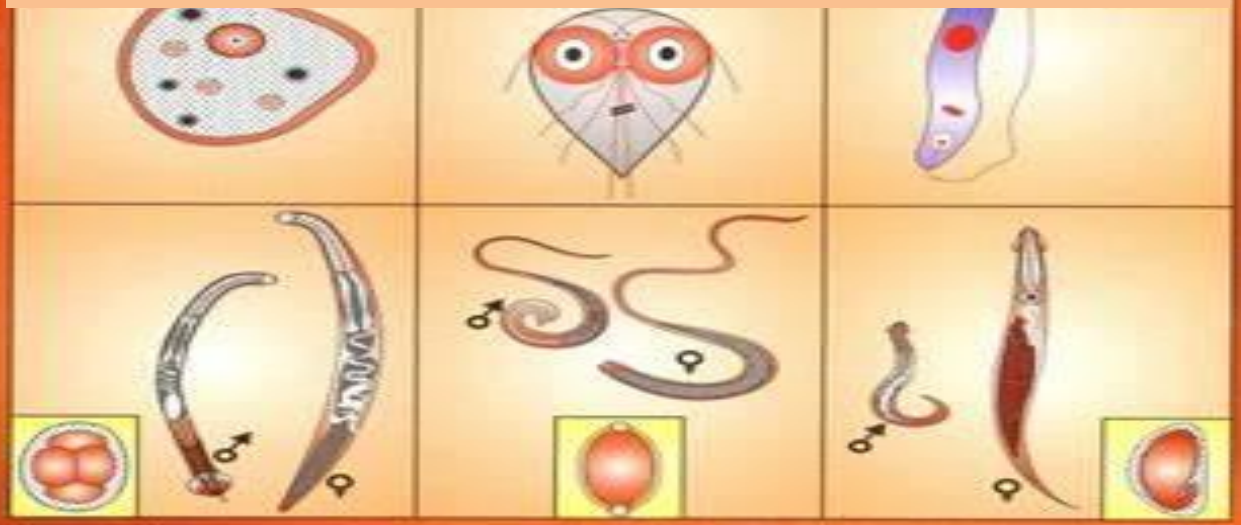
Tikrit University
College of Pharmacy

Microbiology II
-Medical Parasitology

MEDICAL PARASITOLOGY

lec. 5:blood and tissue flagellates

Leishmania



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BLOOD AND TISSUE FLAGELLATES

Blood and tissue flagellates possess a single nucleus, a single kinetoplast and a single flagellum. The kinetoplast consists of parabasal body and an adjacent dot like blepharoplast. The blepharoplast and parabasal body are connected by one or more delicate fibrils.

The flagellum arises from the blepharoplast. The portion of the flagellum extending from the blepharoplast to the surface of the body of the parasite is known as axoneme. Family Trypanosomatidae consists of six genera, of which *Leishmania* and *Trypanosoma* are pathogenic to man. Species of this family may exist in two or more forms.

LEISHMANIA

The genus ***Leishmania*** is widely distributed in nature. It has a number of species that are nearly identical morphologically. Differentiation is, therefore, based on a number of biochemical and epidemiological criteria – use of monoclonal probes to detect specific antigens, promastigote growth patterns *in vitro* in the presence of antisera, and vectors and reservoir hosts.

The parasites of the Old World leishmaniasis **(*L.donovani, L. infantum, L.tropica, L.major* and *L.aethiopica*)** are transmitted to humans by the bite of female sandflies of the genus *Phlebotomus*; while those of the New World leishmaniasis **(*L. peruviana, L.chagasi, L. mexicana* complex and *L.braziliensis* complex)** are carried by sandflies of the genera *Lutzomyia* and *Psychodopygus.* The term ‘New World’refers to the Americas and the ‘Old World’ is used for the rest of the world.

Leishmania pass their life cycle in two hosts – invertebrate hosts and vertebrate hosts. Former are the sandflies and the latter are mammals in which the parasites reside within the phagolysosomal system of mononuclear phagocytic cells, typically macrophages. However, in the invertebrate hosts, the parasites are extracellular, development occurs exclusively in the gut and transmission is via the mouthparts during blood feeding.

