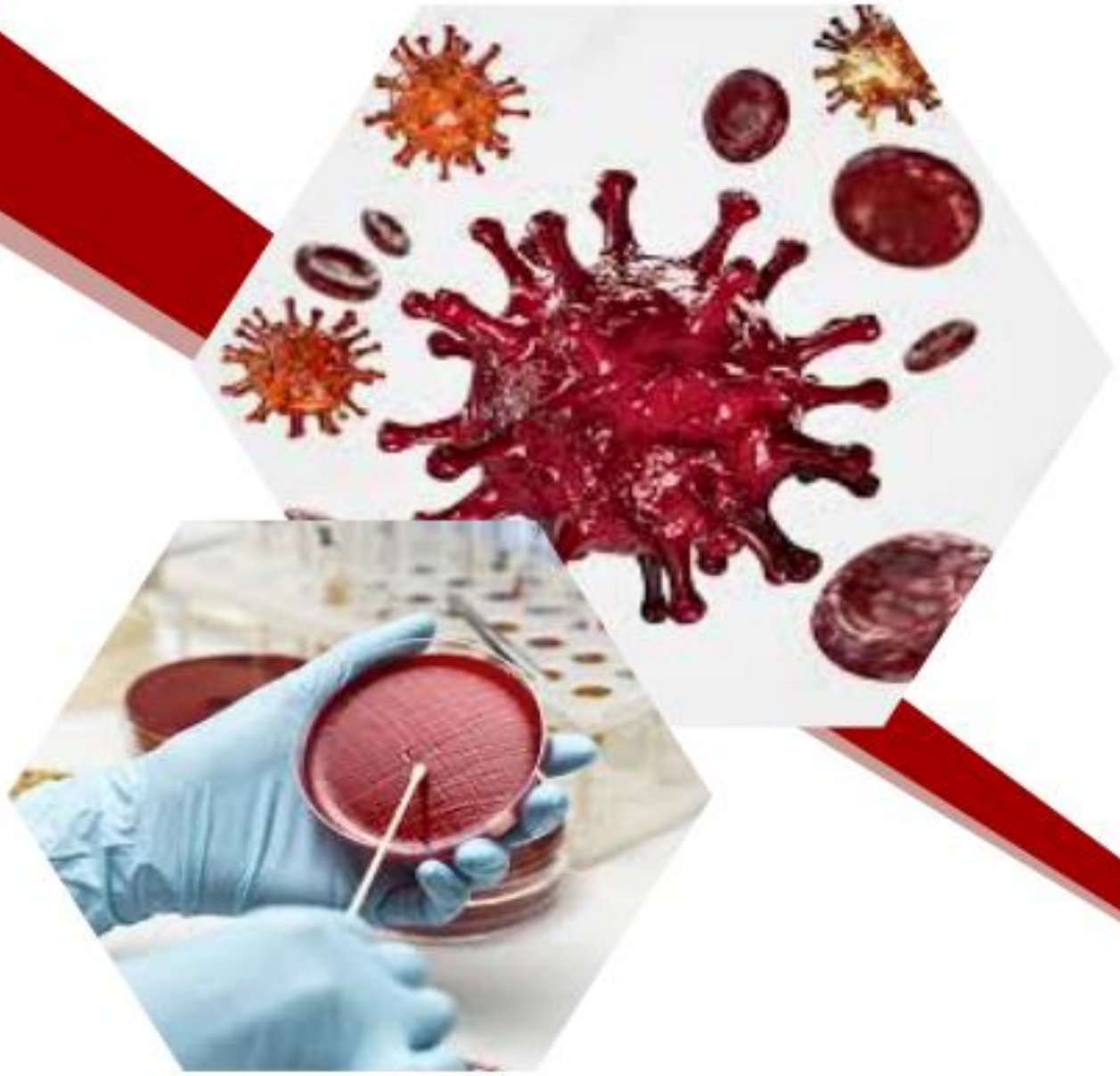
Doctor.021

no.2

HLS MICROBIOLOGY



Writer: Layan Al-Zoubi &Toleen Haddad

Corrector: Toleen Haddad & Layan Al-Zoubi

Doctor: Nader Araidah

HAEMFLAGELLATE

➢ Trypnasoma and Leishmania

- Haemflagellate is a subtype of protozoa (unicellular organisms); they cause blood infections.
- We previously said that the Plasmodium is considered to be part of the phylum Apicomplexia. Meanwhile, haemoflagellates are considered part of kinetoplastida since they share a common feature which is the Kinetoplast (a single complex mitochondrion OR A DNA containing structure).

