HLS

MODIFIED NO. 2

MICROBIOLOGY

كتابة: ميس قشّوع و حسن النويهي

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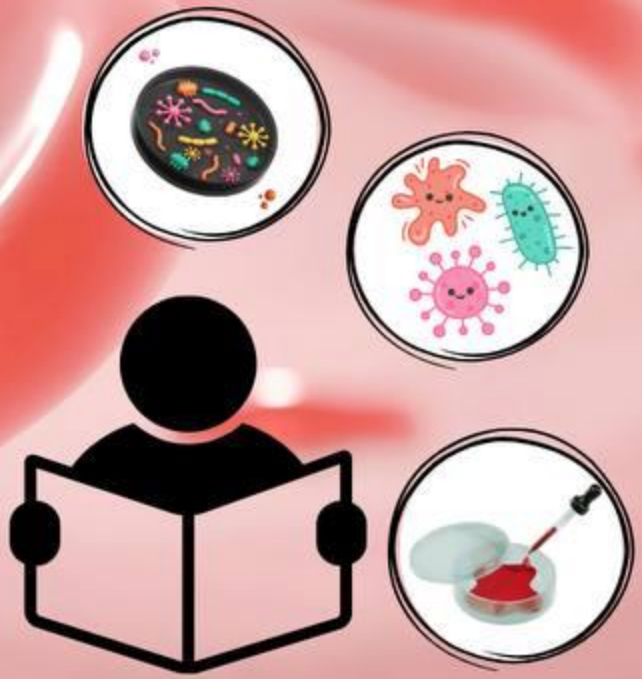


Haemflagellate

TRYPANOSOMA & LEISHMANIA

Color code
Slides
Doctor
Additional info
Important

By: Ass. Prof. Nader Alaridah MD, PhD

To continue from the previous lecture, there are two important pieces of information. First, there is innate immunity in malaria. Some people are protected against infection with Plasmodium species, and this is known as innate immunity. There are many hypotheses regarding this immunity, which include hemoglobinopathies such as hemoglobin S, F, E, sickle cell disease, thalassemia, and G6PD deficiency. These conditions in patients' RBCs are considered a form of innate immunity against Plasmodium infection, specifically Plasmodium falciparum. The second point is that there are two available vaccines against malaria. The first one is RTS, which was approved in 2021, and the second, approved in 2023, is R21. However, the problem with these vaccines is that their protection levels are low, but they are administered in endemic areas because they reduce mortality, particularly in children.

- These two diseases (Trypanosoma, Leishmania) are restricted to certain geographical areas. In Jordan, we have leishmaniasis, specifically a type called cutaneous leishmaniasis, and you will see it.
- They move using flagella.
- Their multiplication is asexual.
- They are vector-borne.
- Trypanosoma infects the blood, while Leishmania infects the reticuloendothelial system.

Haemflagellate

Trypanosoma

leishmania

- The trypomastigote and amastigote are found in blood and extracellular body fluids, while the promastigote and epimastigote are found in the vector and are associated with vector-borne diseases.
- There is an undulating membrane in the trypomastigote that contains the flagella within its body.

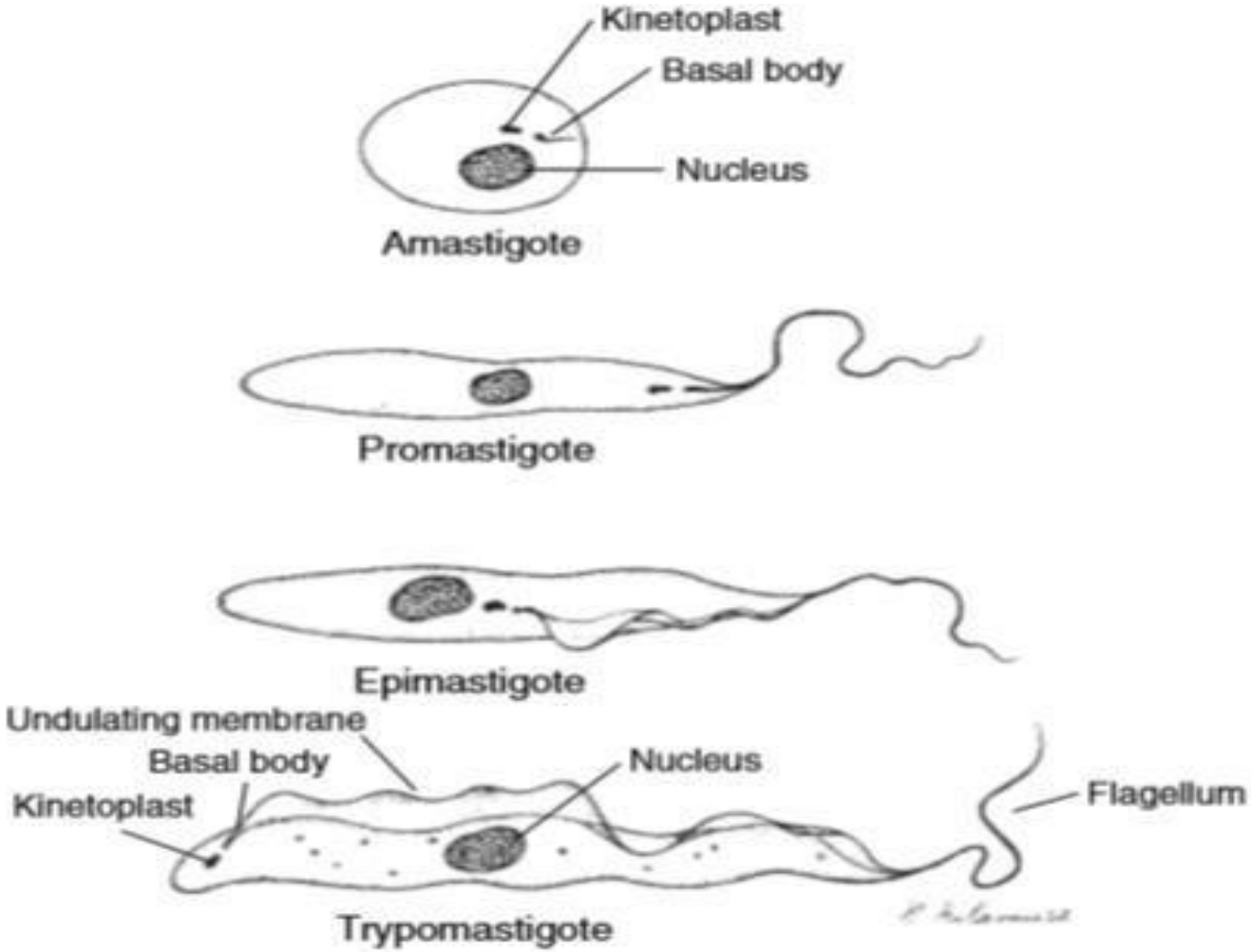


Figure 49-8 Characteristic stages of species of *Leishmania* and *Trypanosoma* in human and insect hosts. (Illustration by Nobuko Kitamura.)

- They are in a class known as **Mastigophora** and belong to the order **Trypanosomatidae** or **Amastigote**. They are characterized by having a unique structure called a kinetoplast in their body, which is a DNA-containing structure that is the origin of the flagella they possess.
- There are 4 developmental stages in the life cycle of Trypanosoma and Leishmania, and we need to know which stage is infective and which stage is diagnostic.
- Note that amastigotes do not have flagella, while all the other stages possess flagella. The amastigote is a round form that occurs in infections involving intracellular pathogens. This is seen in Trypanosoma cruzi (Chagas disease or American trypanosomiasis) and in leishmaniasis, which is completely intracellular.

Trypanosoma

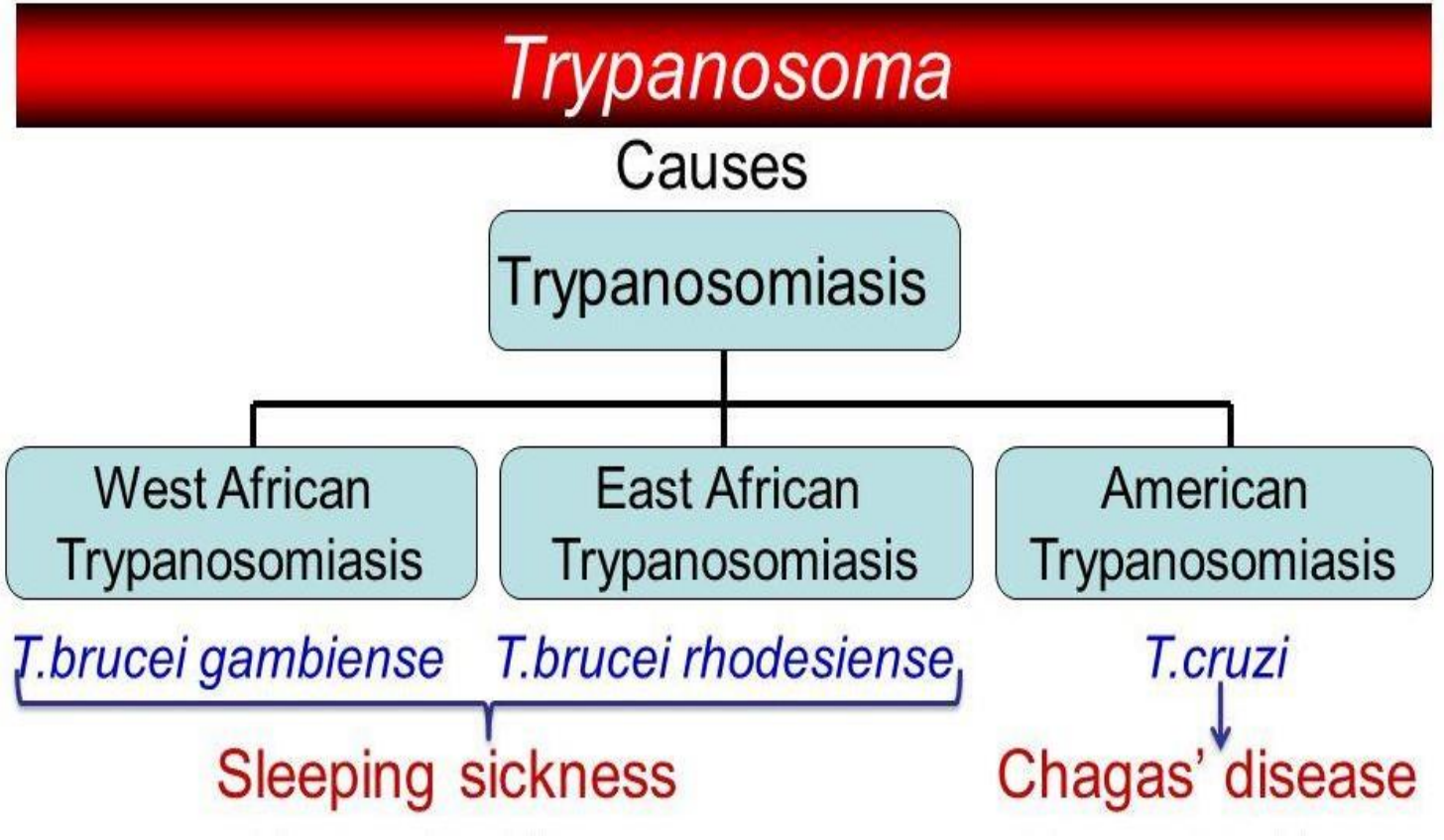
- Causative agents of African trypanosomosis (sleeping sickness) and American trypanosomosis (Chagas disease).

