Leishmania spp.

Classification of *Leishmania* parasite

kingdom: Protista.

sub- kingdom: Protozoa.

phylum: sarcomastigophora.

sub- phylum: Mastigophora.

Class: Zoomastigophorea.

Order: Kinetoplastide

Family: Trypanosomatidae.

Genus: *Leishmania.*

Leishmania spp.

- The leishmaniasis is a group of vector-borne diseases caused by protozoan haemoflagelates of the genus *Leishmania*
- It is spread through females of Sandflies of the genus *Phlebotomus* spp. in the Old World, and of the genus *Lutzomyia* in the New World.
- Leishmaniasis is a zoonotic deadly parasitic disease that affects over 12 million people worldwide, with 350 millions are at risk.
- With more than 2 million new cases reported every year.

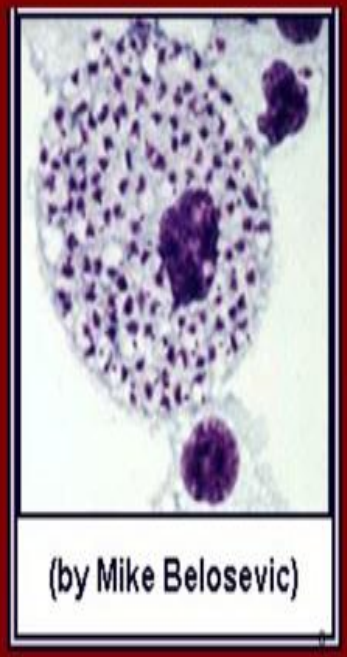
The species of leishmania exist in two forms, amastigote (a flagellar) and promastigote (flagellated) in their life cycle. They are transmitted by certain species of sand flies (Phlebotomus & Lutzomyia)

Leishmania spp.

Amastigote. (Leishmanial form)

- Spherical or Oval in shape
- 2-3 µm in diameter.
- non-motile
- Large eccentrically located nucleus
- Rode shape Kinetoplast
- Usually found in the vertebrate host.

Leishmania spp.

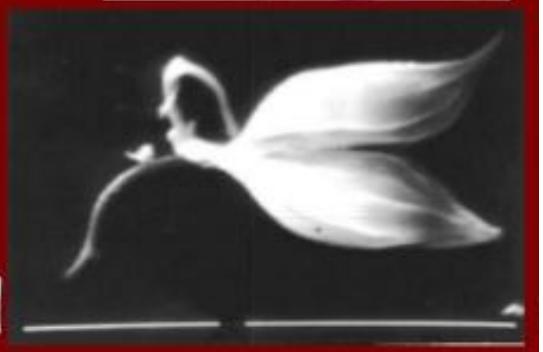


(by Mike Belosevic)

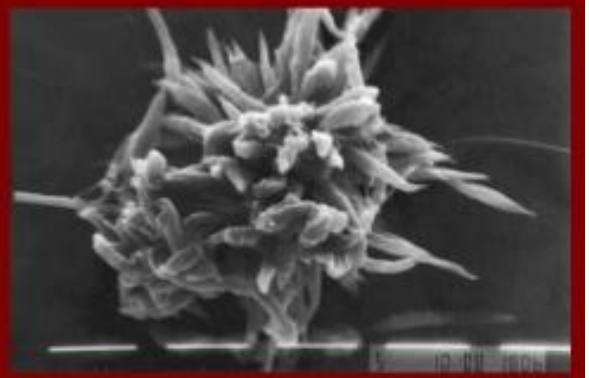
- A macrophage filled with *Leishmania* amastigotes.

Leishmania spp.

Promastigote: (Leptomonad stage)



- Spindle shaped with 14-20 by 1.5 - 4 µm in diameter
- Free anterior flagellum
- Kinetoplast at the anterior end of the body.
- There is no undulating membrane.
- Usually promastigote found in female Sandflies and Culture



Type of Leishmania Parasites and Diseases

SPECIES	**Disease**
*Leishmania tropica** *Leishmania major** *Leishmania aethiopica* *Leishmania mexicana*	Cutaneous leishmaniasis
Leishmania braziliensis	Mucocutaneous leishmaniasis
*Leishmania donovani** *Leishmania infantum** *Leishmania chagasi*	Visceral leishmaniasis

Leishmania spp.

