Microbiology - HLS

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Haemflagellate

TRYPANOSOMA & LEISHMANIA

The agents for Trypanosomiasis and Leishmaniasis

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Haemflagellate

Trypanosoma, leishmania

- Plasmodium come from a class called sporozoan and a special characteristic in that class is that they alternate between sexual and asexual reproduction.
- These agents are from the asexual reproducing type and they are from the flagellates because they have flagella.
- These two flagellated organisms are called kinetoplastida because they contain kinetoplasts which are DNA structures present in the protozoa and this is the origin of the flagella. It represents the single mitochondrion present in the protozoa.
- If it was mature, such as in the case of the trypomastigote, they have an undulating membrane that is connected to the body and continues as the flagella
- There are four different developmental stages; amastigote, promastigote, epimastigote, and trypomastigote.
- The amastigote occurs intracellularly, it doesn't have flagella it is called the round intracellular form.

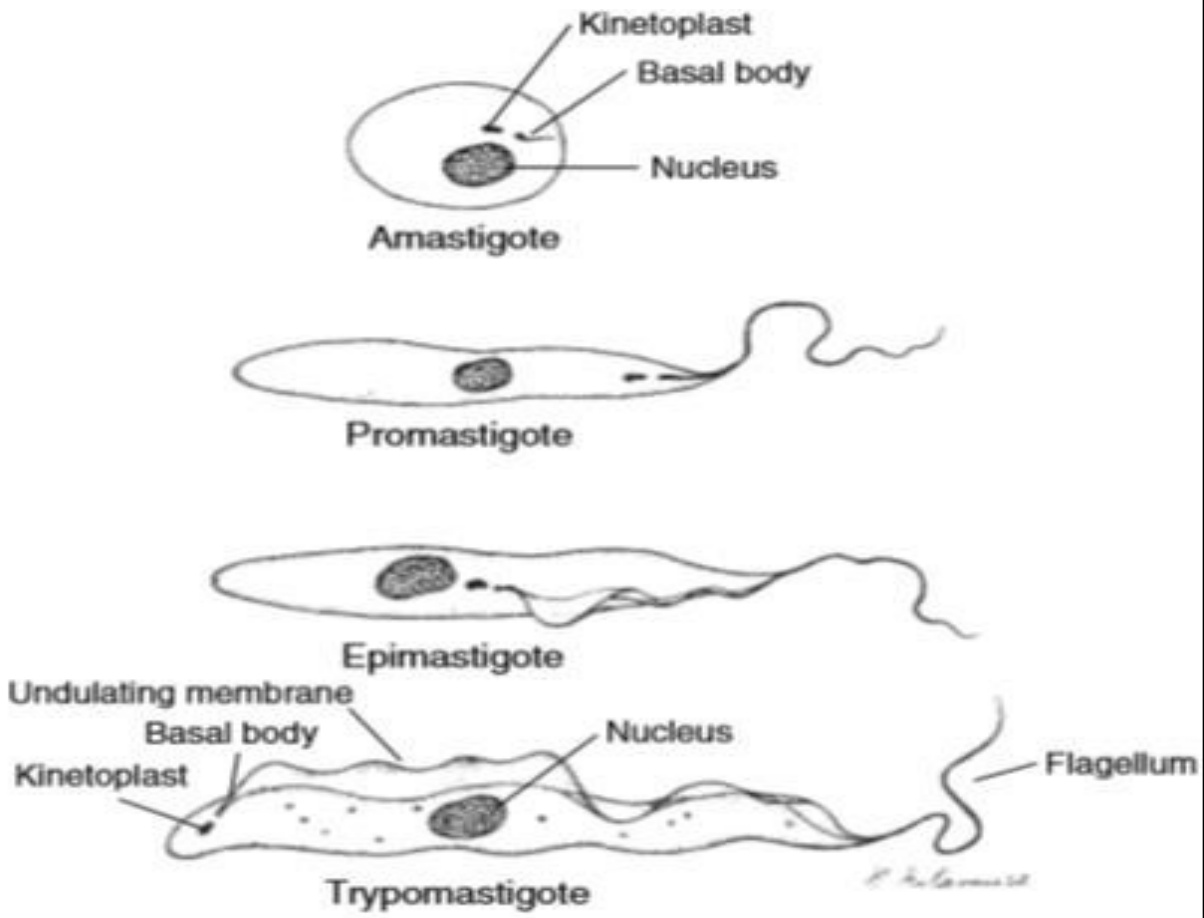


Figure 49-8 Characteristic stages of species of *Leishmania* and *Trypanosoma* in human and insect hosts. (Illustration by Nobuko Kitamura.)

Trypanosoma

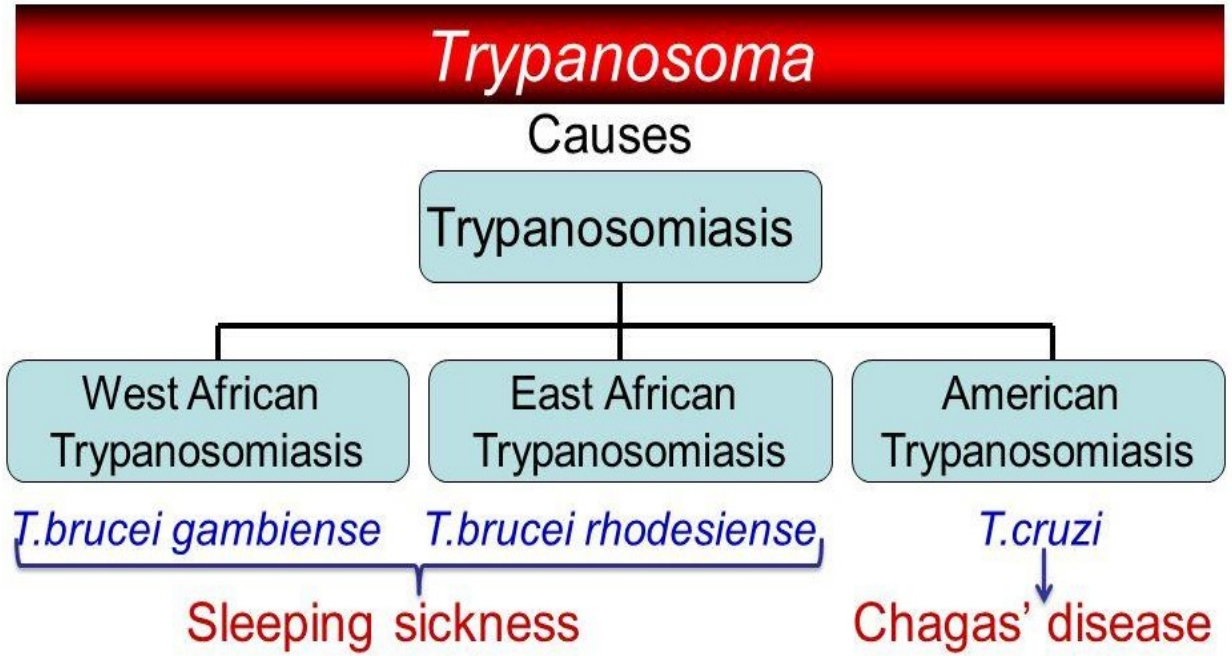
- Causative agents of African trypanosomosis (sleeping sickness) and American trypanosomosis (Chagas disease), they're very different from each others .

- Trypanosoma brucei gambiense and Trypanosoma brucei rhodesiense cause African trypanosomosis (sleeping sickness) in humans.
- Trypanosoma cruzi, the causative agent of American trypanosomosis (Chagas disease) occurs in humans and many vertebrate animals in Central and South America.

TRYPANOSOMA

African trypanosomiasis : African sleeping sickness

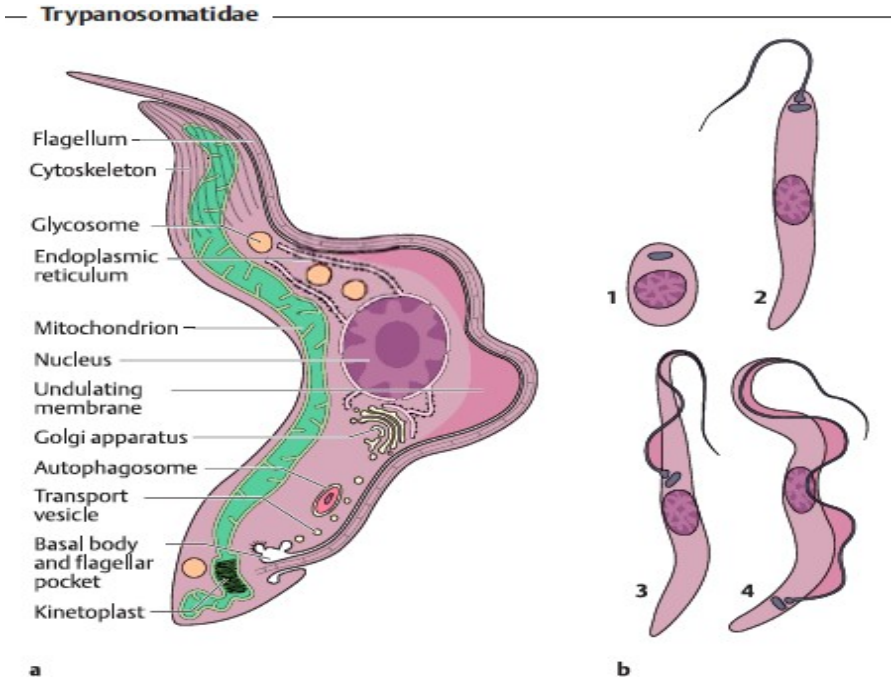
American trypanosomiasis (Chagas' disease)

