جامعة الانبار

كلية: الصيدلة

قسم: العلوم المختبرية السريرية

اسم المادة باللغة العربية: الاحياء المجهرية

اسم المدة باللغة الإنكليزية: **microbiology**

المرحلة: الثانية

التدريسي: سليمان عجاج عبدالله

عنوان المحاضرة باللغة العربية: سوطيات الدم

عنوان المحاضرة باللغة الإنكليزية: **Haemoflagellates**

محتوى المحاضرة

 Medically important haemoflagellates require two hosts to complete their life cycle, some are called (digenetic or heteroxenous).
 They live in the blood and tissue of human and other vertebrate hosts and also in the gut of insect vectors.
 Haemoflagellates infecting human belong to two genera, in the family trypanosomatidae (Trypanosoma and Leishmania).

Morphological stages

Haemoflagellates exist in two or more of the following four morphological stages:

1- Amastigote (leishmanial form):
This stage is rounded or oval shaped without flagellum. This stage found intracellulary in vertebrate hosts.

2. Promastigote (leptomonal form):
This stage is lanceolate the kinetoplast is anterior to the nucleus, from which arises the short flagellum. There is no undulating membrane, this is the infective stage of leishmania found in the mid-gut of insect.

3- Epimastigote (crithidial form)
This stage is elongated with the kinetoplast placed more posteriorly and in front of the nucleus. The flagellum extends alongside the body as a short undulating membrane.

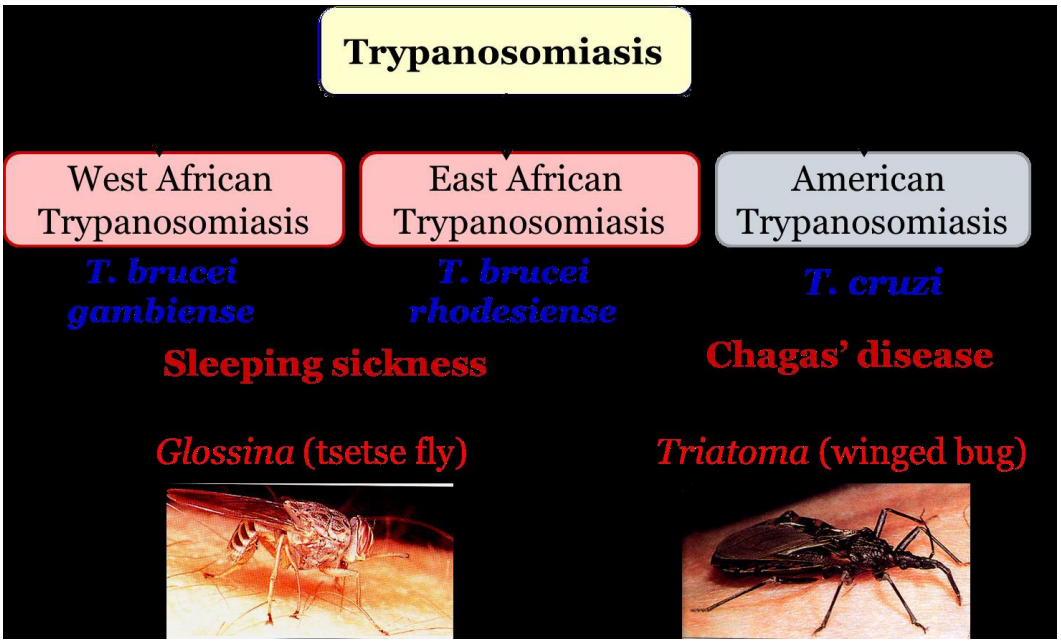
4. Trypomastigote (trypanosomal form):

This stage is more elongated, spindle-shaped with central nucleus and the kinetoplast posterior to the nucleus. The flagellum extends alongside the entire length of the cell to form long undulating membrane before protruding from anterior end. This stage lacking in leishmania

