Seventh lecture. by Dr. Hiro M. Obaid

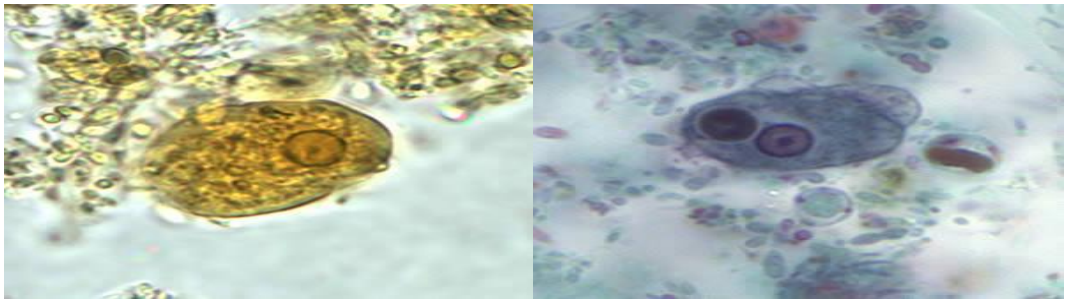
Intestinal and luminal protozoa significant to human health include *Entamoeba histolytica* (Ameba); *Balantidium coli* (Ciliate); *Giardia lamblia* and *Trichomonas vaginalis* (Flagellate); *Cryptosporidium parvum* and *Isospora belli* (Sporozoa).

***Entamoeba* and Amebiasis:**

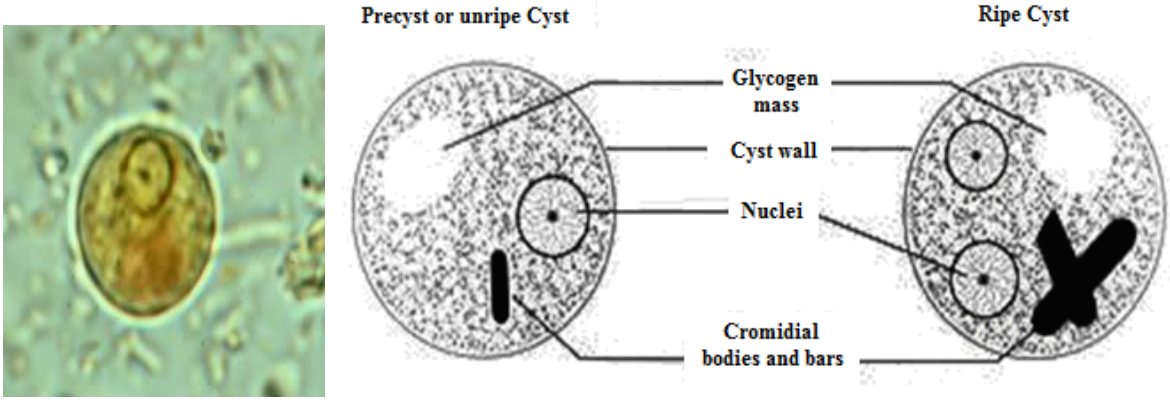
Entamoeba histolytica primitive unicellular microorganisms with a relatively simple life cycle which can be divided into two stages:

• Trophozoite – actively motile feeding stage.

• Cyst – quiescent, resistant, infective stage.