In humans, There are different forms of this disease :

- Visceral leishmaniasis: involving liver, spleen, and bone marrow
- Cutaneous leishmaniasis: involving the skin at the site of a sandfly bite
- mucocutaneous leishmaniasis: involving mucous membranes of the mouth and nose after spread from a nearby cutaneous lesion (very rare).

Different species of *Leishmania* cause different forms of disease

Leishmania donovani

Visceral leishmaniasis (VL)

G.D. Asia, Africa and south America and can affect people of all ages

90% of all visceral leishmaniasis occurs in Bangladesh, Brazil, India, Iraq and the Sudan

- **2893** cases were reported in Iraq in 2001
- **12** visceral leishmania cases were reported in Americans in Desert Storm.

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Leishmania donovani

Visceral leishmaniasis (VL)

Habitat:- internal organ (liver, lymph nodes, spleen ,bone marrow)

Disease Visceral leishmaniasis, Kala azar, Dum Dum fever

Transmission VL. is transmitted chiefly by female Sandflies (*Phlebotomus* spp.)

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L. donovani

This visceral disease has a new and old world form: particularly Brazil, and Mediterranean Europe, North Africa, East Africa, India and China.

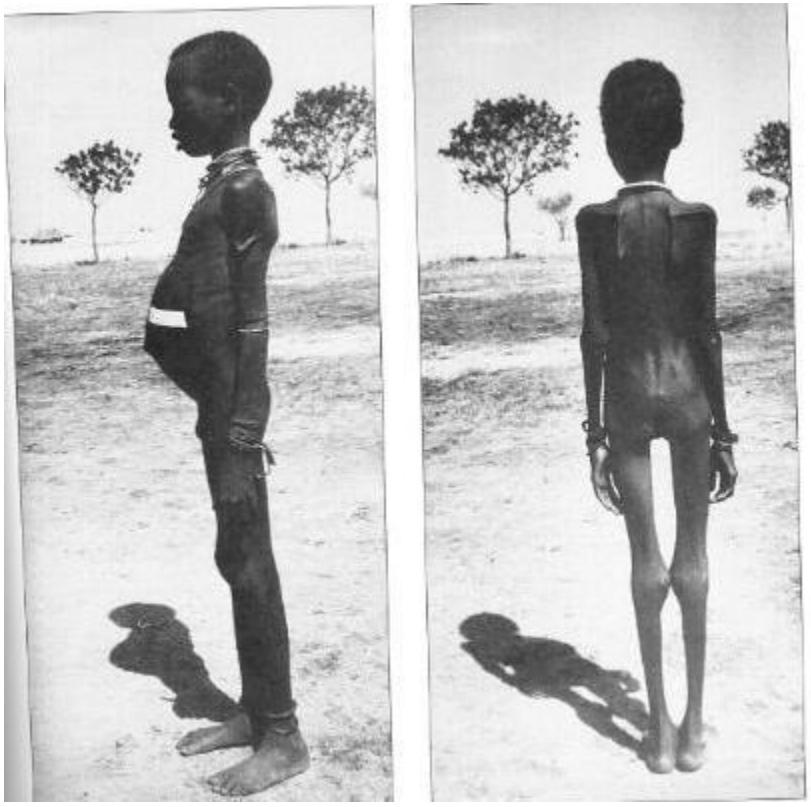
The amastigote forms are found within the reticulo-endothelial cells of the viscera, i.e the spleen, lymph nodes, liver and intestine.

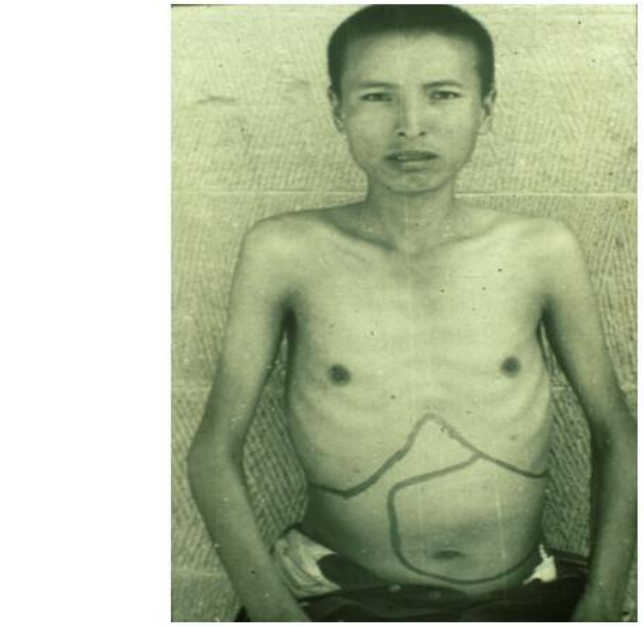
The incubation time of 10 days-a year. The symptoms are a slow developing low grade fever, and general malaise, a progressive wasting of the patient with anaemia. Other classic symptoms as the disease progresses is the protrusion of the abdomen, hepatospenomegaly. If untreated death will occur within 2-3 years of contracting the infection.