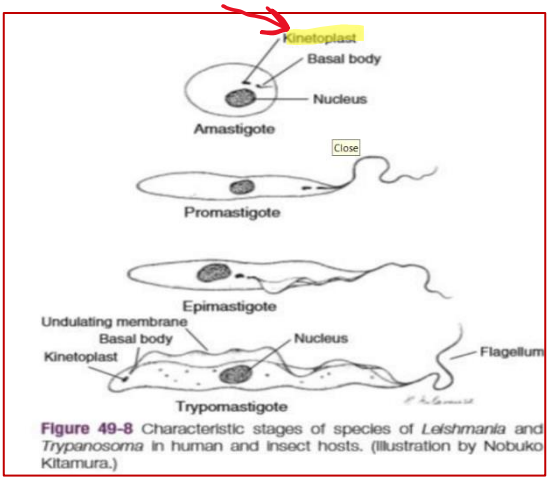
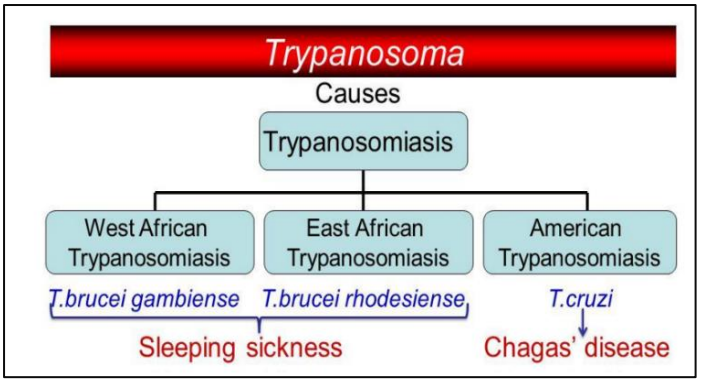


Figure 49-8 Characteristic stages of species of *Leishmania* and *Trypanosoma* in human and insect hosts. (Illustration by Nobuko Kitamura.)

- These parasites have 4 developmental stages (Notice the figure), you won't find them all in Trypnasoma or Leishmania, but keep in mind that the ones that exist in humans are the amastigote and trypomastigote while the other two exist in vectors.
- Flagella are their organ of locomotion.
- When a flagellum is inside the body of Protozoa it is called =Axoneme. While if it's on the border then it is called undulating membrane and a free flagellum when it's projecting outwards.
- Important: The amastigote stage is only intracellular.

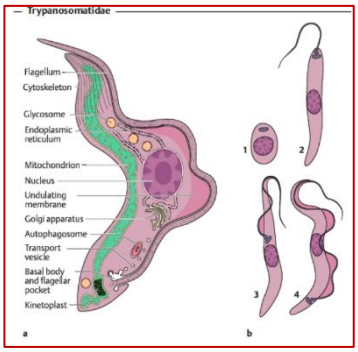
➢ Now we'll begin with Trypanosoma:

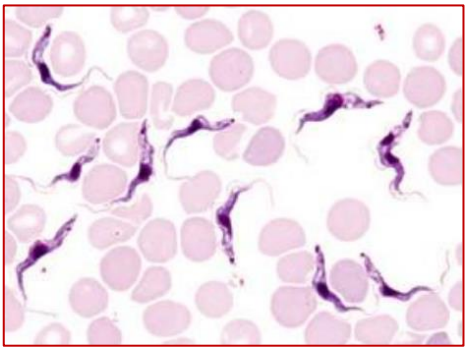
- **Causative agents of African trypanosomosis (sleeping sickness) and American trypanosomosis (Chagas disease).**
- **Trypanosoma brucei gambiense and Trypanosoma brucei rhodesiense cause African trypanosomosis (sleeping sickness) in humans.**
- They are divided into East African trypansomiasis (caused by T. brucei rhodesiense) and West African trypanosomiasis (caused by T. brucei gambiense); it is called sleeping sickness because these patients get an uncontrollable urge to sleep in late stages and it's a fatal disease.Trypanosoma cruzi, the causative agent of American trypanosomosis (Chagas disease)