Trypanosomes

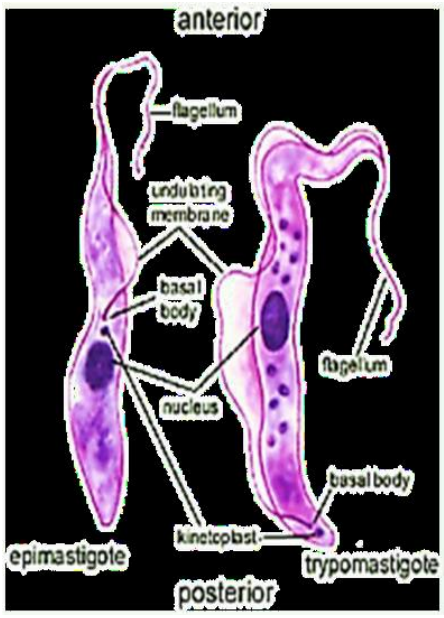
 All members of the genus trypanosoma exist at sometime in their life cycle, as the trypanomastigote (trypanosomal stage).

 Blood sucking insects represent the intermediate host and vector. The vector becomes infective to the vertebrate host only after an external incubation period during which the parasite undergoes development and multiplication.



African sleeping sickness (African Trypanosomiasis)

- **Causal Agent:** *Trypanosoma brucei*
- **Morphology:** Exist into two interchangeable forms:



- **Trypomastigote**

in Blood, and Lymph tissue space of various organs & C.N.S is terminal & fatal

- **Epimastigote** (Vector only)

in salivary gland of vector & Culture media

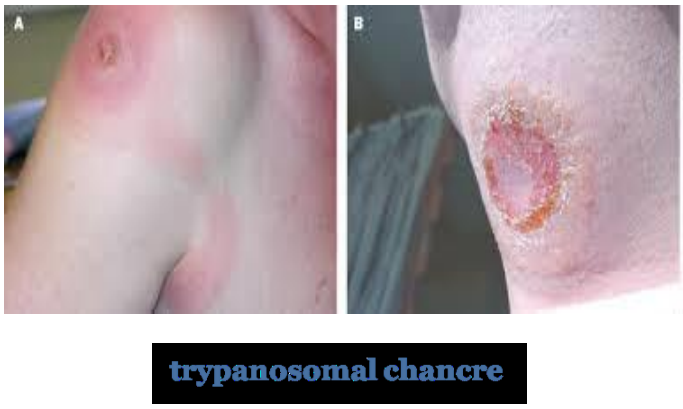
Clinical Features

Infection occurs in 3 stages:

 A trypanosomal chancre can develop on the site of inoculation.

 Hemolymphatic stage with symptoms that include fever, lymphadenopathy, and itching.

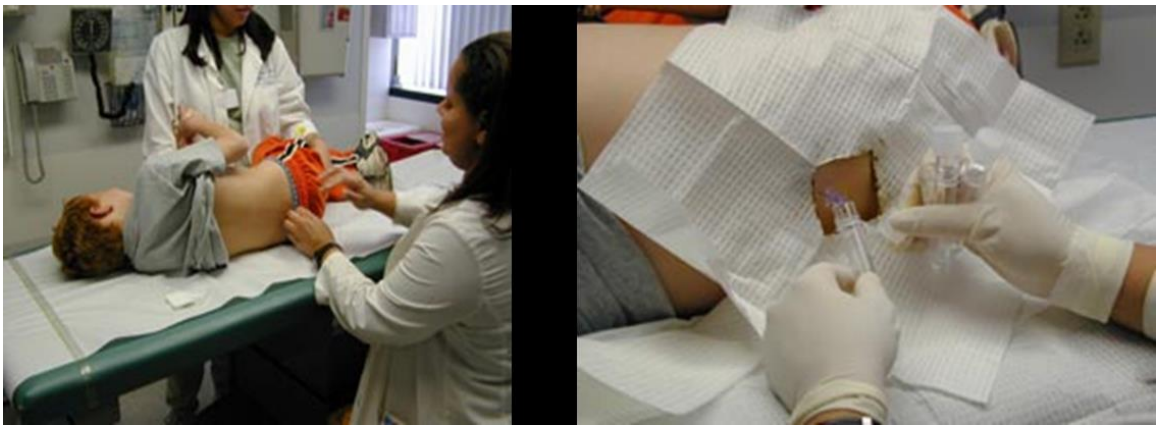
 Meningoencephalitic stage, invasion of the central nervous system can cause headaches, somnolence, abnormal behavior, and lead to coma.