Entamoeba histolytica Trophozoites



Entamoeba histolytica cysts

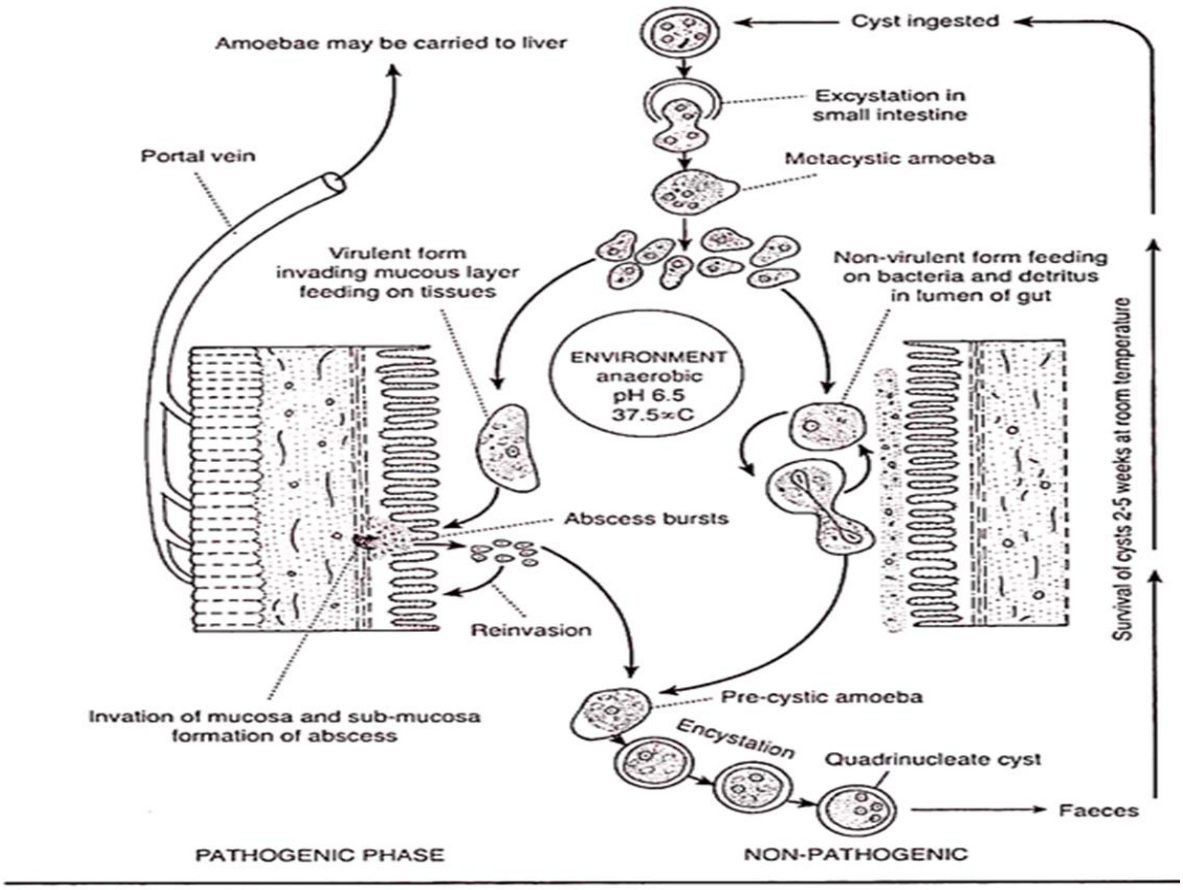
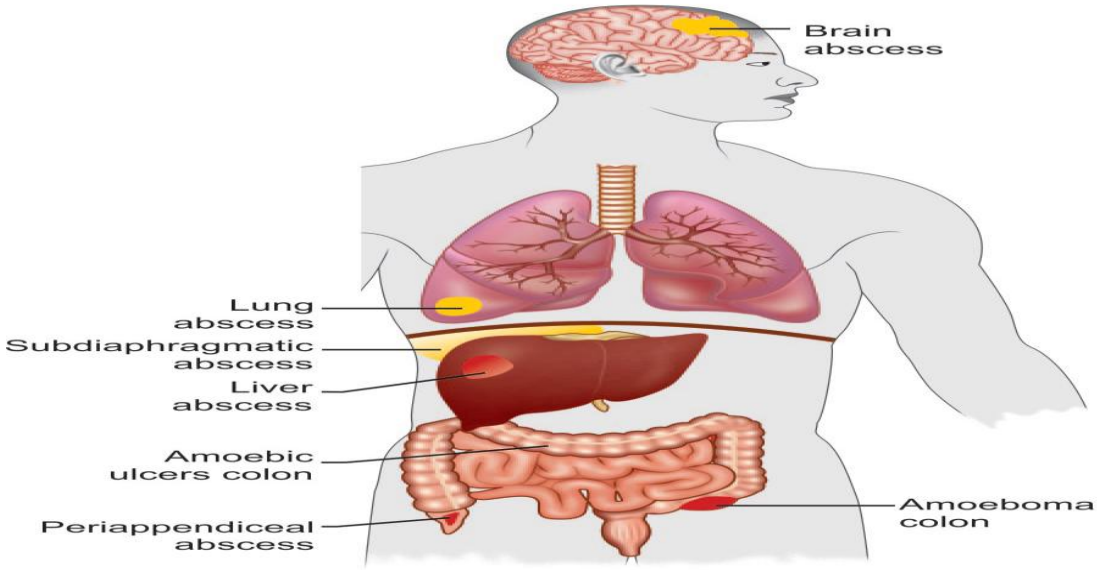


Fig. 6.2: Life cycle of *E. histolytica* in man



1-*E.histolytica* has a worldwide distribution. Although it is found in cold areas, the incidence is highest in tropical and subtropical regions that have poor sanitation and contaminated water.

2-About 90% of infections are asymptomatic, and the remaining produces a spectrum of clinical syndrome.
3-The epidemic form is a result of direct person-to-person faecal-oral spread under conditions of poor personal hygiene.
4- The higher prevalence in areas of lower socioeconomic status is likely due to poor sanitation and a lack of indoor plumbing.