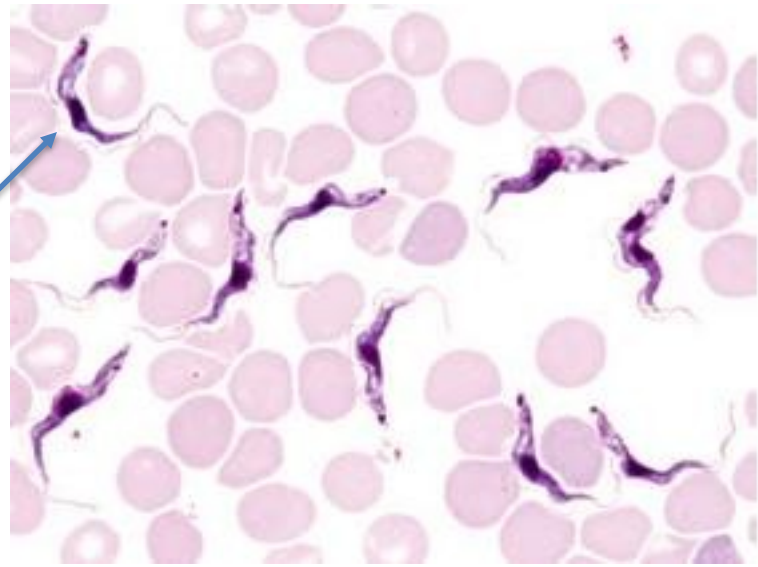


Morphology

- The morphologically differentiated forms include spindly, uniflagellate stages (trypomastigote, epimastigote, promastigote) and a rounded, amastigote form.

- The epimastigote and the promastigote of Trypanosoma are usually present in the vector, and in the human, we can find the trypomastigote or the amastigote if the is presence of the intracellular form.

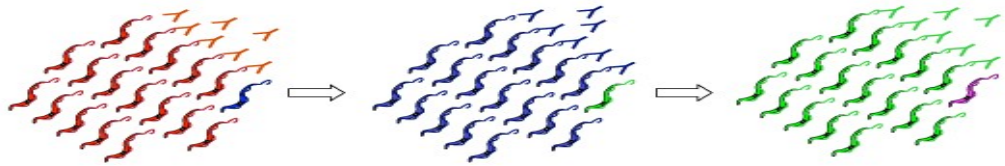




This is **African** trypanosomiasis, and this is the trypomastigote, as you can see it is present extracellularly, between the cells. It is not present intracellularly.

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)
- Every time the immune system recognizes the antigen, the Trypanosoma undergo cyclic fluctuation of their VSG, and this helps them evade the immune system.
- Each time the antigenic coat changes, the host does not recognize the organism and must mount a new immunologic response
- If you take a blood sample, it depends on the time of sample. In blood-borne infections, it is very important to take blood samples repeatedly every certain amount of time, for example every 2-4 hours, in order to find the protozoa, you need to confirm your diagnosis.



AFRICAN TRYPANOSOMIASIS

- Is caused by 2 sub spp. :
- T. brucei gambiense: **West** African trypanosomiasis

Slowly progressive, chronic, and less mortality. Its main reservoir is in humans.

- T. brucei rhodesiense: **East** African trypanosomiasis

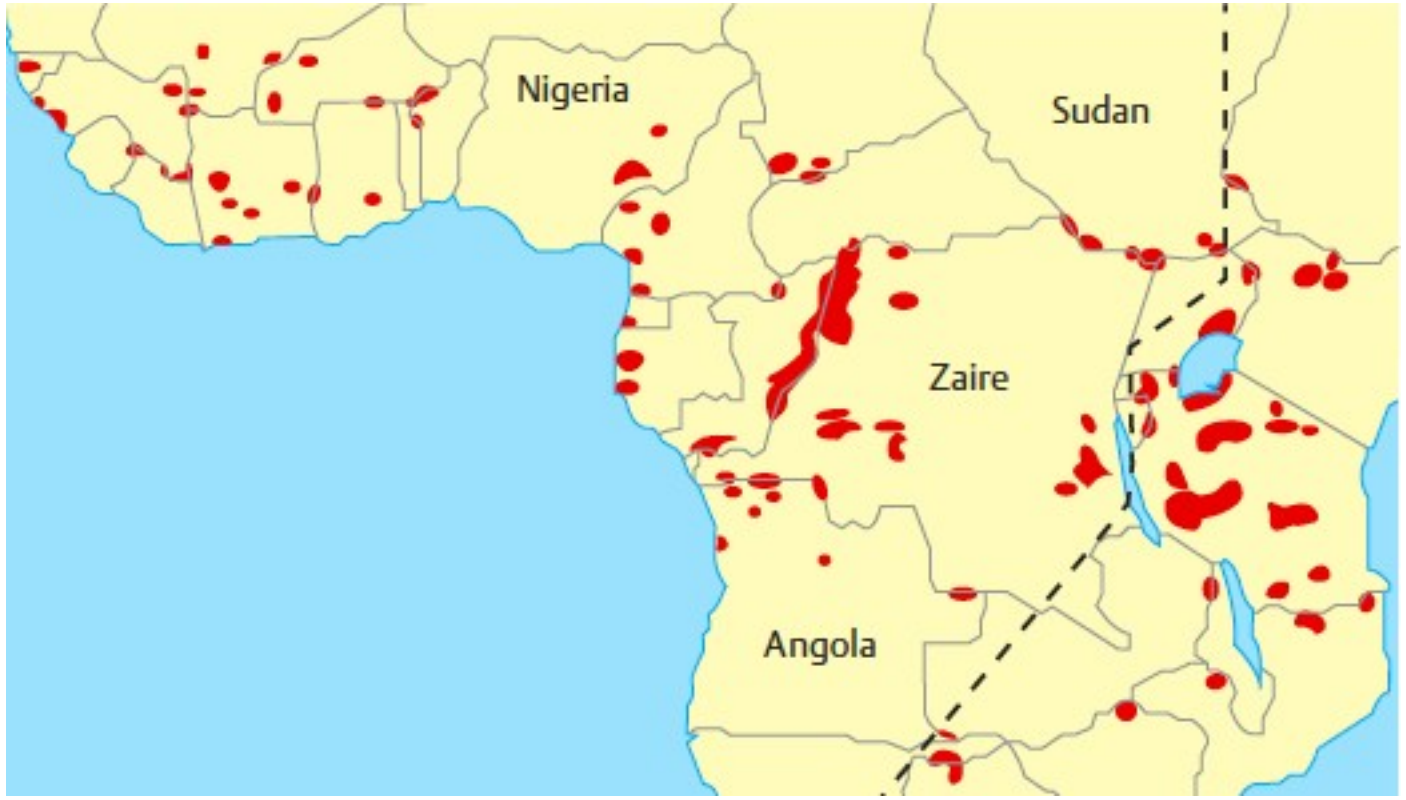
More mortal, acute, rapidly progressing, fatal. Its reservoir is in wild animals, and that's why it's very fatal because of the genetic variations.

- Vector: **tsetse fly** (Glossina spp.) both sexes of the fly can transmit the disease
- Which is found only in rural Africa
- Glossina palpalis transmits T. b. gambiense
- Glossina morsitans transmits T. b. rhodesiense

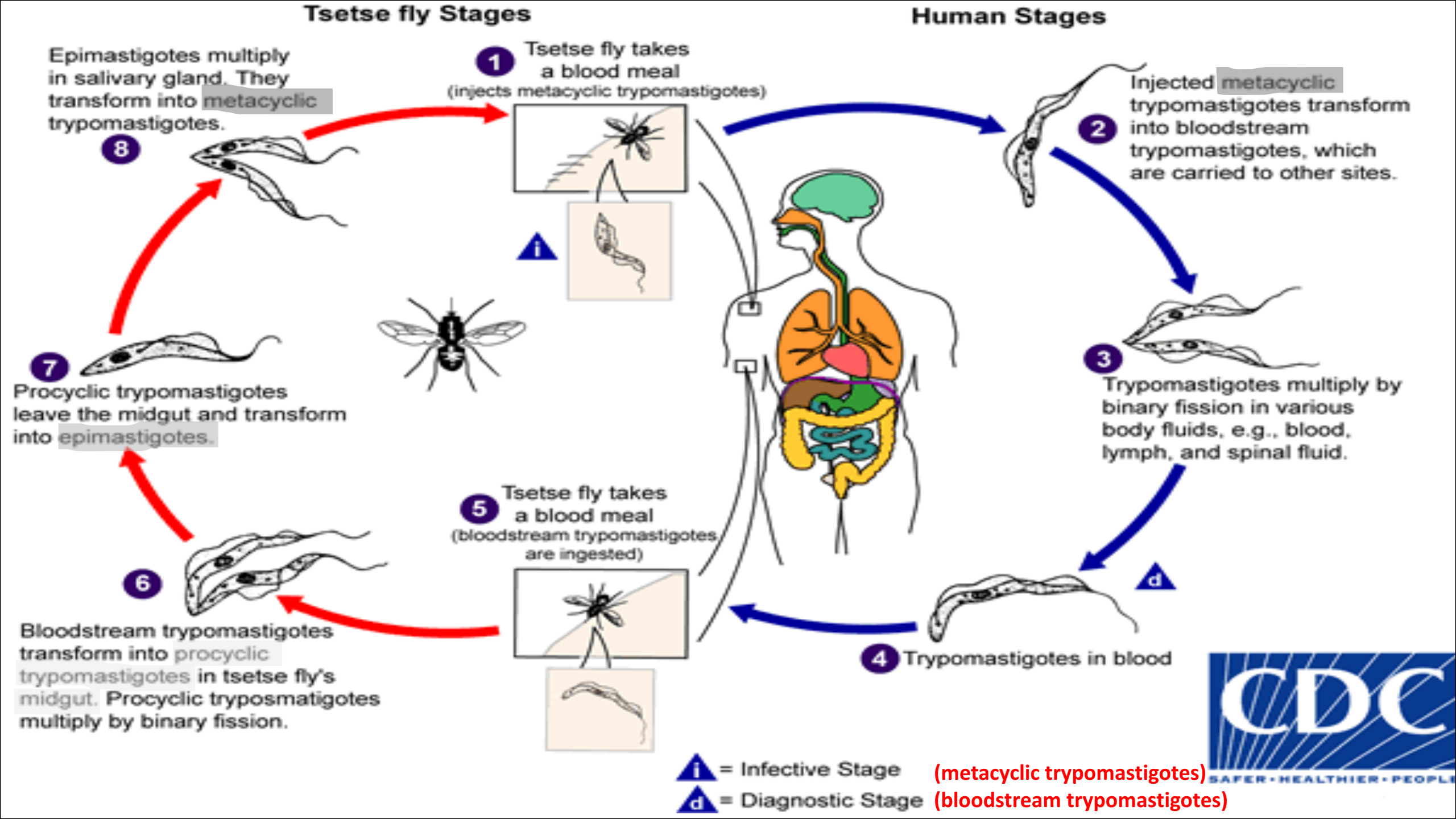
Epidemiology.