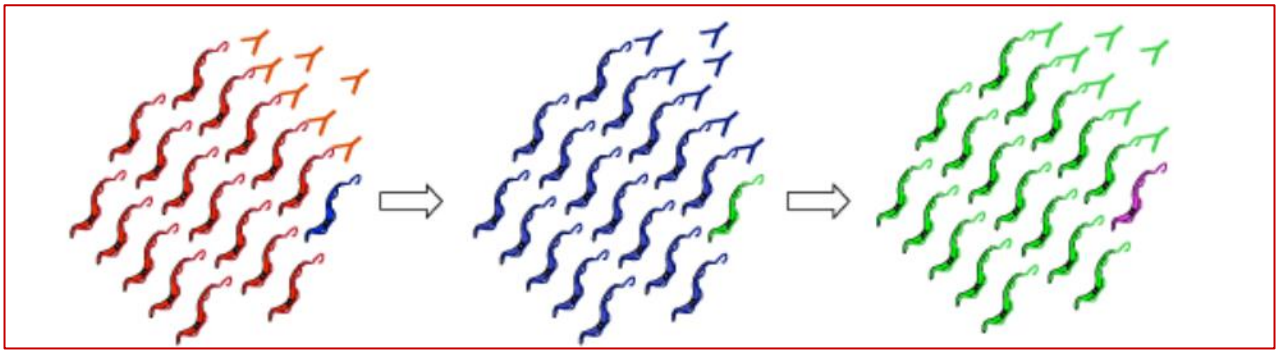
occurs in humans and many vertebrate animals in Central and South America.

- They reproduce asexually.
- **The morphologically differentiated forms include spindly, uniflagellate stages (trypomastigote, epimastigote, promastigote) and a rounded, amastigote form.**
- This blood film shows the diagnostic stage (and infective) of the African sleeping sickness which is called trypomastigote.
- All the life stages of African trypanosomiasis are extracellular (No Amastigote).





- **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) and each time the antigenic coat changes, the host does not recognize the organism and must mount a new immunologic response.**
  - A mosaic of genes (more than a thousand gene) encodes for these VSGs making a variation in their parasitemia causing a dilemma in their diagnosis.
  - This variation also happens in: Influenza virus, HIV, pneumococcus gonorrhea, Borrelia, Group A streptococcus.



AFRICAN TRYPANOSOMOSIS:

- **Is caused by 2 sub spp.:**

1) T. brucei gambiense: West African trypanosomiasis

T. brucei gambiense is a slowly progressing disease; a chronic one.

2) T. brucei rhodesiense: East African trypanosomiasis

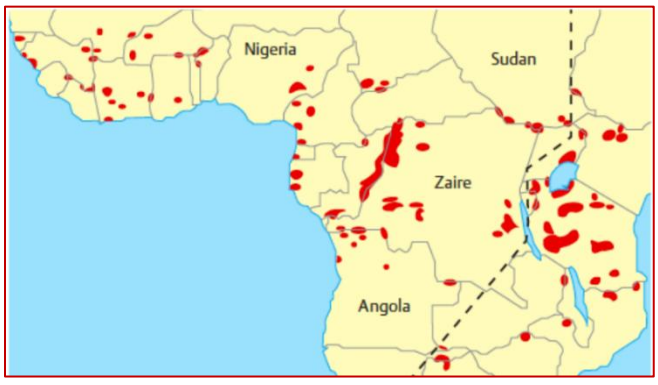
T. brucei rhodesiense is a rapidly progressing disease; infected patients develop neurological manifestations within a year, and one of the explanations for the difference between these two types is the difference in the reservoir host; as in the case of rhodesiense both infected humans and animals serve as reservoir hosts while in gambiense mainly humans serve as such hosts.