In acute forms-the disease can run its course within 6- 12 months. Clinical signs include edema, particularly of the face, bleeding mucus membranes, breathing difficulties and diarrhea.

VISCERAL LEISHMANIASIS

L. donovani causes the classic type found in India. This is a metastatic disease. Rarely is a lesion seen at the site of bite and parasites are only occasionally seen in blood, but are present in the spleen and lymph nodes. Disease is characterized by fever, anemia, splenomgegaly, wasting, imbalance of serum proteins and hyperpigmentation of the skin. The death rate is very high if left untreated. *L. infantum* causes the Mediterranean form of kala azar and has dogs, jackals and foxes as reservoirs. Humans are accidental hosts.





Post Kala-azar dermal Leishmaniasis (PKDL) or Dermal Leishmanoid :

PKDL or dermal leishmanoid is relatively common consequence of therapeutic cure from visceral leishmaniasis caused by *L. donovani* . It is caused by the reversal of the agent from viscerotropic to dermatotropic .It generally develops 1-2 years after completion of antimonial treatment for the original disease in a bout 2-10%of the cases ,when the visceral infection disappears but the skin infection persists.

Patient develops hypopigmented macules any where on the body , especially the upper trunk , arms, thighs, forearms, legs, abdomen and neck in that order and erythematous patches (often having butterfly distribution) on the nose, cheeks and chin . later yellowish-pink nodules appear mostly on the face , especially on the nose, cheeks, lips and ears. The lesion are soft, painless, granulomatous of varying size that of lepromatous leprosy(non-ulcerative).

Diagnosis of PKDL can be established by demonstration of amastigote form of L. donovani by a microscopical examination of leishman- stained smear , prepared from the biopsy material obtained from nodular lesions.

Sign and symptoms

- Sever weight loss
- Pancytopenia
- Hepatosplenomegaly
- Intermitten fever
- Hypergamma globulinemia
- Dark skin

Leishmania tropica

Cutaneous leishmaniasis (CL)

- Most sores develop within a few weeks of the sandfly bite, however they can appear up to months later
- Skin sores of cutaneous leishmaniasis can heal on their own, but this can take months or even years
- Sores can leave significant scars and be disfiguring if they occur on the face

Leishmania tropica

Cutaneous leishmaniasis (CL)

G.D. in Asia, Africa, and Mediterranean
90% of cutaneous leishmaniasis occurs in Afghanistan, Iran, Iraq, Saudi Arabia, Syria, Brazil and Peru

- **8,779** cases were reported in Iraq in **1992**
- At least **20** cases of cutaneous leishmaniasis were reported in Americans from Desert Storm

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Leishmania tropica

Cutaneous leishmaniasis (CL)

Habitat:- Skin
Disease Cutaneous leishmaniasis, Oriental sore, Delhi or Baghdad boils
Transmission female Sandflies (*Phlebotomus* sp.)