Leishmaniasis is a collection of diseases, each with its own clinical manifestations and epidemiology. It is mainly a **zoonosis**, although in certain areas of the world there is primarily human-vector-human transmission. The World Health Organization estimates that 1.5 million cases of cutaneous leishmaniasis and 500,000 cases of visceral leishmaniasis occur every year in 82 countries. Estimates indicate that there are approximately 350 million people at risk of acquiring leishmaniasis, with 12 million currently infected.

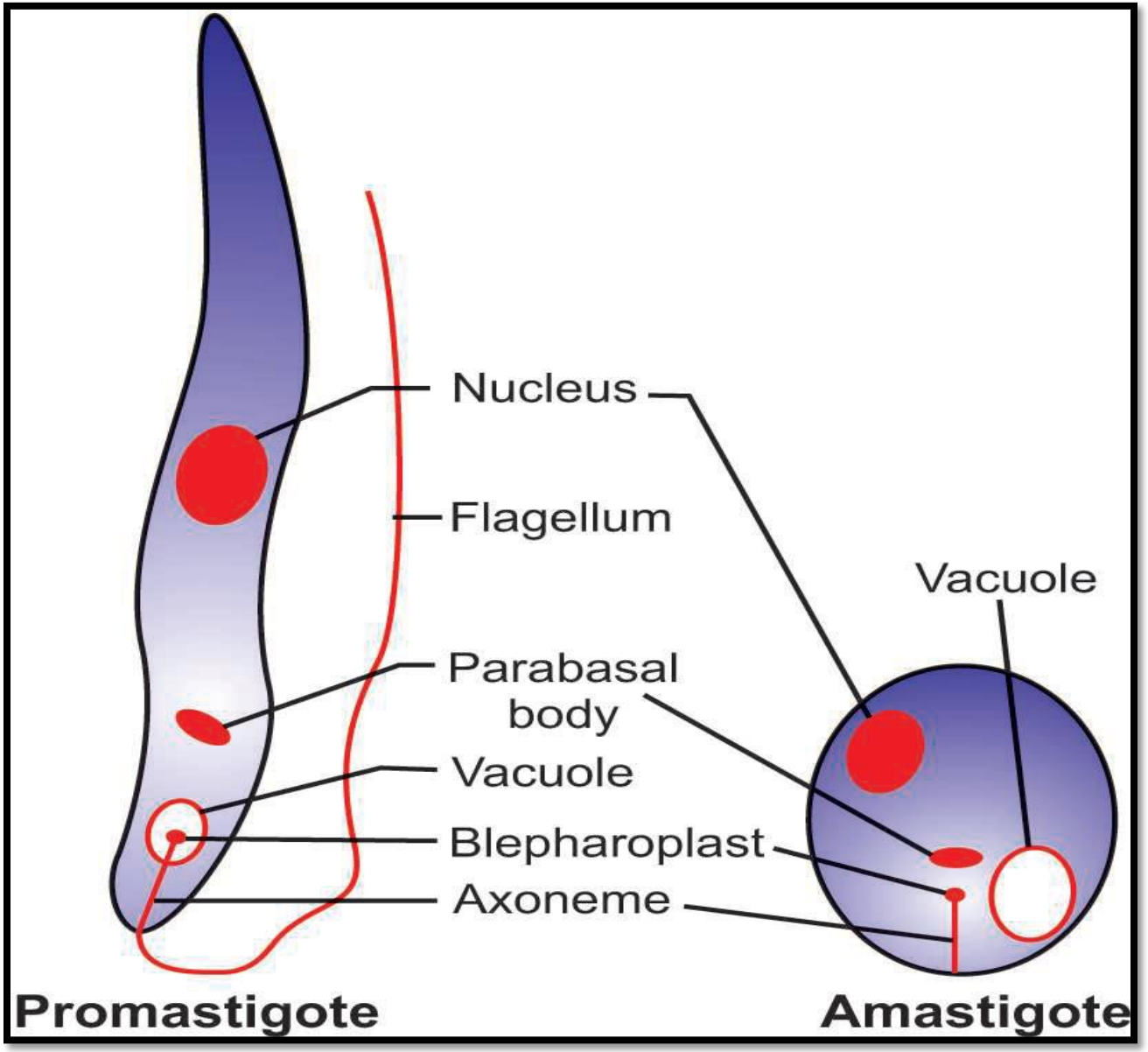
Old world leishmaniasis

1. *Leishmania donovani*

The amastigote form of this parasite is an obligate intracellular parasite of reticuloendothelial cells, predominantly of the liver, spleen, bone marrow and lymph nodes of man and other vertebrate hosts (dog and hamster) where it occurs in amastigote form. **The parasite exists in two morphological forms: amastigote and promastigote.** The **amastigote** form of the parasite resides in the cells of the reticuloendothelial system (macrophages, monocytes, polymorphonuclear leucocytes, or endothelial cells) of the vertebrate hosts. It is a non-motile, round or oval body

measuring 2-41μm in length along the longitudinal axis; it can be demonstrated in fresh specimens only. The nucleus is round or oval, situated in the middle of the cell or along the side of the cell wall. In preparations stained with Giemsa or Wright stain, the cytoplasm appears pale blue, the nucleus red, the parabasal body deep red and the kinetoplast bright red.