A.Laboratory diagnosis of *Entamoeba histolytica*

Intestinal amoebiasis

Stool examination	Stool culture	Mucosal scrapings	Serodiagnosis	Molecular diagnosis
•Microscopy •Macroscopy •Iodine stained preparation •Trichome stained preparations to demonstrate trophozoite or cyst	Media used • Boeck and Drbohlav • NIH polygenic • Craig's • Nelson's • Robinson's	Wet mount Stained preparation	• IHA • ELISA • Latex agglutination test	• DNA probe

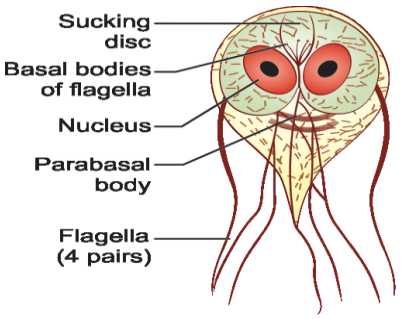
B.Laboratory diagnosis of amoebic liver abscess

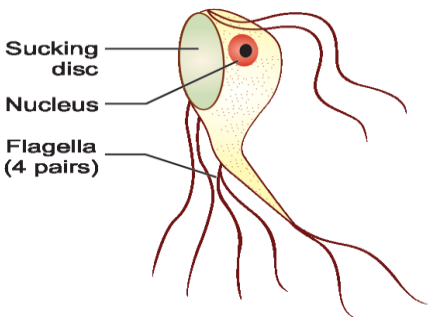
Amoebic liver abscess

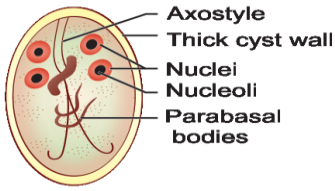
Microscopy	Histopatholoigical examination	Serodiagnosis	Radiological examination	Stool examination
of pus or aspirate	of pus or aspirate	• IHA • ELISA • Latex agglutination test	• X-ray • USG • CT scan • MRI	