- This is the African disease.
- 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.

Epidemiology



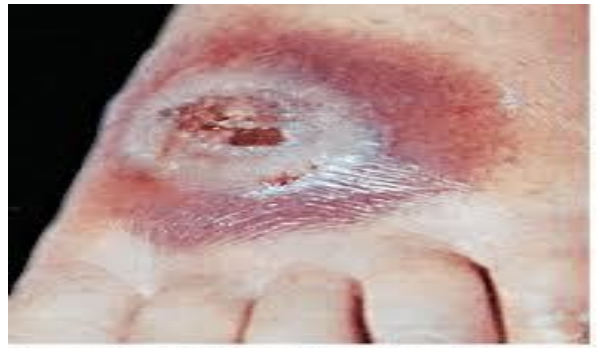
This zig-zag line is the line that splits the distribution of east and west African trypanosomiasis.


Tsetse fly Stages
Human Stages
Epimastigotes multiply in salivary gland. They transform into metacyclic trypomastigotes.
8
1 Tsetse fly takes a blood meal
(injects metacyclic trypomastigotes)
i
2 Injected metacyclic trypomastigotes transform into bloodstream trypomastigotes, which are carried to other sites.
3 Trypomastigotes multiply by binary fission in various body fluids, e.g., blood, lymph, and spinal fluid.
d
4 Trypomastigotes in blood
5 Tsetse fly takes a blood meal
(bloodstream trypomastigotes are ingested)
6
Bloodstream trypomastigotes transform into procyclic trypomastigotes in tsetse fly's midgut. Procyclic tryposmatigotes multiply by binary fission.
7
Procyclic trypomastigotes leave the midgut and transform into epimastigotes.
i = Infective Stage (metacyclic trypomastigotes)
d = Diagnostic Stage (bloodstream trypomastigotes)
CDC
SAFER • HEALTHIER • PEOPLE

- The most important thing to know is the infective and the diagnostic stages.
- The tsetse fly comes and injects the **metacyclic trypomastigote** and this is the infective stage, this trypomastigote gets access to the peripheral blood circulation and it tries to reach other body fluids, such as lymphatic and CSF.
- The diagnostic stage is also the presence of the **trypomastigote in the blood.**
- If another tsetse fly comes to take a blood meal from an infected person, the cycle continues inside the fly. The trypomastigote becomes a procyclic trypomastigote in the midgut and in the hindgut, it turns into an epimastigote which then turns into a metacylic trypomastigote in the salivary glands which is the infective stage.

Trypanosoma brucei gambiense

- **Clinical feature:**
- After the host has been bitten by an infected tsetse fly, **a nodule or chancre** at the site may develop after a few days, on the bite site, a local reaction.



• This is a chancre, it is like an ulcer and it is present at the location of the bite of the tsetse fly and this is where they insert the infective stage protozoa. It could be a chancre or a nodule (a raised papule)

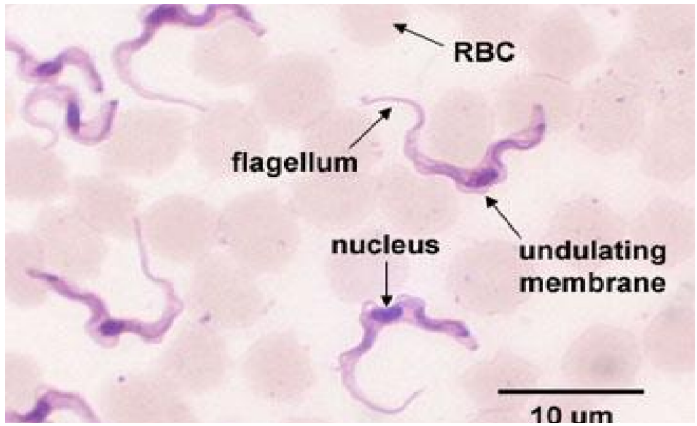
- **stage I:** the patient have systemic trypanosomiasis without CNS involvement. It represents the parasitemia, the parasite is present in the blood.
- The trypomastigotes enter the bloodstream and invade the lymph nodes, and it causes their enlargement.
- The first symptoms appear and include: irregular fevers with night sweats, enlargement to liver and spleen, Winterbottom's sign; posterior cervical lymph node enlargement.

Winterbottom's sign

- **Stage II**: organisms invade the CNS, they get access to the spinal fluid, the traverse the coroidal plexus until they reach the brain, the sleeping sickness stage of the infection is initiated. Therapy differs and the prognosis becomes much worse.
- The patient shows change in character and personality, there is stuttering, slurring of speech, drowsiness, dizziness, seizures, encephalitis and coma.
- Before it reaches the stage of coma and death, the patients have an uncontrollable urge to sleep and that it is why it is called the sleeping sickness.
- The patient becomes emaciated and progresses to profound coma and death

Laboratory Diagnosis

- The microscope is the easiest and cheapest tool for diagnosis in these (African) countries, but it not always easy to find the trypomastigote in the field of the microscope, it depends on the patient and the infecting species and the expertise of the lab technician or doctor so it differs.
- **Specimen: blood, serum, CSF** (if the patient has reached stage II), **aspiration from lymphnode**
- **Routine Methods**: thick and thin blood films
- There are also antigen rapid tests, antigen detecting kits, that are present for trypanosomiasis and the most common one is the CATT test which stands for Card indirect agglutination trypanosomal test. (on google it was Card Agglutination Test with Stained Trypanosomes… idk). The WHO usually gives out these tests to poor countries, so they detect and diagnose trypanosomiasis in Africa. (Check out next slide)



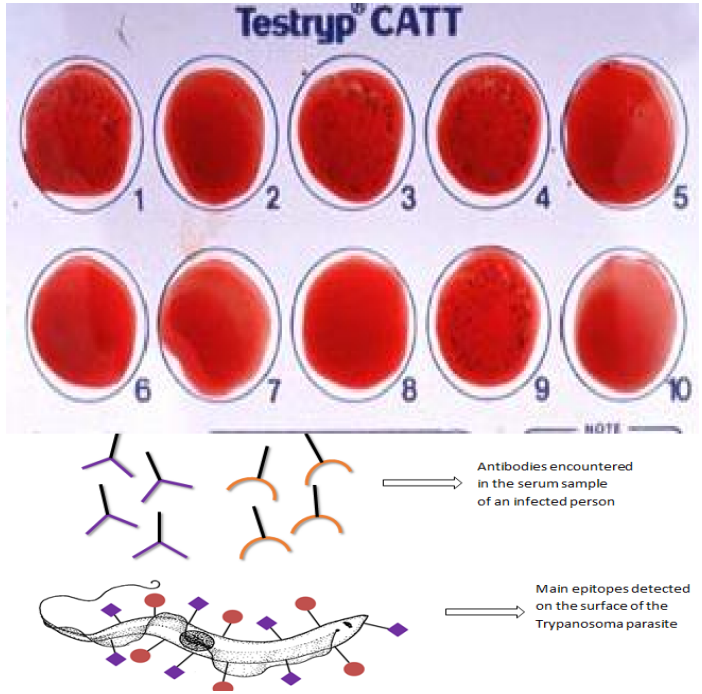
Laboratory Diagnosis

- **Antigen Detection**: simple and rapid test card indirect agglutination

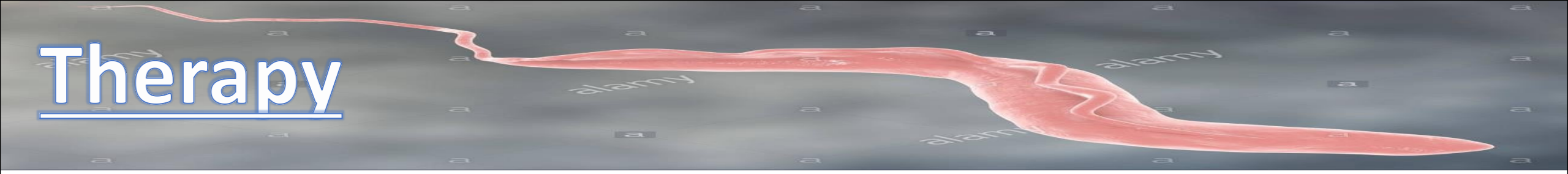
- **Antibody Detection: Serologic** by using ELISA Serum or CSF IgM concentrations

- **Molecular Diagnostics**: **PCR-based** methods to detect infections and differentiate species, but these methods are not routinely used

- But these last two tests are not usually used because the type of machinery required is not available in the countries where the disease is present.



