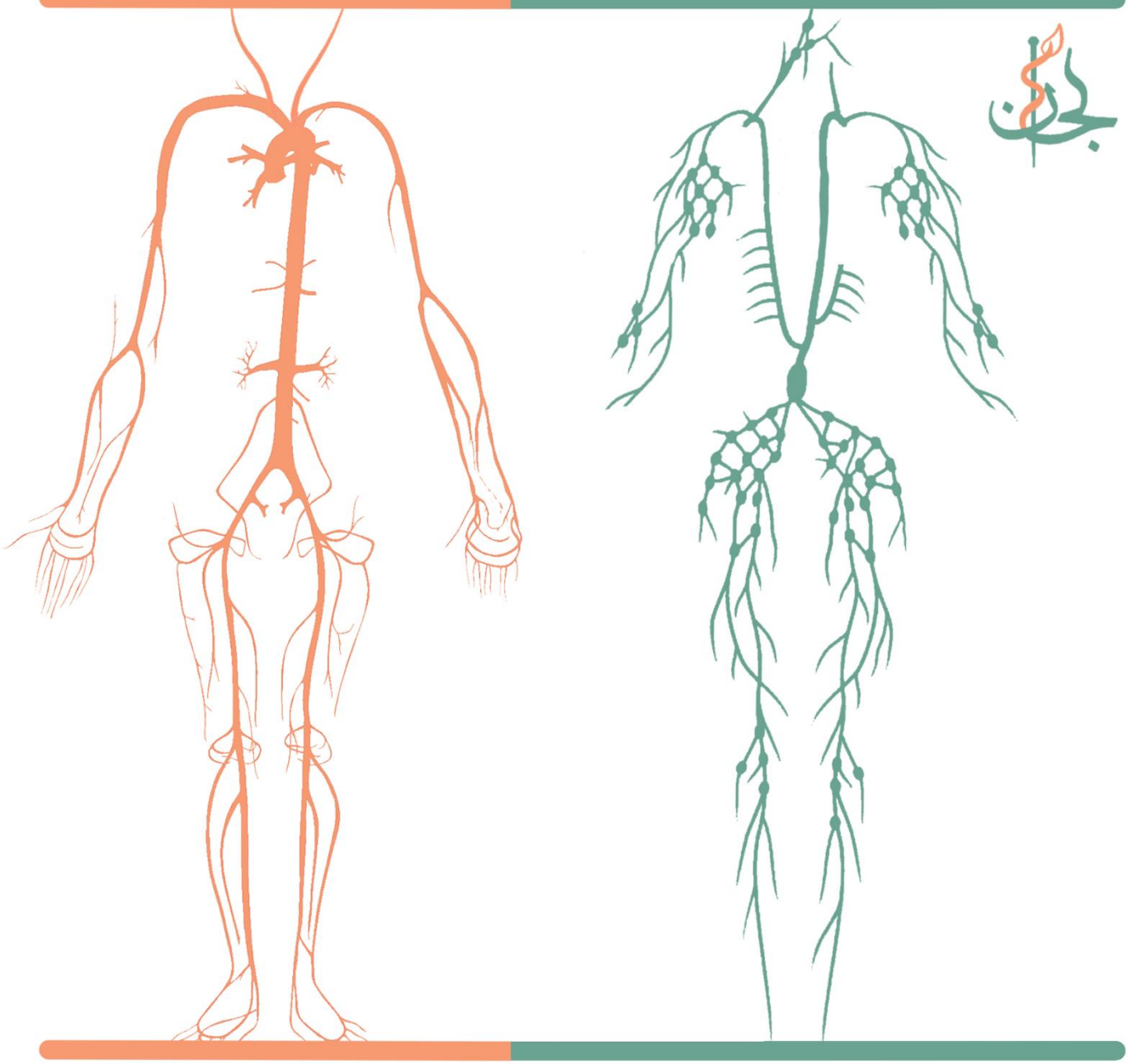
Microbiology

HematoLymphatic



Title: 4 - Hemoflagellates

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Doctor: Dr. Nader Alaridah

Note: Anything that is from the slides that the professor didn't talk about is underlined.