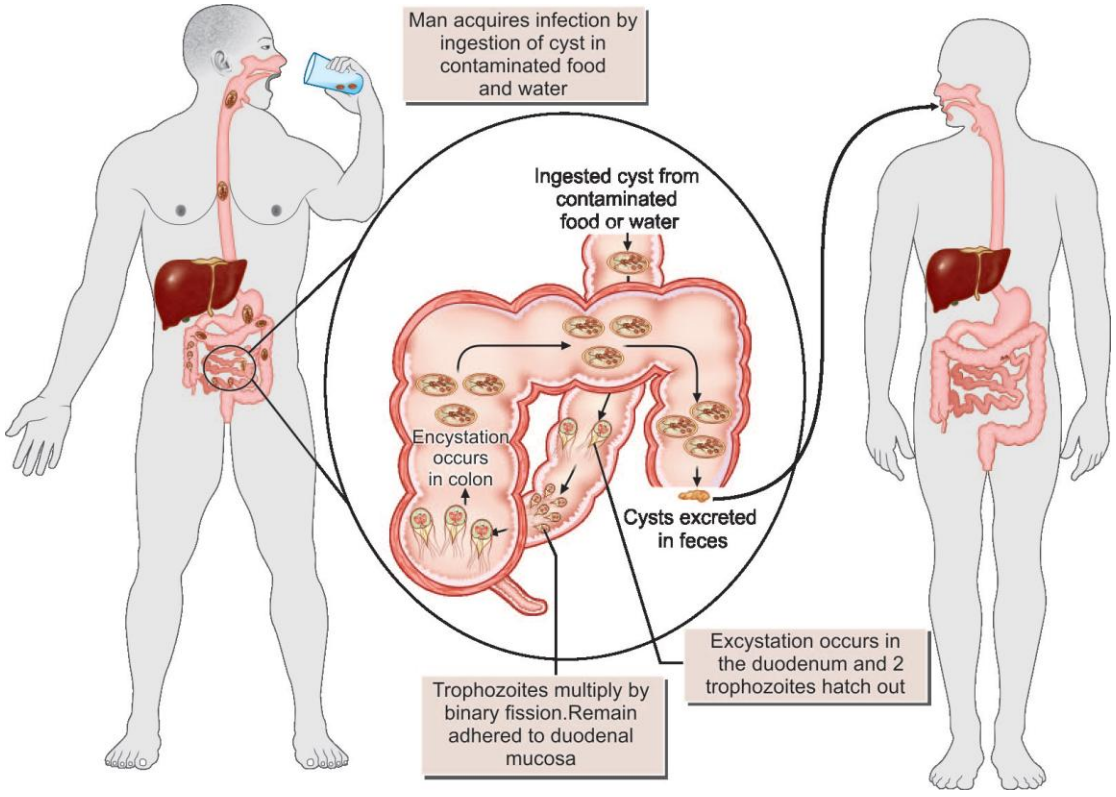
Giardia lamblia

Giardia is the only protozoan parasite found in the lumen of the human small intestine (duodenum and jejunum).
·Trophozoites are pearshaped, bilaterally symmetrical with 2 nuclei, 4 pairs of fl agella, and a ventral concave sucking disc. They exhibit motility resembling a 'falling leaf'.
·Ellipsoid cysts contain 4 nuclei with remnants of fl agella. ·**Infective form:** Ellipsoid cysts. ·**Clinical features:** Mostly asymptomatic but in some cases may cause diarrhea, dull epigastric pain, and malabsorption. Stool contains excess mucus but no blood.
·**Treatment:** Metronidazole and tinidazole are the drugs of choice.

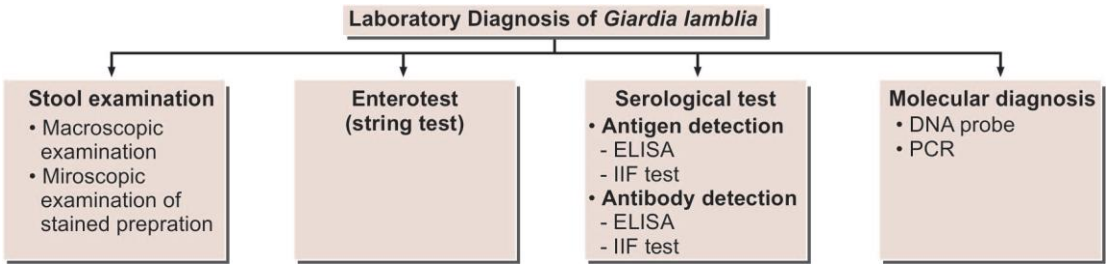








Life cycle of Giardia lamblia



· **Trichomonas vaginalis** occurs only in trophozoite form, which is pearshaped, with 5 fl agella and an undulating membrane.

· The motility is rapid jerky or twitching type.

· **Habitat:** Vagina and cervix in female and urethra in males.

· **Clinical features:** Often asymptomatic in males. In females, it leads to pruritic vaginitis with greenish yellow discharge, strawberry mucosa and dysuria.

· **Treatment:** Metronidazole is the drug of choice and simultaneous treatment of both partners is recommended.

Microscopic examination

Vaginal or urethral discharge is examined microscopically in saline wet mount preparation for characteristic jerky and twitching motility and shape. In males, trophozoites may be found in urine or prostatic secretions.

Fixed smears may be stained with acridine orange, papanicolaou, and Giemsa stains. Direct fluroscent antibody (DFA) is another method of detection of parasite and is more sensitive than the wet mount.

Culture

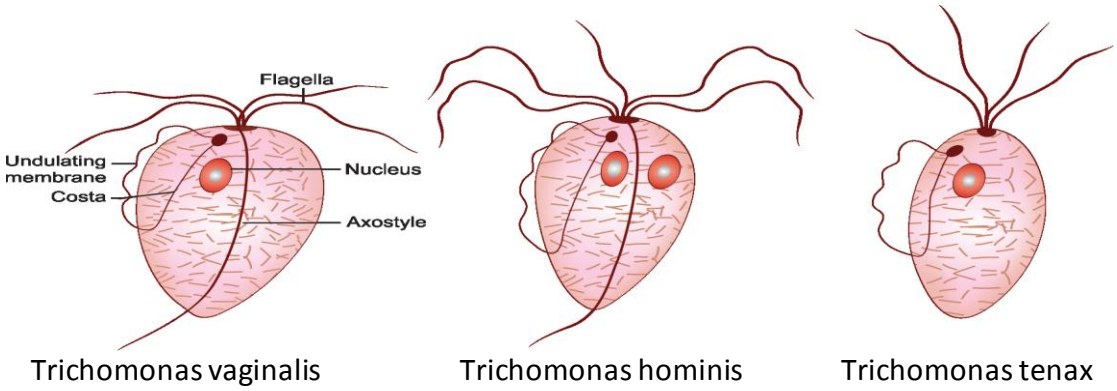
Culture is recommended when direct microscopy is negative and is considered as a '**gold standard**' as well as the most sensitive (95%) method for the diagnosis of *T. vaginalis* infection. It grows best at 35° –37° C under anaerobic conditions. The optimal pH for growth is 5.5–6.0. It can be grown in a variety of solid or liquid media, tissue culture, and eggs. Cysteine-peptone-liver-maltose (CPLM) medium and plastic envelope medium (PEM) are often used.

Serology

ELISA is used for demonstration of *T. vaginalis* antigen in vaginal smear using a monoclonal antibody for 65-KDA surface polypeptide of *T. vaginalis.*

Molecular method

DNA hybridization and PCR are also highly sensitive (97%) and specific (98%) tests for the diagnosis of trichomoniasis.