The **promastigotes** are found in the digestive tract of the insect vector (sand fly) and in the culture media. These are an elongated, motile, extracellular stage of the parasite. Fully developed promastigotes measure 15-25μm in length and 1.5-3.5μm in breadth. The nucleus is situated centrally, and the kinetoplast lies transversely near the anterior end. The flagellum may be of the same length as the body of the parasite or longer. It does not curve around the body of the parasite, therefore, there is no undulating membrane.



Morphological forms of Leishmania donovani.

Life cycle

L. donovani passes its life cycle in two hosts, man and also dog in some areas are the vertebrate hosts, and female sandfly ***Phlebotomus papatasi*** as the invertebrate host. (Important sandfly hosts include *P. argentipes*, *P. orientalis* and *P. martini*. Of these *P.argentipes* is the Indian vector).

The cycle in sand fly

Amastigotes of the parasite are present in the blood stream of the patient, both free as well as phagocytosed by polymorphonuclear leucocytes and monocytes. These are taken up by the sand fly in a blood meal and reach the midgut of the insect. Here the parasite transforms into promastigotes and multiplies producing enormous numbers. The parasites then proceed forwards to the pharynx and buccal cavity which they block between the 6th and 9th day of its infective blood meal.

The cycle in man

Because of the blockage of the pharynx and buccal cavity, the sand fly has difficulty in getting a blood meal, nevertheless, it pricks the skin of the victim and regurgitates the promastigotes in the wound caused by its proboscis. These are engulfed by nearby fixed macrophages and change into amastigotes within the cytoplasm of these host cells. Here the amastigotes multiply slowly and may remain more or less quiescent for weeks or months.