Laboratory Diagnosis

 Clinical picture

 Specimens: The diagnosis depends on demonstrating trypanosomes by microscopic examination of chancre fluid, lymph node aspirates, blood, bone marrow, or, in the late stages of infection, cerebrospinal fluid.



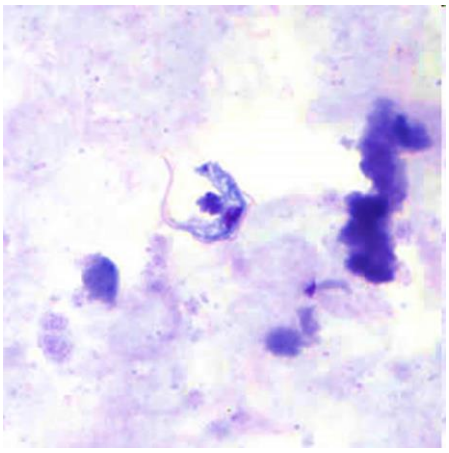
 A wet preparation should be examined for the motile trypanosomes, and in addition a smear should be fixed, stained with Giemsa stain, and examined. Concentration techniques can be used prior to microscopic examination.

 Antibody detection (immunofluorescence and ELISA) has sensitivity and specificity.

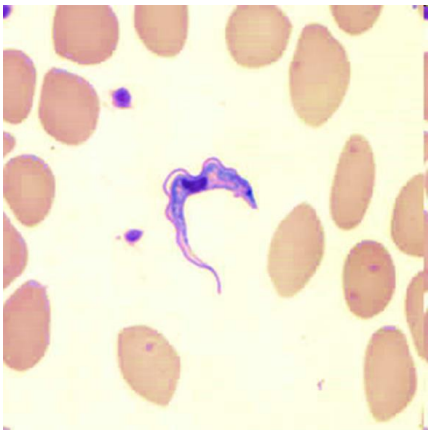
 Culture on suitable medium (Novy-MacNeal-Nicolle OR Weinmann’s media) to detect Epimastigote.

 Animal inoculation

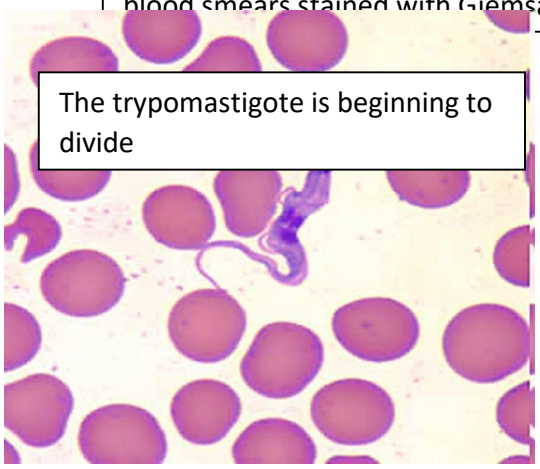
 Molecular methods using (PCR).



Trypansoma brucei ssp. in thick blood smears stained with Giemsa



Trypansoma brucei ssp. in thin blood smears stained with Giemsa



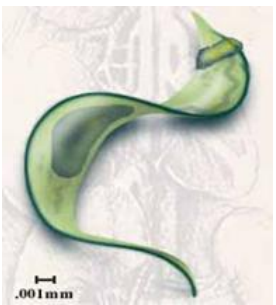
The trypomastigote is beginning to divide

Tsetse fly taking a blood meal

Chagas Disease (American Trypanosomiasis)

- Causal Agent: *Trypanosoma cruzi*

- **Morphology:**

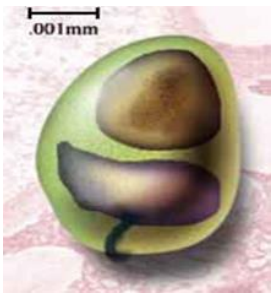


- **Trypomastigote** (Monomorphic)

Slender shaped (20µ) – Central nucleus – C or U-shaped –Free flagellum 1/3 body- length.