Trichomonas vaginalis | Trichomonas hominis | Trichomonas tenax

Blood and Tissue Protozoa: Hemoflagellates

Hemoflagellates belonging to two genera, Leishmania and Trypanosoma, infect humans.

Both require blood-feeding insect vectors in their life cycles. hemoflagellates may assume as many as four distinct morphologic forms.

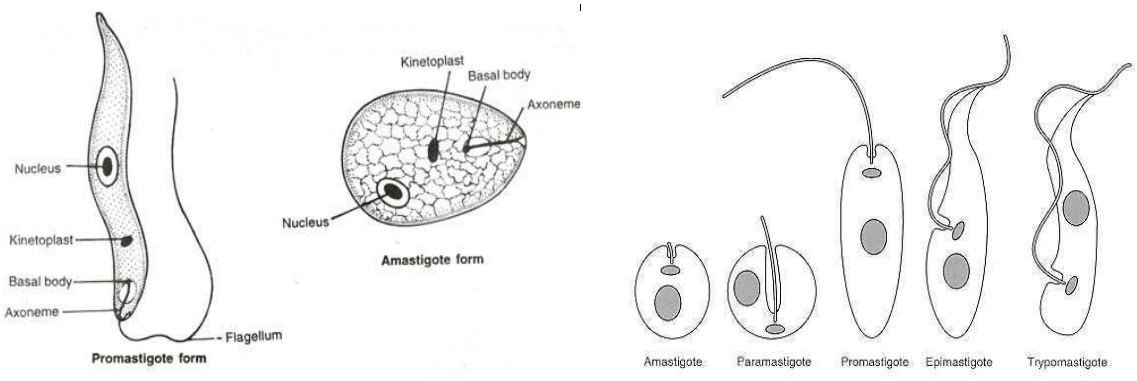
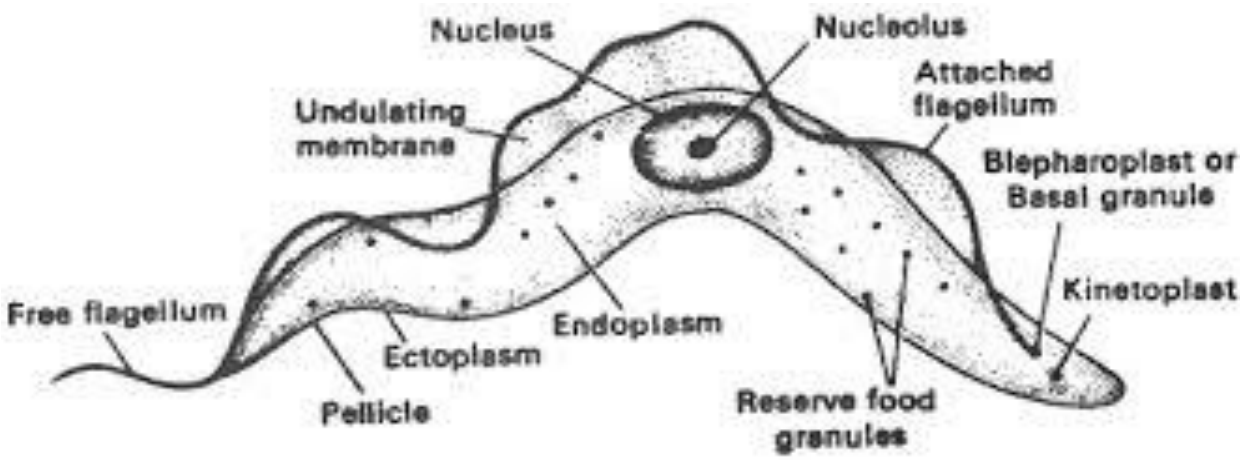


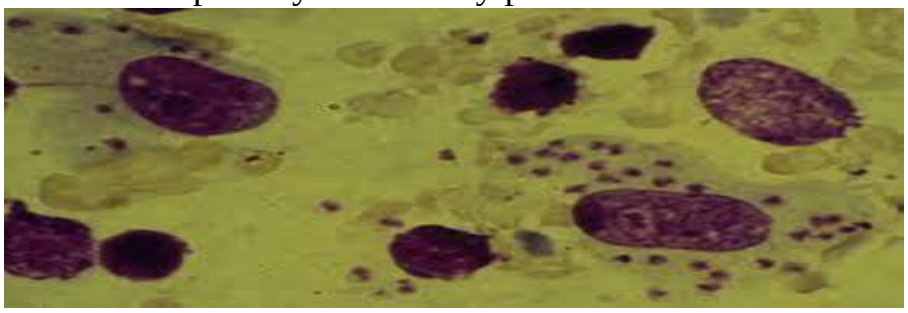
Fig. 178. Morphological forms of *Leishmania donovani*