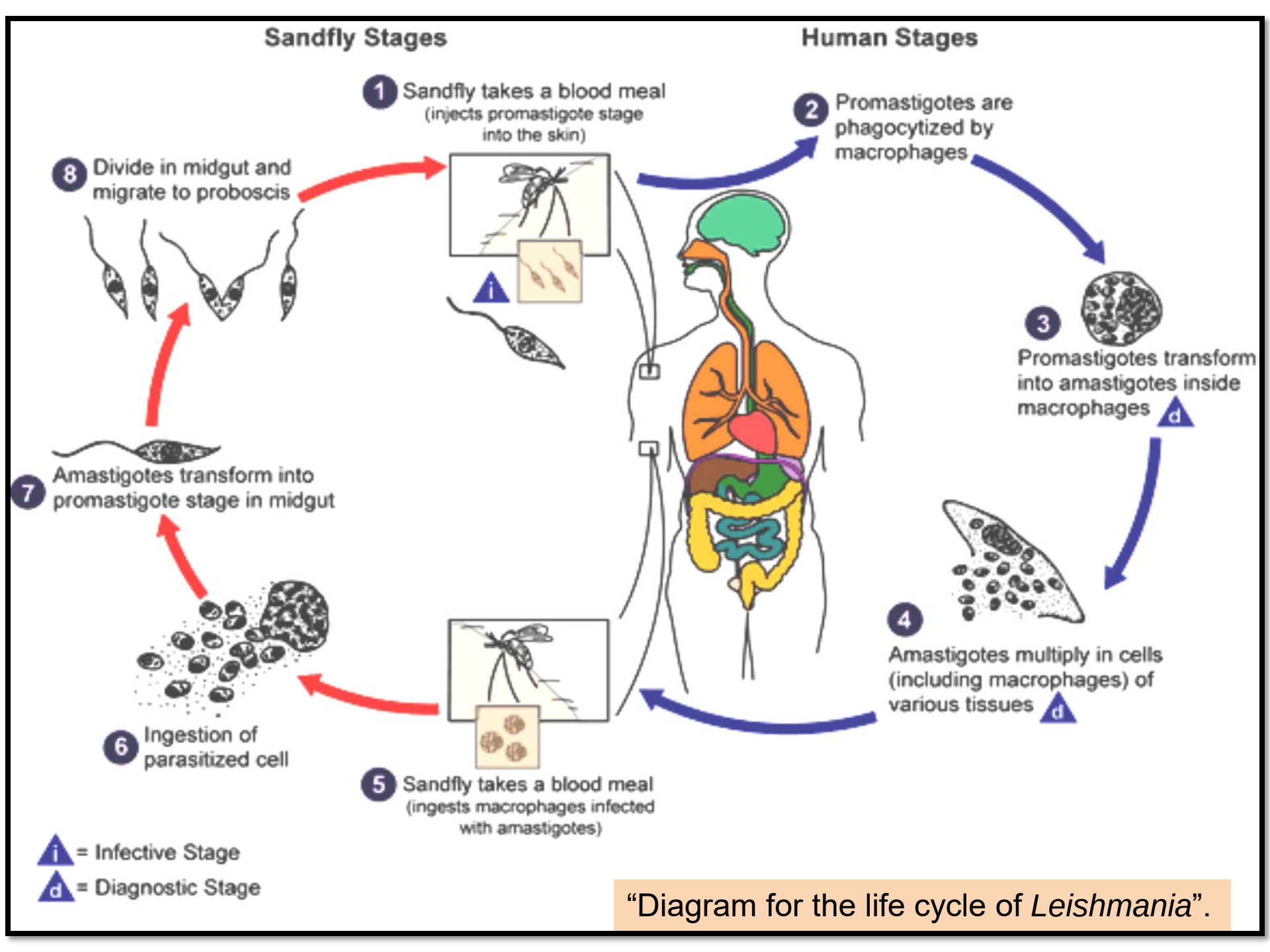
Thereafter, parasitized macrophages are set free into the blood stream and are carried from the skin to the spleen, liver, bone marrow and other centers of reticuloendothelial activity.

The amastigotes are now taken up by fixed phagocytic cells such as Kupffer's cells in the liver, and multiply by simple binary fission till the cells become packed with the parasites (50-200 or more in the cytoplasm of the infected cell).

The infected cell ruptures and the parasites are liberated into the circulation. These are taken up by other reticuloendothelial cells followed by the multiplication of the parasites and rupture of the cells.

In this way the entire reticuloendothelial system becomes progressively infected.

In the blood stream, some of the free amastigotes are phagocytosed by polymorphonuclear leucocytes and monocytes. A blood-sucking insect takes these free amastigotes, as well as those within the cells during its blood meal and the cycle is repeated.



"Diagram for the life cycle of *Leishmania*".

Pathogenicity and Symptoms

L. donovani causes visceral leishmaniasis or kala-azar (kala meaning black and azar meaning disease), Dum Dum fever or tropical splenomegaly. The incubation period generally varies from 3-6 months, but it may be as short as 10 days or as long as 2 years. As the parasite multiplies in the reticuloendothelial cells, especially in the spleen, liver, lymph nodes and bone marrow, this leads to progressive enlargement of these organs and hypersplenism contributes to the production of anaemia.

The host cellular and humoral defence mechanisms are stimulated. The primary result is a marked proliferation of macrophages. These cellular elements make up a large part of the bone marrow, compromising both the erythropoietic and granulocytic activity.