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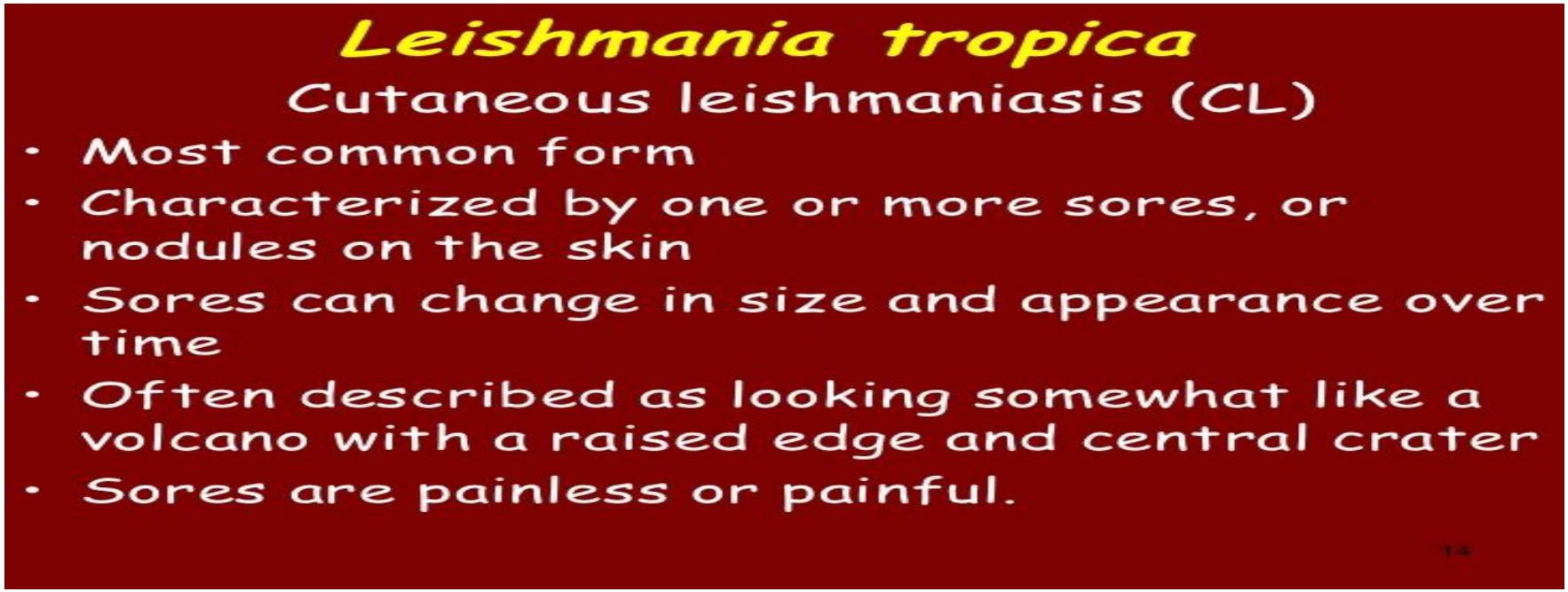


These are parasites of the skin found in endothelial cells of the capillaries of theinfected site, nearby lymph nodes, within large mononuclear cells, in neutrophilic leukocytes, and free in the serum exuding from the ulcerative site. Metastasis toother site or invasion of the viscera is rare.

Pathogenesis and Signs

In neutrophilic leukocytes, phagocytosis is usually successful, but inmacrophages the introduced parasites round up to form amastigote and multiply.

In the early stage, the lesion is characterized by the proliferation of macrophagesthat contain numerous amastigotes. There is a variable infiltration of lymphocytesand plasma cell. The overlying epithelium shows acanthosis and hyperkeratosis,which is usually followed by necrosis and ulceration.

The first sign, a red papule, appears at the site of the fly's bite. This lesion becomes irritated, with intense itching, and begins to enlarge & ulcerate.

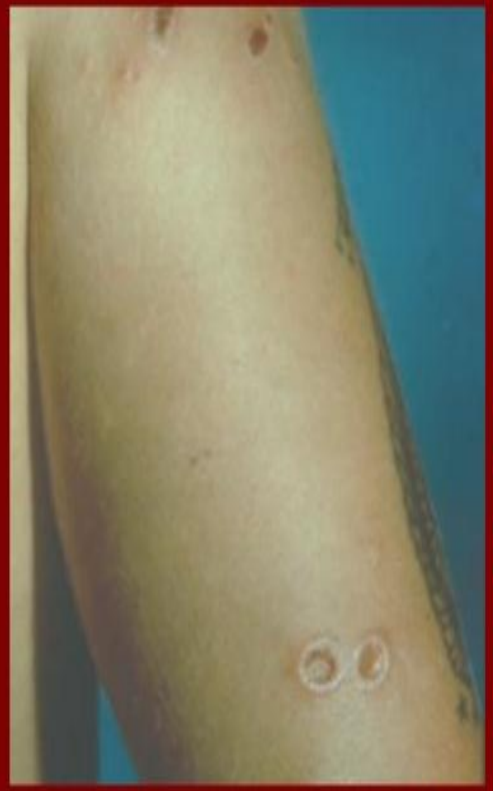
Gradually the ulcer becomes hard and crusted and exudes a thin, serous material. At this stage, secondary bacterial infection may complicate the disease.