- 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)



- The causative agent is called Trypanosoma, and the disease it causes is known as trypanosomiasis.
- The first type is Trypanosoma brucei complex (Africana trypanosomiasis – sleeping sickness), which includes Trypanosoma brucei gambiense (west African) and Trypanosoma brucei rhodesiense (east African). In the late stages, patients experience uncontrollable daytime sleep, which is a poor prognostic factor.

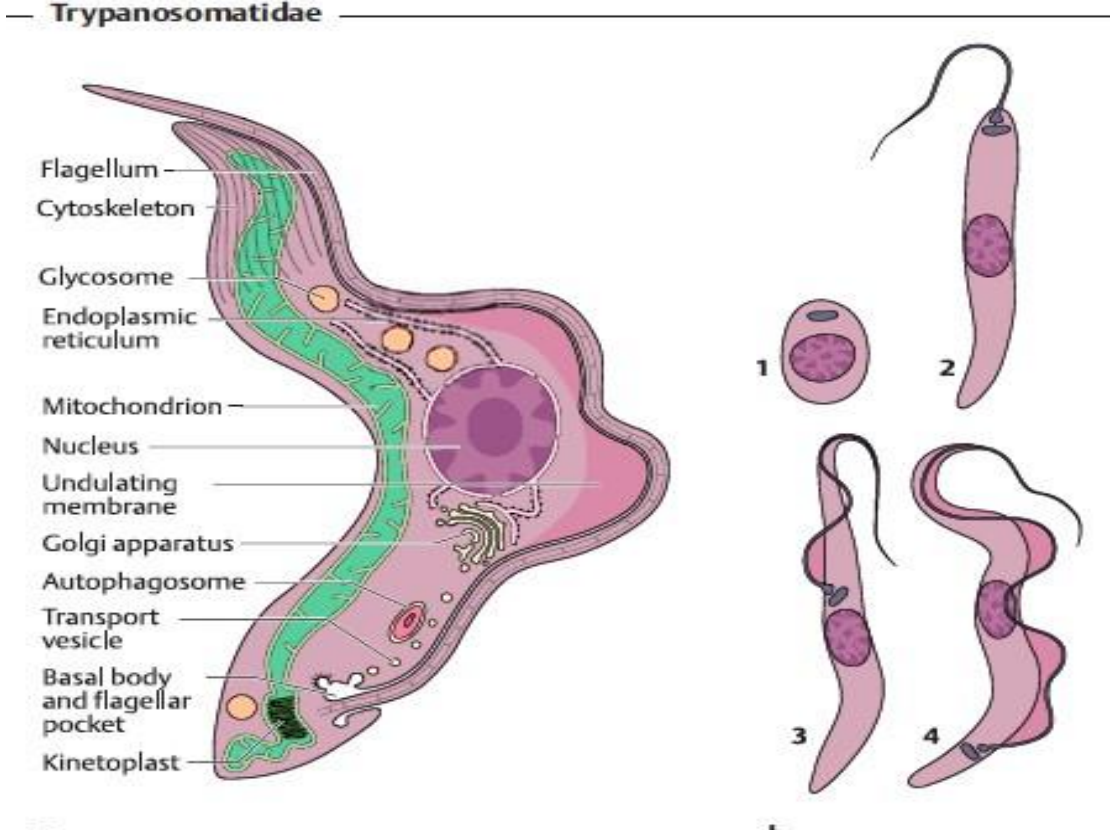
- Trypanosomiasis is a fatal disease if left untreated.

- There is a difference between the two African diseases: Trypanosoma brucei rhodesiense is more severe, rapidly progressive , and can turn fatal within a short period of time, while Trypanosoma brucei gambiense develops slowly and takes a longer duration.

- The other type is American trypanosomiasis – Chagas disease, caused by Trypanosoma cruzi

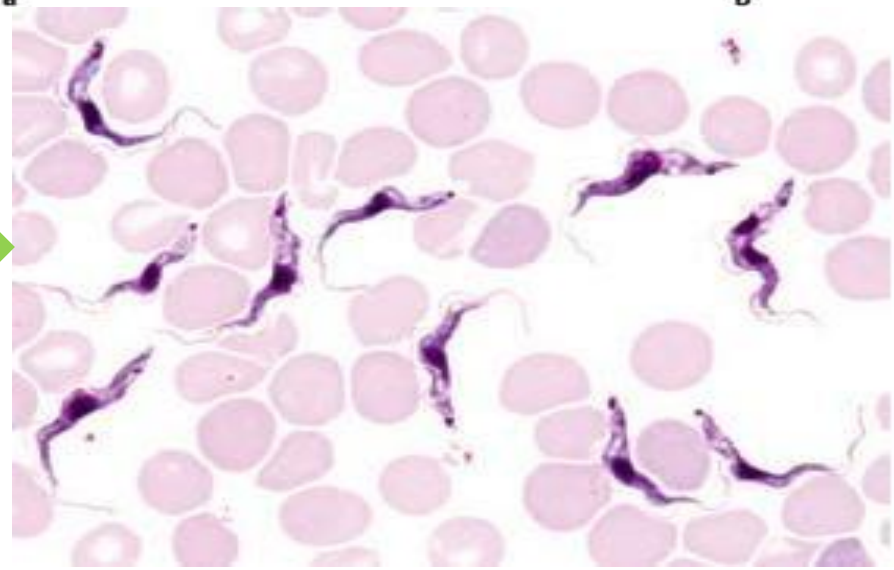
Morphology

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



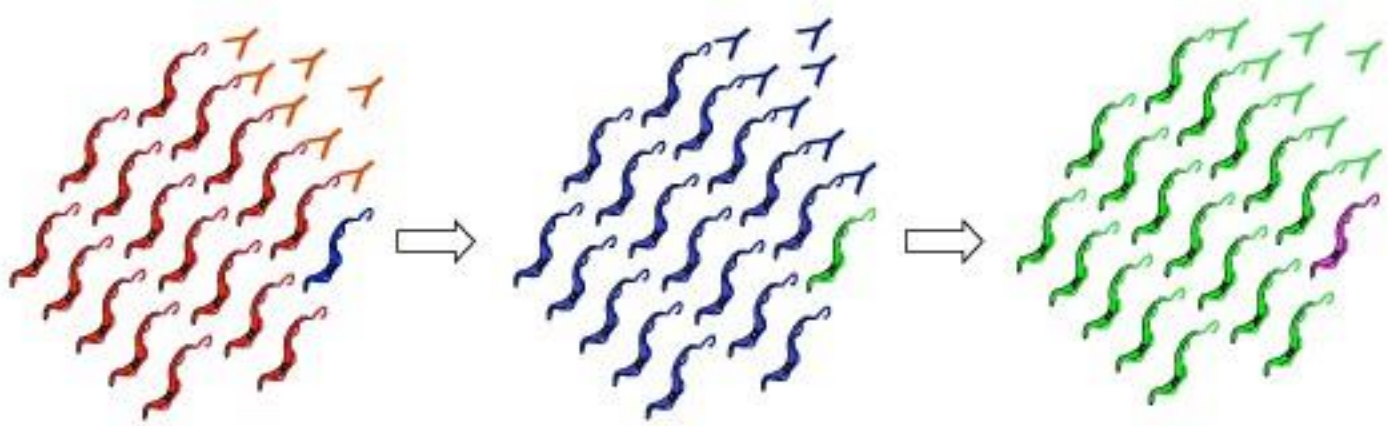
- This is a blood film from a patient with African sleeping sickness, also known as African trypanosomiasis. Note that all the life cycle stages of African trypanosomiasis are presented extracellularly. The diagnostic stage is called the trypomastigote, which is extracellular, as we mentioned before :).

- On the other hand, American Chagas disease features an extracellular trypomastigote and an intracellular amastigote . Found mainly in the heart, as well as in the esophagus, colon, and possibly the brain.



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



- They have a genetic mosaic of about a thousand genes, and the genetic control is called VSG . These genes continually change the coating of trypanosomes, which is one of the problems in diagnosing them and developing methods for their diagnosis.
- This antigenic variation is also seen in viruses, such as influenza.