- **Vector: tsetse fly (Glossina spp.); which is found only in rural Africa.**
  - **Glossina palpalis transmits T. b. gambiense.**
  - **Glossina morsitans transmits T. b. rhodesiense.**

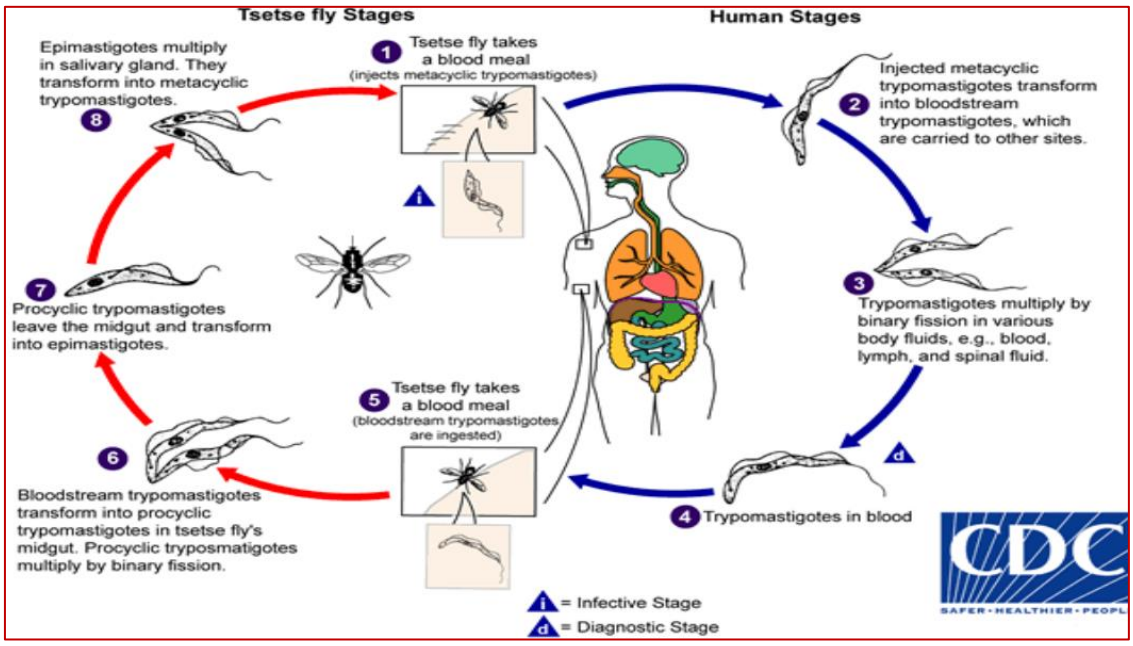
-Both sexes of this fly participate in the transmission.

- **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.**

  - Everything that lies to the east of the dotted line is T. rhodesiense and to its west is T. Gambiense.



- Ponder this figure well, it shows the life cycle of African Trypanosomiasis.

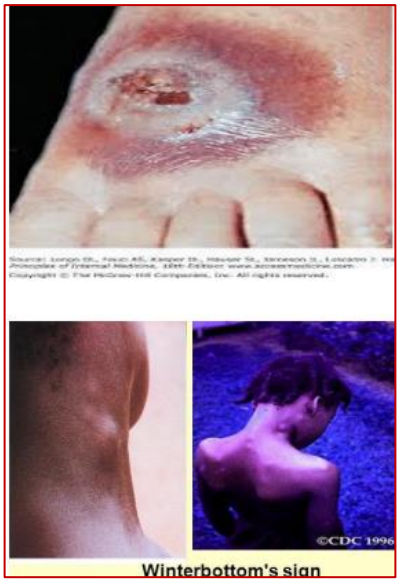


- Infective stage: Trypanomastigote.
- Diagnostic stage: Trypanomastigote.

- Trypanomastigote stage is extracellular and it can reach various body fluids; it doesn't have to stay in blood.
- Notice the reproduction process of the trypanomastigote inside the tsetse fly when it ingests it again.

CLINICAL FEATURES:

After the host has been bitten by an infected tsetse fly, a nodule or chancre at the site may develop after a few days.



Winterbottom's sign

- Chancre: A localized reaction; it begins as a papule then becomes a nodule and eventually it undergoes ulceration and becomes oozing, it's painless and it's seen in syphilis as well.

***Stage I: the patient has 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 (Trypanomastigotes' invasion of blood and body fluids causes enlargement of the lymph nodes in the posterior cervical trunk)**.**

*** Stage II: organisms invade the CNS; the sleeping sickness stage of the infection is initiated.**

- The patient becomes emaciated and progresses to profound coma and death.

- The first symptom in these patients is a change in the personality (character).

- Knowing what stage the patient falls in is very important as the treatment and the prognosis both differ between the 2 stages (Stage II is fatal).

