes enter the human body through breaks in the skin and are transformed into amastigotes.
- can be transmitted via blood transfusions.
- has been transmitted in utero, and is associated with fetal demise and fetal abnormalities.
Acute Chagas’ disease

- active infection
- 1-4 months
- majority asymptomatic
- a small, indurated papule with erythema and local lymphadenopathy occurs at the site of invasion by the organism - *chagoma*.
- when contact is made from the organism to the conjunctiva, periocular edema occurs (Romaña sign), or swelling and closure of the eye.
- fevers, constitutional symptoms, lymphadenopathy, and splenomegaly can occur and usually resolve within weeks.

*Fig. 1 - Inoculation chagoma on the dorsal surface of the left hand at the base of the thumb, 21 days after accidental inoculation with blood trypomastigote forms of *Trypanosoma cruzi* in a 42-year-old female patient.*
Chagas’ disease - indeterminate phase

- 10-30 years of latency
- no detectable parasitemia
- relatively asymptomatic
- seropositive

Armadillo

Opossum
Chagas’ disease - chronic phase

• ~1/3 of infected persons
devlops years after the initial infection
• patients develop megaesophagus and dysphagia.
• colonic dysfunction with megacolon occurs.
• cardiomyopathy develops and is associated with heart failure and/or arrhythmias, which are often fatal.
Acute Chagas’ disease – diagnosis

- finding circulating parasites in the blood confirms the diagnosis.
- wet preparations and Giemsa stains of anticoagulated blood (thin and thick smears) should be obtained.
- the organisms are motile. Detection occurs 50% of the time.

*Trypanosoma cruzi* seen in a human blood smear. Flagellated tryptomsastigote is in mid-right portion of the smear.
Chronic Chagas’ disease – diagnosis

- Serology is used to detect antibodies to the organism.
- Many false-positive tests occur.
- Xenodiagnosis – laboratory-bred reduviid bugs can be allowed to feed on the patient suspected of having Chagas’ disease; subsequent dissection of the bugs will reveal parasites in the hindgut.
Leishmania spp.
Leishmaniosis

• *Leishmaniasis* refers to the spectrum of disease caused by the protozoa *Leishmania* and transmitted by a sandfly vector.

• Clinically, leishmaniasis is divided into
  - visceral
  - cutaneous
  - mucosal syndromes
  - diffuse mucosal
Leishmaniosis - vector

Transmitted by a hematophagous arthropod:

- *Phlebotomus* (Old World)
- *Lutzomyia* (New World)
Leishmaniosis - morphology

- Promastigotes in sand fly saliva

- Amastigotes intracellularly in macrophages
Leishmaniosis – life cycle

- Promastigotes are transferred to the vertebrate host during sand fly feeding
- Within the vertebrate host, the promastigotes are phagocytosed by macrophages
<table>
<thead>
<tr>
<th>Leishmaniosis</th>
<th>Causative organism</th>
<th>Clinical manifestations</th>
<th>Location</th>
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</thead>
<tbody>
<tr>
<td>Visceral leishmaniosis</td>
<td><em>L. d. donovani</em></td>
<td>VL with rare cases of post kala-azar CL</td>
<td>China India, Iran, Sudan, Kenya, Ethiopia</td>
</tr>
<tr>
<td></td>
<td><em>L. d. infantum</em></td>
<td>VL or CL depending on strain</td>
<td>Mediterranean basin</td>
</tr>
<tr>
<td></td>
<td><em>L. d. chagasi</em></td>
<td>VL and some atypical CL</td>
<td>Brazil, Columbia, Venezuela, Argentina</td>
</tr>
<tr>
<td>Cutaneous leishmaniosis</td>
<td><em>L. tropica</em></td>
<td>CL (dry) and rare cases of recidiva CL</td>
<td>Mediterranean basin, Afghanistan</td>
</tr>
<tr>
<td></td>
<td><em>L. major</em></td>
<td>CL (wet)</td>
<td>Middle East, W &amp; N Africa, Kenya, Ethiopia</td>
</tr>
<tr>
<td></td>
<td><em>L. aethiopica</em></td>
<td>CL and rare cases of DCL</td>
<td>Ethiopia</td>
</tr>
<tr>
<td></td>
<td><em>L. mexicana</em></td>
<td>CL and rare cases of MCL and DCL</td>
<td>Central America &amp; Amazon basin</td>
</tr>
<tr>
<td>Mucocutaneous leishmaniosis</td>
<td><em>L. braziliensis</em></td>
<td>CL with some cases developing MCL later</td>
<td>Brazil, Peru, Ecuador, Venezuela, Columbia</td>
</tr>
<tr>
<td></td>
<td><em>complex</em></td>
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</tbody>
</table>
Leishmania donovani