Therapy



- All drugs used in the therapy of African trypanosomiasis are toxic and require prolonged administration
- 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 recommended for treatment of late-stage sleeping sickness. At this point it is too late for the patient.

- These drugs usually don't have many other indications, they are given under strict monitoring and over a long period of time, at least for two years.

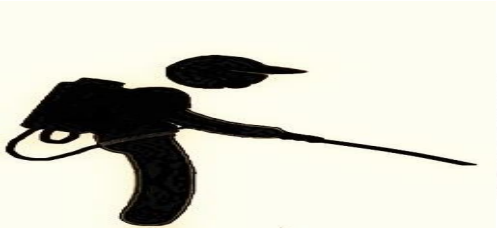
- There is a new drug that was recently FDA approved called **Fluoranthene**, and it is called the resurrection drug.

prevention

- Usually, for vector-borne diseases, all the preventive measures are concerned around preventing the vector from carrying the infecting protozoa to the susceptible host.

1. Preventing flies from biting through the use of insecticide will reduce the transmission of the parasite. Also, the use of repellents and nets.
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 is no chemical prophylaxis and no vaccine.



AMERICAN TRYPANOSOMIASIS

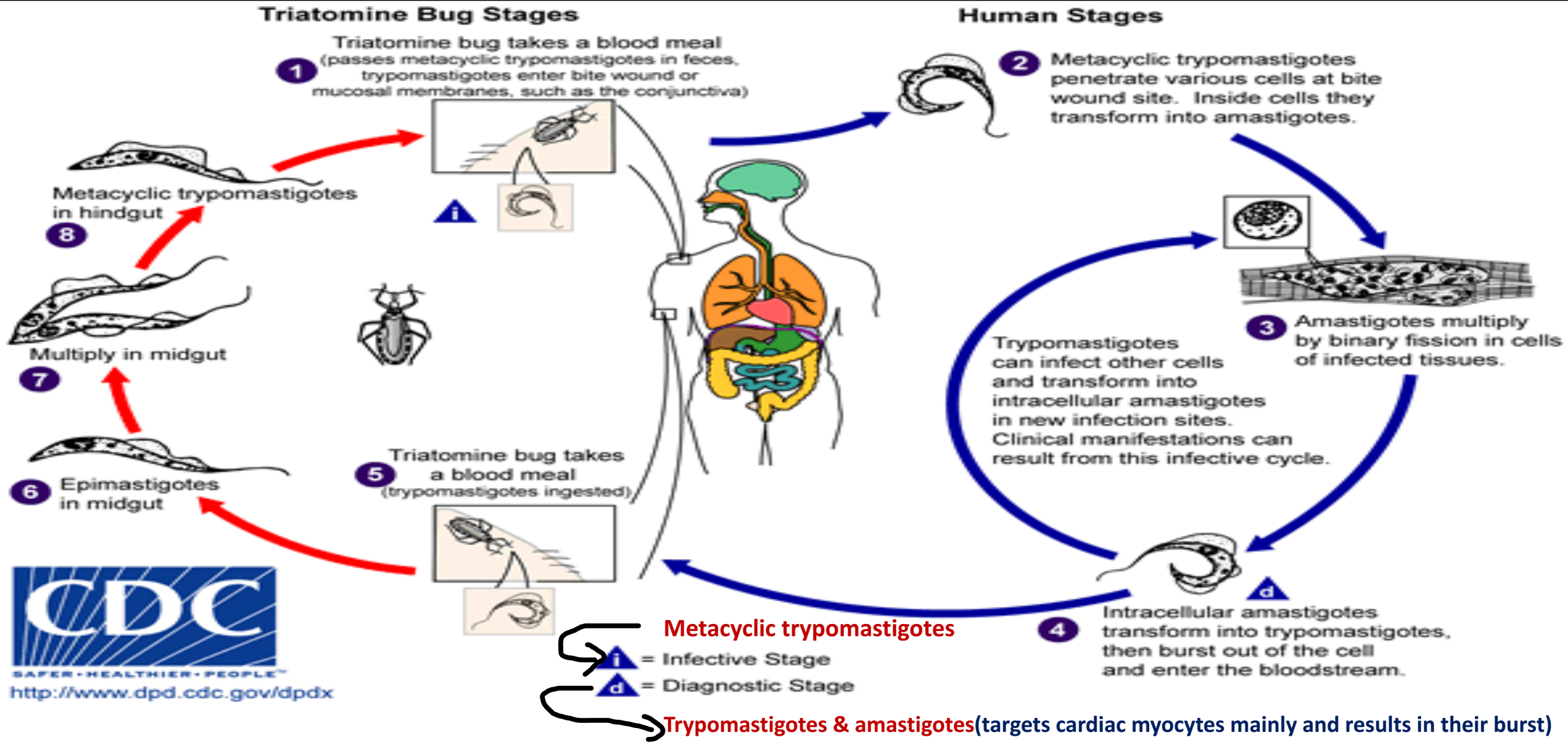
- Trypanosoma cruzi **(Chagas' disease)**
- Zoonosis
- Transmitted by vector : reduviid bugs, kissing bug (u will know why later), tritomine/triatoma
- Reduviid bug defecates while taking a blood meal

NOTE: here u have an intracellular form (amastigote)

- **Definitive host:**
- Human, dog, cat, rats…etc.
- **Habitat** in the Definitive host:
- Trypomastigote in blood
- Amstigote in tissue

Epidemiology

Through out central and south America



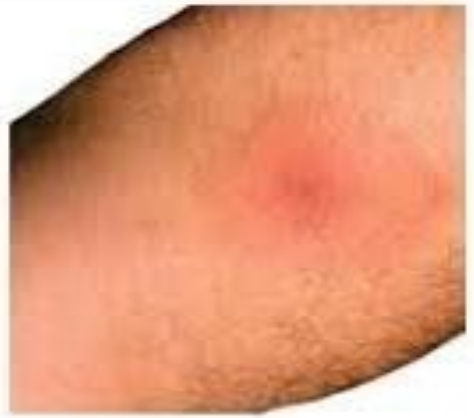
Metacyclic trypomastigotes

Trypomastigotes & amastigotes(targets cardiac myocytes mainly and results in their burst)

Notice here that also after their bite, they will leave their feces behind, and because the site becomes itchy, u will start Rubbing, after rubbing their feces will enter the circulation, such an example for the site is the face; this parasite loves the face and exactly the eye, so when they leave their feces around ur eye and u start rubbing this area, they will get access through mucous membranes and especially through conjunctiva

Pathogenesis

- Chagas' disease are categorized as acute, indeterminate, and chronic
- Nodule chagoma: near the bite



Chagoma de inoculación

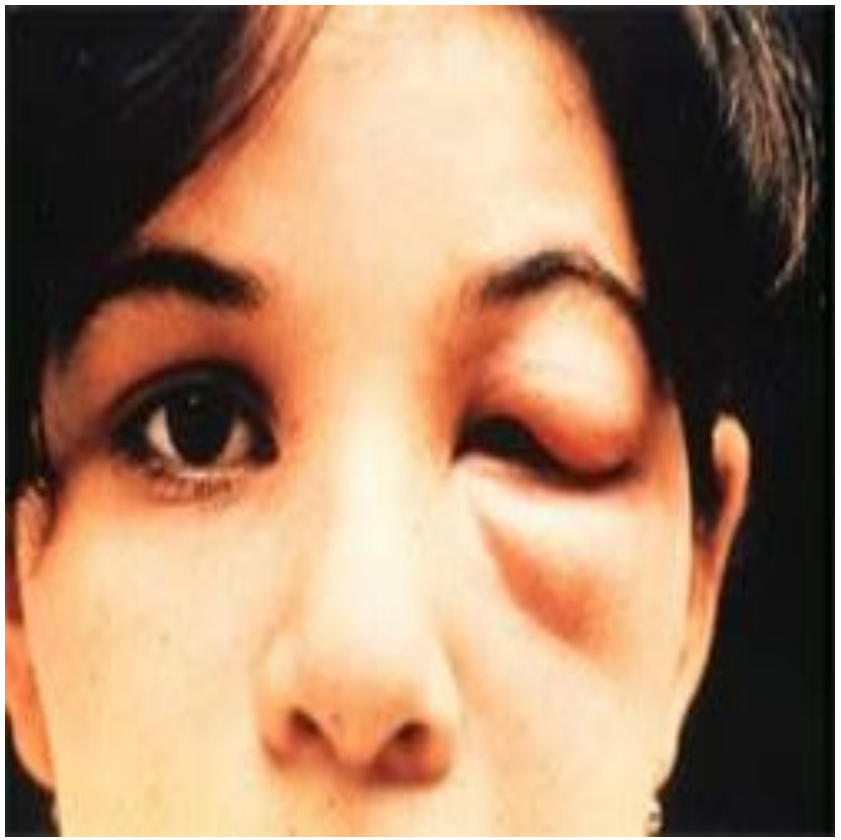
Trypanosoma cruzi

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

•Acute phase:

- Start 1 week after infection
- Fever
- Lymph node enlargement
- Enlarge liver and spleen(hepatosplenomegaly)
- Unilateral swelling of eyelids romana’s sign
- Acute myocarditis