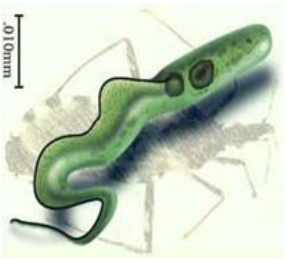
o Amastigote

Obligatory intracellular – mainly in cardiac & Skeletal muscles – Brain meninges – Nerve ganglia – cells of GIT …. etc



o **Epimastigote** (Vector only)

Spindle shape– Kinetoplast anterior to central nucleus– Undulating membrane is short – terminal free flagellum



Clinical Features

 The acute phase is usually asymptomatic, but can present with manifestations that include fever, anorexia, lymphadenopathy, mild hepatosplenomegaly, and myocarditis.

Chagoma: (Romaña's sign)

 Romaña′s sign may appear as a result of conjunctival contamination with the vector′s feces.

 A nodular lesion, usually called chagoma, can appear at the site of inoculation.

 The symptomatic chronic form may occur for years or even decades after initial infection. Its manifestations include cardiomyopathy (the most serious manifestation); pathologies of the digestive tract such as megaesophagus and megacolon; and weight loss.

Laboratory Diagnosis

 Microscopic examination:

 of fresh anticoagulated blood, or its buffy coat, for motile parasites

 of thin and thick blood smears stained with Giemsa, for visualization of trypomastigote .

 Biopsy from lymph node, liver or spleen (amastigotes) are found

 Isolation of the agent:

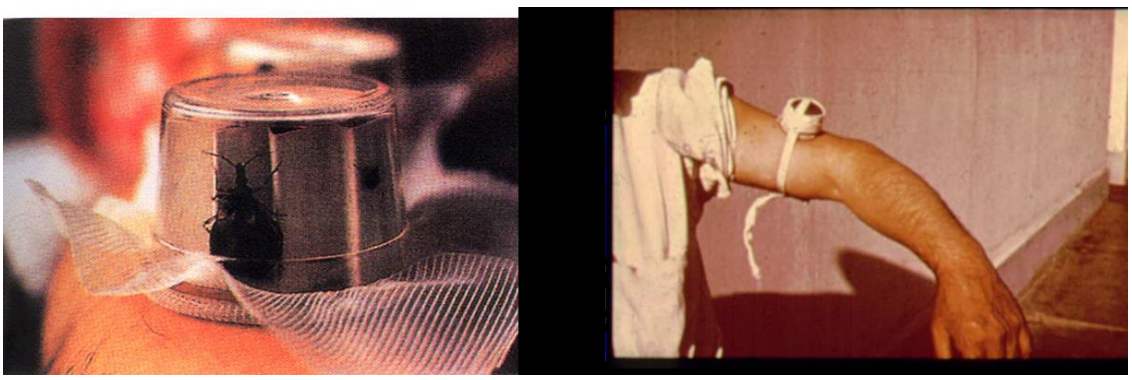
 inoculation on N.N.N. medium (Epimastigotes)

 inoculation on N.N.N. medium (Epimastigotes)

 Polymerase chain reaction (PCR).

Xenodiagnoses

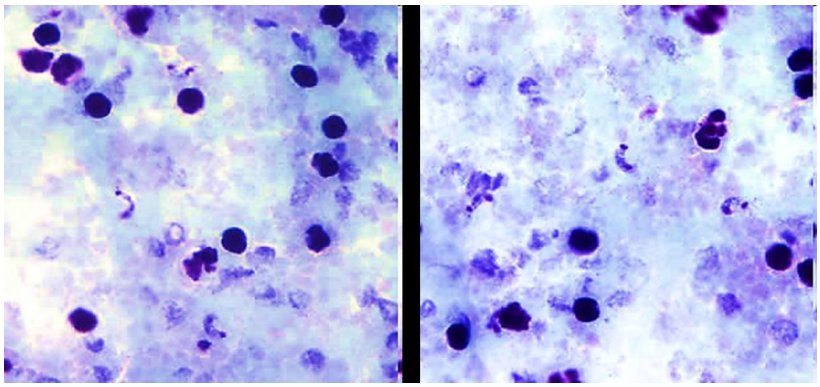
 Highly efficient – demonstrate low level of parasite in blood