AFRICAN TRYPANOSOMIASIS

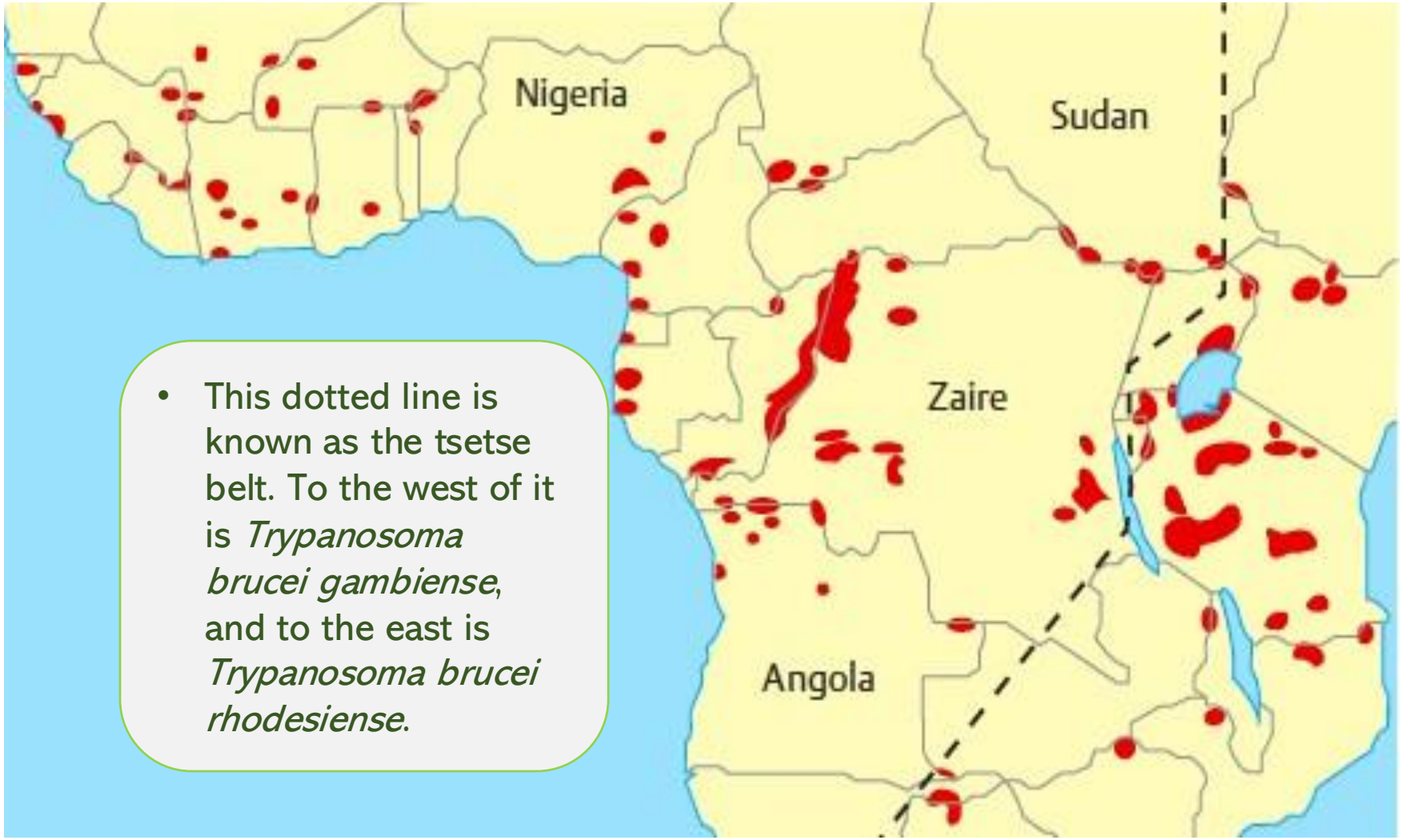
- Is caused by 2 sub spp. :
- T. brucei gambiense :West African trypanosomiasis
- T. brucei rhodesiense: East African trypanosomiasis
- Vector: **tsetse fly** (Glossina spp.)
- Which is found only in rural Africa
- Glossina palpalis transmits T. b. gambiense
- Glossina morsitans transmits T. b. rhodesiense

- They differ in their reservoir hosts: in Trypanosoma brucei gambiense, the infected hosts are humans and some domestic animals, while in Trypanosoma brucei rhodesiense, the reservoir hosts are wild animals and wild cattle. This is one of the explanations for why patients infected with rhodesiense tend to have more severe disease.

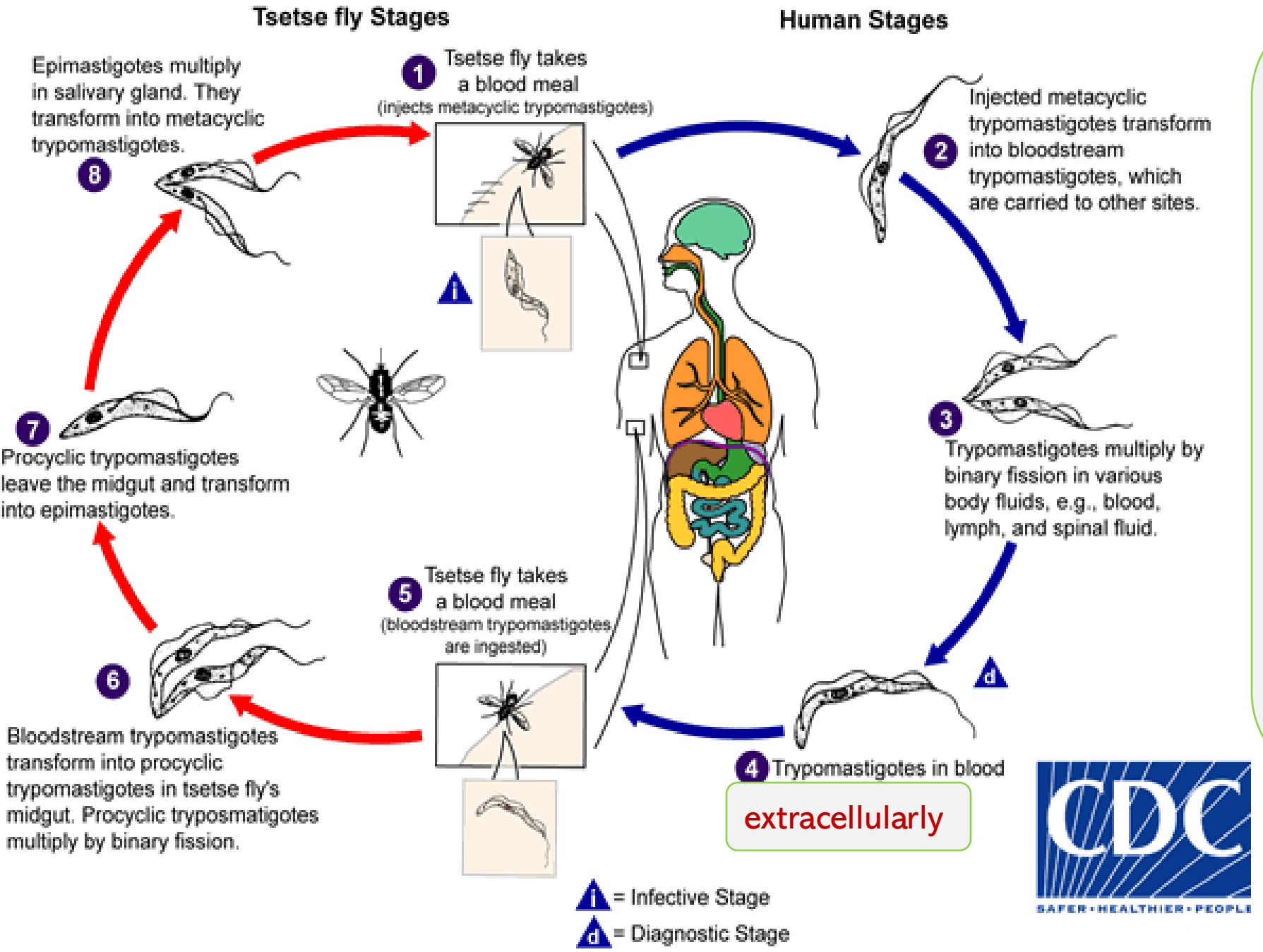
Epidemiology.

- 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.

Epidemiology



- This dotted line is known as the tsetse belt. To the west of it is *Trypanosoma brucei gambiense*, and to the east is *Trypanosoma brucei rhodesiense*.



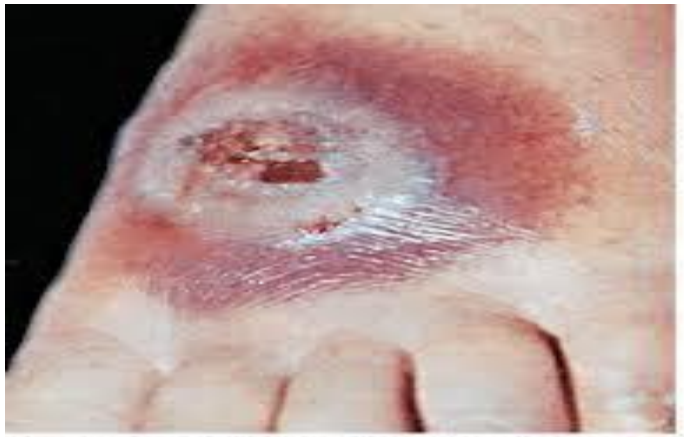
extracellularly

- This lifecycle is called the salivaria cycle because the trypomastigotes are found in the salivary glands of the tsetse fly, which transmit the infective stage when they bite. This is one of the differences between African and American trypanosomiasis. In American trypanosomiasis, the cycle is called the stercoraria cycle, and the trypomastigotes are in the hindgut (of the vector bug) with the infective stage present in the stool of the tsetse fly when they defecate.