The effect of this in the peripheral blood is leucopenia with granulocytopenia and relative monocytosis, anaemia (usually normocytic, **normocytic mean that there is fewer red blood cells than normal, and those blood cells don't have the normal amount of hemoglobin**) and thrombocytopenia. It has been suggested that the erythrocytes adsorb immune complexes and become subject to enhanced phagocytosis by the macrophages of the liver and spleen.

Lymphadenopathy is also produced. The production of globulin is greatly increased, and this leads to the reversal of the albumin/ globulin ratio. The invasion of *L. donovani* parasites to the reticuloendothelial cells results in **reticuloendotheliosis**, where reticuloendothelial cells of various organs are proliferated.

The disease manifests clinically with fever, malaise, headache, progressive enlargement of the spleen, liver and lymph nodes, anaemia, leucopenia and emaciation. Skin changes are often seen on the face, hands, feet, and abdomen, where patients acquire an earth-gray color. If left untreated 75-95% of patients die within 2 years. **Death in kala-azar is due to secondary infections**.

Visceral leishmaniasis and human immunodeficiency virus (HIV) together are synergistic infections because visceral leishmaniasis accelerates the development of acquired immunodeficiency syndrome (AIDS) and the presence of HIV infection enhances the spread of visceral disease. In contrast to cutaneous leishmaniasis, cell-mediated immunity is impaired in active kala-azar patients who consequently lack a delayed type hypersensitivity response, but this can be demonstrated after the cure.

Post Kala-Azar dermal Leishmaniasis

Post kala-azar dermal leishmaniasis (PKDL) was first described in patients with visceral leishmaniasis caused by *L. donovani* in India. It occurs in up to 20% of these patients. Skin lesions may appear 2-10 years after being partially treated, untreated or even those considered adequately treated for visceral leishmaniasis. In East Africa, lesions appear within a few months. Individuals with PKDL may be very important reservoirs for maintaining the infection in the population because of the high concentration of organisms in the skin.

Diagnosis

Non-specific tests

1.Blood count: Total and differential leucocyte count which reveals pancytopenia, mainly neutropenia and decreased erythrocyte count.

2. Haemoglobin estimation: It reveals anaemia.

3. Estimation of serum proteins: It reveals raised serum proteins with reversal of the albumin/ globulin ratio due to greatly raised IgG levels. Immunological tests are used, but these are difficult to evaluate since post-recovery cases are indistinguishable from active cases.