In the case of the Ethiopian cutaneous leishmaniasis, there are similar developments of lesions, but they may also give rise to diffuse cutaneous leishmaniasis (DCL) in patients who produce little or no cell mediated immunity against the parasite. This leads to the formation of disfiguring nodules over the surface of the body.

Leishmania tropica

Cutaneous leishmaniasis (CL)

Multiple lesions on arm with a variety of appearances.



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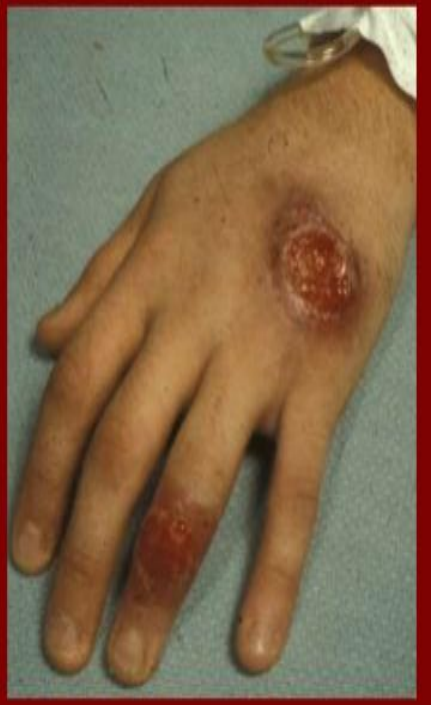
Leishmania tropica

Cutaneous leishmaniasis (CL)

Both lesions are leishmaniasis.

Note the raised border and wet appearance of the sore on the back of the hand.



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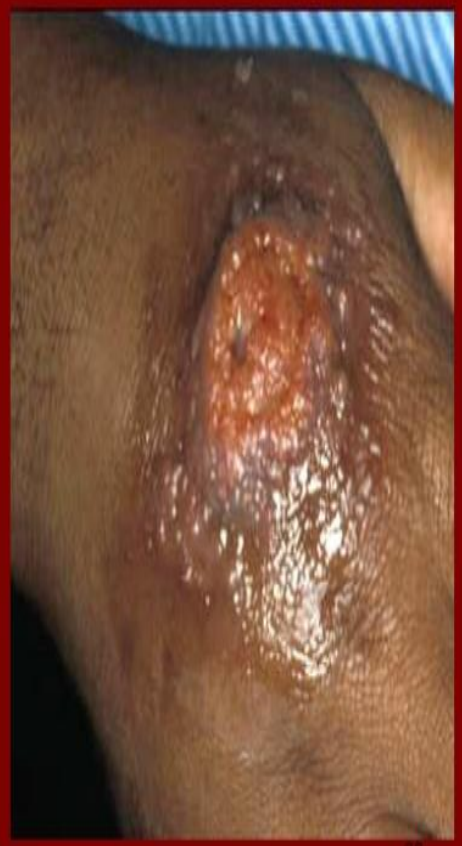
Leishmania tropica

Cutaneous leishmaniasis (CL)

Back of hand.
Note raised border and wet appearance.
Patient has bacitracin ointment applied to lesion.



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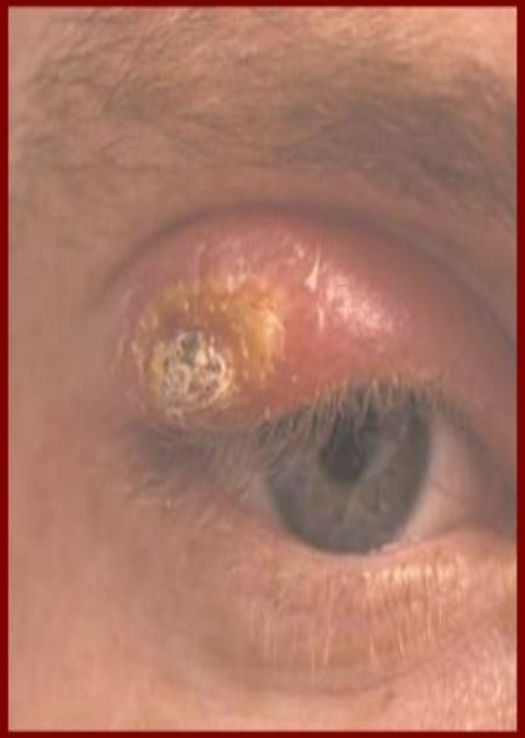
Leishmania tropica