- Infective stage: Trypanomastigote.
- Diagnostic stage: Trypanomastigote

Trypanosoma brucei gambiense

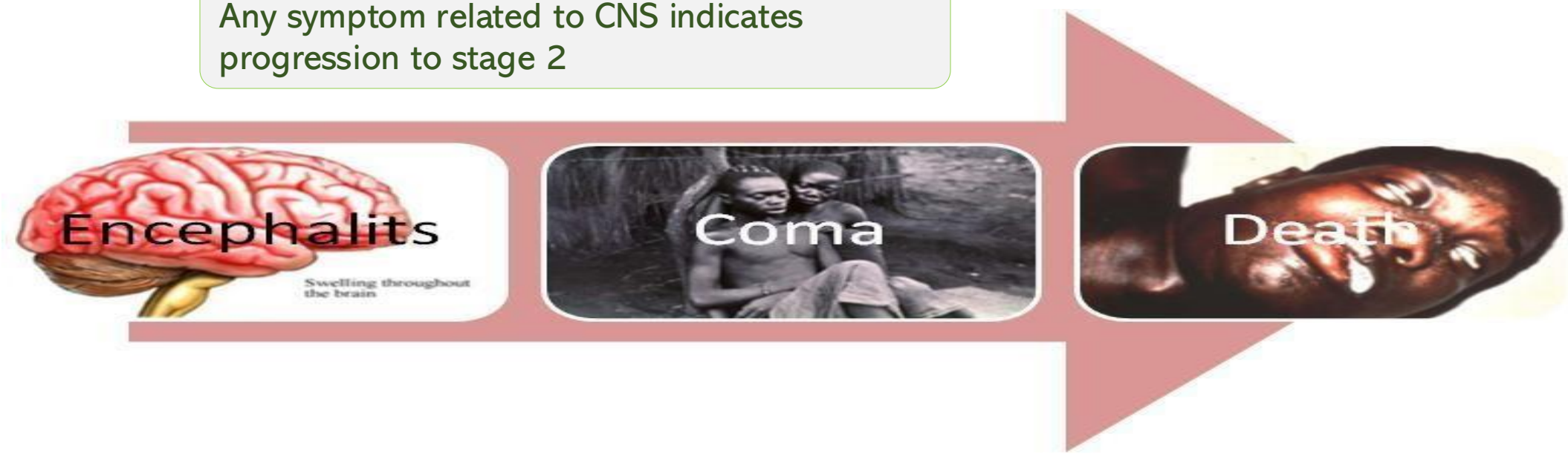
- **Clinical feature:**
- After the host has been bitten by an infected tsetse fly, **a nodule or chancre** at the site may develop after a few days.
- **stage I:** the patient have systemic trypanosomiasis without CNS involvement.
- The trypomastigotes enter the bloodstream and invade the lymph nodes
- The first symptoms appear and include: irregular fevers with night sweats, enlargement to liver and spleen, Winterbottom's sign –> Enlargement of the lymph nodes in the posterior cervical lymph nodes.
- Once the trypomastigote starts crossing the blood-brain barrier, stage two occurs.
- The outcome for the patient is completely different if you treat them in stage one or stage two and the outcome in stage two is unfavorable.



Winterbottom's sign

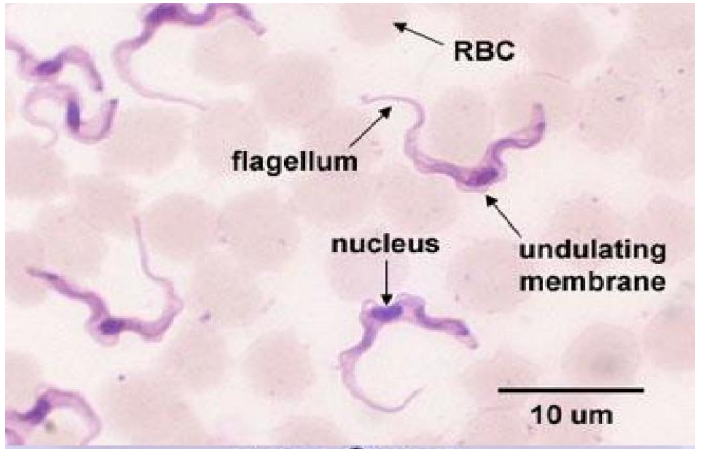
- **Stage II**: organisms invade the CNS, the sleeping sickness stage of the infection is initiated
- The first signs are changes in personality and character, followed by all the symptoms related to the CNS, indicating that the patient is entering stage two. This is followed by delirium, and eventually, they begin to experience excessive sleepiness etc .
- The patient becomes emaciated and progresses to profound coma and death

Any symptom related to CNS indicates progression to stage 2



Laboratory Diagnosis

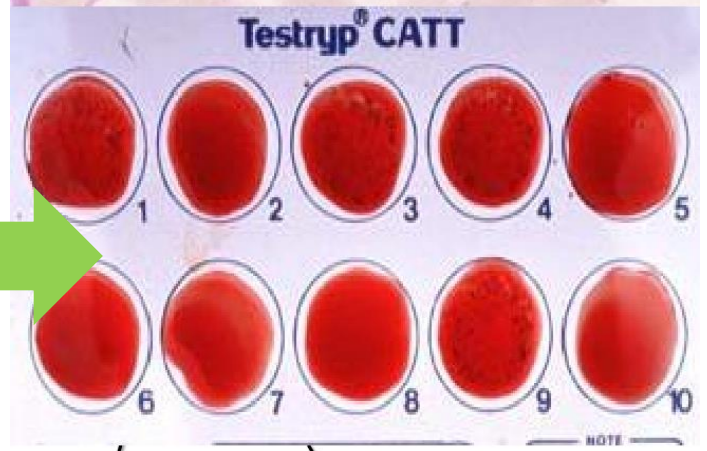
- **Specimen: blood, serum,CSF, aspiration from lymphnode**
- **Routine Methods**: thick and thin blood films



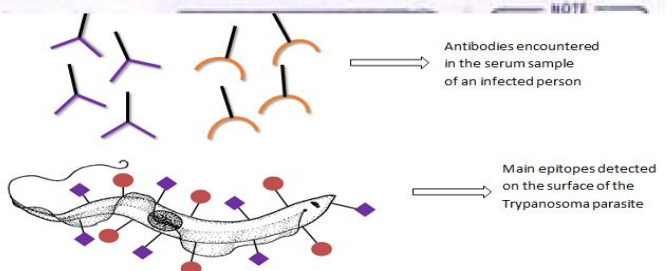
- **Antigen Detection**: simple and rapid test
  card indirect agglutination

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

This test is common in areas with endemicity for trypanosomiasis: the CATT test. This is a screening test; it has low sensitivity but is used for screening suspected cases.



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



Therapy

- All drugs used in the therapy of African trypanosomiasis are toxic and require prolonged administration So that is a problem :(.

- 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

prevention

1. preventing flies from biting through the use of insecticide 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.

AMERICAN TRYPANOSOMIASIS

- Trypanosoma cruzi **(Chagas' disease)**
- Zoonosis
- Transmitted by vector : reduviid bugs (kissing bug/triatomine bug).
- Reduviid bug defecates while taking a blood meal (They don't bite and leave , instead they bite and defecate).

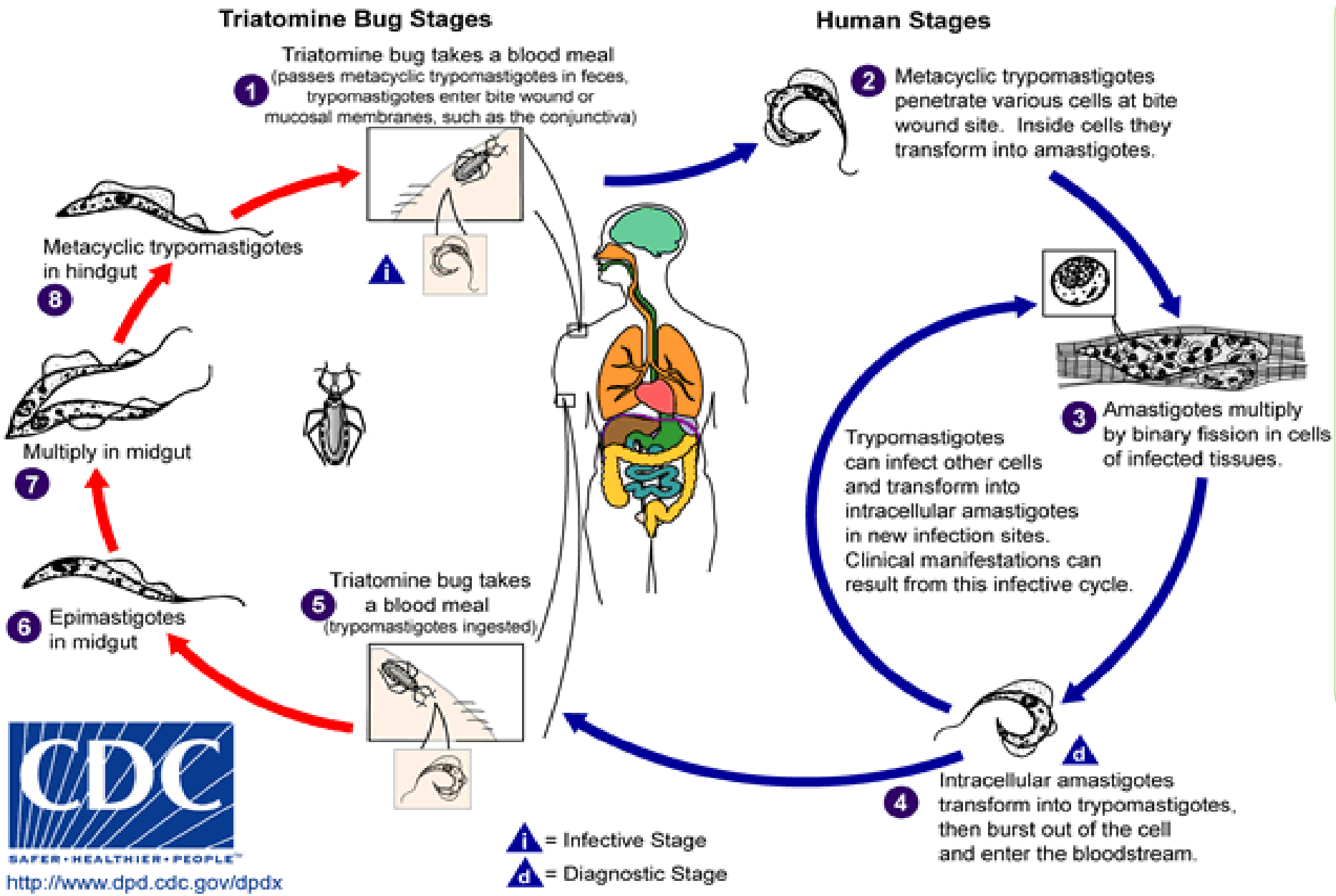
- **Definitive host:**
- Human, dog, cat, rats…etc.(these are reservoir hosts)
- **Habitat** in the Definitive host:
- Trypomastigote in blood(Diagnostic stage)
- Amstigote in tissue (Diagnostic stage)

