Sheet No.
2

HLS MICROBIOLOGY

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Haemoflagellate

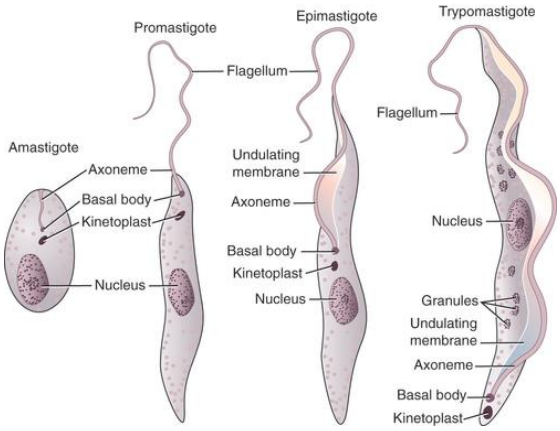
Trypanosoma & Leishmania

- ❖ This lecture is about blood flagellates, they multiply **asexually** and they move by **flagella** as their organ of locomotion.
- ❖ 2 species:

I. Trypanosoma: causative agent of "Trypanosomiasis", (African sleeping sickness) and (Chagas' disease or American Trypanosomiasis).

II. Leishmania: causative agent of "Leishmaniasis".

General concepts about their morphology:



- These haemoflagellates belong to a class called "Kinetoplastida" they contain this special structure (kinetoplast) which is a DNA containing structure you can consider it as the mitochondria of protozoa and it is the origin of the flagellum, that's why they're called Kinetoplastida.

- This class has four morphological forms:

- Promastigote + Epimastigote are found in the vector (vector mediated diseases similar to Plasmodium).
- Trypomastigote + Amastigote are found in the vertebrate host (humans).

- They poses single nucleus.

- The flagellum while it's inside the protozoa called "Axoneme" after it projects out it becomes "flagellum" or "undulating membrane".

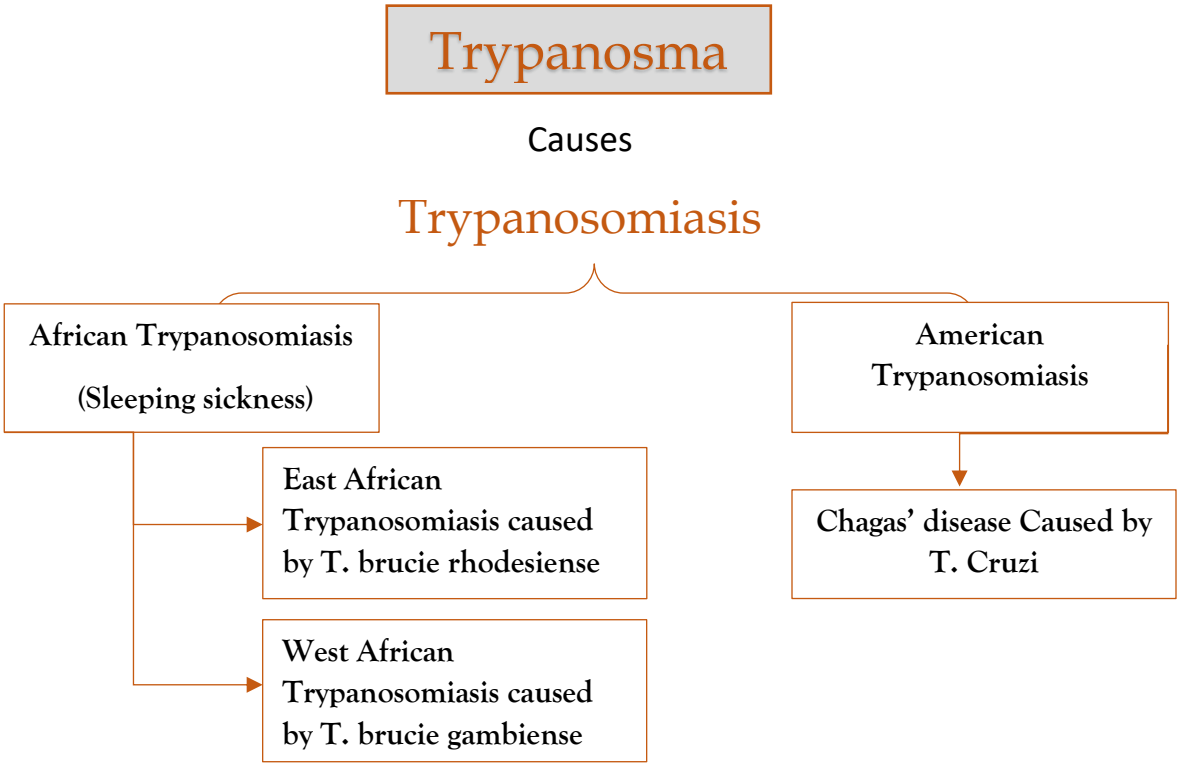
- The most important form -for the doctor at least- is the Amastigote the round intracellular form which is non-motile unlike other forms and it's the diagnostic stage for Trypanosoma as well as Leishmania.