Parasitological diagnosis

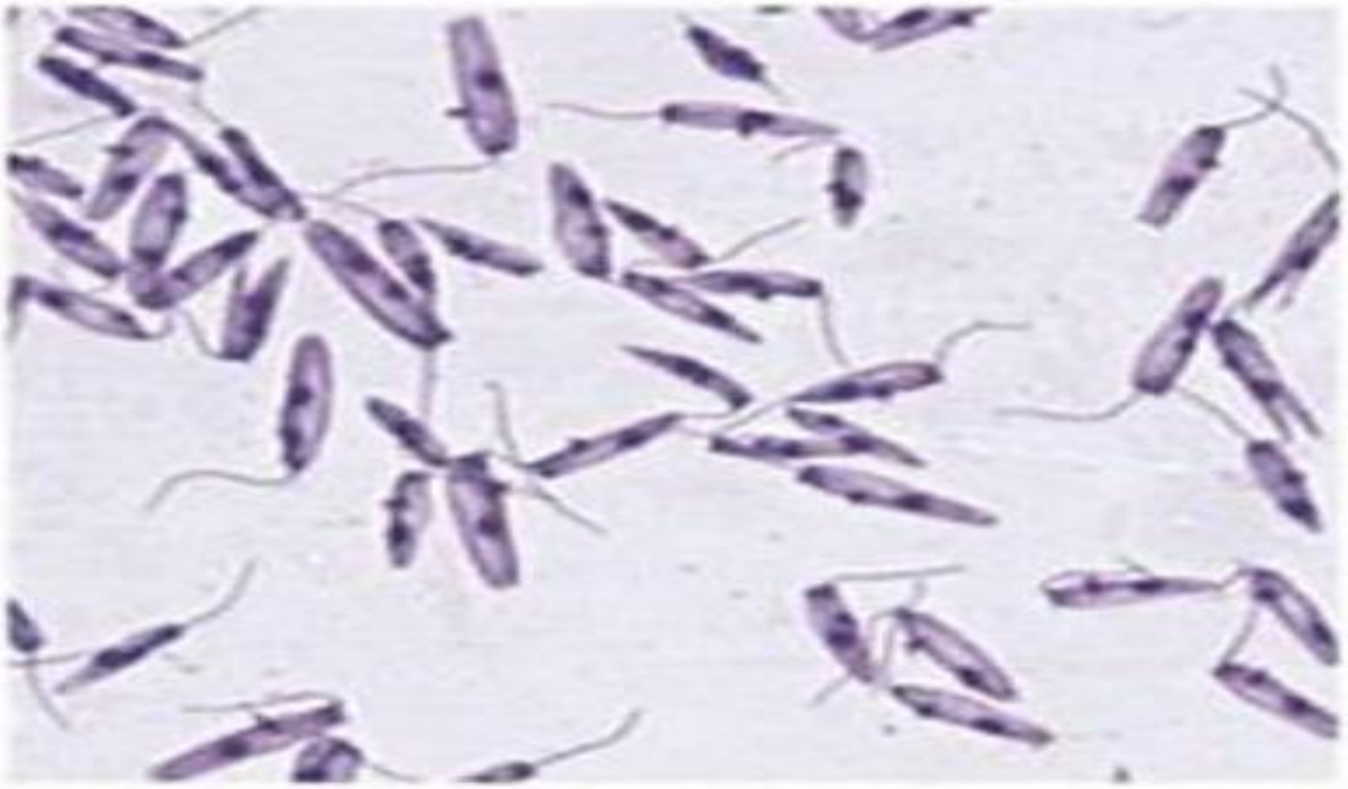
1. Peripheral blood film

The amastigote form of the parasite may be demonstrated inside circulating monocytes and less often in neutrophils, in the stained peripheral blood film with Leishman or Giemsa stain in the thick film method. Owing to the small number of *Leishmania* parasites present in the peripheral blood, an examination of a thin film is often negative.

2. Needle biopsy/aspiration

Deeper tissues, e.g., lymph nodes, bone marrow, liver and spleen may be sampled by needle biopsy/ aspiration. Amastigote forms of the parasite can be demonstrated, within reticuloendothelial cells, in touch preparations or smears stained with Giemsa stain.

Sample of the new parasite isolated from skin lesion



3. Culture

Whatever material is collected (blood and biopsy/ aspiration material from various organs), it should be inoculated into the water of condensation of NNN medium and incubated at 22°-25°C and examined microscopically twice a week for the first 2 weeks and once a week thereafter for up to 4 weeks before they are reported as negative. Promastigote stages can be detected microscopically in wet mounts taken from centrifuged culture fluid. The material can also be stained with Giemsa stain to facilitate observation at a higher magnification.

4. Molecular methods

A number of molecular methods have been developed for species identification of the promastigotes, using DNA probes and polymerase chain reaction (PCR) test.

5. Animal inoculation

Aspirate or biopsy material obtained from lymph nodes, spleen, liver, bone marrow, etc. is inoculated intraperitoneally in young hamster 2-4 months old.

Immunological tests

These include non-specific and specific tests. Specific tests include complement fixation test, indirect fluorescent antibody test (IFAT), indirect haemagglutination assay (IHA), and enzyme-linked immunosorbent assay (ELISA).

Flowchart 4.1. Laboratory diagnosis of kala-azar

Non-specific tests

- Blood count (pancytopenia mainly neutropenia and decreased erythrocyte count)
- Haemoglobin estimation (anaemia)
- Estimation of serum proteins (raised serum proteins with reversal of albumin:globulin ratio due to greatly increased IgG level)

Parasitological diagnosis

- Peripheral blood film by thick film method (amastigote form)
- Blood culture in NNN medium (promastigote form)
- Needle biopsy/aspiration

Lymph node | Bone marrow | Liver | Spleen

– By touch preparation or smear stained with Giemsa stain (amastigote form)