- In the African type , amastigotes are not present, whereas in the American form they appear, they multiply within cells, particularly in hollow organs such as the heart (most commonly),esophagus, and brain.

Epidemiology

Through out central, south and some parts of north America

Ponder the figure well !!



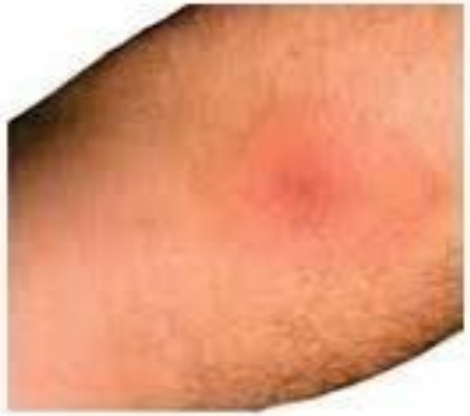
- **Diagnostic stage:**
  1- Trypomastigote in blood (extracellular).
  2- Amastigote (round intracellular form).

- **Infective stage:**
  Trypomastigote [These trypomastigotes either remain extracellular in the blood or enter organs, causing intracellular infections].

Pathogenesis

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

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



Chagoma de inoculación

Trypanosoma cruzi

- Remember we call it kissing bug , why?

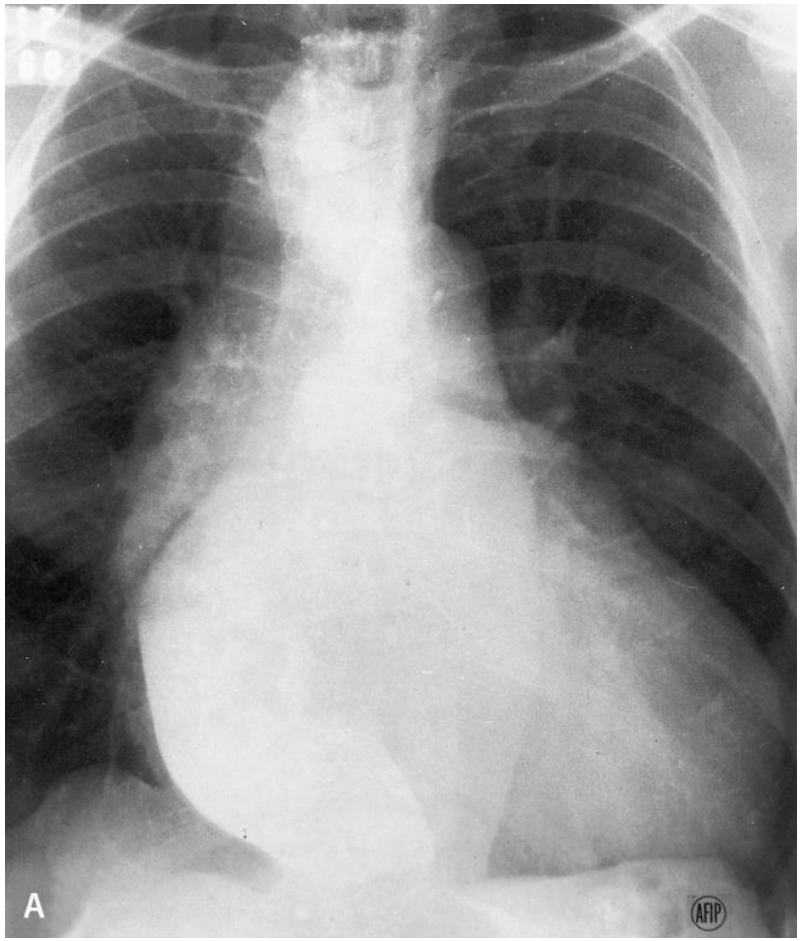
- Because the bug bites then excretes its feces which contains the infective stage; since the bite results in an allergic reaction, the patient would most probably scrub his face and allow access to the feces into the wound or mucous membranes like the conjunctiva causing unilateral swelling of eyelids that is called romana's eye.(see next slide).

- **Acute phase:** systemic , invlovement of lymphatic organs
- Start 1 week after infection
- Fever
- Lymph node enlargement
- Enlarge liver and spleen (hepatosplenomegaly)
- Unilateral swelling of eyelids romana's sign (in case the bite was in the face)
- Acute myocarditis, Pericarditis and endocarditis.

•Chronic phase: involvement of hollow organs

- 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
- Patients could have cardiomegaly, megaesophagus, megacolon; these are due to the amastigote (round intracellular form).
- The patient might not know he is infected; he would come to the emergency department due **to arrhythmia** most probably

- The multiplication of trypanosomes occurs intracellularly, where they transform into amastigotes.



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.

- A new drug in America called eflornithine is used in Chagas disease treatment and is also applied topically, especially by women, to prevent facial hair growth.

- Remember, these vector-borne infections are most commonly transmitted by vectors, but they are also blood-borne so they can be transmitted through blood transfusions, organ transplants, and from mother to child, among other routes.

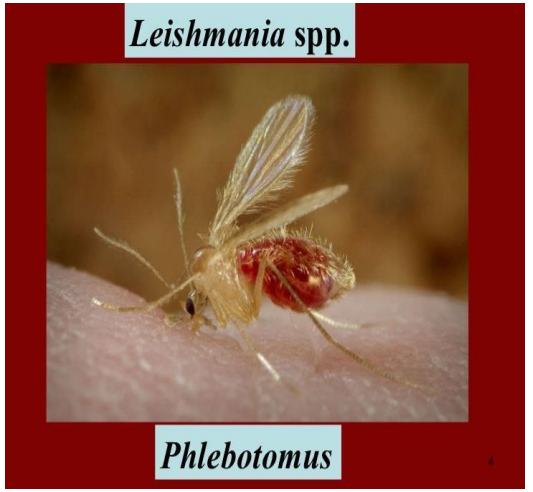
Prevention

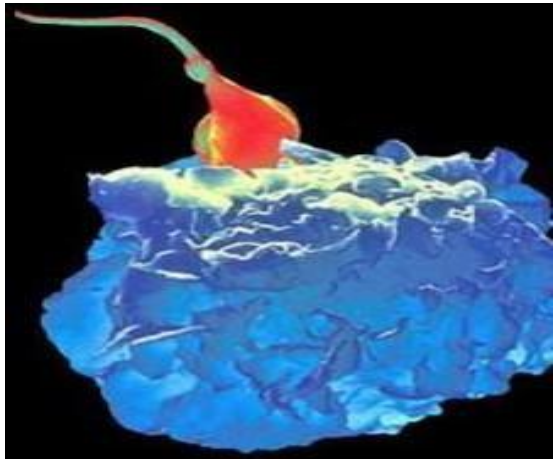
1. Vector control
2. Transfusion control and screening of blood donors
3. testing of organ, tissue or cell donors and receivers

LEISHMANIA

The causative agent of leishmaniasis

- It is a flagellated protozoan
- Life cycle requires two hosts :
  - **a) vertebrate** ; human (anthroponotic) and mammalian host (zoonotic).
  - **b) Invertebrate vector** ; female sand fly [phlebotomus] (why female ? Because they need it for egg maturation).
- Obligate intracellular organism
- Infects primarily phagocytic cells and macrophages (there are no extracellular stages)
- The incubation period ranges from 10 days to 2 years
- In Jordan, it is prevalent in Wadi Araba, Irbid, and Ajloun.





LEISHMANIA SPP.

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

1. Cutaneous Leishmaniasis infection is confined to the superficial surface of the skin **(L.tropica,Leishmania major and L. infantum) >>** Old world spp.