Trypanosoma

- As stated previously they cause Trypanosomiasis which include 2 major types:

- American Trypanosomiasis: causative agent called *Trypanosoma cruzi* and the disease called "American Trypanosomiasis" or "Chagas' disease", occurs in humans and many vertebrate animals in Central and South America.
- African Trypanosomiasis: caused by another species called *Trypanosoma brucei* or *Trypanosoma brucei complex*, and the disease called "African sleeping sickness" which is subdivided into:

- East African Sleeping Sickness caused by *Trypanosoma brucei rhodesiense.*
- West African Sleeping Sickness caused by *Trypanosoma brucei gambiense.*



Morphology

- The morphologically differentiated forms include spindly, uniflagellate stages (trypomastigote, epimastigote, promastigote) and a rounded, amastigote form that exist intracellularly and doesn't poses a flagellum which means it's non motile.

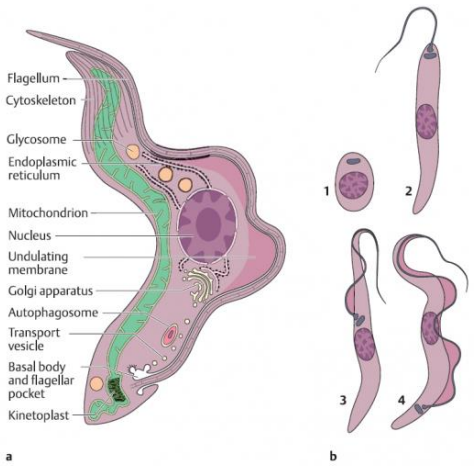
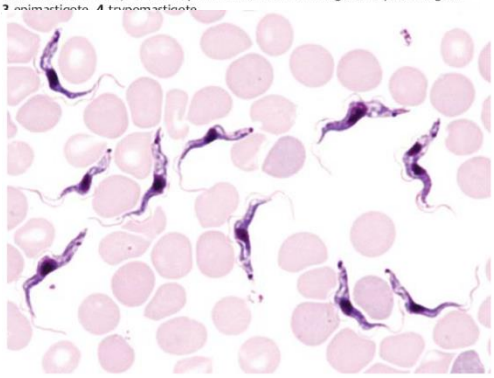


Fig. 9.3 **a** Ultrastructure of *Trypanosoma* (trypomastigotic form) (according to Warren KS ed. *Immunology and Molecular Biology of Parasitic Infections*. 3rd ed. Boston: Blackwell; 1993). **b** Developmental forms: **1** amastigote, **2** promastigote, **3** epimastigote, **4** trypomastigote

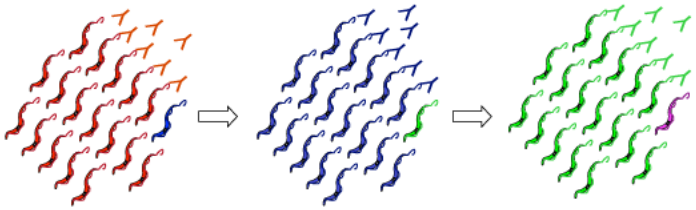
- This is a blood film from African Trypanosomiasis's patient, notice that the protozoa are extracellular unlike Plasmodium and Babesia which were discussed in the last lecture



- in Chagas' disease (American Trypanosomiasis) it's possible to see the protozoa intracellularly but they will be inside cardiac muscle cells not RBCs while the <u>African Trypanosomiasis is always extracellular.</u>

❖ 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.



-The trypomastigote surface is covered with a dense coat of variant surface glycoprotein (VSG).

- Each time the antigenic coat changes, the host does not recognize the organism and must mount a new immunologic response.

Antigenic variations causes cyclic fluctuations in parasitemia levels which affects trypomastigote's detection in blood, they become hard to be detected in blood.

❖ African Trypanosomiasis

-Is caused by 2 sub spp.:

T. brucei gambiense: West African trypanosomiasis → more chronic but not as fatal as East African Trypanosomiasis

T. brucei rhodesiense: East African trypanosomiasis → more acute, more progressive and more fatal.

- Vector: tsetse fly (Glossina spp.) there are more than 50 types of Glossina that transmit Trypanosoma, which is found only in rural Africa

- Glossina palpalis transmits T. b. gambiense

- Glossina morsitans transmits T. b. rhodesiense

Although these blood borne infections are mainly vector mediated diseases but don't forget the possibility of congenital transmission or transmission through blood transfusions, organ transplantation and sharing syringes between drug abusers.

- ## Epidemiology

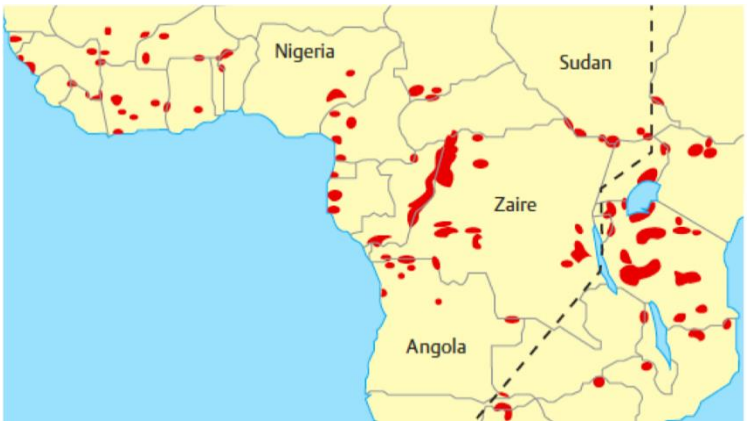
There are epidemiological differences between T. gambiense and T. rhodesiense), the main one being that T. rhodesiense persists in a latent enzootic cycle in wild and domestic animals and is normally transmitted by Glossina from animal to animal, more rarely to humans.