Method:

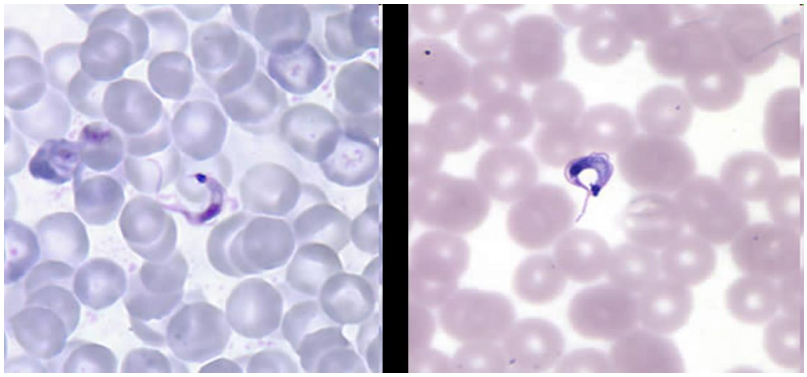
A Laboratory bred winged bug is starved for 2 weeks then fed on suspected patient's blood – 30 days later, it feces & gut examined for trypanosomes.

T. cruzi trypomastigotes in a thick blood smear stained with Giemsa



T. cruzi trypomastigotes in thin blood smears stained with Giemsa

Note the typical C-shape of the trypomastigote that characterizes *T. cruzi in fixed blood smears*



Triatomine bugs

Leishmaniasis

☐ Leishmaniasis is a zoonosis disease, transmitted among mammalian hosts by female sand flies.

☐ Human leishmanial infections can result in 2 main forms of diseases:

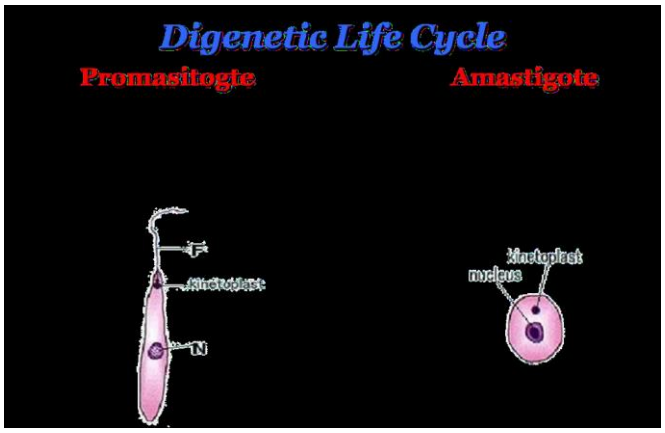
- ***Leishmania donovani*** (Visceral leishmaniasis)

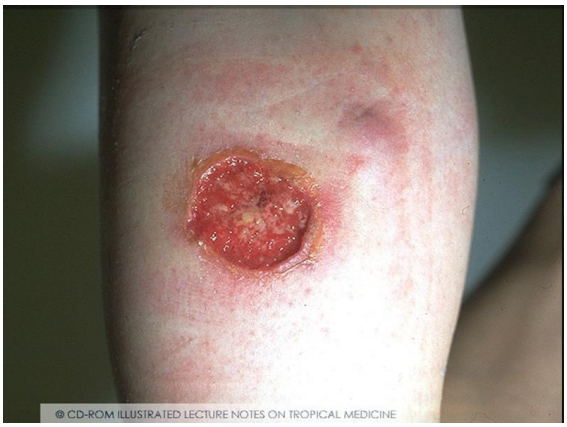
kala azar (Liver, spleen and bone marrow)

- ***Leishmania tropica*** (Cutaneous leishmaniasis)