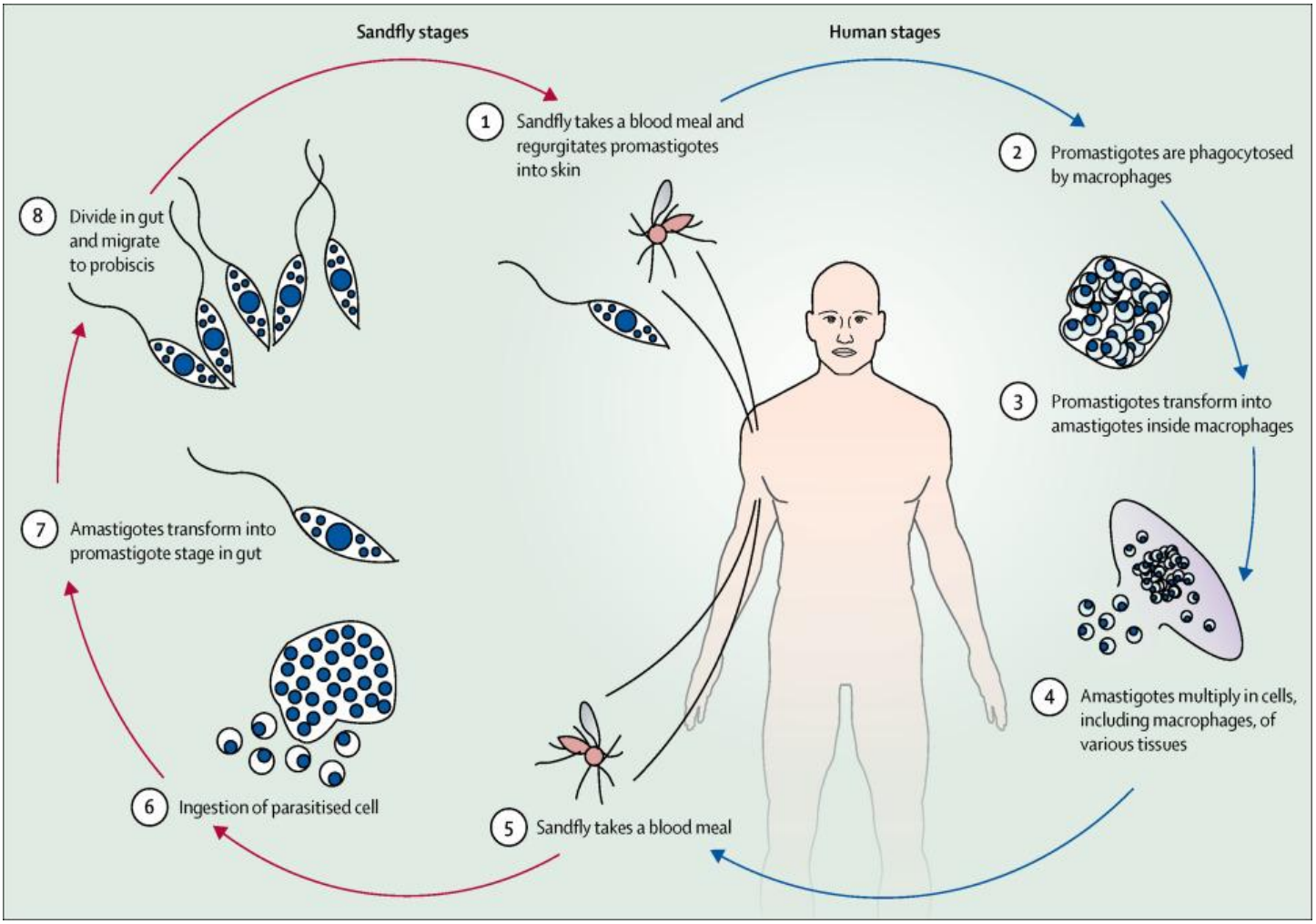


Trypanosoma gambiense

Genus Leishmania

A number of species and subspecies of Leishmania have been partially characterized. Of those that infect humans, three clinical manifestations are evident: visceral, cutaneous, and mucocutaneous leishmaniasis. While their life cycles are identical and they are morphologically indistinguishable, they differ in the type and location of primary lesions they produce in the human host.