– Culture in NNN medium (promastigote form)

- Molecular methods
  - DNA probes
  - Polymerase chain reaction
- Animal inoculation

Immunological tests

Non-specific tests

- Aldehyde test
- Antimony test

Indicate greatly increased serum proteins

- Complement fixation test with WKK antigen

Specific tests

- Indirect fluorescent antibody test
- Indirect haem-agglutination test
- Enzyme-linked immunosorbent assay
- Direct agglutination test
- Leishmanin or Montenegro skin test

Leishmania infantum

It is another type of visceral leishmaniasis; caused by *L. infantum*, distributed in the Mediterranean basin, and Central and Western Asia. The main vertebrate host is the domestic dog, which develops an acute or chronic disease. Canine visceral leishmaniasis presents abundant parasites in the skin available for transmission.

The invertebrate hosts or vectors are ***Phlebotomus ariasi*** and ***P. perniciosus***.

In man it causes **infantile visceral leishmaniasis** classically restricted to children, especially those below the age of 2 years. However, it may also involve adults, particularly those infected with HIV. The PKDL is not seen with *L. infantum* infection.

2. *Leishmania tropica*

The amastigote form occurs in man, whereas the promastigote form is found in sand fly and in cultures. Morphologically, both forms of *L. tropica* are indistinguishable from those of *L. donovani*. Regarding the life cycle, the vertebrate host is man and the invertebrate host is the **sand fly** (***Phlebotomus sergenti***).

The life cycle of *L. tropica*, in both vertebrate and invertebrate hosts, is similar to that of *L. donovani* except that in man amastigote form of the *L. tropica* resides in the reticuloendothelial cells of the skin and not in the viscera. One factor restricting the parasite causing cutaneous leishmaniasis to the skin may simply be temperature, to which some species of *Leishmania* are particularly sensitive.

Pathogenicity and Symptoms

L. tropica causes **urban anthroponotic cutaneous leishmaniasis** or **Oriental sore** or **Delhi boil (or Baghdad boil)**. The infection is transmitted to man either by the direct inoculation of the promastigotes of *L. tropica* through the bite of the sand fly or by crushing of the infected sandfly into the punctured wound caused by the bite.

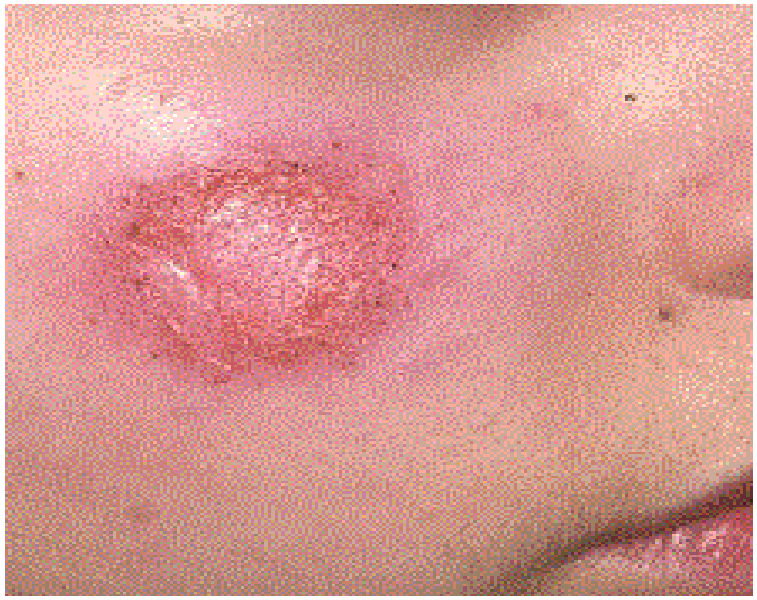
At the site of inoculation, the promastigotes are phagocytosed by reticuloendothelial cells of the skin and are transformed into amastigotes. The cutaneous lesion or leishmanioma develops at the site of the infective sand fly bite. It is characterized by a chronic infective granuloma with fibrosis. In the early stage, the lesion is due to the proliferation of reticuloendothelial cells of the skin that contain a large number of amastigotes.