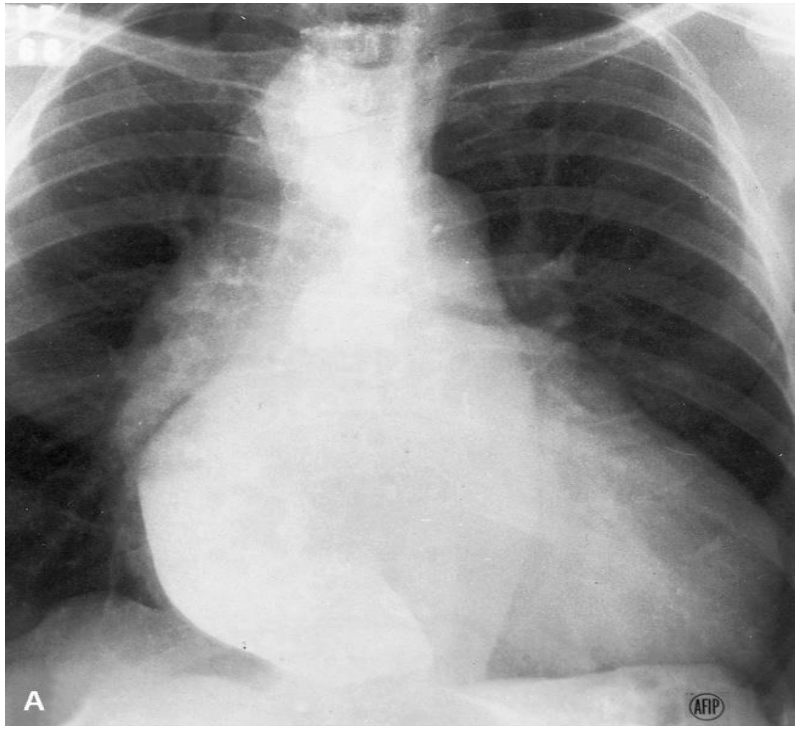
ROMANA'S SIGN



•Chronic phase:

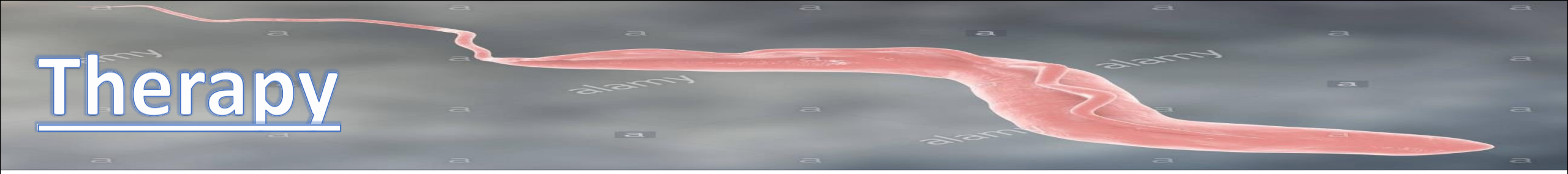
- Develop years after the diagnosis of acute disease

- Most frequent clinical signs of chronic Chagas' disease involve the heart, where enlargement of the heart , including cardiac changes
- Enlargement of the colon(mega-colon), esophagus(mega-esophagus), and they can be easy perforated.



Chest X-ray showing so enlarged heart as a compensation, also they can have arrhythmia

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.

In chronic stage it is too late, here we only care about symptomatic treatment.

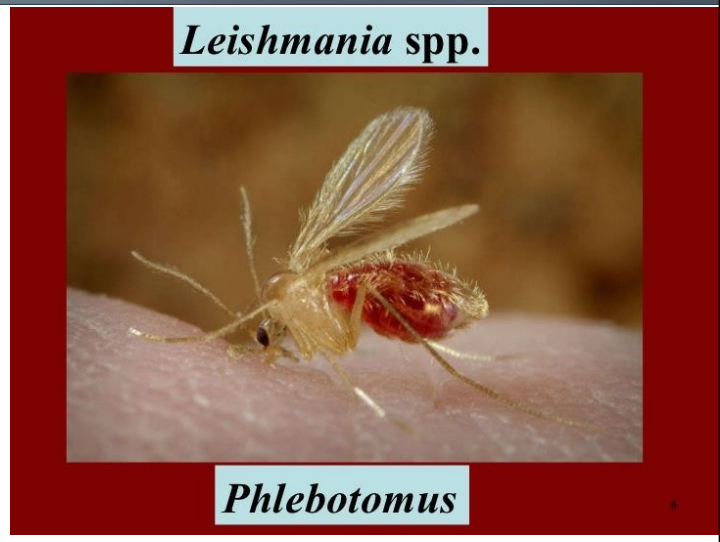
Prevention

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

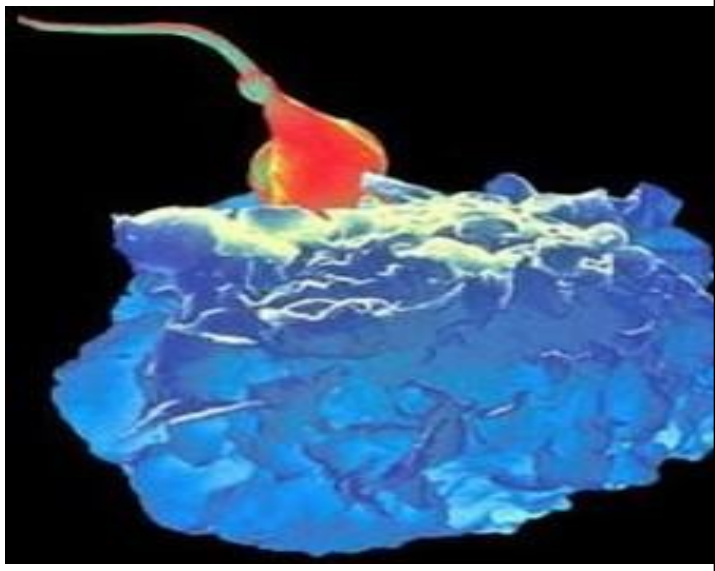
No vaccine nor chemo-prophylaxis

LEISHMANIA

- It is a flagellated protozoan
- Life cycle requires two hosts :
  - **a) vertebrate** ; mammalian host
  - **b) Invertbrate vector** ; female sand fly (found in Jordan), endemic in middle east.



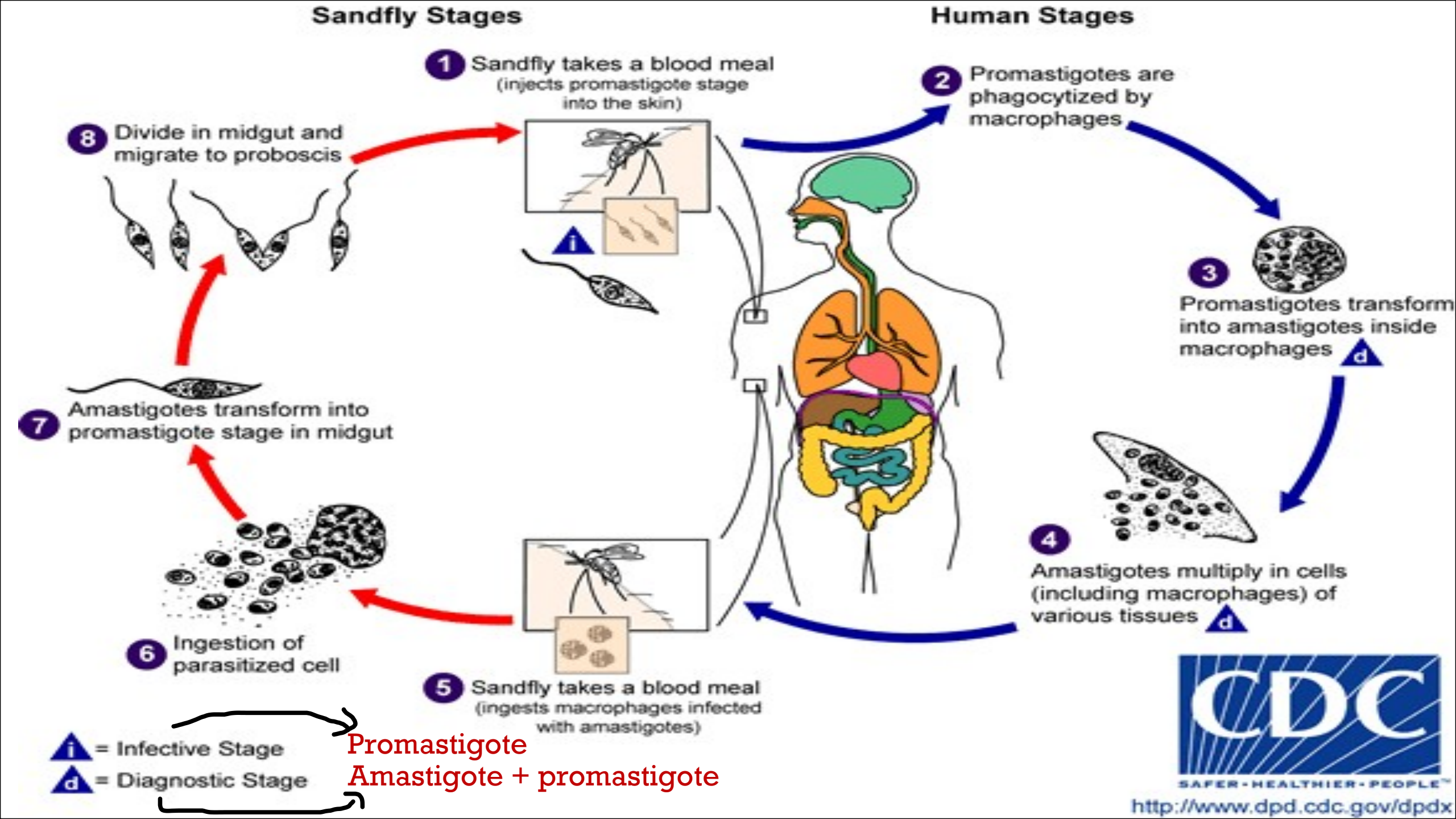
- Obligate intracellular organism
- Infects primarily phagocytic cells(monocytic cells)and macrophages
- The incubation period ranges from 10 days to 2 years,



LEISHMANIA SPP.