Hemoflagellates (of the mastigophora group) move, evidently, by the mean of a single flagella. They reproduce through asexual reproduction *only* through division by binary fission. This includes two protozoa:

1. Trypanosoma – Causes the disease Trypanosomiasis.
2. Leishmania – Causes the disease Leishmaniasis.

In the image to the right, we can see the different developmental stages of blood flagellates:

1. Amastigote – The round, intracellular form.
2. Promastigote
3. Epimastigote
4. Trypomastigote

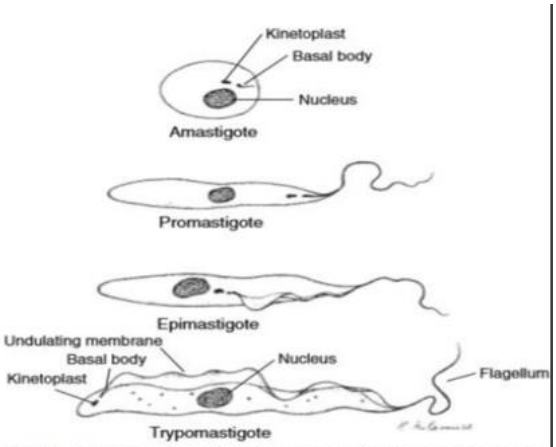


Figure 49-8 Characteristic stages of species of *Leishmania* and *Trypanosoma* in human and insect hosts. (Illustration by Nobuko Kitamura.)

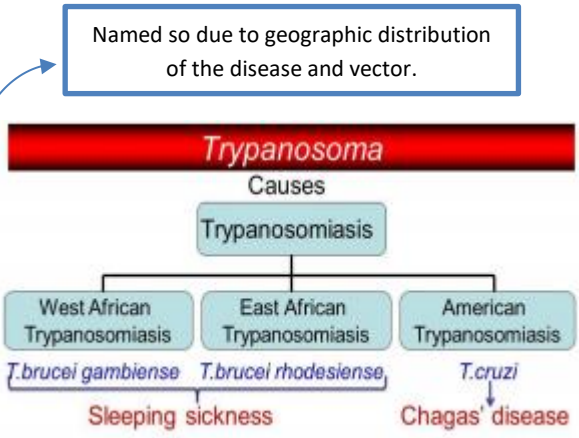
In the developmental stages there is a kinetoplast. It consists of the DNA and the mitochondria. The flagella and undulating membrane originate from the kinetoplast. This is why both Trypanosoma and Leishmania fall under the order **Kinetoplastida**.

Along with the appearance of the flagella, there is an undulating membrane found on the same axis of the protozoa. It is seen in the trypomastigote stage.

Trypanosoma

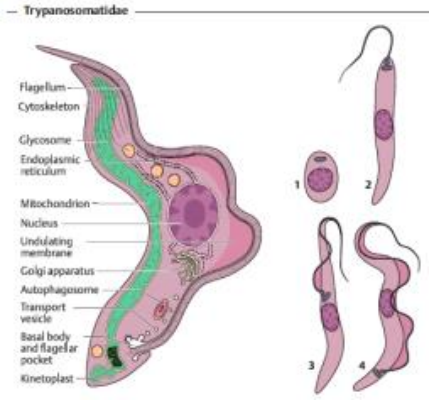
Clinically, there are two very different forms of trypanosomiasis, both of which are vector-borne:

1. African Trypanosomosis (sleeping sickness)
    - Causative agent: Trypanosoma Brucei complex
        i. West African: T. brucei gambiense
        ii. East African: T. brucei rhodesiense
    - Vector: Tsetse fly (Glossina) (both sexes)
2. American Trypanosomosis (Chagas disease)
    - Causative agent: Trypanosoma cruzi.



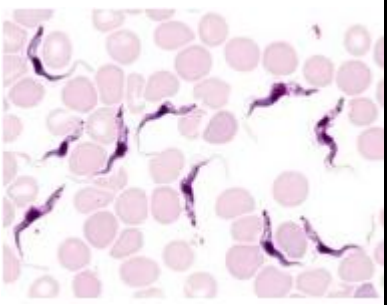
- Occurs in humans and many vertebrate animals in Central and South America (it is endemic there).
- Vector: Triatomine bug (Kissing bug)

Morphology



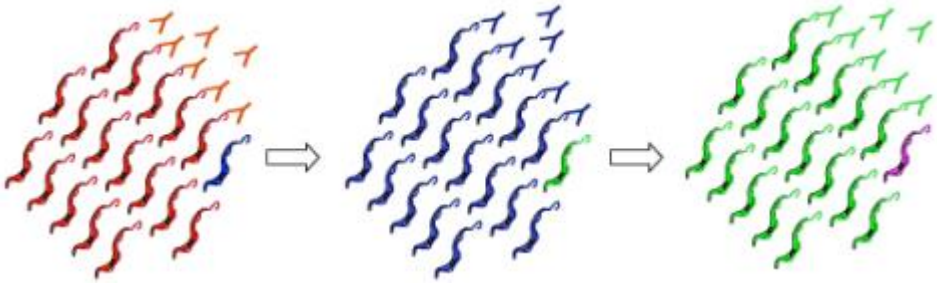
- The morphologically differentiated forms include spindly, uniflagellate stages (trypomastigote, epimastigote, promastigote) and a rounded, amastigote form.
- The trypomastigote and amastigote stages are seen in the human.
- The promastigote is seen in the vector.