T. gambiense, on the other hand, is transmitted mainly from human to human by the tsetse flies, although various animal species have also been identified as reservoir hosts for T. gambiense strains.

- Differences between East and West African Trypanosomiasis:

	East African Trypanosomiasis	West African Trypanosmiasis
Causative agent	T. brucei rhodesiense	T. brucei gambiense
Vector	Glossina morsitans	Glossina palpalis
Reservoir	Humans and wild animals (Anthroponotic & zoonotic)*	Mainly humans (anthroponotic) but also domestic animals can act as a reservoir
Disease presentation	More acute, fast progression, severe and fatal	Begins subacute, slow progression and not fatal as East African Trypanosomiasis

*from infected human to susceptible human as well as from animal to human that's why it's more severe because it has wide range of reservoir hosts so there will be more variant surface glycoprotein (VSG).

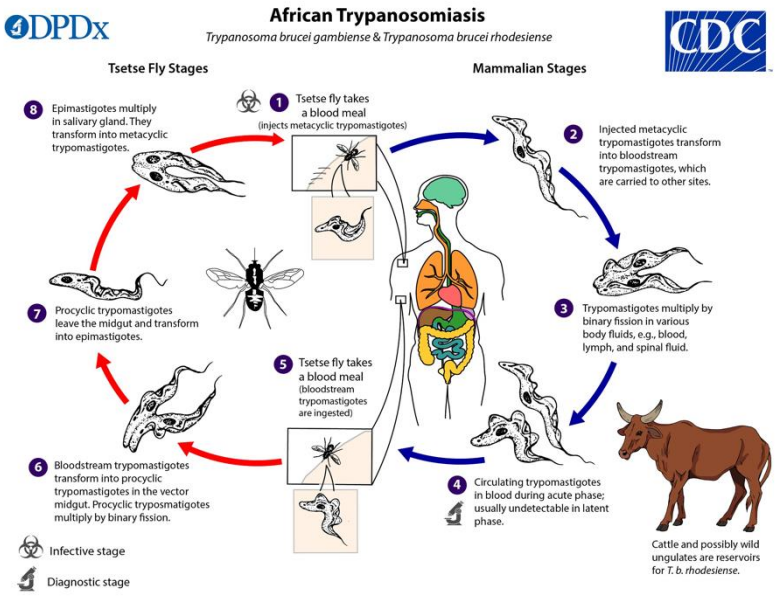


- Notice the dotted line, east to it T. brucei rhodesiense and west to it T. brucei gambiense, first case in the west was in Gambia that's why it's called "gambiense".

- There are no Trypanosomiasis cases in Jordan -unlike Leishmania- in our circumference trypanosomiasis presents in Sudan (West African Trypanosomiasis).

- ## Life Cycle of T. brucei

- In trypanosomiasis both sexes of Tsetse fly can transmit the parasite unlike anopheline mosquito vector of Malaria which is transmitted only through the female because it has to take a blood meal for the maturation of the parasite and this can't be done by the male.



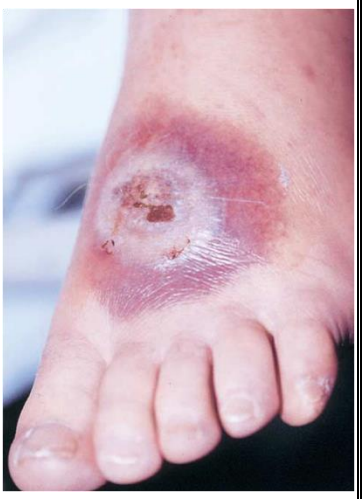
Infective stage: metacyclic trypomastigote, injected by Tsetse fly from their salivary gland.

Then these trypomastigote either head to the lymph nodes or they would be in the blood circulation after that another Tsetse fly takes a blood meal contains trypomastigote and it continues there in the vector where it develops into procyclic trypomastigote → epimastigote→ metacyclic trypomastigote.

Diagnostic stage: trypomastigote in blood (extracellular, there is no intracellular stage in African Trypanosomiasis).

- ## Clinical Features

After the host has been bitten by an infected tsetse fly they will develop after a few days an acute local reaction at the site of the bite known as a nodule or chancre.



* Papule → macule → ulceration → chancre (painless)

Stage I:

Then this trypomastigote would move from the site of the bite to the peripheral circulation, which will lead to systemic trypanosmiasis with manifestations like lymph nodes enlargement (winterbottom's sign)*, hepatosplenomegaly, irregular fevers and night sweats but there are no CNS symptoms in stage I.