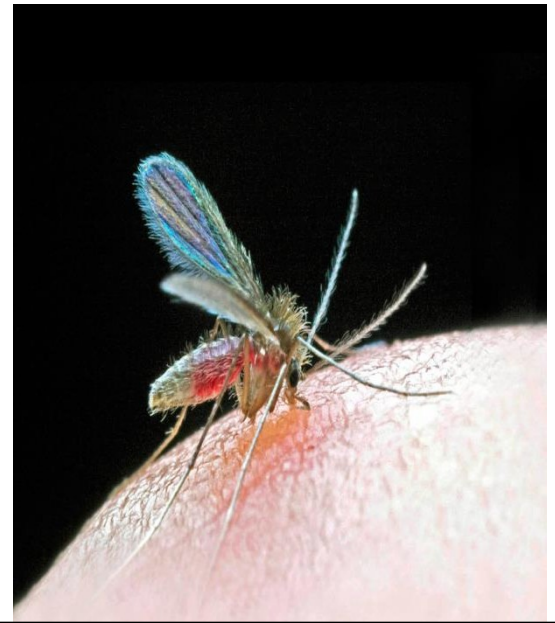
- Leishmaniasis is divided into clinical syndromes according to what part of the body is affected most.

1. **Cutaneous Leishmaniasis(L.tropica, L.major, L.infantum)**
2. **Mucocutaneous leishmaniasis(L. Braziliensis), known as nasopharyngeal leishmaniasis.**
3. **Visceral Leishmaniasis(L.donovani), the worst one, known as black disease or kala azar.**


Sandfly Stages
Human Stages
1 Sandfly takes a blood meal
(injects promastigote stage
into the skin)
2 Promastigotes are
phagocytized by
macrophages
3 Promastigotes transform
into amastigotes inside
macrophages
d
4 Amastigotes multiply in cells
(including macrophages) of
various tissues
d
5 Sandfly takes a blood meal
(ingests macrophages infected
with amastigotes)
6 Ingestion of
parasitized cell
7 Amastigotes transform into
promastigote stage in midgut
8 Divide in midgut and
migrate to proboscis
i
i = Infective Stage
d = Diagnostic Stage
Promastigote
Amastigote + promastigote
CDC
SAFER·HEALTHIER·PEOPLE™
http://www.dpd.cdc.gov/dpdx

Transmission

1. Bite of sand fly (mainly)
2. Transfusion blood and transplantation
3. Mother to baby
4. Direct contact; from man to man through nasal secretion, in nasopharyngeal (debated)



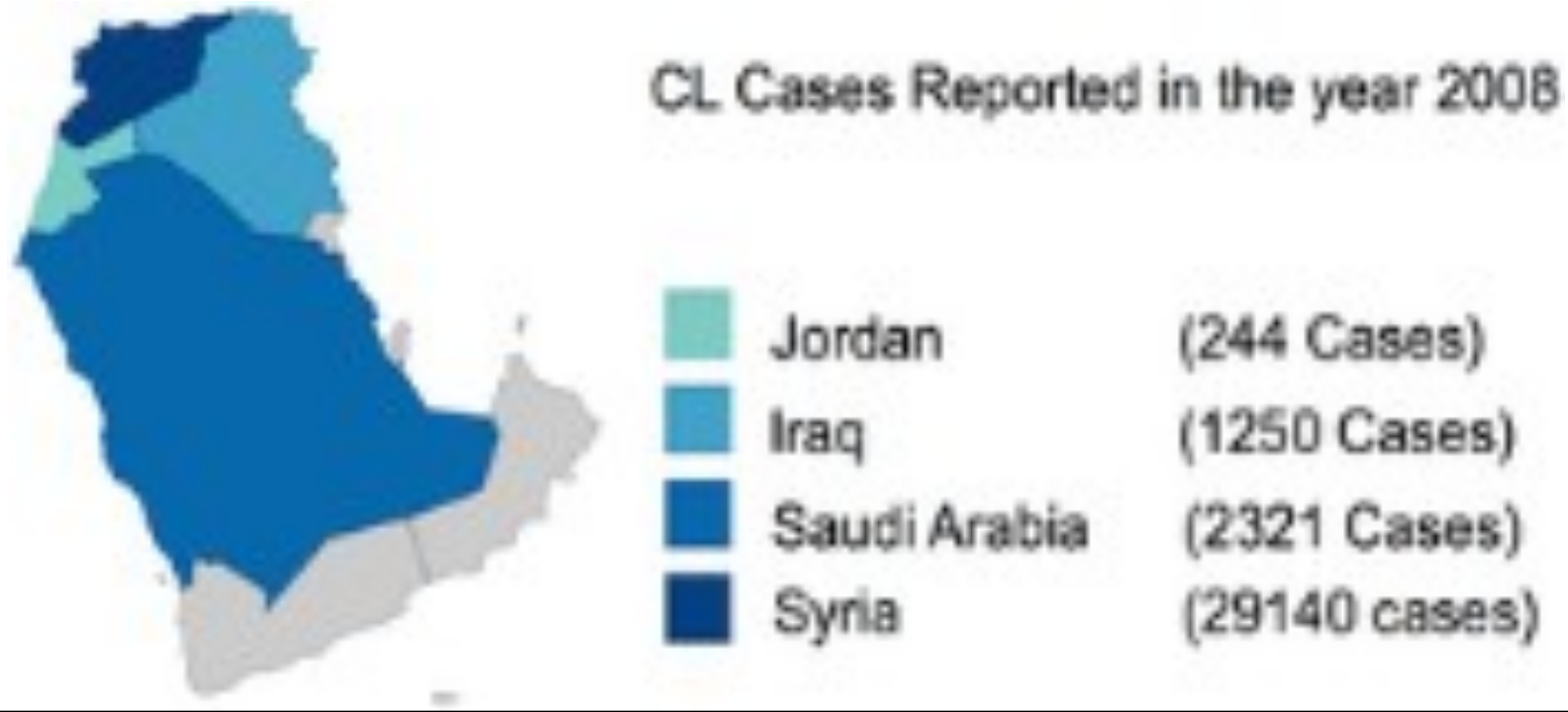
Cutaneous Leishmaniasis : Leishmania tropica, L major, L infantum

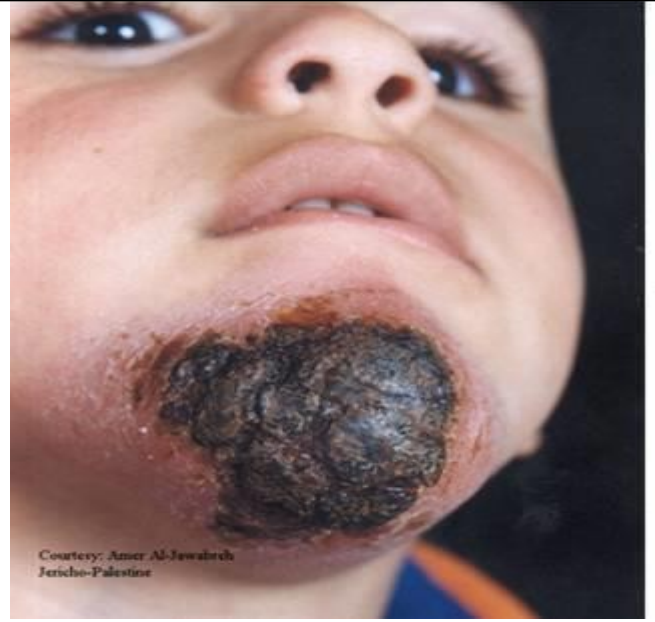
Known as Aleppo boil, or Baghdad boil

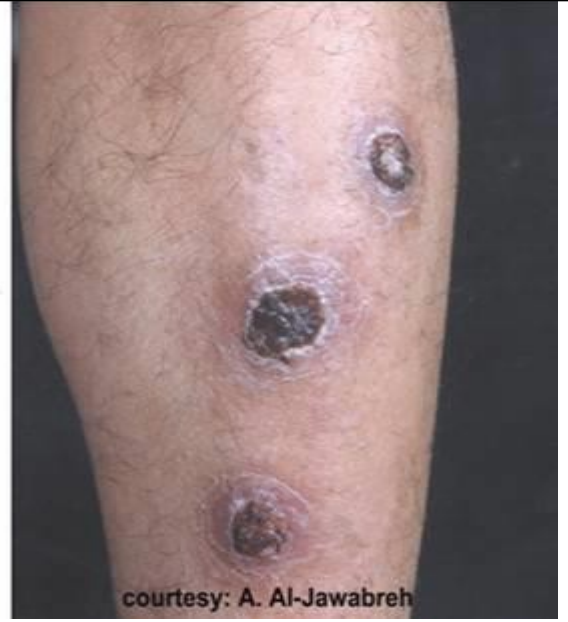
- **Habitat**: skin
- **Disease**: Cutaneous leishmaniasis
- **Clinical feature** : first sign is a **lesion** (generally a firm, The lesions begin as reddish , soft itchy papular , gradually enlarges ,raised and firm , with serous discharge at the bite site, then convertes into ulcer.
- **Epidemiology**: the Middle East , south America