The image to the right shows a blood film, and we can see extracellular parasites only. This would indicate African sleeping sickness, which has no intracellular form. An intracellular form is seen in American trypanosomiasis, where the parasite also prefers cardiac myocytes. So, people with Chagas disease have cardiac problems.



Antigenic Variation

- A unique feature of African trypanosomes is their ability to change the antigenic surface coat of the outer membrane of the trypomastigote, helping to evade the host immune response. By the time the host recognizes a certain antigen and mounts a response against it, the surface antigen gets replaced.
- The trypomastigote surface is covered with a dense coat of variant surface glycoprotein (VSG). The VSGs are controlled by over a hundred genes, so the result is a mosaic of various surface glycoproteins.
- Each time the antigenic coat changes, the host does not recognize the organism and must mount a new immunologic response.



So, as explained by this image, the body begins to mount a response against the first VSG, in this case the red. But by the time they are cleared a new variant- the blue glycoprotein -appears, so new antibodies against it must be created, but by then the new green glycoprotein appears, and so on the process continues.

African Trypanosomiasis

This disease is also known as African sleeping sickness, because in the late stages of the disease, as the patient has serious manifestations (especially changes in character and personality), they keep sleeping.

As discussed earlier, there are two forms of this disease caused by two subspecies:

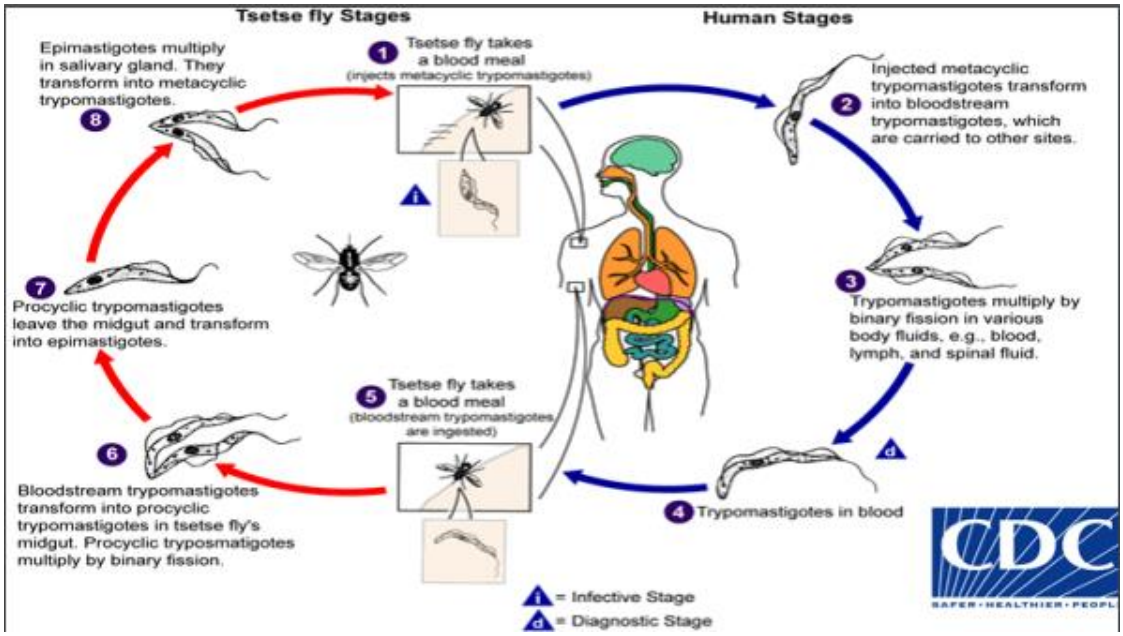
Disease	West African Trypanosomiasis	East African Trypanosomiasis
Parasite	T. brucei gambiense	T. brucei rhodesiense
Disease	Chronic, slow progression	Acute, quick progression
Frequency	More common	Less frequent
 Vector (Tsetse fly) species (found only in rural Africa)	Glossinia palpalis	Glossinia morsitans
Reservoir	Humans	Animals
Epidemiology	Is transmitted mainly from human to human by the tsetse flies, although various animal species have also been identified as reservoir hosts.	Persists in a latent enzootic cycle in wild and domestic animals and is normally transmitted by Glossinia from animal to animal, more rarely to humans.