(Skin and mucous membranes)

Morphology





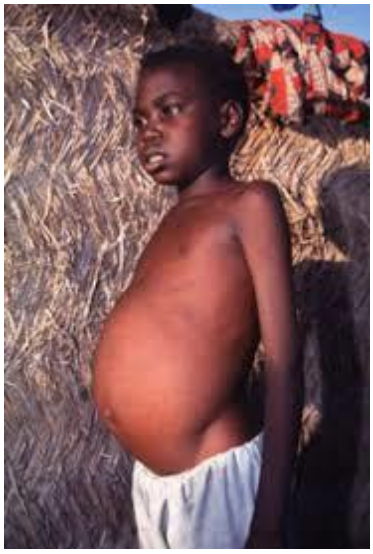
Cutaneous leishmaniasis

Visceral leishmaniasis

- Persons who have visceral leishmaniasis usually have fever, weight loss, and an **enlarged spleen and liver**. Some patients have swollen glands.

- Certain blood tests are abnormal. For example, patients usually have low blood counts, including a low red blood cell count (anemia), low white blood cell count, and low platelet count. Some patients develop post kala-azar dermal leishmaniasis.

Visceral leishmaniasis



Laboratory Diagnosis

 Specimens:

 Cutaneous leishmaniasis :(scraping, aspirate or biopsy).

 Visceral leishmaniasis: Bone marrow biopsy or splenic aspirate

 Examination of Giemsa stained slides of the relevant tissue is still the technique most commonly used to detect the parasite.

 Isolation of the organism in culture (N.N.N medium)

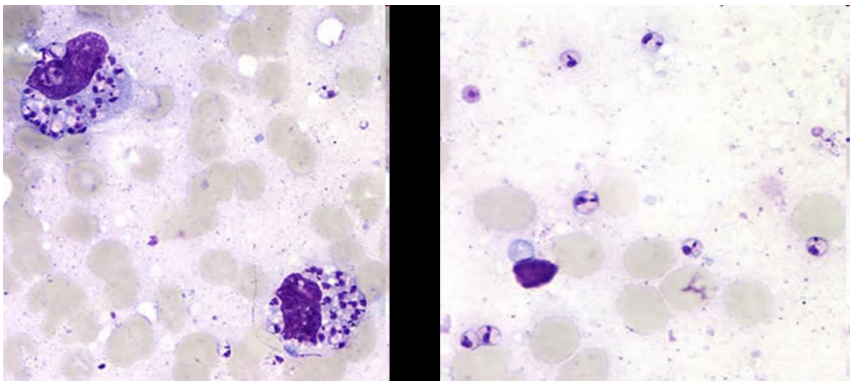
 Inoculate serum of infected person in lab. animals.

 Antibody detection can prove useful in visceral leishmaniasis but is of limited value in cutaneous disease.

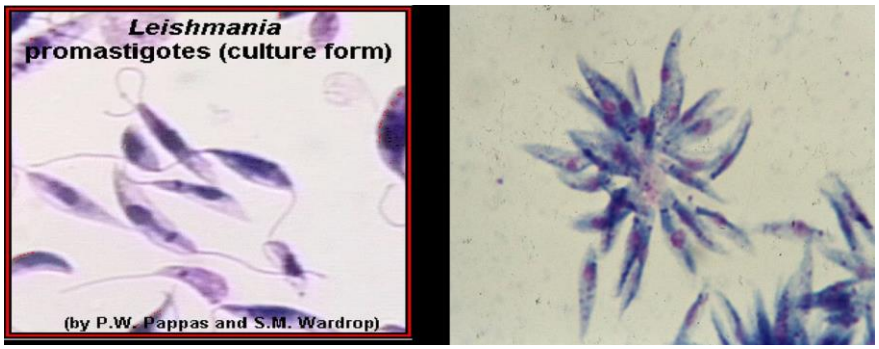
 PCR

 Skin test

Leishmania spp. amastigotes
Ovoid small intracellular parasites in a bone marrow aspirate.



Promastigotes in rosettes in a culture on N.N.N. medium (Giemsa stain)



Sandfly