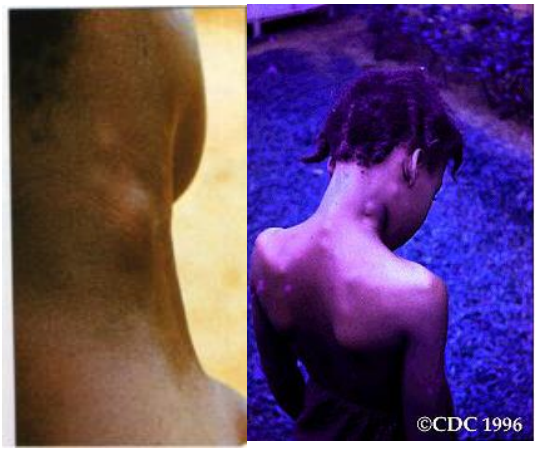
stage I→ Acute→ systemic manifestations **without CNS involvement**.

Stage II:

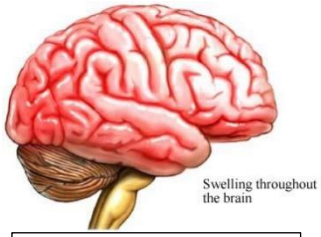
Whenever this trypomastigote crosses brain blood barrier → choroid plexus→ meninges, the patient will enter in stage II of the disease where they will start to show neurological symptoms and signs.

Beginning with meningoencephalitis→ coma (uncontrollable urge to sleep and that's where the name of the disease came from) → death (if it was T. brucie rhodesiense since it's fatal).

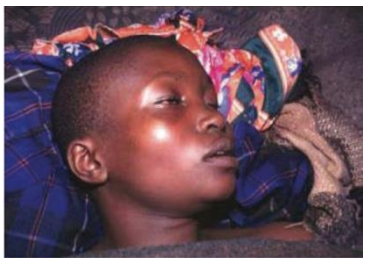
Any neurological sign → Stage II



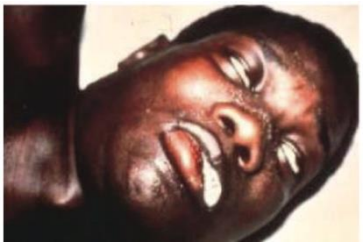
*Winterbottom's sign: a special sign in African Trypanosoma patients in stage1 where there will be enlargement of the posterior cervical lymph nodes.



Encephalitis



Coma



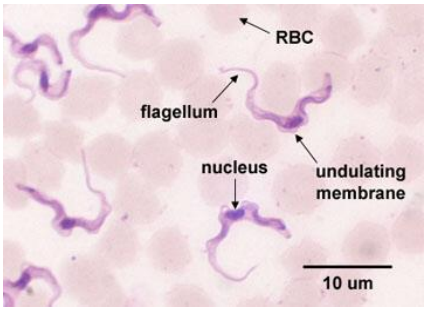
Death

Stage II: The patient becomes emaciated and progresses to profound coma and death

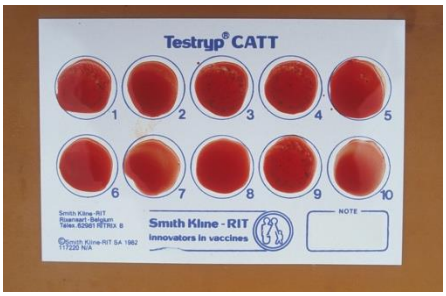
- Laboratory Diagnosis

- Specimen: blood, serum, CSF, aspiration from lymph node or chancre.You'll look in the microscope for trypomastigote (diagnostic stage) but they're not easily found sometimes you have to perform concentration techniques.

- Routine Methods: thick and thin blood films

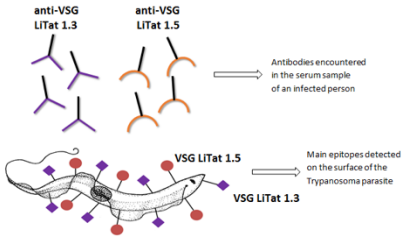


- Antigen Detection: simple and rapid test card indirect agglutination test (CATT) and it's highly sensitive especially in endemic regions.



  - In CATT there will be latex beads contain antibody against Trypanosoma antigen, you mix them with serum or blood sample of the patient if there's agglutination it means that Trypanosoma is present in the patient's blood.

-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 routinely used.

• Therapy

- usually patients in stage1 get well after treatment, but stage2 has bad prognosis and shortened life expectancy, so anti parasitic drug selected depends on whether the CNS is infected.

- All drugs used in the therapy of African trypanosomiasis are toxic and require prolonged administration

- **Suramin** or **pentamidine** isethionate can be used when the CNS is not infected (**stage1**).

-**Melarsoprol**, a toxic trivalent arsenic derivative can cross blood brain barrier, is effective for both blood and CNS stages but is recommended for treatment of late-stage sleeping sickness (**stage 2**).

- Recently Trypanosomiasis patients have been given DFMO (resurrection drug) and the results seems good.

- ## Prevention

1. Preventing flies from biting through the use of insecticide, repellents, bed nets and protective clothes will reduce the transmission of the parasite.

2. Screening of people at risk helps identify patients at an early stage.

3. Treatment cases and should be monitored for 2 years after completion of therapy.

- There were trials to stop off-spread Tsetse fly by genetics techniques to decrease their population but they didn't continue with this approach.

❖ American Trypanosomiasis

-Caused by Trypanosoma cruzi (chagas' disease)

- Zoonosis

- Transmitted by vector: reduviid bugs or triatomine bug or kissing bug (because it bites the host's face mostly).

