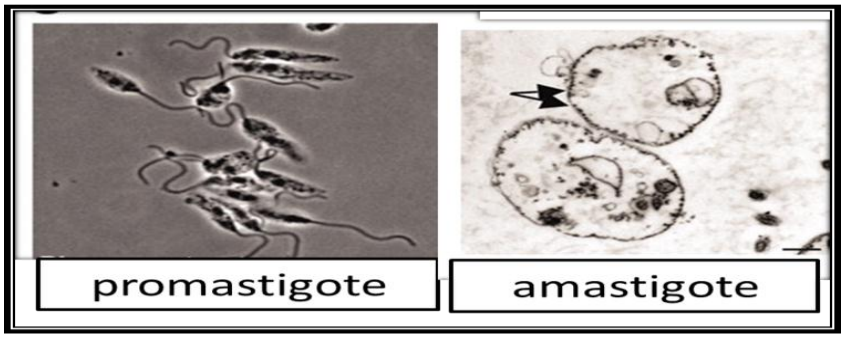
***leishmania* laboratory diagnosis ,routine methods, immunological assays and molecular assays**



There are three types

-Cutaneous leishmaniasis : results from *Leishmania tropica*. ,lesions appear on the skin. This is the most common type of leishmaniasis.

-Mucocutaneous leishmaniasis : caused by *Leishmania braziliensis*. Classic occurs secondary to cutaneous lesions. It consists of the continuous destruction of the nose and pharynx, resulting in destruction in the midfacial.

- **Visceral leishmaniasis** : the main cause is *Leishmania donovani* and *Leishmania infantum* . It is the most dangerous type because it may lead to death if it is not treated.

Diagnosis and identification of leishmaniasis

leishmaniasis is complex to diagnose because its clinical symptoms are shared by a host of other common diseases, such as typhoid, malaria and tuberculosis.

1-Detection of the parasite can be induced through direct evidence (amastigotes in tissues) of the liver, bone marrow,

peripheral blood, or splenic aspirates.

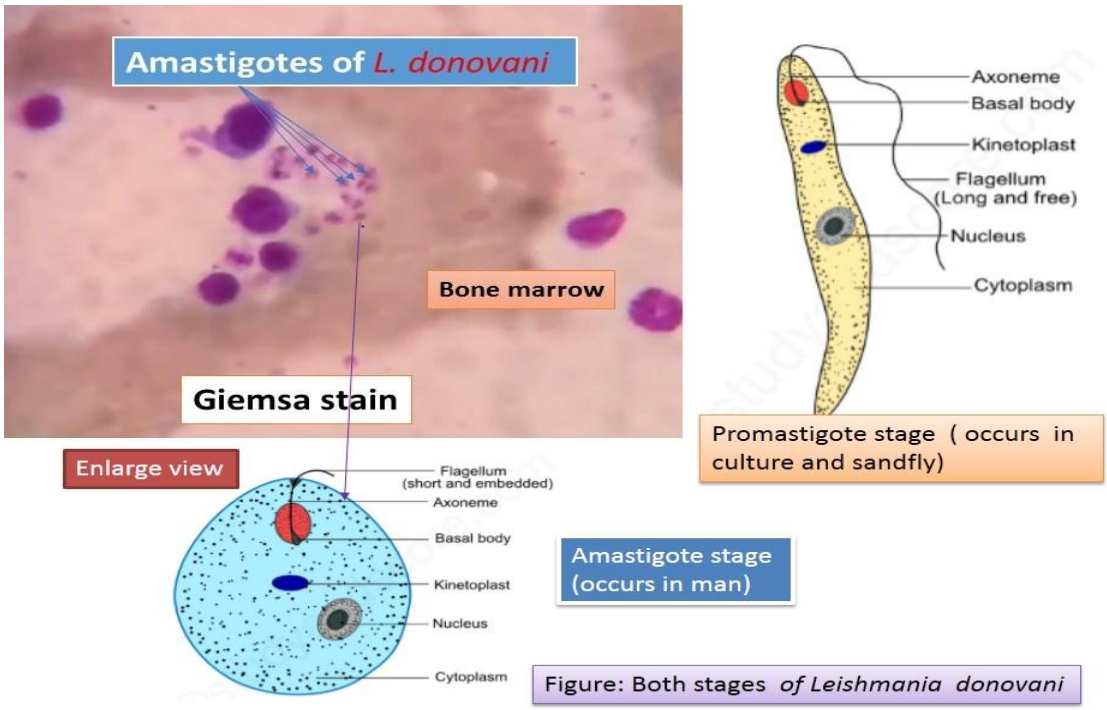


Figure: Both stages *of Leishmania donovani*

2-Laboratory diagnosis of leishmaniasis can be made by showing the parasite in related tissues through light microscopy of the stained sample, in vitro culture, animal inoculation