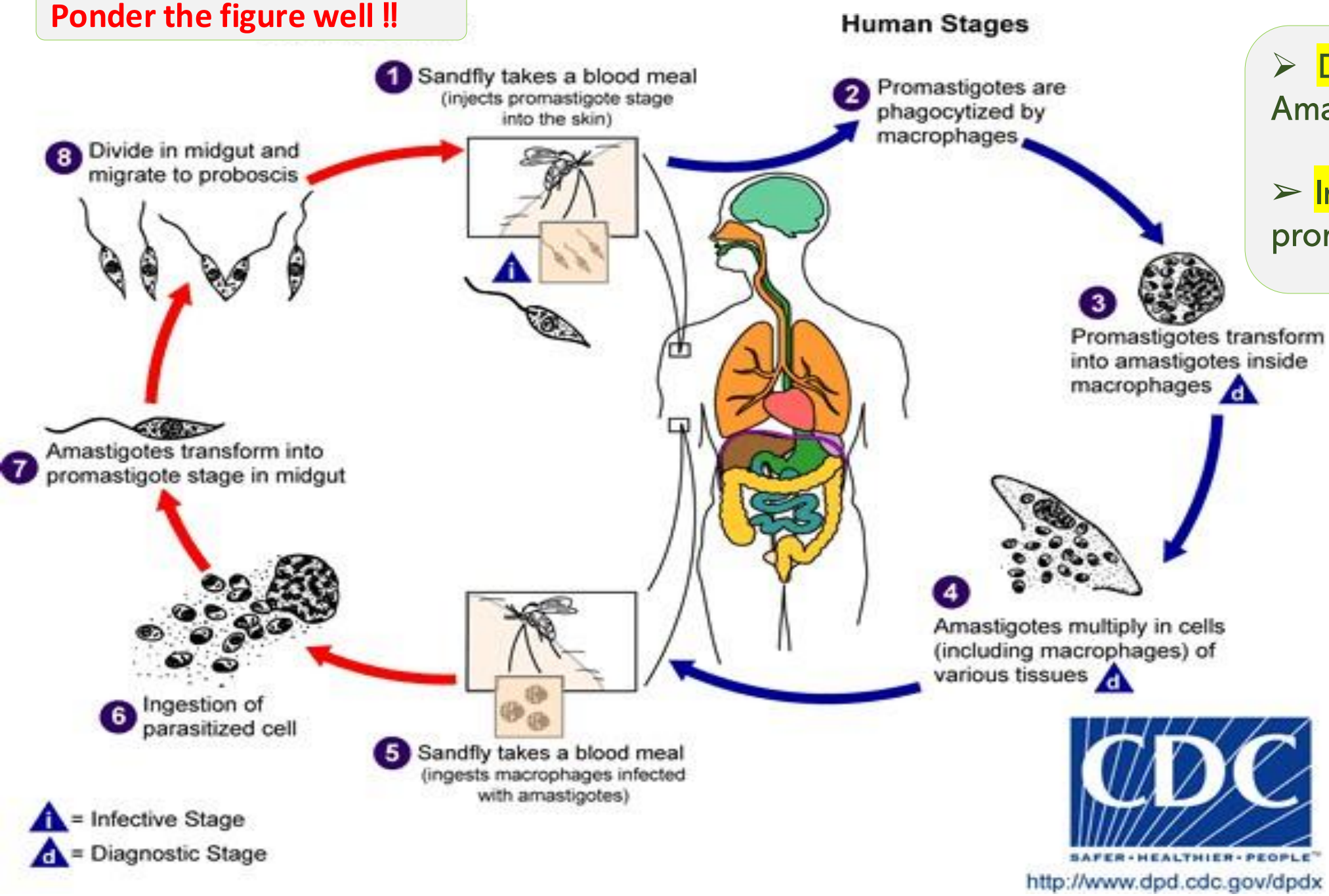
- The New world is called L. ethiopia

- Other names for cutaneous leishmaniasis: Baghdad boil/aleppo boil/ oriental sores/delhi boi(to show you how it is very endemic).

2. Mucocutaneous leishmaniasis (also called Espundia , Nasopharyngeal leishmaniasis **)(L. braziliensis)**

3. Visceral Leishmaniasis (if they entered lymphoid organs (liver, spleen, bone marrow)**(L.donovani).** Also called kala azar, black fever and dom dom fever in india.

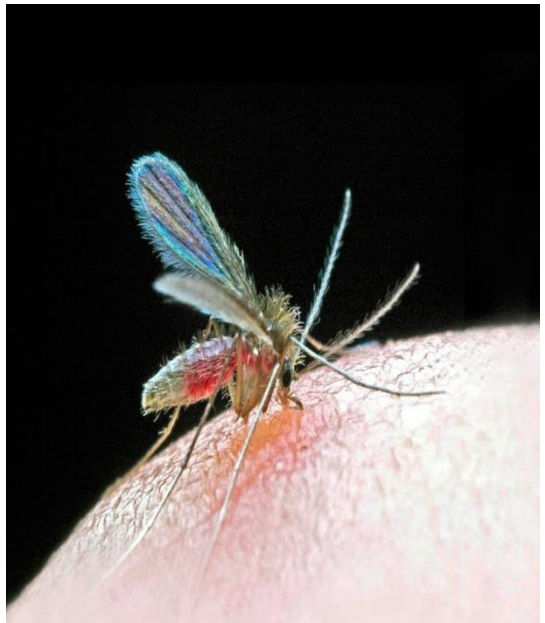
Ponder the figure well !!



- Diagnostic stage: Amastigote
- Infective stage: promastigote

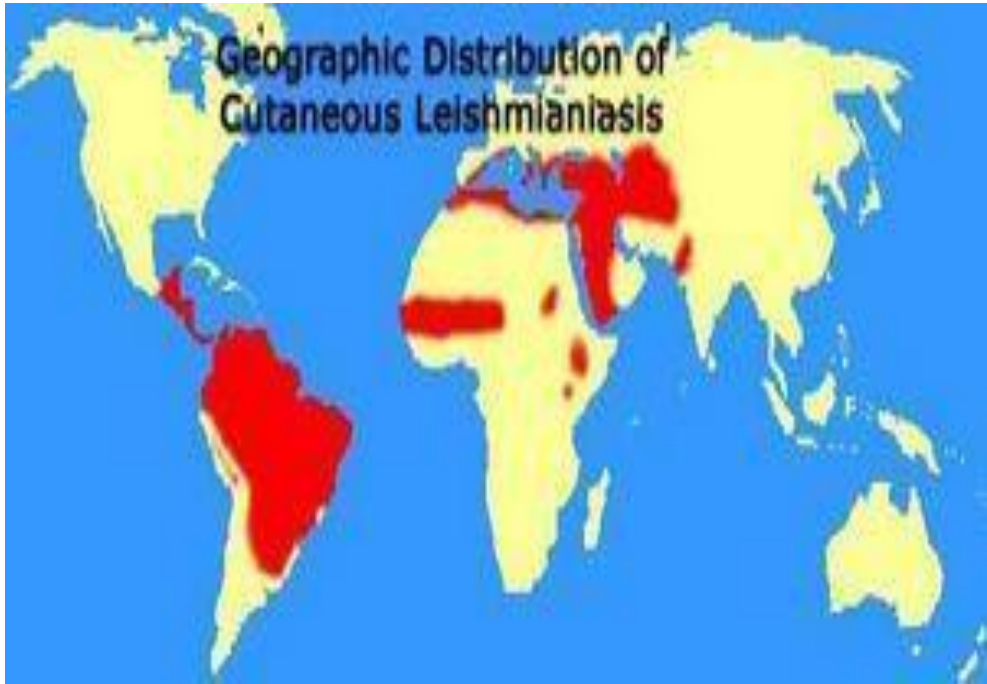
Transmission

1. Bite of sand fly (most common)
2. Transfusion blood and transplantation
3. Mother to baby
4. Direct contact; from man to man through nasal secretion (espicially Mucocutaneous leishmaniasis).



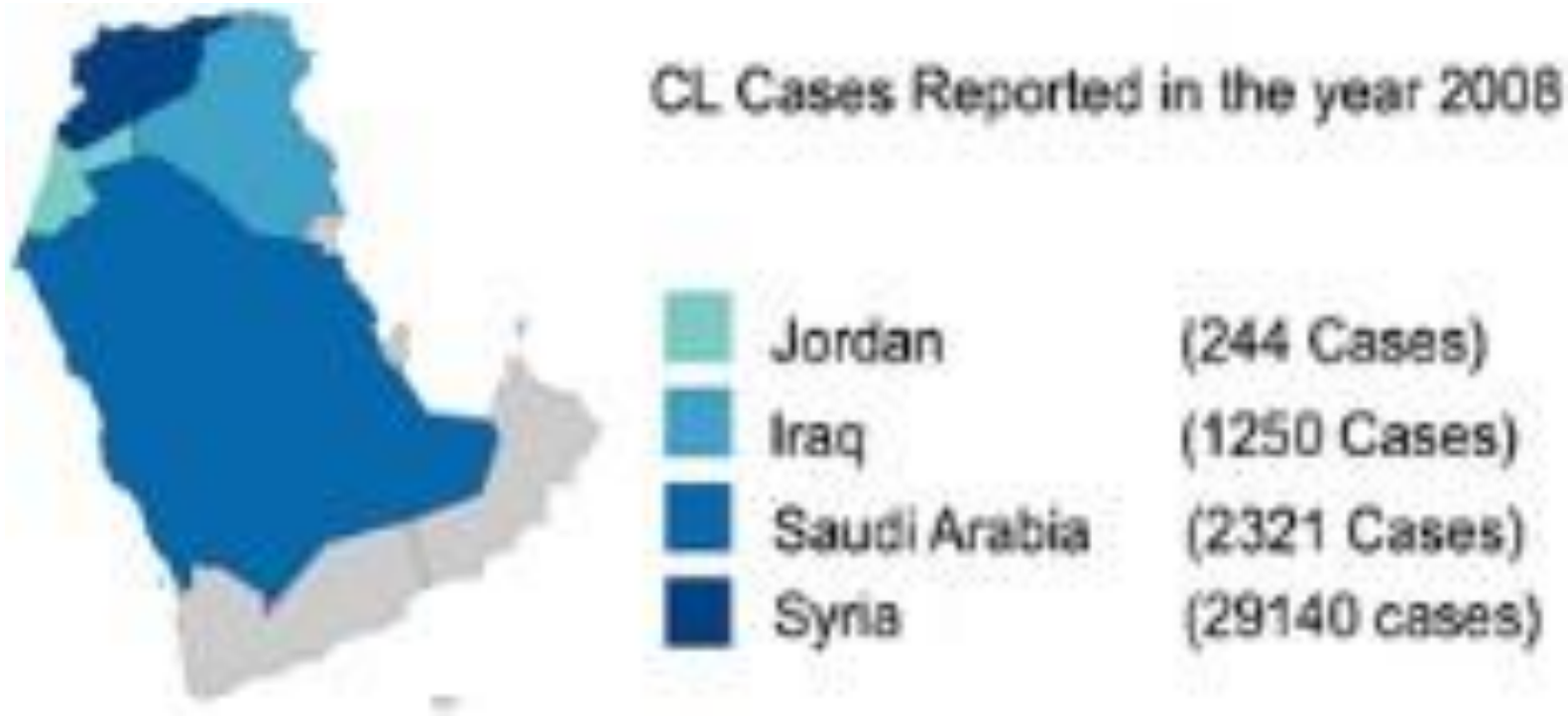
Cutaneous Leishmaniasis : Leishmania tropica, L major, L infantum