Reduviid bug defecates while taking a blood meal, it does bite the host like Tsetse fly but doesn't inject the infective stage it rather leave it in their feces. The feces of the bug causes allergy so the host might itch or rub their skin then the trypomastigote (infective stage) might get access to the blood through the bite wound and if it was in the face might get in through the conjunctiva causing (Romana sign).

- Definitive host:

Human, dog, cat, rats…etc.

- Habitat in the Definitive host:

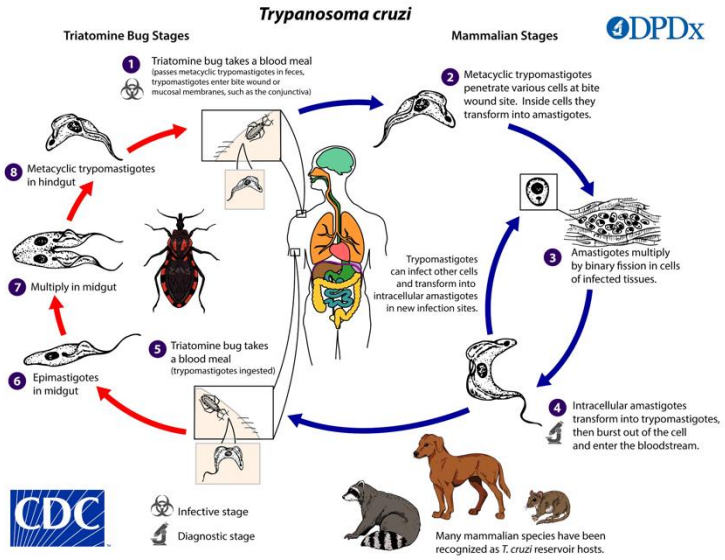
Trypomastigote in blood

Amastigote in tissue, found intracellularly mostly in cardiac muscle cells but can be anywhere also like liver or brain cells.

- ## Epidemiology

- Endemic in Central and South American

- ## Life Cycle of T. Cruzi



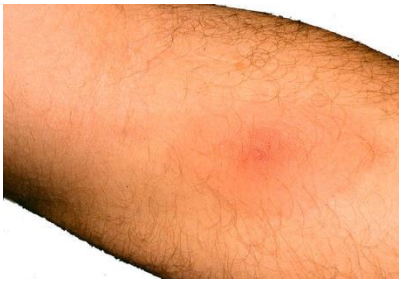
- In the life cycle is similar to the African Trypanosoma with some differences in their:

1- Mechanism of infection: reduviid bug defecates and then the infective stage will get access to the host's body through the ways mentioned previously not injected.

(infective stage: metacyclic trypomastigote)

2- Diagnostic stage: here it could be trypomastigote in blood in the acute phase or it could be amastigote intracellularly.

- ## Pathogenesis



- In T. Cruzi near the site of the bite and the site where feces get access to the body after it becomes itchy it develops into something called "Chagoma".

First there will be erythema→nodule chagoma (which is a raised red lump surrounded by hard edema "induration"), you can't depend on its color only to diagnose the induration you should also palpate it to feel the hardness of it because it's not only erythema.

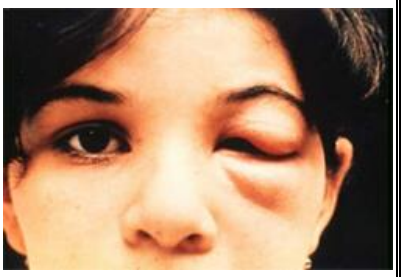
- Chagas' disease are categorized as acute, indeterminate, and chronic

- In the acute stage trypomastigote (trypomastigoyte) exists in the blood.

- But if the patient reaches the chronic stage, amastigote would be intracellularly inside tissues' cells mainly cardiac muscle cells.

- The incubation period in humans is about 7-14 days.

- Acute Phase



- Start 1 week after infection.

- Irregular fever and night sweats

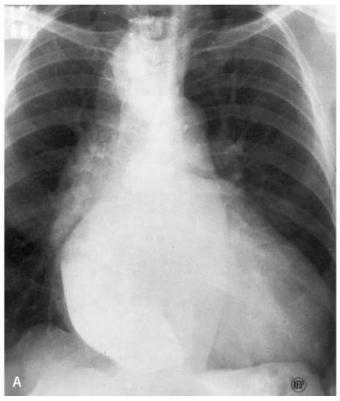
- Lymph node enlargement

- Enlarge liver and spleen

- Unilateral swelling of eyelids romana's sign, in case the trypomastigote got acces through mucus membranes mostly conjunctiva.

- Acute myocarditis, this is inflammatory process due to the presence of parasite in the blood not related to the chronic stage where amastigote gets intracellularly inside cardiac cells.

- Chronic Phase



- Parasite lives intracellularly in amastigote form and their most preferable site is the cardiac muscle, so to diagnose the chronic phase you have to take a biopsy from the heart muscle to look for amastigotes.

- Disturbance in holo organs' functions (including the heart)

- Most frequent clinical signs of chronic Chagas' disease involve the heart, where enlargement of the heart, including cardiac changes

-Most frequent clinical signs of chronic Chagas' disease is arrhythmia,

signs involve the heart, where enlargement of the heart , including cardiac changes.