KALA-AZAR (visceral laishmaniosis)

• *L. donovani donovani* (Indian kala-azar)
  ➢ men → Phlebotomus → men

• *L. donovani infantum* (Mediterranean kala-azar)
  ➢ dog → Plebotomus → dog  
    (human infection accidentally)

• AIDS – *L. donovani* like oportunist
Visceral leishmaniosi (kala-azar)

Old-world cutaneous leishmaniasis

- a local lesion starts as a papule at the site where promastigotes are inoculated.
- the papule gradually increases in size, becomes crusted, and finally ulcerates.
- the ulcer is usually shallow and circular with well-defined, raised, erythematous borders and a bed of granulation tissue.
- it gradually increases in size and may reach a diameter of 2 cm or more.
- satellite lesions may be present.
- there is frequently a serous or seropurulent discharge.
Mucocutaneous leishmaniasis (MCL)

- a small proportion (< 5%) of patients with simple cutaneous leishmaniasis will develop mucocutaneous leishmaniasis (MCL).
- this manifestation is primarily due to members of the *L. braziliensis* complex.
- mucocutaneous disease begins as simple skin lesions that metastasize via the blood stream or lymphatics, particularly to the mucosae of the nose and mouth.
Diffuse cutaneous leishmaniasis (DCL)

- Diffuse cutaneous leishmaniasis (DCL) and leishmaniasis recidivans are two rare manifestations of cutaneous leishmaniasis.
- DCL is characterized by disseminated nodular lesions that resemble lepromatous leprosy.
- Leishmaniasis recidivans is a chronic recurrence of nodular lesions or a rash associated with *L. tropica* infections.
## Diagnosis of Leshmaniasis

<table>
<thead>
<tr>
<th>Suspected</th>
<th>Dermal</th>
<th>Visceral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• geographical presence of parasite</td>
<td>• prolonged fever, splenomegaly, hepatomegaly, anemia, leukopenia, hyperglobulinemia</td>
</tr>
<tr>
<td></td>
<td>• history of sand fly bite</td>
<td></td>
</tr>
<tr>
<td>Clinical Symptoms</td>
<td>• chronic, painless, “clean” ulcer (CL), nasopharyngeal lesions (MCL), or nodular lesions (DL)</td>
<td>• amastigotes in bone marrow aspirate or biopsy</td>
</tr>
<tr>
<td>Parasite Detection</td>
<td>• amastigotes in scrapings or aspirates</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• culture of promastigotes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• inoculation of hamsters</td>
<td></td>
</tr>
<tr>
<td>Immunodiagnosis</td>
<td>• Montenegro skin test</td>
<td>• serological tests (direct agglutination, dipstick)</td>
</tr>
</tbody>
</table>
Leishmaniasis - diagnosis


Amastigotes in bone marrow aspirate or biopsy

Culture of promastigotes (NNN)
Leishmaniasis - diagnosis


- **Montenegro test**
- **Immunofluorescence test**
- **Formol-gel test (unspecific)**
Plasmodium spp. - Malaria
PLASMODIUM SPP.

- *Plasmodium falciparum*
- *Plasmodium vivax*
- *Plasmodium malariae*
- *Plasmodium ovale*

MALARIJA (*paludismus* – latin: palus = swamp, swamp fever)

- Vector – *Anopheles spp.*

Malaria infects 200 to 300 million people per year
- Malaria is responsible for as many as 2 million deaths a year.
- *P. falciparum* *i* *P. vivax* - 95% INFECTION
Apicomplexan life cycle

- Sporogony occurs immediately after a sexual phase → production of sporozoites.
- Sporozoites are an invasive form that will invade cells and develop into forms that undergo another asexual replication known as merogony.
- Merogony and the resulting merozoites are known by many different names depending on the species.
- As an alternative to asexual replication merozoites can develop into gametes through a process variously called gametogony.
Malaria – life cycle
Liver stage

• human infection is initiated when **sporozoites** are injected with the saliva during mosquito feeding
• the sporozoites enter the circulatory system and within 30-60 minutes will invade a liver cell
• after invading the hepatocyte, the parasite undergoes an asexual replication - **exoerythrocytic (or pre-erythrocytic) schizogony**
• **merozoites**, are released into the circulatory system following rupture of the host hepatocyte
Malaria – life cycle
Blood stage