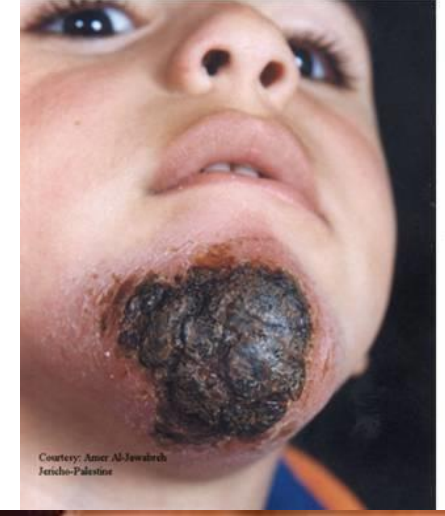
- **Habitat**: skin
- **Disease**: Cutaneous leishmaniasis
- **Clinical feature** : first sign is a **lesion** (generally a firm, The lesions begin as reddish , soft itchy papular , gradually enlarges ,raised and firm , with serous discharge at the bite site, ulceration could happen and it will be wet or dry and some immunosuppressed patients may have diffuse Cutaneous Leishmaniasis
- **Epidemiology**: the Middle East , south America

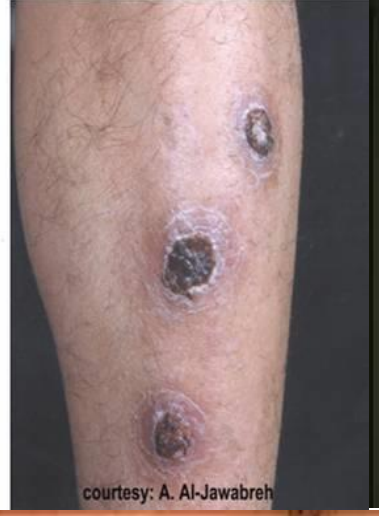


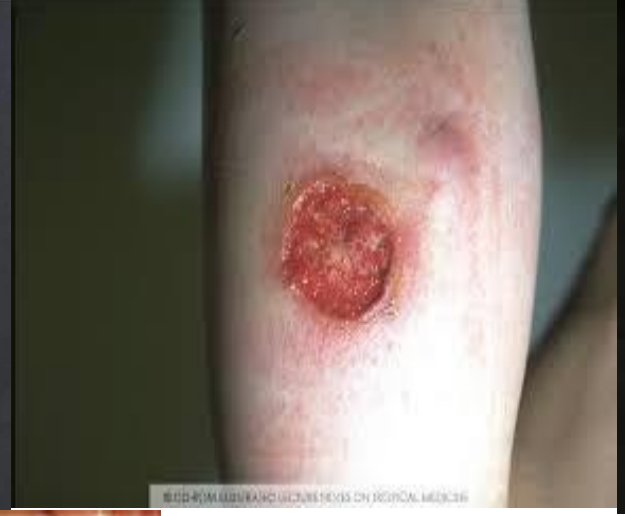
Leishmania In Jordan

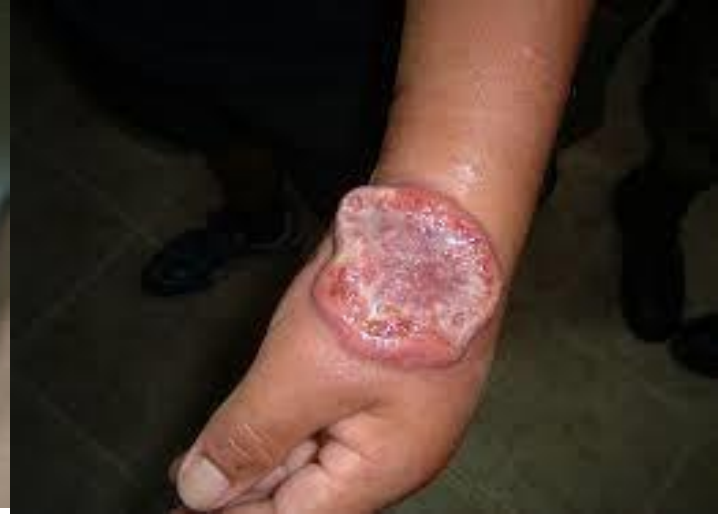
- In Jordan there are several species of Leishmania; Leishmania infantum, Leishmania tropica, and Leishmania major.
- Leishmania major is the major species of Leishmania parasite in Jordan (200-300)cases every year.











- The lesions caused by leishmaniasis begin as papules, progress to nodules, and eventually ulcerate (either as dry or wet ulcers), affecting any part of the body.

- They usually heal spontaneously but take months to heal and they might leave a scar and might not (Depends on the immune status of the individual).

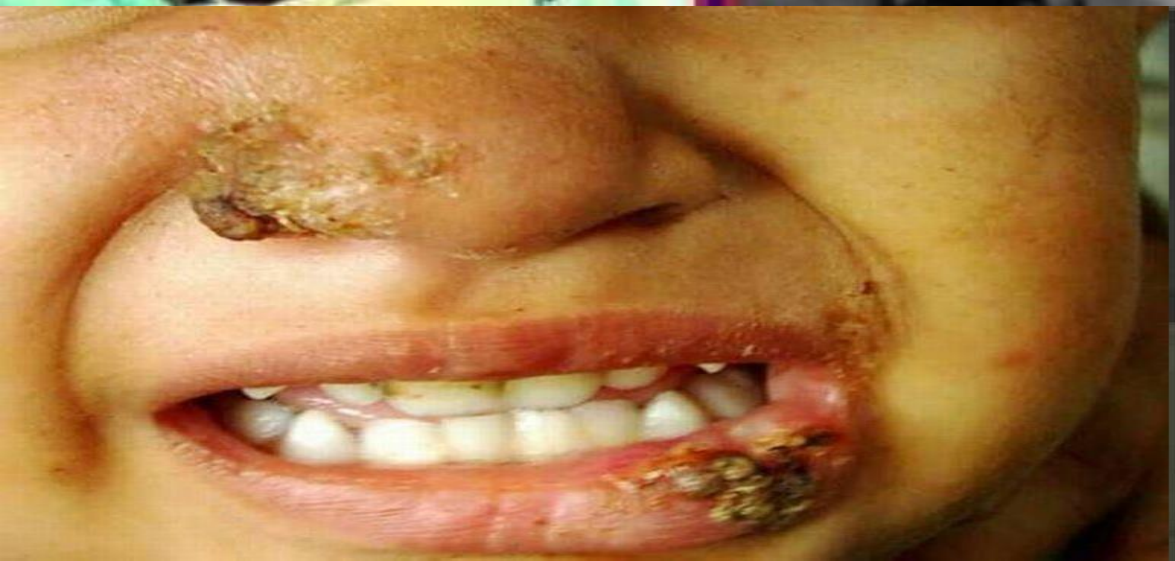
Mucocutaneous leishmaniasis(*L. braziliensis*)

(Also called Espundia , Nasopharyngeal leishmaniasis (this name is important because the pathology mainly happens in the nasopharynx).

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

✓ Mucocutaneous leishmaniasis might turn into visceral Leishmaniasis (immunosuppressed patients).
✓ Notice they have all lost their nasal septum due to destruction of cartilage

Mucocutaneous of American leishmaniasis



Visceral Leishmaniasis(*L.donovani*)

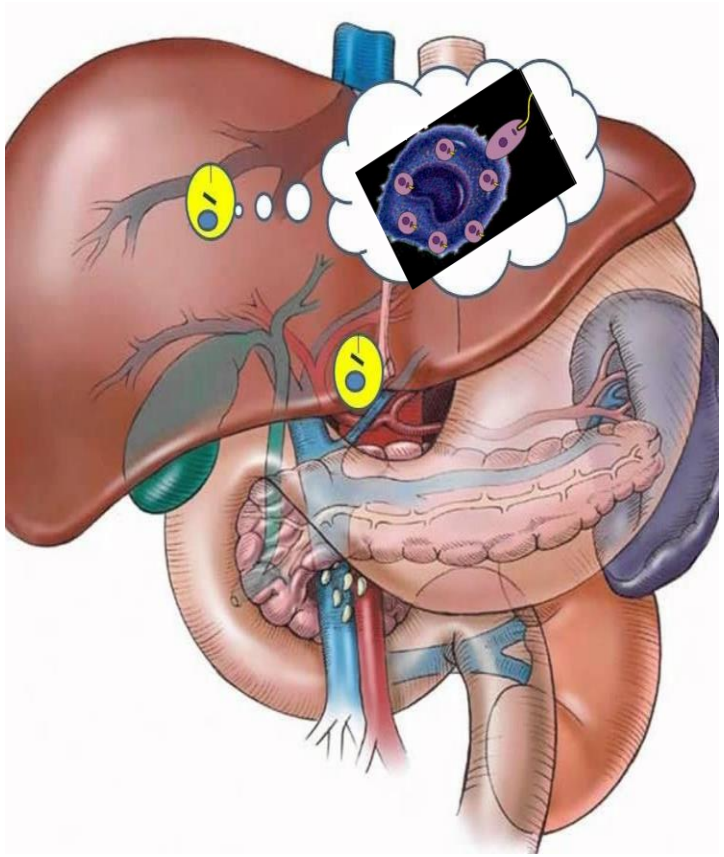
- Is the most severe form of leishmaniasis (fatal disease).
- The parasite migrates to the internal organs such as the liver, spleen (hence "visceral"), and bone marrow

- **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.(Not present in Jordan).