3- Detection of parasite DNA in tissue samples or immunological diagnosis by detecting the parasite antigen in tissue, blood or urine samples, by detecting either non-specific or specific antibodies to leishmaniasis or by assay for leishmania-specific cell-mediated immunity.

-Nonspecific tests: such as formal gel tests were used in the past, but they should be abandoned due to poor specificity and sensitivity.

-Specific tests: 1)Immunoblotting, 2)Indirect Fluorescent Antibody Test (IFAT), 3)Immunochromatographic strip test (ICT), 4)The antigen-based latex agglutination test (KAtex), 5)Enzyme-Linked Immunosorbent Assay (ELISA), 6)Antigen Detection, dip-stick assays 7) Molecular Diagnosis PCR

- **Methods**: **Biphasic Culture media**
- It is also called NNN (Novy-MCNeal-Nicolle), It consists of two phases:

A-Solid phase

Ingredients	Weight
Brain heart infusion	37 gm
Agar	20 gm
Dextrose	10 gm
Blood	200 mL
Gentamicin (80 mg/mL)	2.5 mL
Distilled water	1000 mL

1-All the ingredients were added to distilled water excepted antibiotic

2- after dissolving , the blood was added and mixed well

3-Autoclave at 121C° for 15 minutes.

4-Cool to about 50-55C°, added antibiotic, then 4mL was dispensed into sterile screw-cap culture tube, incubate at 37C° for 24 hours to make sure the media free contamination then stored at 4C°.

B-Liquid phase (lock solution):

Ingredients	**Weight**
Sodium chloride (NaCl)	9.0 gm
Potassium chloride (KCl)	0.42 gm
Inhydrate Calcium chloride (CaCl2.2H2O)	0.322 gm
Sodium bicarbonate (NaHCO3)	0.2 gm
Glucose (C6H12O6)	2.0 gm
Distilled water	1000 mL

- 1-All ingredients were dissolved in distilled water,
- 2-autoclave at 121C° in 15 min,
- 3-store at 4C°. Dispense 5mL into each tube contains solid media before used

Vaccine against leishmaniasis

Vaccination strategy is the most economical method for the prevention of infectious diseases. Generally, there are different forms of vaccines: killed, attenuated, recombinant, subunit, VLP (virus-like particle), and DNA vaccines.

Preventive vaccines with induction immune responses can produce **memory lymphocytes** toward the immunity pathway for controlling infections.

However, such vaccines for preventing leishmaniasis are yet to be found.

Properties of ideal vaccines , leishmania vaccine in trail

1)First-generation vaccines

Prophylaxis

First-generation vaccines against leishmaniasis include vaccines made of whole killed parasites.

the benefits:

1-These vaccines can be produced with low cost in developing countries.

2-there are many potential obstacles to the registration of standardization of vaccines derived from cultured parasites.

Efficacy trials of first-generation vaccines

The perceived results of this clinical trial study indicated that this vaccination was immunogenic and safe, but did not provide protection.

Note / To produce the first-generation vaccine, a 10- to 12-year period is required with a total cost of US$2–3 million.

2)Second-generation vaccine

Live vaccines

This classification includes genetically modified vaccines in which essential genes were knocked out. These parasites can generate adaptive immune responses adequately, resulting in inactivated infection and subsequently, disease does not occur in vaccinated people.

3)Third-generation vaccine

Studies have shown that DNA vaccines are much more stable than recombinant protein vaccines and they also have a lower cost of production compared with other vaccines.

The mode of action in DNA vaccines

The mode of action in DNA vaccines is performed by the generation of immune responses through activation of innate immunity.