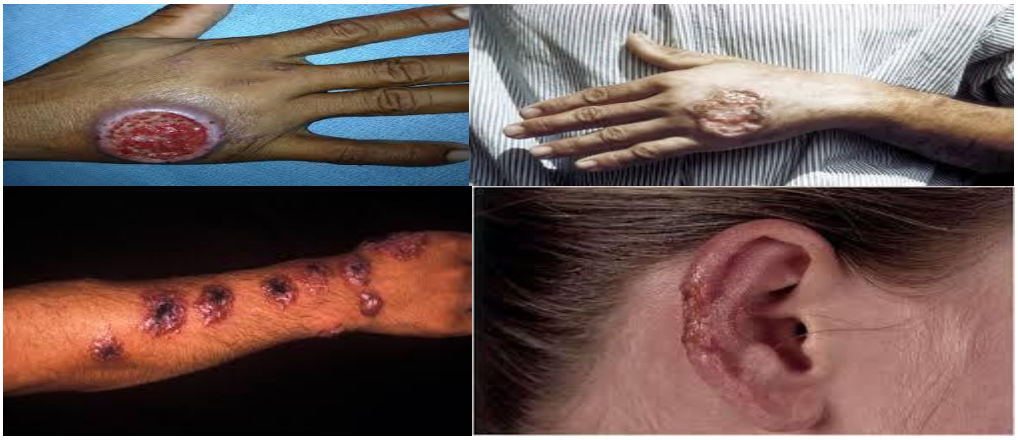




Cutaneous Leishmaniasis (Leishmania tropica and Leishmania mexicana).
Cutaneous leishmaniasis, a relatively mild skin disease commonly known as oriental sore, is caused by Leishmania tropica in the Old World and Leishmania mexicana in the New World. It is the most common of the leishmaniases. Unlike the amastigote of L. donovani, those of L. tropica and L. mexicana are found primarily in macrophages around cutaneous sores. Sandflies must feed at these sites in order to acquire the infective amastigotes.

In humans, the initial sign of the infection is the appearance of a vascularized papule or nodule on the skin at the feeding site of the insect. The papule becomes ulcerated after a few weeks, erupts, and spreads, forming cutaneous lesions most commonly on the hands, feet, legs, and face. incubation period varies from 1 to 2 weeks up to several months or even several years. Two types of oriental sore are produced by different strains of the protozoan:
(1) the chronic, dry (or urban) type (L. minor, which is often considered a different species), producing delayed ulceration with numerous amastigotes.

(2) the acute, moist (or rural) type (L. major), characterized by early ulceration with few amastigotes.
In the absence of secondary bacterial contamination, sores tend to heal within a year, but disfiguring scars often remain.
The lesions in diffuse cutaneous leishmaniasis, however, differ from those accompanying the usual infection in that they are disseminated as multiple nodules under the skin and contain numerous parasites in the associated macrophages, in this manner resembling lepromatous leprosy. This form of cutaneous leishmaniasis is found in patients with deficiency in their cell-mediated immune processes.
Pathogenesis;
When the bite of an infected sandfly liberates promastigotes into the skin, the parasites proliferate as amastigotes in the macrophages and the endothelium of the capillaries and other small blood vessels of the immediate area. Lysis of the amastigotes occurs following activation of the macrophages by sensitized lymphocytes. A granulomatous reaction results in formation of a localized nodule, which ulcerates when the blood supply to the area is compromised by parasite-induced damage. A pyogenic infection, generally a trivial one, develops in the open ulcer bed, and as host immunity increases, the ulcer heals.



Diagnosis
Positive diagnosis requires identification of amastigotes in infected cells of lesion by scrapings or aspirates and staining with lieshman stain for amastigote identification.
The most reliable diagnosis is achieved by in vitro culturing of lesion scrapings or aspirates and subsequent identification of promastigotes in the medium.
An immunological test is available, but, as in L. donovani, its diagnostic value is limited.
Montenegro "leishmanin" test: ID test using Ag from cultured promastigote gives delayed reaction after 3 days +ve in 95 % in cutaneous L.

Mucocutaneous Leishmaniasis (Leishmania braziliensis)

Leishmania braziliensis causes mucocutaneous leishmaniasis. Amastigotes are found in macrophages in ulcerations at mucocutaneous junctures of the skin. This disease is also known by various other names, including American leishmaniasis, espundia, uta, pian bois, and chiclero ulcer.
The disease is common in humans in an area extending from the Yucatan peninsula in Mexico south to Argentina. While human infections have occurred in the Sudan, Kenya, Italy, China, and India, the disease is far more common in the Western Hemisphere, hence the name American leishmaniasis.
in Mexico and Central America the secondary lesion usually appears on:
1- ear, causing chiclero ulcer, a condition common among the chicleros, forest-dwelling natives who harvest the gum of chicle trees.
2- lesions erupt at the mucocutaneous junctures of the skin, with nasal and buccal tissues most often affected. In these geographic areas the disease is commonly called espundia or uta.
First generation antileishmanial vaccines comprises of three main subgroups: whole-killed parasites (i), fractionated *Leishmania* antigen (ii), Live-attenuated pathogens. Whole-killed parasites