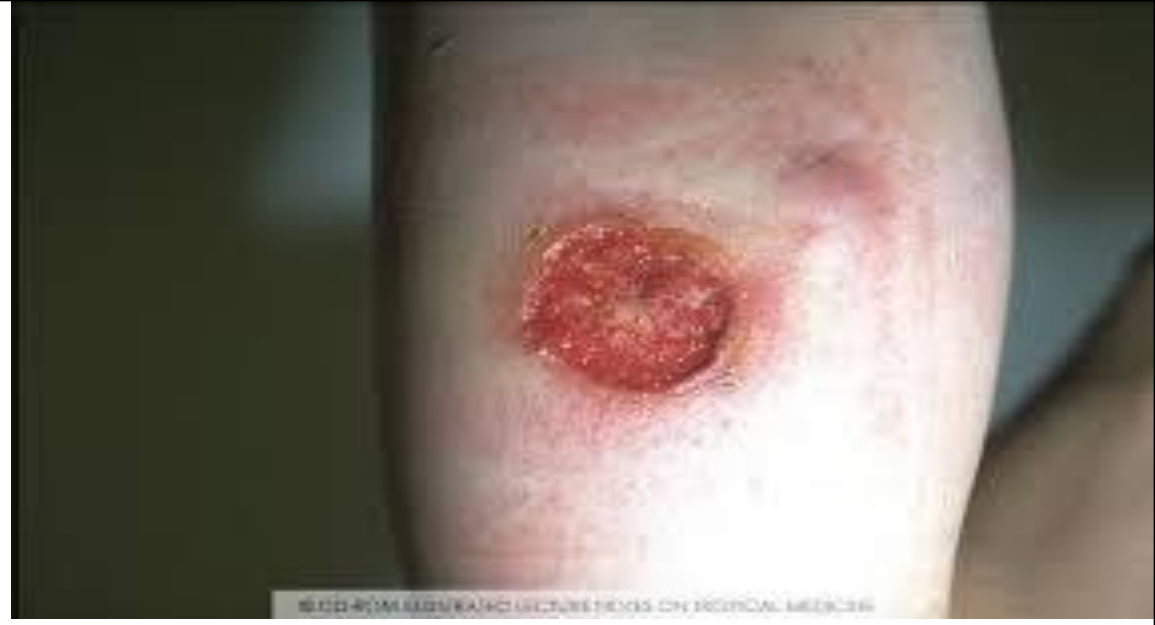
Leishmania In Jordan

- In Jordan there are several species of Leishmania; Leishmania infantum, Leishmania tropica, and Leishmania major.
- Leishmania major (like it is name, major)is the major species of Leishmania parasite in Jordan .

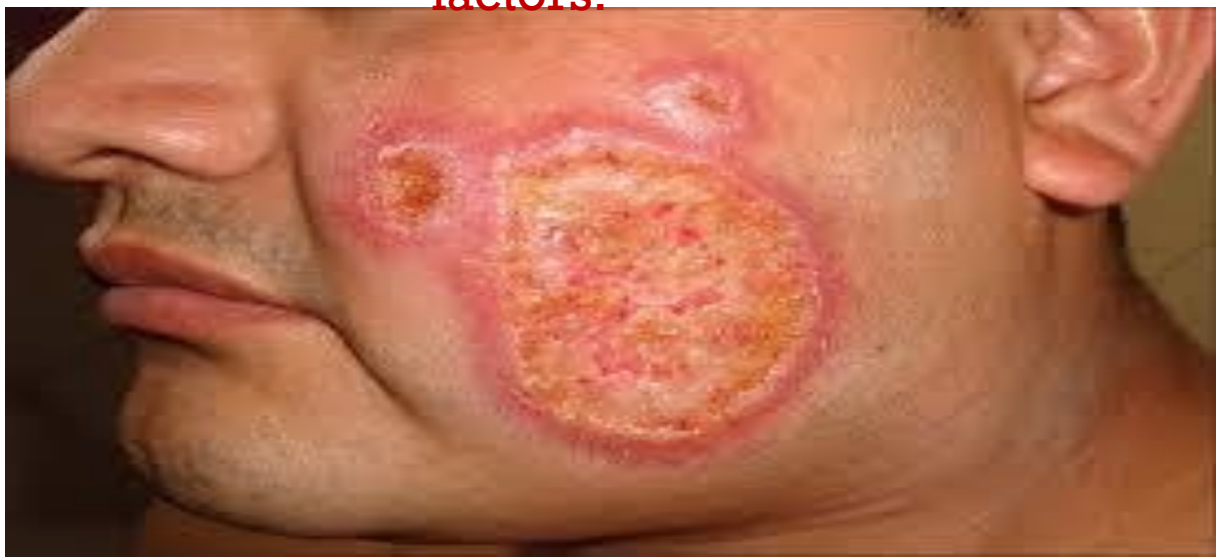


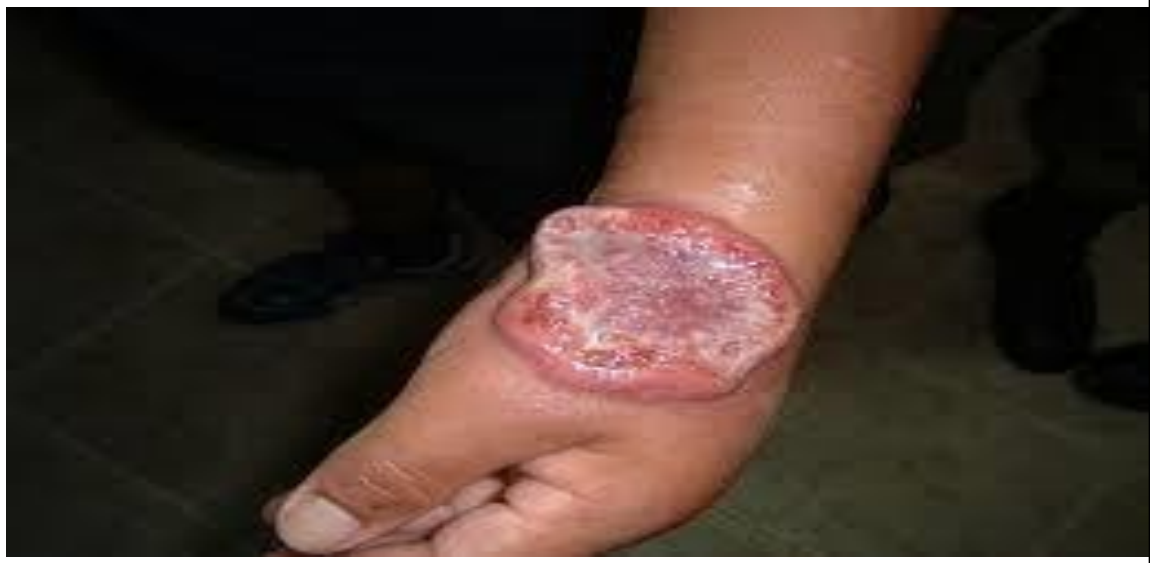






It is very ugly, but it is painless, and doesn't need a treatment, meaning that it resolves spontaneously, without leaving a scar, but, it might leave a scar depending on many factors.





Mucocutaneous leishmaniasis(*L. braziliensis*)

Know as Nasopharyngeal leishmaniasis

- The primary lesions are similar to those found in cutaneous leishmaniasis, either it starts there or disseminates from another area(cutaneous).

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

They are painful, don't heal spontaneously so they need treatment, u will have erosions, and destruction of nasal septum.
Those patient might have dissemination toward liver and spleen, and have visceral leishmaniasis(kala azar)

Visceral Leishmaniasis(*L.donovani*)

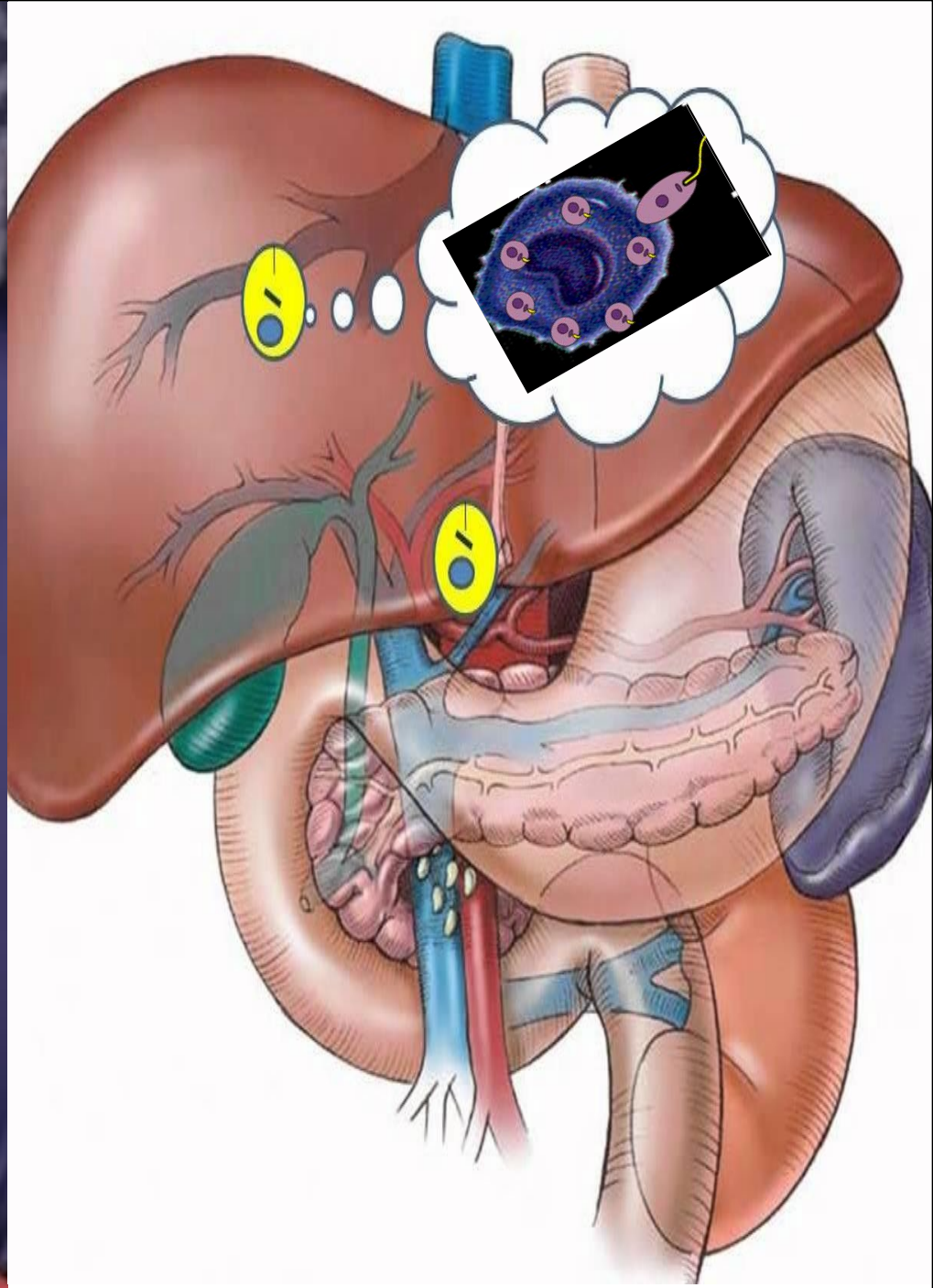
Known as black disease(because they have hyper-pigmentation of the skin) or Kala Azar