Cutaneous leishmaniasis (CL)



Upper Eyelid. Note the dry, crusted appearance which is different than previous sores shown.

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Leishmania tropica

Cutaneous leishmaniasis (CL)

Three lesions on face. Raised and dry.
Another different presentation.

Leishmania braziliensis

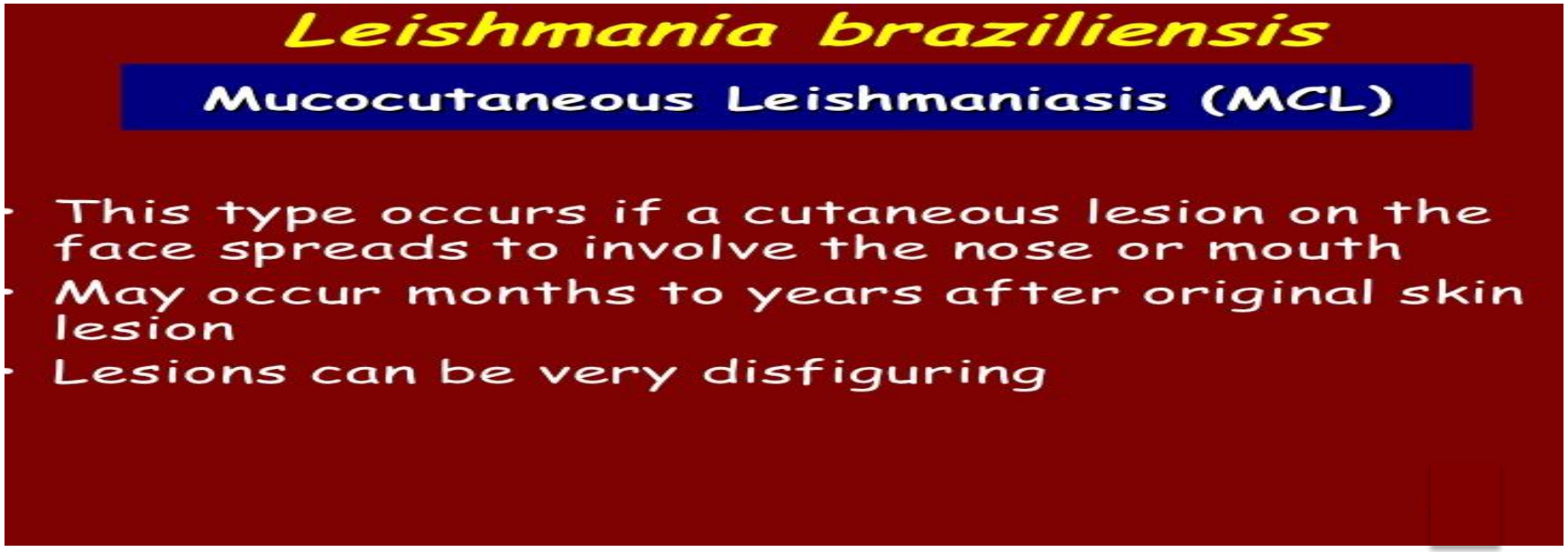
Mucocutaneous Leishmaniasis (MCL)

G.D. In South and Central America (Brazil, Bolivia, and Peru)

Habitat:- Skin and mucosa

Disease mucocutaneous leishmaniasis, espundia, Uta, American leishmaniasis

Transmission: female Sandflies *Lutzomyia*



The American cutaneous leishmeniasis is the same as oriental sore. But some ofthe strains tend to invade the mucous membranes of the mouth, nose, pharynx,and larynx either initially by direct extension or by metastasis. The metastasis is usually via lymphatic channels but occasionally may be the bloodstream.