Killed *Leishmania* vaccines in new world

Whole-killed *Leishmania* vaccines have low cost and achieved the first senior success in animal modeling; nevertheless, none of the human vaccines in this subgroup has accomplished the World Health Organization (WHO) validity. For instance, Leish. vaccine, which comprised whole-killed promastigotes of *Leishmania amazonensis* (*L. amazonensis*) strain (IFLA/BR/1967/PH8) and Bacillus Calmette–Guérin (BCG), could play a prominent role in the protection of canine Leishmaniasis. In fact, this vaccine induced a significant increase in a mixed cytokine pattern. The vaccine stimulated innate immunity (especially neutrophils and eosinophils) and activated CD4+T, CD8+T, and B cells. Leishvaccine in human was successfully applied in Phase I and II of clinical trials, which well documented its safety and immunogenicity; however, this vaccine failed to achieve satisfactory results in Phase III of the randomized clinical trial (RCT).

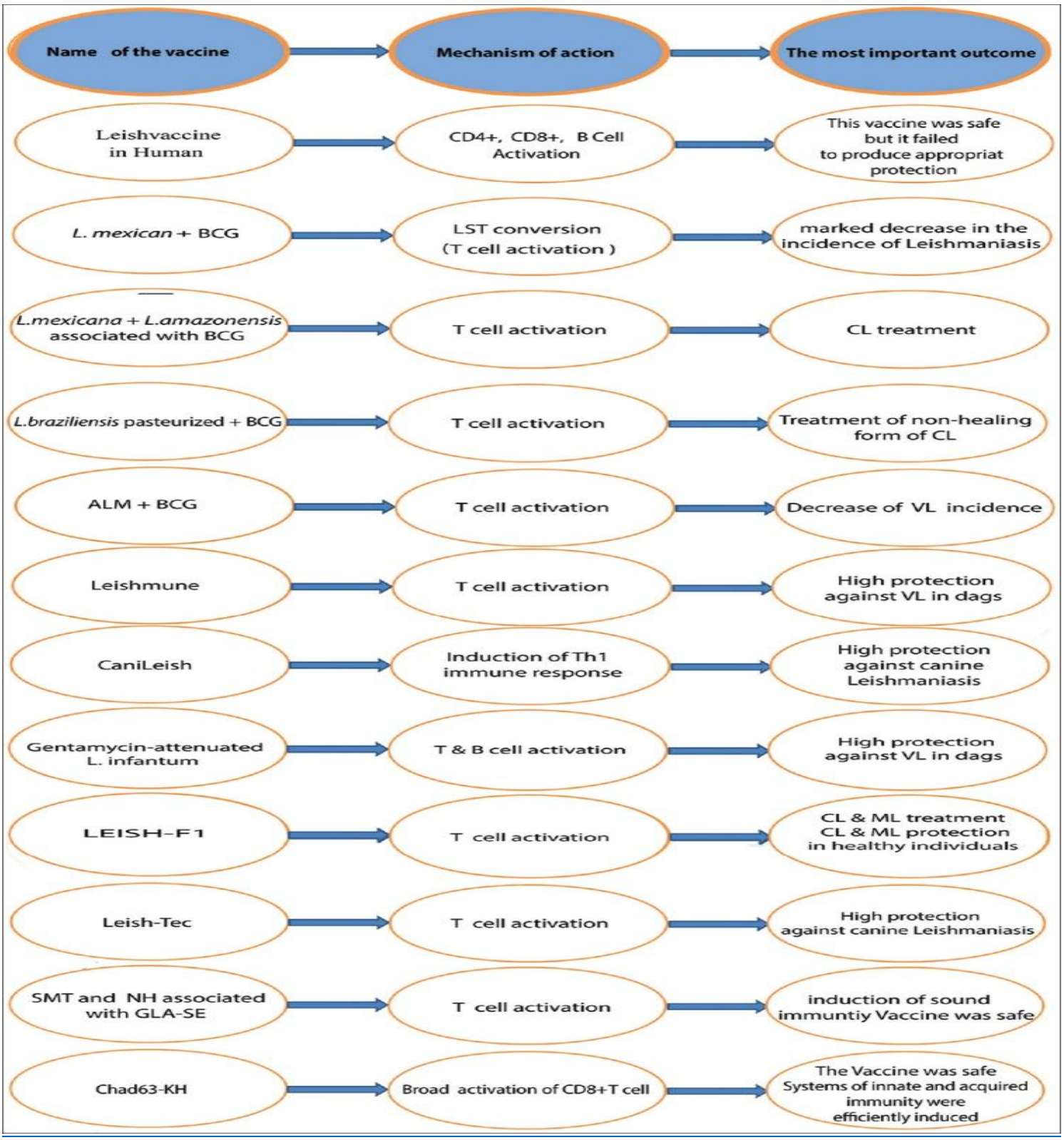


Diagram showing mechanism of action and the most important outcome of the vaccines