- 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.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LABORATORY DIAGNOSIS

1) **Stained blood smear**: aspiration(intracellular), scraping(from lesions)
2) **Cultured** :cultured using special techniques

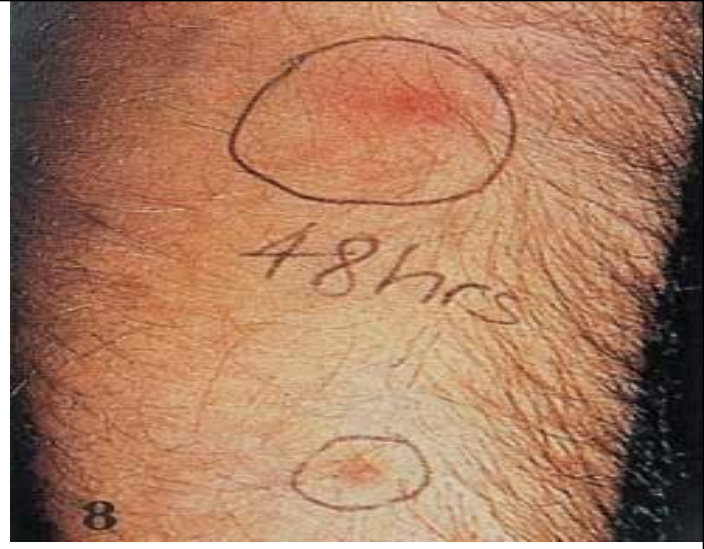
3) **ElISA ,IFA or direct agglutination** give useful indication of active or recent kala-azar, but in cutaneous leishman... they are negative , in mucocutaneous, border line; sometimes positive sometimes negatives.

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

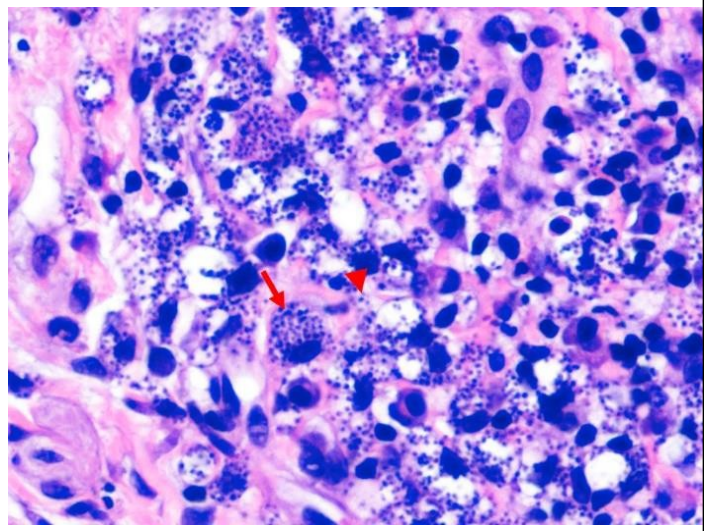
5-Intradermal Montenegro test :

Injection of intradermal antigen prepared from cultured promastigotes of Leishmanian spp but they are attenuated, then u ask the patient to return after 48 hours and measure the induration, this test if it is positive, it tells u that the patient has been infected or not, just that :)

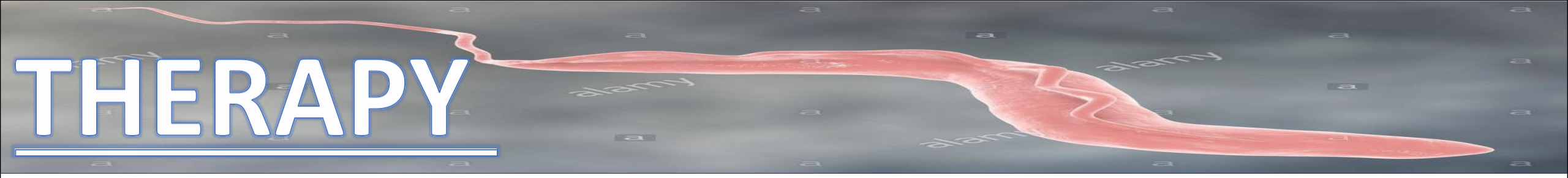
This produces a typical cell-mediated response .



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



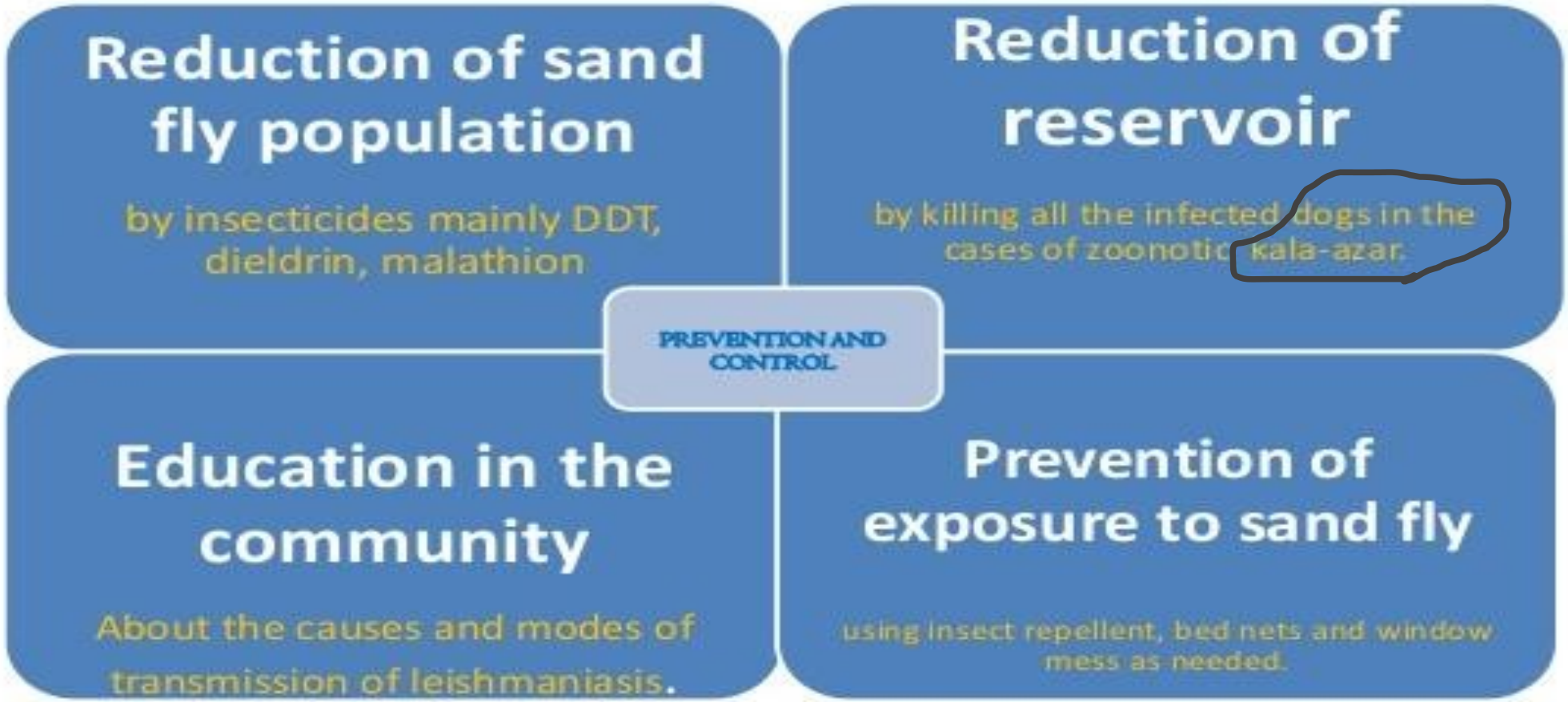
THERAPY



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

- In simple cutaneous leishmaniasis, lesions usually heal spontaneously
- **Antimony, sodium stibogluconate** drugs of choice for the treatment of visceral and mucocutaneous leishmaniasis.

PREVENTION



There are No Vaccines to prevent leishmaniasis.

The End