Geographical Distribution: To the west of the dotted line you see Trypanosoma brucei gambiense (West African), and to the east of the dotted line East African Trypanosomiasis.

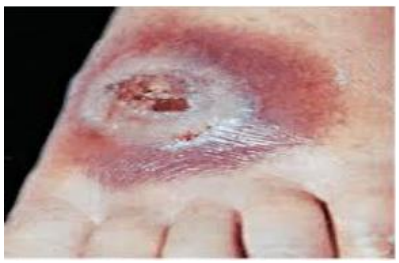
Life Cycle

1. When the tsetse fly bites a human, they transfer from their salivary glands metacyclic trypomastigotes. **This is the infective stage.**
2. The metacyclic trypomastigote transforms into bloodstream trypomastigotes. This is what we look for in the peripheral blood sample, therefore, **this is the diagnostic stage.**
3. When another tsetse fly takes a blood meal, it gets infected by the bloodstream trypomastigotes.
4. The trypomastigote transforms into a procyclic trypomastigote (promastigote) in the vector.

Something extra the doctor said: Knowing the infective and diagnostic stage helps in understanding the disease course.



Trypanosoma Brucei Gambiense

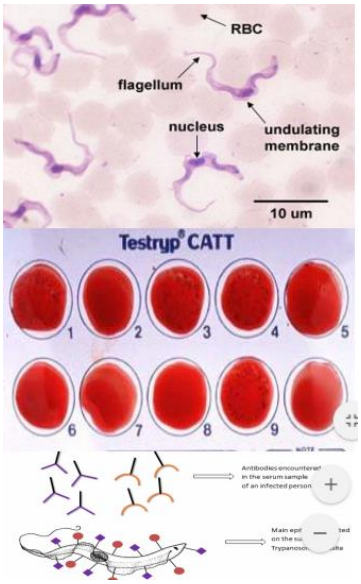


- After the host has been bitten by an infected tsetse fly, a local reaction occurs at the site, in which a painless nodule or chancre (called a Trypanosoma chancre) may develop after a few days.
- Then, as the trypomastigote invades the blood (parasitemia) and lymphatics, symptoms start to appear and are characterized by 2 stages:
  1. Stage 1: Systemic trypanosomiasis without CNS involvement:
     - It can invade lymph nodes.
     - <u>The first symptoms appear and include: irregular fevers with night sweats, enlargement to liver and spleen, and Winterbottom's sign.</u>
     - Winterbottom's sign is the enlargement of posterior cervical lymph nodes.
     - **Only parasitemia, NO CNS involvement**

Winterbottom's sign

  2. Stage 2: Organisms invade the CNS:
     - By entering the cerebral spinal fluid, the parasite can enter the CNS via the choroid plexus (extra: a blood-CSF barrier).
     - CNS manifestations occur, including change in character in personality. They start with restlessness, trouble sleeping, agitation, and then issues with over-sleeping occur.
     - The sleeping sickness stage of the infection is initiated.
     - <u>The patient becomes emaciated (abnormally thin or weak) due to encephalitis</u> and progresses to profound coma and death.

Laboratory Diagnosis



- Similar to American trypanosomiasis
- The definitive diagnosis is seeing the trypomastigote in the peripheral blood.
- Specimens that can be used include blood, serum, CSF, and an aspiration from a lymph node.
- Routine Method: Thick and thin blood film.
- Antigen Detection: Simple and rapid test: the card indirect agglutination test aims to look for a Trypanosoma antigen.
- Antibody Detection: Serologic by using ELISA serum or CSF IgM concentrations.
- Molecular Diagnostics: PCR-based methods to detect infections and differentiate species, but these methods are not frequently used.

Therapy

- If untreated, the disease course will result in the death of the patient.
- The best results are achieved if the patient begins treatment before CNS symptoms appear as once it reaches the CNS the prognosis worsens. At that rate, treatment is not curative.
- All drugs used in the therapy of African trypanosomiasis are toxic and require prolonged administration.
- The anti-parasitic drug selected depends on whether the CNS is infected.
- Suramin or pentamidine isethionate can be used when the CNS is not infected.
- Melarsoprol, a toxic trivalent arsenic derivative, is effective for both blood and CNS stages but is the drug of choice recommended for treatment of late-stage sleeping sickness.
- These drugs are unique and usually only used for this disease.
- A new drug, elfornithine has been approved by the FDA but it's not fully recommended.