➢ **Laboratory diagnosis:**

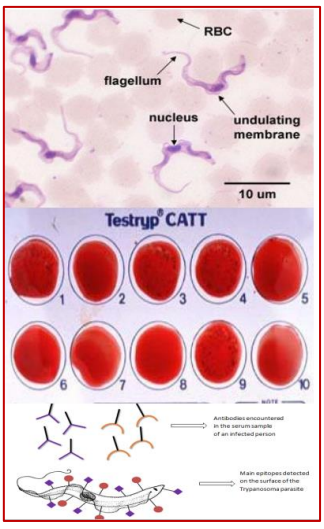
- **Specimen: blood, serum,CSF, aspiration from lymphnode**

1) Routine Methods: thick and thin blood films (This is the gold standard for diagnosis; the microscope).

2) Antigen Detection: simple and rapid test card indirect agglutination (So many constraints on their interpretation; considering the antigenic variation).

3) Antibody Detection: Serologic by using ELISA Serum or CSF IgM concentrations (Shows past exposure mainly).

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



Definitive diagnosis: Trypomastigote

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 (old one) can be used when the CNS is not infected (Stage I).**
- **Melarsoprol, a toxic trivalent arsenic derivative, and** Eflornithine **are effective for both blood and CNS stages (they can cross the blood-brain barrier) but is recommended for treatment of late-stage sleeping sickness.**
- These drugs are highly toxic, and called Resurrection drugs, they cause severe side effects.

PREVENTION:

- **Preventing flies from biting through the use of insecticide will reduce the transmission of the parasite.**
- **Screening of people at risk helps identify patients at an early stage.**
- **Treatment cases and should be monitored for 2 years after completion of therapy.**
- No vaccine is currently available for trypanosomiasis.

AMERICAN TRYPANOMOSIASIS

Trypanosoma cruzi (Chagas' disease)

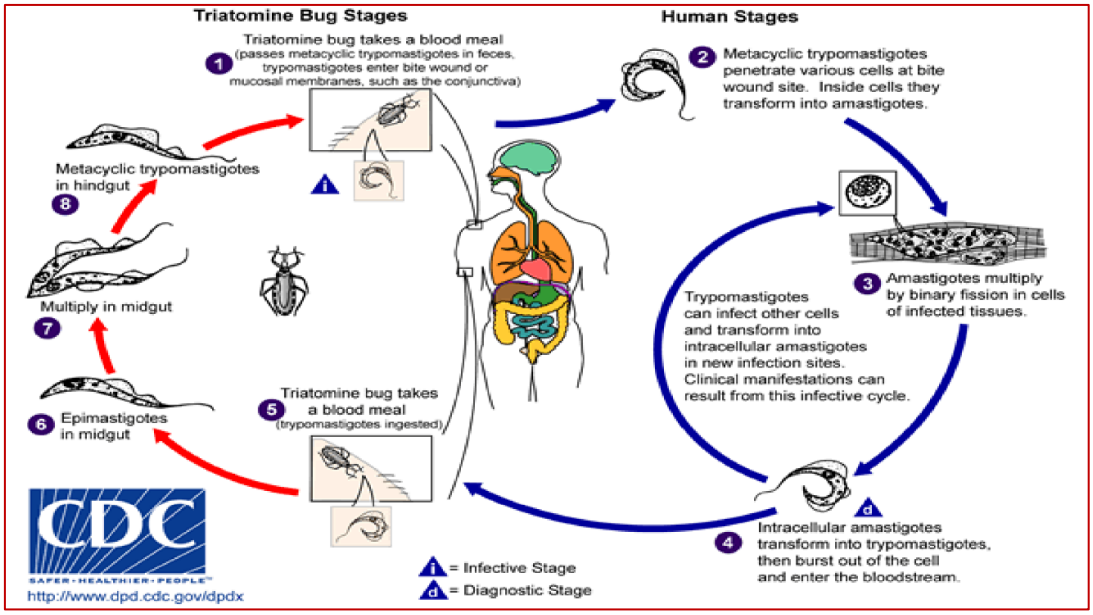
- **Zoonosis**
- **Transmitted by vector: reduviid bugs**
  - Other names: kissing bug/triatomine bug
- **Reduviid bug defecates while taking a blood meal**
  - The bug bites then excretes its feces which contains the infective stage; since the bite results in an allergic reaction, the patient would 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.
  - It defecates on the face around the mouth.