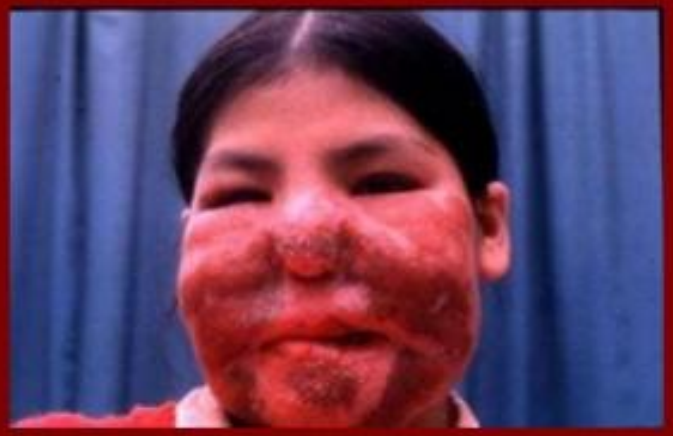
Pathogenesis and Clinical Signs

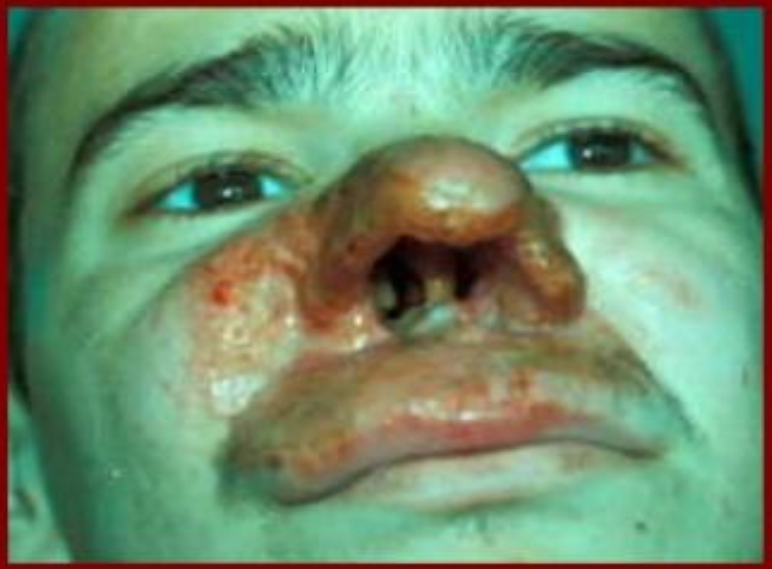
The lesions are confined to the skin in cutaneous leishmaiasis and to the mucous membranes, cartilage, and skin in mucocutaneous leishmaniasis. A granulomatous response occurs, and a necrotic ulcer forms at the bite site. The lesions tend to become superinfected with bacteria. Secondary lesions occur on the skin as wellas in mucous membranes. Nasal, oral, and pharyngeal lesions may be polypoid initially, and then erode to form ulcers that expand to destroy the soft tissue andcartilage about the face and larynx. Regional lymphadenopathy is common.

The types of lesions are more varied than those of oriental sore and includeChiclero ulcer, Uta, Espundia, and Disseminated Cutaneous Leishmaniasis

Leishmania braziliensis

Mucocutaneous Leishmaniasis (MCL)





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Life cycle of *leishmania*

leishmaniasis is transmitted by the bite of infected female phlebotomine sandflies. the sandflies inject the infective stage (i.e., promastigotes) from their proboscis during blood meals the number 1. promastigotes that reach the puncture wound are phagocytized by macrophages the number 2and other types of mononuclear phagocytic cells. progmastigotes transform in these cells into the tissue stage of the parasite (i.e., amastigotes) the number 3, which multiply by simple division and proceed to infect other mononuclear phagocytic cells the number 4. parasite, host, and other factors affect whether the infection becomes symptomatic and whether cutaneous or visceral leishmaniasis results. sandflies become infected by ingesting infected cells during blood meals (the number 5, the number 6). in sandflies, amastigotes transform into promastigotes, develop in the gut the number 7 (in the hindgut for leishmanial organisms in the viannia subgenus; in the midgut for 8) organisms in the leishmania subgenus), and migrate to the proboscis the