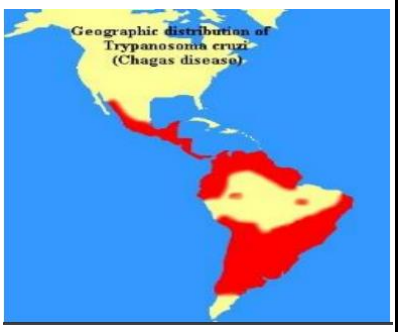
Prevention (focused on vector control)

- Preventing flies from biting through the use of insecticide, suitable clothing (cover exposed skin), and bed nets will reduce the transmission of the parasite.
- Screening of people at risk helps identify patients at an early stage.
- Treatment cases should be monitored for 2 years after completion of therapy.

American Trypanosomiasis

- Causative agent: Trypanosoma cruzi
- Vector: Triatoma bug/kissing bug/Reduviid bug
    - It is called the kissing bug as it tends to bite the face.
    - This vector is different from the vectors we took before, as the infective stage is not the actual bite from the insect but the feces it leaves as it is taking its blood meal. So, the life cycle may start if the feces enters through the bite or if you have a skin abrasion/cut elsewhere (such as the hand) that comes into contact with the feces.

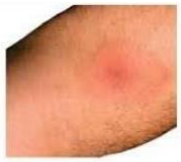
- Definitive Host: Human, dog, cat, rats, etc.
- Habitat in the definitive host:
    - Trypomastigote in blood.
    - Amastigote in tissue.
- It is distributed throughout Central and South America.
- The life expectancy of individuals with this disease is shorter as they suffer from cardiac problems. The amastigote tends to select cardiac muscle, which causes cardiac myopathy, arrythmia, and heart failure later on.



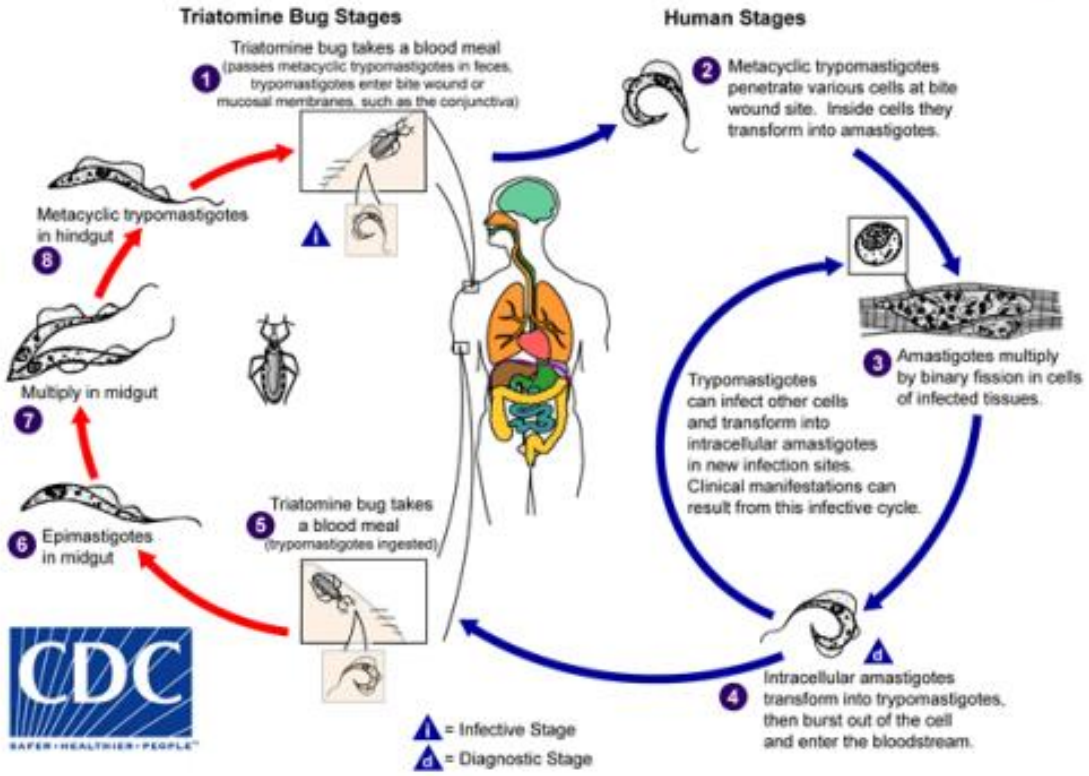
Life Cycle **(image seen on next page)**

- The infective form of Trypanosoma cruzi enters the human through the feces left by the vector. **This is the infective stage.**
    - This will usually cause the patient to rub their eyes, causing unilateral conjunctivitis (Romana's sign).
- The amastigote is intracellular, so it is diagnosed through a cardiac biopsy.
- Since this is a blood parasitic infection, recall that while they are vector-borne and they need a tick/bug for their life cycle, they can also be spread through blood transfusion or transplacentally.
- Note: The professor said the diagnostic stage is important but did not specifically mention it in the lecture. In an email, he said in the blood it is the trypomastigote and intracellularly it is the amastigote (both are diagnostic).