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

1- trypomastigote in blood (extracellular)

2- amastigote (round intracellular form; can be found in biopsy from hollow organs)

- They are mostly found in the heart muscle followed by the liver and the brain.
- Infective stage: trypomastigote (The bug doesn't inject it; it rather defecates the trypomastigote since it goes to the hindgut of the vector during its development).



- ponder the figure well.

EPIDEMIOLOGY

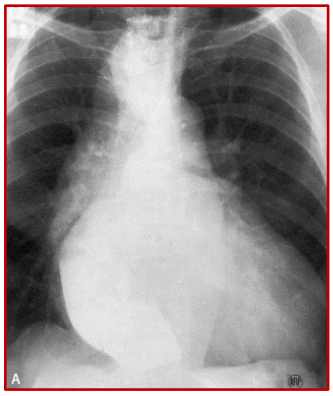
Throughout central and south America.

PATHOGENESIS

- **Chagas' disease is categorized as acute, indeterminate, and chronic**
- **Nodule chagoma: near the bite (Appears in body parts other than the face).**
  - Keep in mind the difference between nodule and papule; nodule is hard edema.
- **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**
- **Unilateral swelling of eyelids romana's sign** (in case the bite was in the face)
- **Acute myocarditis (The chief complaint when these parasites access the heart muscle).**



CHRONIC PHASE (MORE DANGEROUS):

- **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.
- Their life expectancy is reduced.
- They get tired a lot.

THERAPY

- **Nifurtimox and benznidazole reduce the severity of acute Chagas' disease.**
  - These 2 medications are shown to be effective when given at the acute phase.

- **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.**

PREVENTION

1. Vector control

2. Transfusion control and screening of blood donors.

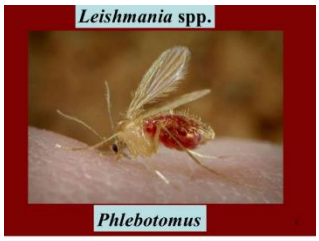
3. testing of organ, tissue or cell donors and receivers.

* Can be transmitted vertically.

- Remember; these parasites have other routes of transmission, not just through vectors.



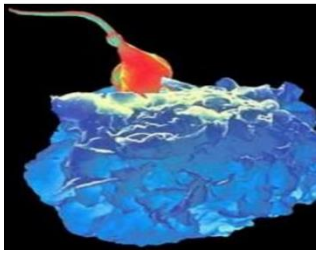
LEISHMANIA



- **It is a flagellated protozoan**
- **Life cycle requires two hosts:**

  a) vertebrate; mammalian host

  b) Invertebrate vector; female sand fly /phlebotomus.



- **Obligate intracellular organism;** therefore, diagnostic stage is amastigote only.
- **Infects primarily phagocytic cells and macrophages;** these phagocytic cells either remain in the organ they're in or go to other lymphoid organs such as liver, spleen and bone marrow.
- **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.**

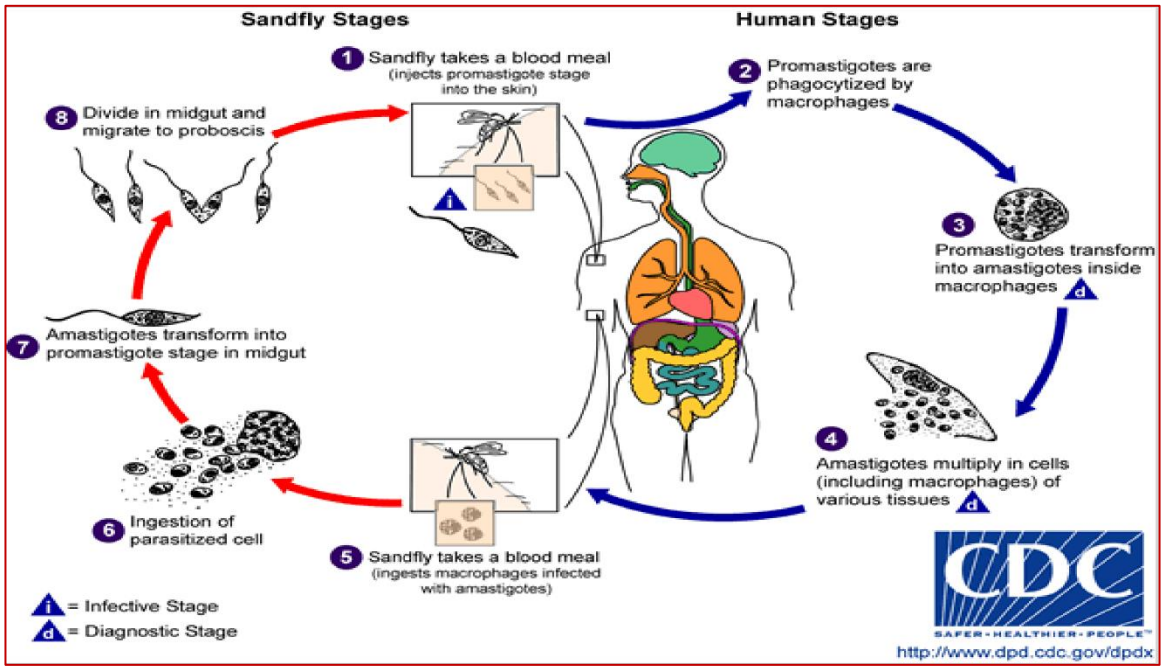
1. **Cutaneous Leishmaniasis** → In case the macrophages remained confined to the superficial surface of the skin.