-Enlargement of the colon and esophagus.

Each myocyte has amastigote inside it becomes dysfunctional so the heart tries to compensate by enlargement of other healthy cells that's why it becomes enlarged.

- Patients die because of functional disability, their life expectancy and productivity get shortened significantly.

- ## Therapy

- 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 the cases of congenital transmission, but still an unknown proportion of the treated patients develop into the chronic phase despite the treatment.

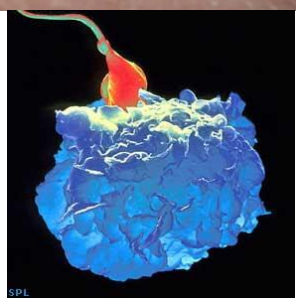
Those that develop the chronic phase have take additional medications to treat arrhythmias, hypertension and heart failure.

- ## Prevention

1. Vector control.
2. Transfusion control and screening of blood donors.
3. testing of organ, tissue or cell donors and receivers.

* Another possible mode of transmission is through ingesting contaminated food but it's debated whether to consider it as a route of transmission or not because it's very rarely to happen

Leishmania



- It is a flagellated protozoan.

- Common in our region especially cutaneous Leishmaniasis, and endemic in Syria, Iraq and Iran.

- Vector mediated infection, life cycle requires two hosts:

a) vertebrate ; mammalian host

b) Invertebrate vector; female sand fly (Phlebtomus)

C) reservoir host ; dogs and wild foxes.

- Sand fly does exist in Jordan specifically Jordan valley.

- Obligate intracellular organism, as soon as the infective stage (promastigote) enters the body gets engulfed by macrophages, inside the macrophages it becomes amastigote. It infects primarily phagocytic cells and macrophages.

- The incubation period ranges from 10 days to 2 years.

• Leishmania Species

- Leishmaniasis is divided into clinical syndromes according to what part of the body is affected most.

1. Cutaneous Leishmaniasis (L.tropica, Leishmania major), affects the skin only.

↳Other names for cutaneous leishmaniasis: Baghdad boil, oriental sore and Aleppo sore.

2. Mucocutaneous leishmaniasis (L. braziliensis -old world- **and L. mexicana)**

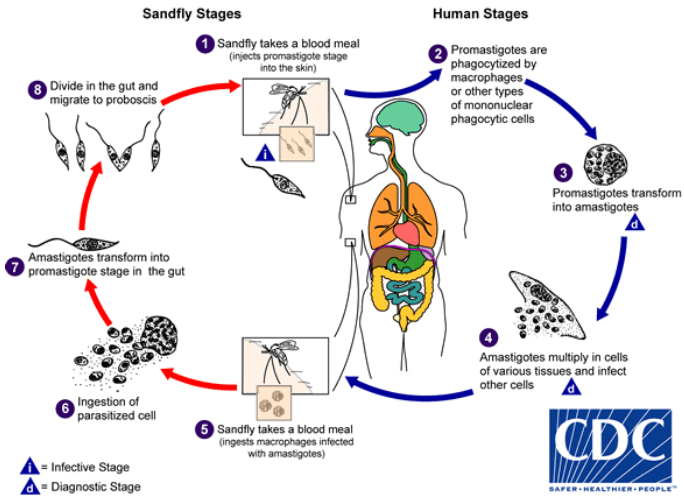
↳Other names: nasopharyngeal leishmaniasis and espundia, and found in South America.

3. Visceral Leishmaniasis(L.donovani).

↳Also called “kala azar” which means “black fever”, most dangerous form found mainly in India, Pakistan and Bangladesh.

- **Leishmania infantum**; it’s common in our region and a part of L. tropica complex so originally causes cutaneous leishmaniasis but might progress to visceral leishmaniasis.

- Life cycle of Leishmania



- Since they're haemoflagellates their life cycle is similar to those of African and American trypanosomiasis.

- Sand fly bites the host and inject promastigote into the skin.

*Infective stage: promastigote

-Then this promastigote get engulfed by a macrophage where there it becomes amastigote.

*Diagnostic stage: amastigote; round intracellular form.

- Transmission

1. Bite of sand fly
2. Transfusion blood, transplantation and sharing syringes
3. Mother to baby
4. Direct contact; from man to man through nasal secretions (in espundia) but very rarely.

- Cutaneous Leishmaniasis : Leishmania tropica, L major, L infantum

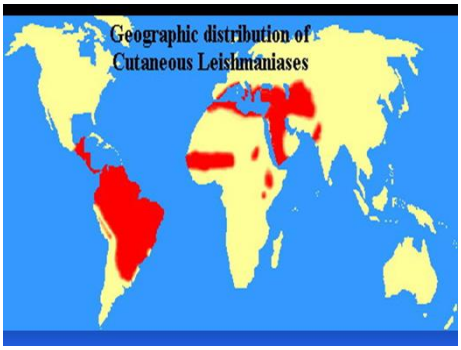
- Most common form of leishmania and common in our region

- Habitat (affected part of the body): skin

- Disease: Cutaneous leishmaniasis

- Clinical feature : first sign is a lesion (generally a firm, The lesions begin as reddish , soft itchy papular , gradually enlarges ,raised and firm , with serous discharge at the bite site.

↳Lesions will ulcerate later on initiating a granulomatous reaction then leave scars.