Pathogenesis

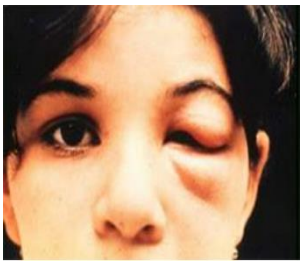

Chagoma de inoculación
Trypanosoma cruzi

- Chagas disease is categorized as an acute, indeterminate, and chronic disease.
- At the site of injection, T. cruzi causes a local reaction. It is known as a Chagoma nodule (skin has erythema).
- The incubation in period in humans is about 7-14 days.



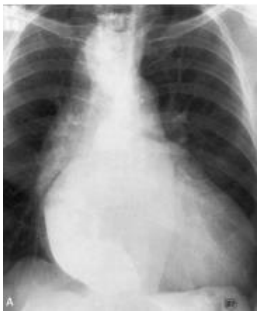
The classical appearance of the disease occurs after a bite by the kissing bug on the face, and so symptoms appear there such as Romana's sign.

Acute Phase:



- Starts one week after infection.
- Unilateral conjunctivitis (swelling of the eyelid): Romana's sign.
- Fever
- Lymph node enlargement
- Enlarged liver and spleen
- Acute myocarditis

Chronic Phase:



- Develops years after the diagnosis of acute disease.
- Our fear stems from the selection by the parasite of the cardiac muscle. So, the most frequent clinical signs of chronic Chagas disease involve the heart, including cardiac changes such as enlargement of the heart (cardiac enlargement) as seen in the chest X-ray, cardiomyopathies, and heart failure.
- Other organs can be affected and enlarged, including the colon (megacolon) and esophagus (mega-esophagus).

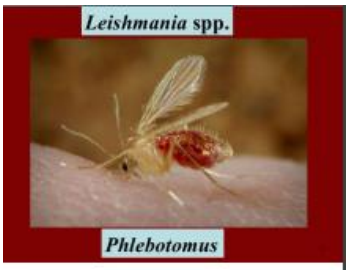
Treatment

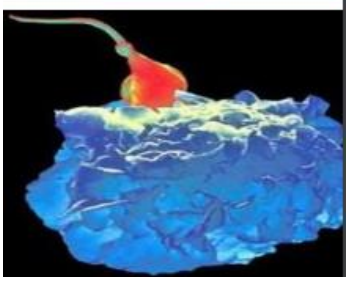
- Nifurtimox and benznidazole reduce the severity of acute Chagas disease.
- Both medicines are almost 100% effective in curing the disease if given soon after infection at the onset of the acute phase, including in cases of congenital transmission.

Prevention

- Once again, vector control is the mainstay of preventing infection.
- Transfusion control and screening of blood donors.
- Testing of organ, tissue, or cell donors and receivers.

Leishmania



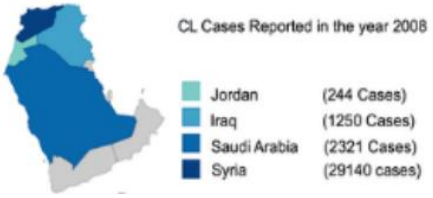


- It is a flagellated protozoan.
- The vector is a female sand fly.
- The life cycle requires two hosts:
  - A vertebrate mammalian host.
  - The invertebrate vector: the female sand fly.
- It is an obligate intracellular organism.
- It infects/targets primarily immune cells, mainly APCs/phagocytic cells and especially **macrophages**.
- The incubation period ranges from 10 days to 2 years.

The Leishmania genus has many species that can cause Leishmaniasis. The disease is divided into clinical syndromes according to what part of the body is affected most:

- **Cutaneous Leishmaniasis (L. tropica, L. major, L. infantum):** The infection is limited to the skin and dermis.
  - As we mentioned, the parasite mainly infects macrophages. If these macrophages reach the skin, this leads to cutaneous Leishmaniasis.
- **Mucocutaneous Leishmaniasis (L. braziliensis**): Also called nasopharyngeal leishmaniasis as it mainly occurs in the nasopharynx.
- **Visceral Leishmaniasis (L. donovani):** Also known as Kala azar leishmaniasis – The name means black fever, in which the patients have hyperpigmentation.
  - Once the macrophages reach reticuloendothelial sites (e.g. liver, spleen, bone marrow) this leads to visceral leishmaniasis.