(L.tropica, Leishmania major, L. infantum)

- Other names for cutaneous leishmaniasis: Baghdad boil/aleppo boil/ oriental sores/delhi boil

Old world	New world
L.tropica	L. mexicana
L. major	L. ethiopia
L. infantum	

2. **Mucocutaneous leishmaniasis (L. braziliensis)** → if they went deeper.
   - These are mainly naso-oral and they call them espundia/naso-oral.
3. **Visceral Leishmaniasis (L.donovani)** -also called **kala azar or Black fever**→ if they entered lymphoid organs (liver, spleen, bone marrow)



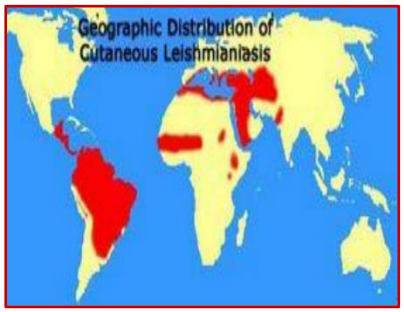
- Infective stage: promastigote.
- Promastigote gets picked up immediately by a circulating monocyte and is called amastigote which is the diagnostic stage. Then it continues its life cycle in the sandfly.

TRANSMISSION

1. **Bite of sand fly**
2. **Transfusion blood and transplantation**
3. **Mother to baby**
4. **Direct contact; from man to man through nasal secretion**→ This one is controversial.

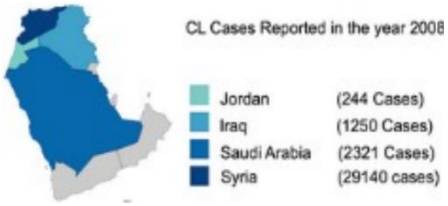
CUTANEOUS LEISHMANIASIS: LEISHMANIA TROPICA, L MAJOR, L INFANTUM

- **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)**.**
- **Epidemiology: The Middle East, south America**

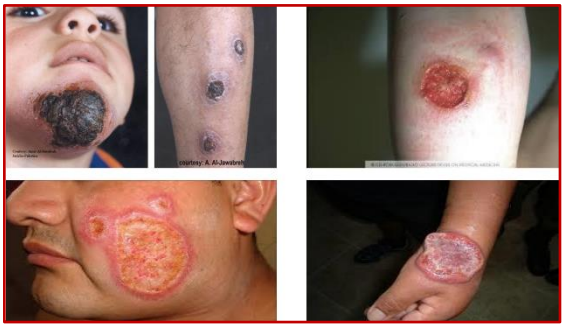


LEISHMANIA IN JORDAN

- **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.**



Oriental sores can be either wet or dry:



1- Look at the child's chin in the image; that's a dry sore.
   Anthroponotic transmission: which is transmitted from human to vector to human.
2- Look at the image right below it; that's a wet (oozing) one. Zoonotic transmission: which is transmitted from animal to vector to human, worse progression.

- 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).
- They are painless.