- Epidemiology: the Middle East, south America

• Leishmania in Jordan

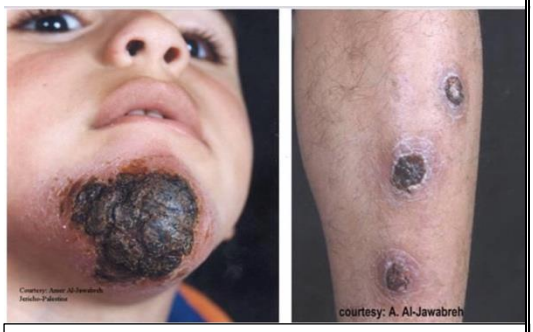
- In Jordan there are several species of Leishmania; Leishmania infantum, Leishmania tropica, and Leishmania major.

- Leishmania major is the major species of Leishmania parasite in Jordan .

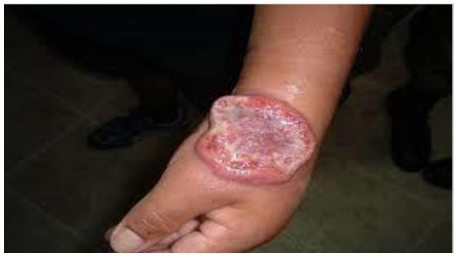
• Stages of Cutaneous Leishmaniasis

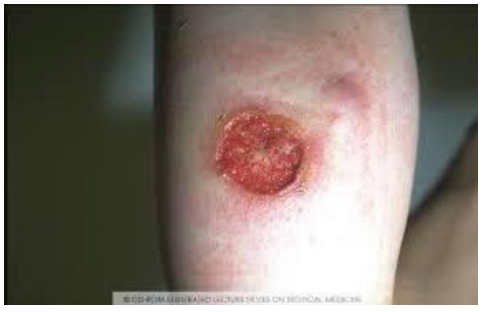
Lesions start as macule→ papule→ ulceration→ granulmatous reaction.

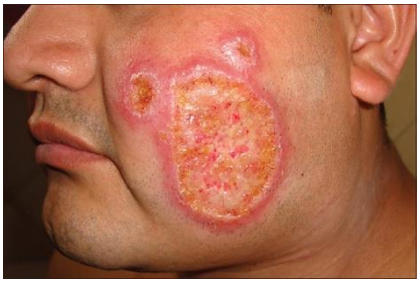
- Lesions are painless but itchy and most of them heal spontaneously -depending on the patient's immune status- if not, we give patients antimoniate compounds, sodium stibogluconate but results are unsatisfactory especially if it was for cosmetic reasons.



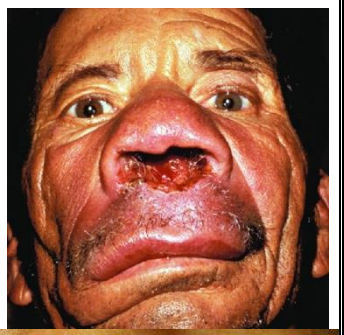
This might be the last stage↑

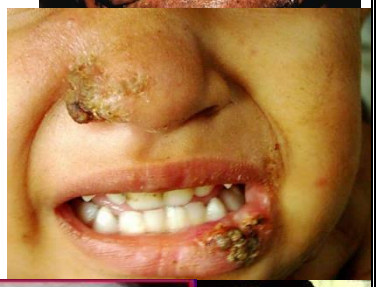


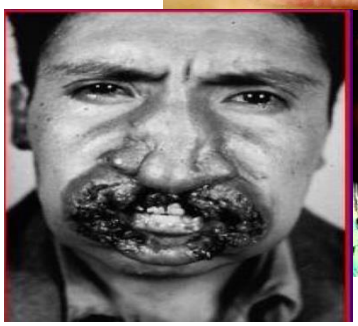




- Mucocutaneous leishmaniasis(L. braziliensis)







- These mucosal lesions do not heal spontaneously, and secondary bacterial infections are common and may be fatal.

- There will be destruction of the nasal septum involving cartilage but not the bones.

- It might progress into visceral leishmaniasis.

- Visceral Leishmaniasis(L.donovani).

- Is the most severe form of leishmaniasis

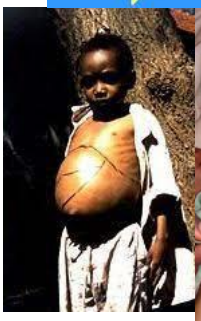
- The parasite migrates to the internal organs such as the liver, spleen (hence "visceral"), and bone marrow

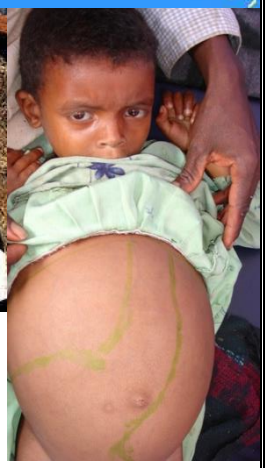
- The incubation period : 10 days to 2 years, usually

- 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, will almost always result in the death of the host

Epidemiology: Bangladesh, Brazil, Ethiopia, India, South Sudan and Sudan.





- Special sign in visceral leishmaniasis patients >> abdominal distention, dark pigmentation of skin of the abdomen and they become anaemic as well.