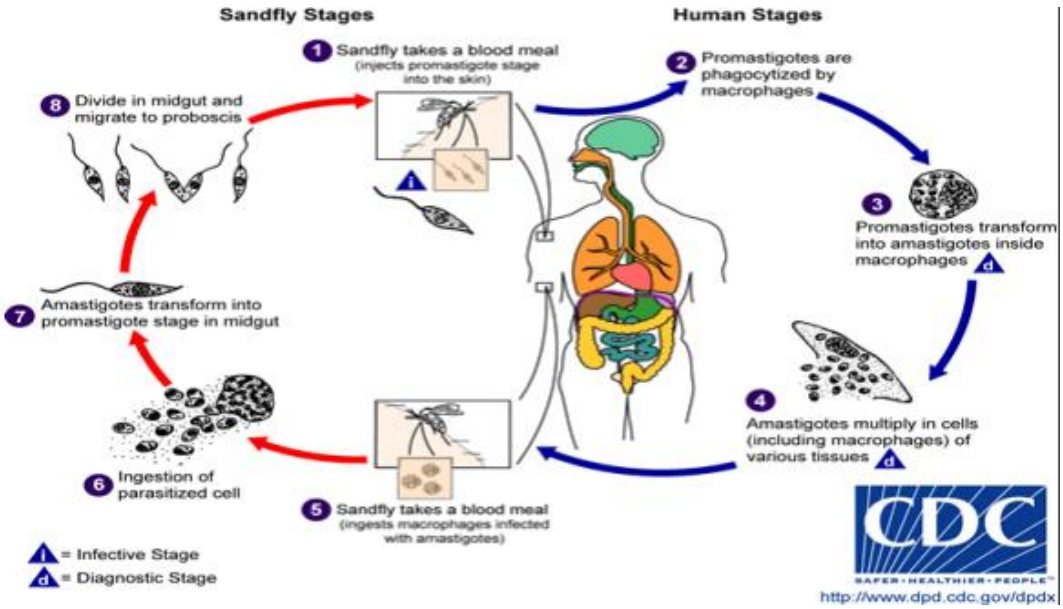
Leishmania in Jordan



- It used to be endemic, but with advances in medicine it isn't so anymore.
- In Jordan you mainly see the cutaneous (more common) and mucocutaneous forms.
- In Jordan there are several species of Leishmania: Leishmania infantum, Leishmania tropica, and Leishmania major.
- Leishmania major is the most common species of the Leishmania parasite in Jordan.

Life Cycle

- The **infective stage** are the promastigotes, which are injected by the sand fly.
- Amastigotes are found intracellularly (in macrophages). The amastigotes are the **diagnostic stage**.

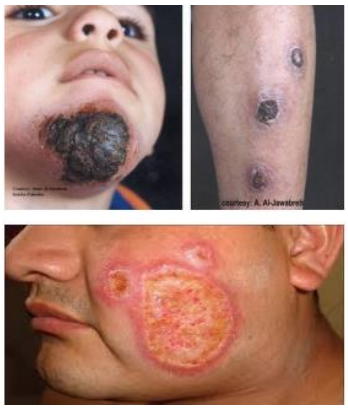


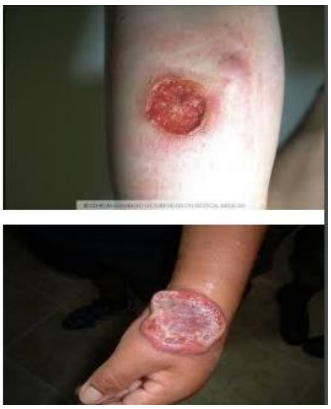
Transmission

- Vector-Borne – A bite from the sand fly (most common).
- Blood transfusion and transplantation (if the organ is contaminated by amastigotes).
- From mother to baby.
- Direct contact; from man to man through nasal secretions.

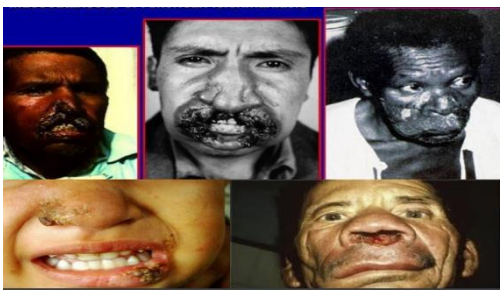
Cutaneous Leishmaniasis (L. tropica, L. major, L. infantum)

- Clinical Features: The first sign is a lesion. It begins as a reddish, soft, and itchy papular lesion and then gradually enlarges, becomes raised, firm, and has serous discharge at the bite site.
- The lesions are ulcerated (as seen in images below).
- Usually they are painless, but can cause extreme disability.
- These lesions can heal spontaneously, but it may take months and it depends on the immune status of the patient. Otherwise, it may scar.
- Usually patients seek medical care for cosmetic purposes.
- Epidemiology: It is distributed in the Middle East and South America.