MUCOCUTANEOUS LEISHMANIASIS (L. BRAZILIENSIS)

- **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.**
- Notice they have all lost their nasal septum due to destruction of cartilage☹

VISCERAL LEISHMANIASIS (L. DONOVANI)

- Not present in Jordan.
- **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 (only thing they complain of) followed by hepatosplenomegaly without an obvious cause… if left untreated, will almost always result in the death of the host.**

- **Epidemiology: Bangladesh (mostly), Brazil, Ethiopia, India, South Sudan and Sudan**
- Dermal lesions start to appear PKDL (post kala azar dermal leishmaniasis) in some patients after they're treated for it by months. One might think these lesions are cutaneous if they don't take history.

LABORATORY DIAGNOSIS

1) Stained blood smear: aspiration, scraping (for cutaneous use scraping and look for amastigote)

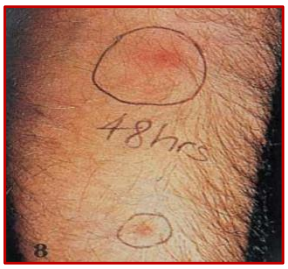
2) Cultured: cultured using special techniques (generally difficult but there is medium for them called triple N)

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

*Definitive diagnosis: Amastigote

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

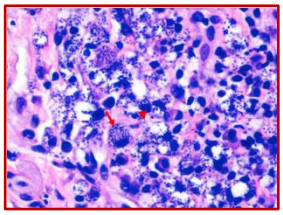
5) Intradermal Montenegro test: Injection of intradermal antigen prepared from cultured promastigotes of Leishmanian spp. This produces a typical cell-mediated response.



Then it's put in their skin, you ask them to come back later (you look at the induration after 48 h). This is Type 4 hypersensitivity, cell mediated immunity, delayed type.

It indicates past exposure.

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

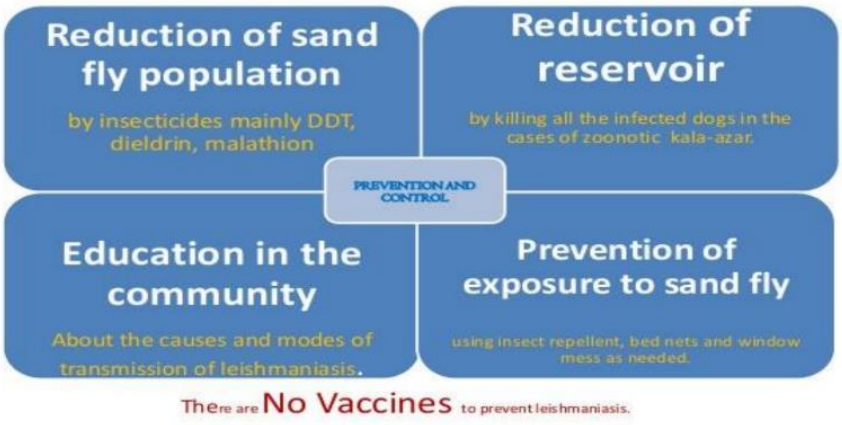


7) Xeno- diagnosis: taking a specimen from the patient and putting it in a rat or mouse.

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 and mucocutaneous leishemeniasis.**

PREVENTION



يا رب، إخواننا في غزة مستضعفين، يستفرد بهم أعداؤك وليس لهم إلا أنت، فاللهم بقدرتك رد عنهم بأس الذين كفروا، وأنت أشد بأسا، وأشد تنكيلا..... اللهم لا تجعل للكافرين علينا سبيلا.

" إن يمسسكم قرح فقد مس القوم قرح مثله وتلك الأيام نداولها بين الناس".

V3

- Page 1: When a flagellum is inside the body of Protozoa it is called =Axoneme. While if it's on the border then it is called undulating membrane and a free flagellum when it's projecting outwards.
- Page 7: Definitive diagnosis: Trypomastigote
- Page 8: When the feces of the sand fly get access to the conjunctiva; the patient will have unilateral swelling of the eye.
- Page 11: visceral leishmaniasis is also called black fever.
- Page 13: 1) oriental sores are painless.
  2) Notice they have all lost their nasal septum due to destruction of cartilage.
  3) Clinical signs: enlarged liver and spleen swollen lymph nodes occasional acute abdominal pain (only thing they complain of) followed by hepatosplenomegaly without an obvious cause.
  4) Definitive diagnosis: Amastigote

- Page 14: 1) The antigen used in the Montenegro test is promastigote not amastigote.