L.infantum can cause visceral type



- Abdominal distention, hepatosplenomegaly, and dry, rough, darkly pigmented skin are common features.
- In some patients, dermal lesions of post-kala-azar dermal leishmaniasis (PKDL) begin to appear two years after treatment occurring in 10-15% of patients.(it begins as visceral leishmaniasis and later progresses to dermal (cutaneous) leishmaniasis.

LABORATORY DIAGNOSIS

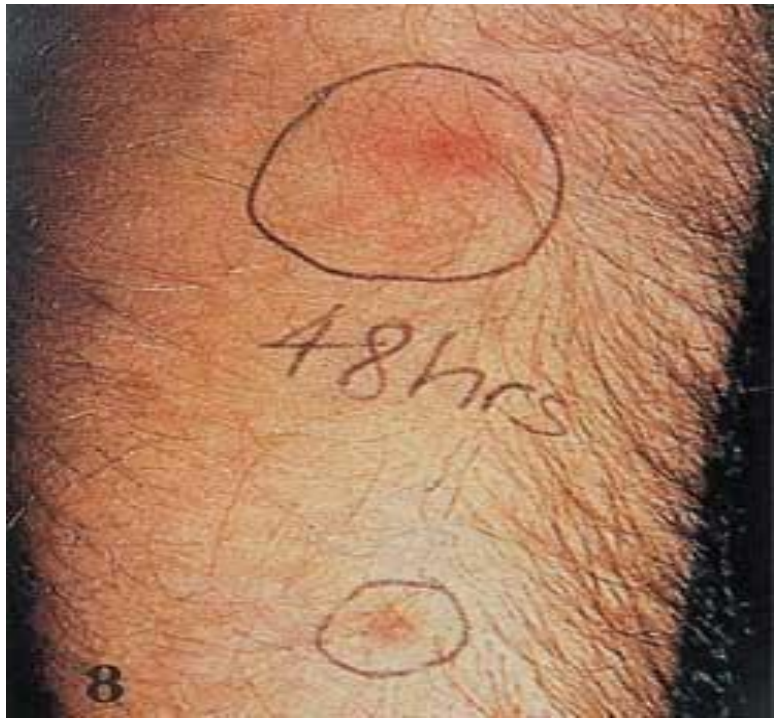
1) **Stained blood smear**: aspiration, scraping (looking for diagnostic stage which is amastigote)
2) **Cultured** :cultured using special techniques (triple N and Schneider media)

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

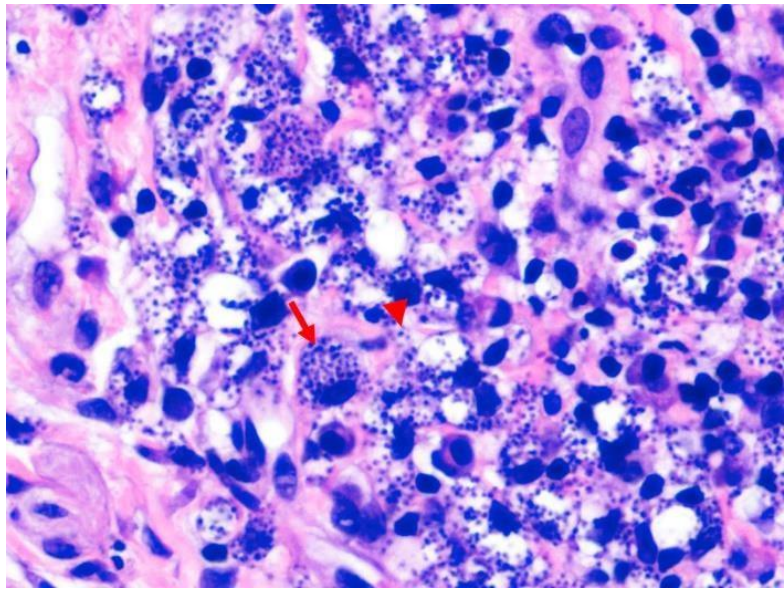
5-Intradermal Montenegro test: Injection of intradermal antigen prepared from cultured promastigotes of Leishmanian spp. This produces a typical cell-mediated response (you look at the induration after 48 h).

It indicates past exposure, or current activity of th disease, you can't tell.

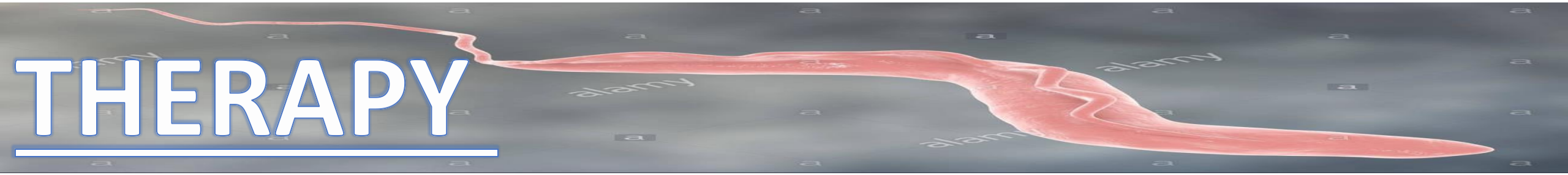


6-Histologic examination by biopsy from tissue to demonstrate the presence of organism in the tissue.

- In histopathology, Leishman-Donovan (LD) bodies appear when a biopsy or aspirate is taken from a lymphoid organ.



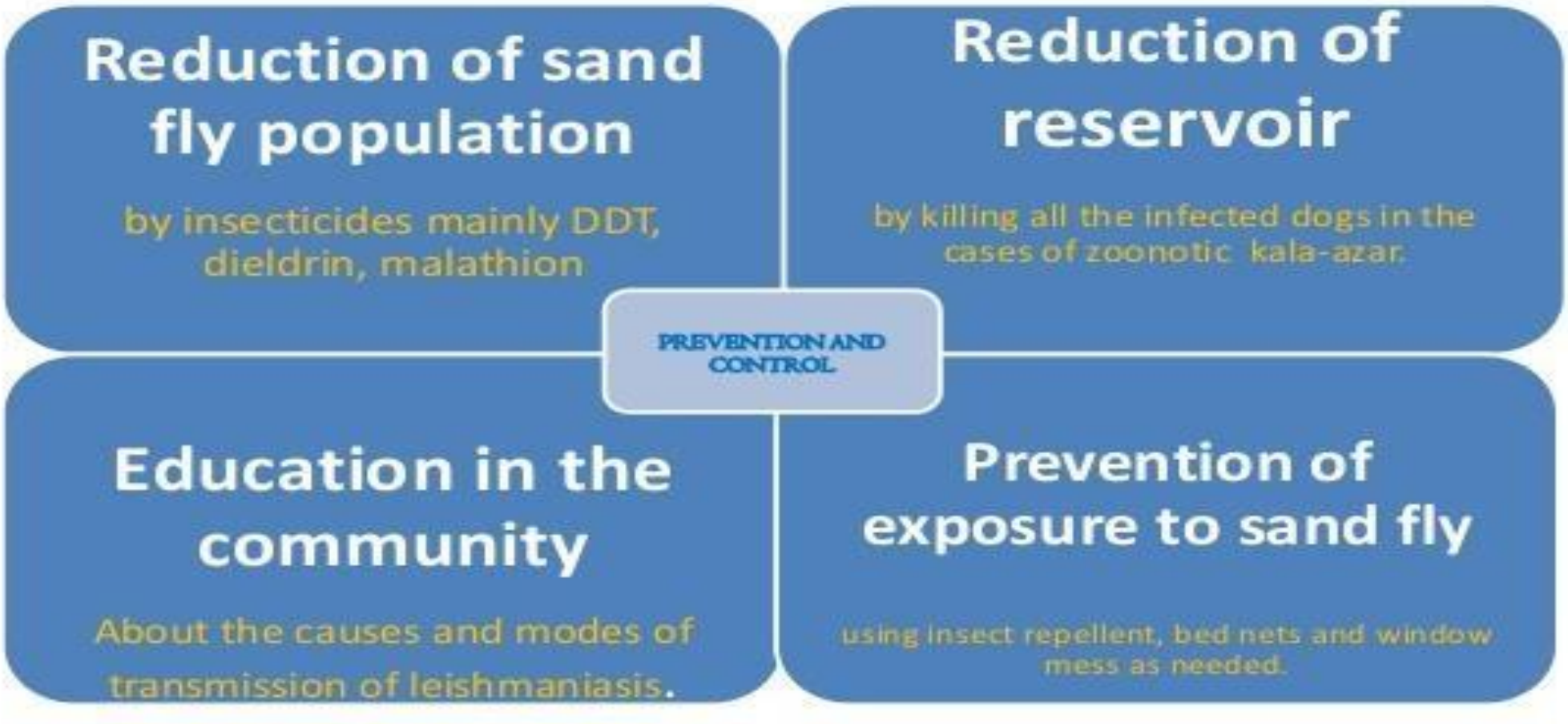
THERAPY



- 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 leishmaniasis.

PREVENTION



There are No Vaccines to prevent leishmaniasis.

The End

"اللَّهُمَّ إِنَّكَ عَفُوٌّ تُحِبُّ الْعَفْوَ فَاعْفُ عَنِّى"

VERSIONS	SLIDE #	BEFORE CORRECTION	AFTER CORRECTION
V1→ V2			
V2→V3			

امسح الرمز و شاركنا بأفكارك لتحسين أدائنا !!