Mucocutaneous Leishmaniasis (Nasopharyngeal leishmaniasis)

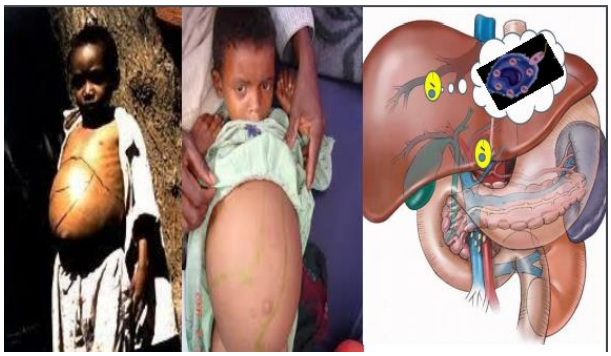


- Causative species include L. braziliensis (South America) and L. Mexicana (Central America).
- The primary lesions are similar to those found in cutaneous leishmaniasis.
- Dissemination to the nasal or oral mucosa may occur from the active primary lesion or may occur years later after the original lesion has healed.
- May destruct the nasal septum if not treated.

- These mucosal lesions do not heal spontaneously, and secondary bacterial infections are common and may be fatal.
- For diagnosis, samples are taken from the edges of the erosions.

Visceral Leishmaniasis (Kala Azar)

- The most severe form of Leishmaniasis.
- The parasite migrates to the internal organs such as the liver, spleen (hence "visceral"), and bone marrow. The lymph nodes may be affected as well.
- Incubation period: 10 days to 2 years, usually.
- Causes hyperpigmentation of the skin.
- Symptoms: Fever, anorexia, malaise, weight loss, and, frequently, diarrhea.
- Clinical signs: Enlarged liver and spleen, swollen lymph nodes, occasional acute abdominal pain.
- If left untreated it will almost always result in the death of the host.
- Epidemiology: Bangladesh, Brazil, Ethiopia, India, South Sudan, and Sudan.
- For diagnosis, the sample must be taken from the bone marrow, such as through aspiration from the sternum.

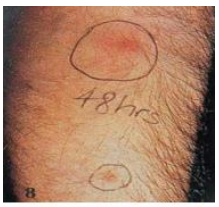


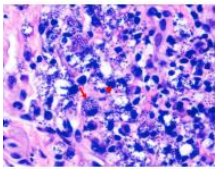
In the image to the left we can see the typical presentation of visceral Leishmania of abdominal distention (due to hepatomegaly and splenomegaly).

Laboratory Diagnosis

- Definitive Diagnosis: Visualizing the parasite (developmental stage: amastigotes).
- Stained blood smear: Aspiration, Scraping
- You may need to do lymph node aspiration or biopsy of the involved organ (such as splenic puncture to diagnosis visceral Leishmaniasis)
- Culture: Cultured using special techniques
- ELISA, IFA (indirect immunofluorescence assay) or direct agglutination give useful indication of active or recent kala-azar.
- PCR methods have excellent sensitivity and specificity for direct detection.

- **Intradermal Montenegro Test**: The patient is injected intradermally with an antigen prepared from cultured promastigotes of Leishmania spp. This produces a typical cell-mediated response. After 48 hours we measure the induration around the injection site. A ruler is used, and there are cutoff values to determine whether the patient has been exposed to Leishmania or not.
    - This test is used for screening, but it does not give a definitive diagnosis.
    - Immunologically wise, we are testing for a delayed type 4 hypersensitivity reaction.
    - This is similar to the screening done for Mycobacterium Tuberculosis (TB PPD test)





- Histologic examination by biopsy from tissue to demonstrate the presence of organism in the tissue.

Therapy

- The patient response varies depending on the Leishmania species and type of disease.
- In simple cutaneous leishmaniasis lesions usually heal spontaneously. Drugs are prescribed for cosmetic purposes.
- Drugs are prescribed for mucocutaneous and visceral Leishmaniasis.
- Antimony sodium stibogluconate is the drug of choice for the treatment of visceral and mucocutaneous leishmaniasis.
- Pentamidine can also be used. It is used in endemic areas as a drug of choice for serious visceral leishmaniasis.

Prevention