• **merozoites** released from the infected liver cells invade erythrocytes
• the merozoites recognize specific proteins on the surface of the erythrocyte
• after entering the erythrocyte the parasite undergoes a trophic period followed by an asexual replication
• the young trophozoite is often called a **ring form** due to its morphology in Geimsa-stained blood smears
• as the parasite increases in size this 'ring' morphology disappears and it is called a **trophozoite**
• **Sexual Stage.** As an alternative to schizogony some of the parasites will undergo a sexual cycle and terminally differentiate into either **micro- or macrogametocytes**. The factors involved in the induction of gametocytogenesis are not known.
Malaria – life cycle

- in *P. vivax* and *P. ovale* some of the sporozoites do not immediately undergo asexual replication, but enter a dormant phase known as the **hypnozoite**
- this hypnozoite can reactivate and undergo schizogony at a later time resulting in a relapse
Malaria – course of infection

• **relapse** has a specific meaning in regards to malaria and refers to the reactivation of the infection via hypnozoites

• **recrudescence** is used to describe the situation in which parasitemia falls below detectable levels and then later increases to a patent parasitemia

Diagram representing the course of malaria infection. The black line depicts the blood-stage parasitemia following sporozoite infection (sp). There is prepatent period (p) between sporozoite inoculation and the detection of parasites in the blood. The blue line depicts the microscopic threshold (ie, limit of detection) and the yellow area represents a subpatent parasitemia. The orange area represents an asymptomatic patent parasitemia. The red line depicts a clinical threshold, or the parasitemia which produces paroxysms or other clinical symptoms (pink area). As immunity develops this clinical threshold increases. The incubation period (i) is the time between infection and the appearance of symptoms.
Malaria – course of infection

• Symptoms of malaria usually start to appear 10-15 days after the bite of an infected mosquito.

• The **prepatent period** is defined as the time between sporozoite inoculation and the appearance of parasites in the blood and represents the duration of the liver stage and the number of merozoites produced.

• **Incubation periods** tend to be a little longer and are defined as the time between sporozoite inoculation and the onset of symptoms.
Malaria – clinical manifestations

• All four species can exhibit non-specific prodromal symptoms a few days before the first febrile attack.
• These prodromal symptoms are generally described as 'flu-like' and include: headache, slight fever, muscle pain, anorexia, nausea and lassitude (the symptoms tend to correlate with increasing numbers of parasites).
• These prodromal symptoms will be followed by febrile attacks also known as the malarial paroxysms.
# Malarial paroxysms

<table>
<thead>
<tr>
<th>The Malarial Paroxysm</th>
<th>Cold stage</th>
<th>Hot stage</th>
<th>Sweating stage</th>
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<tr>
<td><strong>Cold stage</strong></td>
<td>•feeling of intense cold</td>
<td>•intense heat</td>
<td>•profuse sweating</td>
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<td>•vigorous shivering</td>
<td>•dry burning skin</td>
<td>•declining temperature</td>
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<td>•lasts 15-60 minutes</td>
<td>•throbbing headache</td>
<td>•exhausted and weak → sleep</td>
<td></td>
</tr>
<tr>
<td>•lasts 2-6 hours</td>
<td>•lasts 2-4 hours</td>
<td></td>
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- Feeling of intense cold
- Intense heat
- Profuse sweating
- Vigorous shivering
- Dry burning skin
- Declining temperature
- Throbbing headache
- Exhausted and weak → sleep
- Lasts 15-60 minutes
- Lasts 2-6 hours
- Lasts 2-4 hours
Malarial paroxysms

- Paroxysms will exhibit periodicities of 48 hours for *P. vivax*, *P. ovale*, and *P. falciparum*, and a 72-hour periodicity for *P. malariae*
- Patients may also exhibit splenomegaly, hepatomegaly (slight jaundice), and hemolytic anemia during the period in which the malaria paroxysms occur
Malaria – transmission

• the most common way to obtain malaria is through the natural transmission by mosquitoes
• malaria can also be transmitted via blood transfusions
• mechanical transmission of infected blood will result in a shorter incubation period since there will be no liver stage
• congenital transmission has also been documented, but is believed to be relatively rare despite the heavy infection of the placenta.
Cerebral Malaria

• complication of severe falciparum malaria
• a diffuse encephalopathy with loss of consciousness
  ➢ consciousness ranges from stupor to coma
  ➢ onset can be gradual or rapid
  ➢ unresponsive to pain, visual, and verbal stimuli
• associated with sequestration in cerebral microvasculature

A brain affected by cerebral malaria

A blocked cerebral capillary
Malaria - diagnosis

Samples: peripheral blood, bone marrow, cord blood or placental impression

Principles of blood collecting and examination:

• It is recommended that blood be collected every 6 to 12 hours, for up to 48 hours before considering a patient free of malaria parasites
• Multiple sets of blood films are necessary in order to rule out malaria
• Both tick and thin Giemsa stained blood smears are examined with a X100 oil immersion objective
• A minimum of 100 oil immersion fields must be examined per film
Malaria - diagnosis

**Thin blood smear**
- Red cell morphology is maintained
- Malaria parasites are found inside erythrocytes

**Thick blood smear**
- Red cells are completely lysed
- Malaria parasites are found between white blood cells

B. Molecular biology techniques – DNA probes, PCR
C. Rapid tests – detection of specific parasite protein
Thin blood smear and Thick blood smear
Plasmodium falciparum
(thin blood smear)

Trophozoite – ring form

Gametocyte
Plasmodium falciparum
(thick blood smear)

Trophozoite

Gametocyte
Rapid tests – detection of specific parasite protein
Toxoplasma gondii
Toxoplasma gondii

• *Toxoplasma gondii* is a coccidian parasite which infects humans as well as a wide variety of mammals and birds

• Toxoplasmosis is found throughout the world (except extremely cold or dry climates)

• Toxoplasmosis is most often a benign disease

• Noted exceptions are in the cases of **congenital infection** or **immunocompromised individuals**
Toxoplasma gondii – life cycle

- *Toxoplasma* has a complex life cycle consisting of intestinal and tissue phases.
- The intestinal phase of the infection occurs only in felines (the parasites invade intestinal epithelial cells and undergo merogony).
- The resulting merozoites can then either undergo additional rounds of merogony or undergo gametogony.
- Thus, the cat is considered the definitive host since this is the host in which the sexual cycle occurs.
- Intermediate hosts, such as rodents and birds, become infected through the ingestion of sporulated oocysts.
Toxoplasma gondii – human transmission

- ingest oocysts
- ingest tissue stages
- raw goat's milk
- congenital
- organ transplant
- blood transfusion

> humans are not a natural part of the predator-prey life cycle and represent an accidental host
T. gondii – clinical features

- Asymptomatic in 80% to 90% of cases.
- Symptomatic patients: lymphadenopathy is the most common presentation.
- Symptoms are self-limiting and resolve within weeks to months.
Congenital Toxoplasmosis

• transmission only possible during acute stage (i.e., primary infection must occur during pregnancy)
  – can only occur once
  – one-third of mothers seroconverting during pregnancy will pass on infection to fetus
• incidence between 1 per 1000 and 1 per 10,000 live births
• severity varies with age of fetus (more severe early in pregnancy)
  – transmission is more frequent later in pregnancy
• infection can result in: spontaneous abortion, premature birth, or full-term with or without progressive disease
• typical disease manifestations include: retinochoroiditis, intracerebral calcification, hydrocephaly, microcephaly, psychomotor disturbances, mental retardation, blindness and other visual defects
Congenital Infection Outcomes

- 5-10% death (abortion or still birth)
- 8-10% severe brain or eye damage
- 10-13% moderate-to-severe visual handicaps
- 58-72% asymptomatic at birth, developing retinochoroiditis or neurological symptoms later
Toxoplasma gondii - diagnosis

Serology: is the method of choice for diagnosing the infection!

- A positive IgM antibody titer indicates early primary infection and rising (fourfold rise) IgG antibody titer indicates an active infection
- The antibody response usually peaks 4-8 weeks postinfection
- Also, if IgM antibodies are produced after reactivation of latent disease, a single high titer of IgM established the presence of acute infection or reactivation
- Fetal blood (cordocentesis) and amniotic fluid (amniocentesis) examination for the presence of IgM levels; less than half of the infected infants will have detectable IgM
Toxoplasma gondii - diagnosis

Microscopic examination of biopsy specimen:

- Smears and section stained with Giemsa, periodic-acid Schiff stain (detection of cysts in the muscles or brain suggests chronic infection)
- Detection of parasites in Giemsa stained slides of bronchoalveolar lavage in AIDS patients suspected on pulmonary toxoplasmosis
- Vary useful procedure in case of lymph node tissue
- Detection of parasites in placental biopsy sample by examination of Giemsa stained smears
T. gondii - trophozoites

Giemsa stain

Giemsa stain
T. gondii - prevention

RAW MEAT
• cook thoroughly (66C, 150F)
• wear gloves when handling
• wash hands after handling
• wash cutting boards, counter tops, utensils, etc

CAT FECES
• clean litter box promptly (<24 hr)
• wear gloves while gardening
• wash hands after gardening or cleaning litter box
• wash and peel fruits and vegetables
• cover sand box
• always keep cat in house
• control strays
• do not acquire new cats during pregnancy