- Some of patients after recovery from visceral leishmaniasis they develop cutaneous leishmaniasis, we call this condition Post Kala-azar Dermal Leishmaniasis (PKDL).

• Laboratory Diagnosis

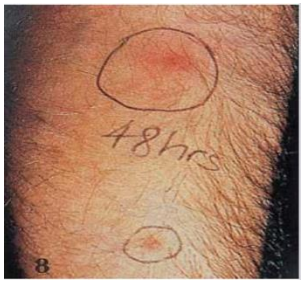
1) Stained blood smear: aspiration, scraping

2) Cultured: cultured using special techniques; Triple N medium (NNN)

3) ELISA, IFA or direct agglutination give useful indication of active or recent kala-azar .

4) PCR methods have excellent sensitivity and specificity for direct detection

5-Intradermal Montenegro test:

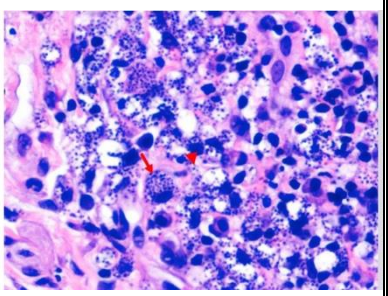


*Injection of intradermal antigen prepared from

cultured promastigotes (infective stage) of Leishmanian spp .

*This produces a typical cell-mediated response results in induration after 48 hrs and as we said previously induration isn't only erythema.
—The test doesn't tell whether the patient is currently infected with Leishmania or from a past exposure, it only gives an indication that the patient's immune system recognized Leishmania (if the test results were positive).

(Type IV hypersensitivity reaction)



6-Histologic examination by biopsy from tissue to demonstrate the presence of organism (amastigote) in the tissue.

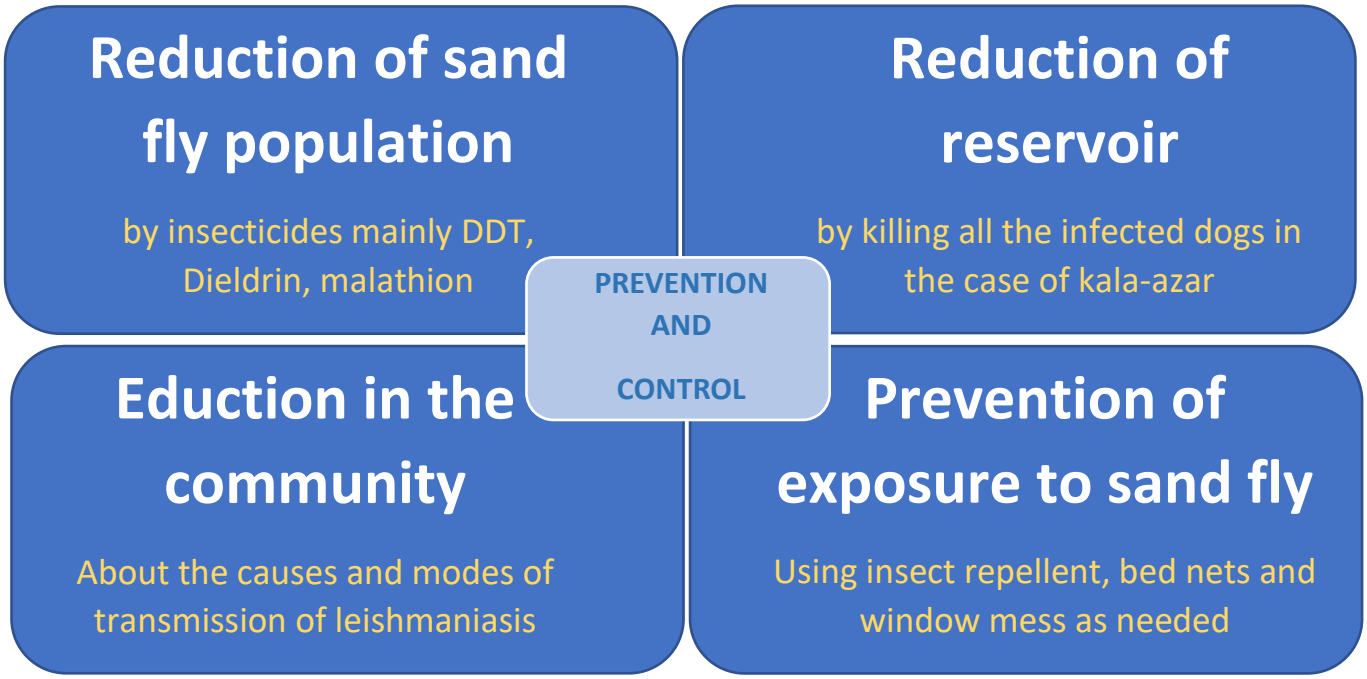
- Therapy

- The patient response varies depending on the Leishmania species and type of disease.

- In simple cutaneous leishmaniasis, lesions usually heal spontaneously but it takes months but some conditions might require treatment

- Antimony, sodium stibogluconate drugs of choice for the treatment of visceral and mucocutaneous leishmaniasis but results are unsatisfactory.

- Prevention



There are **NO Vaccines** to prevent leishmaniasis