Sandfly Stages

Human Stages

1. Sandfly takes a blood meal (injects promastigote stage into the skin)
2. Promastigotes are phagocytized by macrophages
3. Promastigotes transform into amastigotes inside macrophages (d)
4. Amastigotes multiply in cells (including macrophages) of various tissues (d)
5. Sandfly takes a blood meal (ingests macrophages infected with amastigotes)
6. Ingestion of parasitized cell
7. Amastigotes transform into promastigote stage in midgut
8. Divide in midgut and migrate to proboscis

i = Infective Stage

d = Diagnostic Stage

LIFE CYCLE

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Laboratory Diagnosis:

1. Microscopy:
   (slit-skin smear, splenic aspirate, liver biopsy or bone marrow biopsy). Examination of Giemsa and Leishman stained slides of the relevant tissue is still the technique most commonly used to detect the parasite.
2. **Culture:**

The aspirates can be cultured in NNN. In culture the **amastigote** stage converts to the **promastigote** stage. However, this is not a rapid technique, as the parasites may take from 10 - 21 days to grow.

NNN medium

Laboratory Diagnosis:

3. Serodiagnosis:
VL produces large amounts of specific IgG which can be used for diagnosis. Currently the most used serodiagnostic tests Enzyme Linked Immunosorbent Assay (ELISA).
4. Molecular techniques: PCR, such technique, however, are not readily available in general diagnostic laboratories.

Leishmanian or Montenegro test:

It is a delayed hypersensitivity reaction to intradermal crude Leishmania antigen . The test is read after 48-72 hours. A positive test shows an area of erythema and induration 5 mm or more in diameter. The test becomes positive 6-8 weeks after cure from Kala-azar . Cell –mediated immunity is impaired in active Kala- azar patients who consequently lake a delayed hypersensitivity response. Therefore, leishmanin test is negative in active cases of Kala- azar.

This test is of great value in epidemiological studies but is of little clinical use.

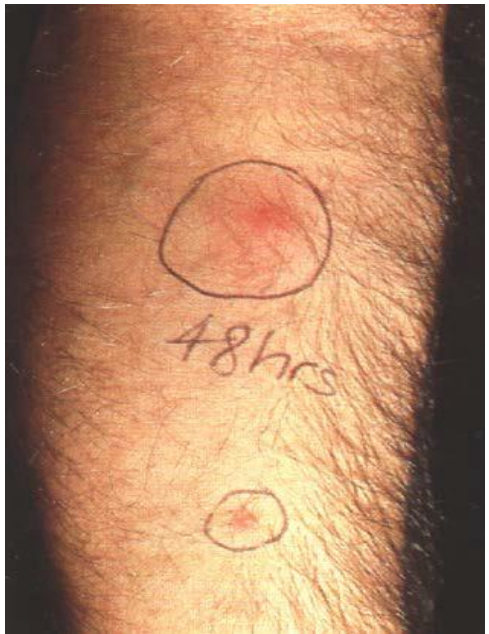
* An intradermal test for delayed hypersensitivity.

*Most reliable for cutaneous leishmaniasis.

*A suspension of 107 /ml promastigotes

in 0.5% phenol/saline intradermally.

*Test is read after 2-3 days.



Treatment

- Sodium antimony stibo gluconate
- pentamidine isthionate
- amphotericin-b
- miltefosine
- phase iii trials with a first-generation vaccine (killed *Leishmania* organism mixed with a low concentration of BCG as an adjuvant) have also yielded promising results
- *Leishmania major* mixed with BCG have been successful in preventing infection with *Leishmania donovani.*

Epidemiology&Risk factor

- Children are the greater risk than adult in endemic area
- Malnutrition has been showen to contribute to development the disease
- Person with AIDS are at 100-1000 times greater risk of development visceral leishmaniasis
- Incomplete therapy of initial disease is risk factor for recurrence of leishmaniasis
- The bite of one infected sand fly isufficient to cause the disease(and exist of reservior animal)

Prevention&Control

- Reductionn of sand fly population(by insecticide mainly DDT)
- Reduction of reservior(by killing all the infected dogs in the cases of zoonotic kala azar).
- .prevent exposure to sand fly(use window mess,bed net)
- Education in the community(about the causes and mode of transmission)
- Treatment of active cases