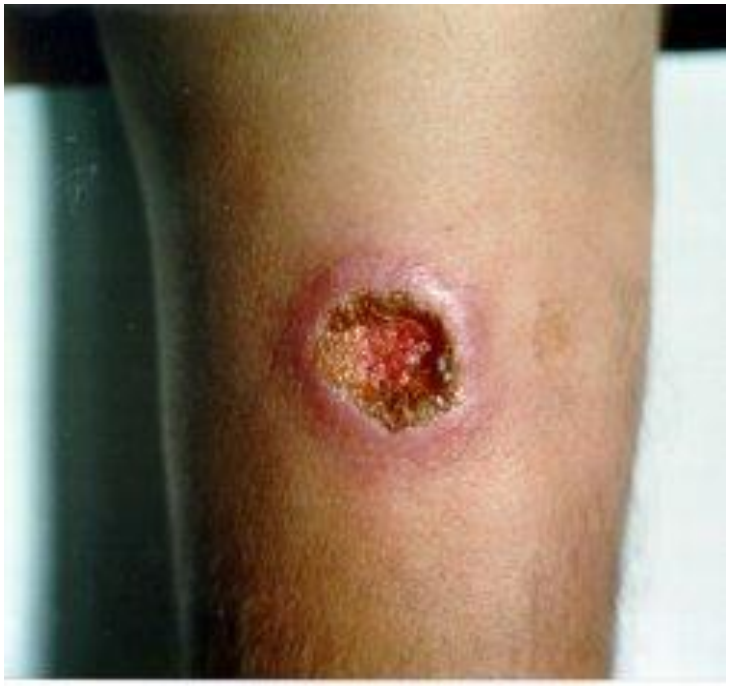
Later, infiltration of lymphocytes and plasma cells associated with a marked reduction in the number of parasites and development of a delayed hypersensitivity skin reaction (leishmanin reaction) occur.

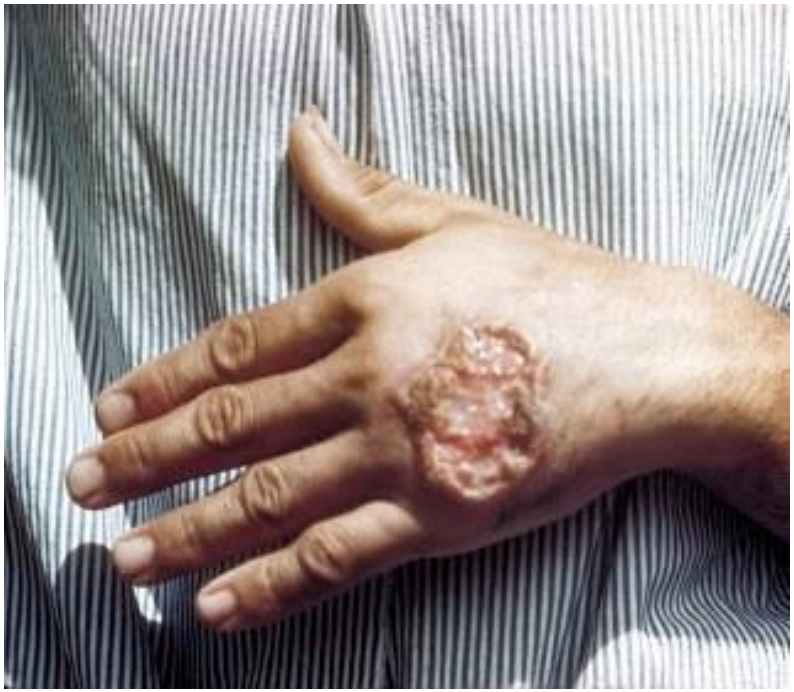
The incubation period varies from a few weeks to 6 months and in some cases it may be 1-2 years. Clinically, the lesion begins as a raised papule about 2.5cm in diameter. In the majority of cases, it ulcerates. The ulcer has a clean-cut margin with a raised indurated edge, surrounded by a red areola. At this stage, the parasite is found along the red margin and not on the floor of the ulcer. The ulcer heals spontaneously in about 6 months, leaving a depressed scar and solid immunity.

There is a marked development of cell-mediated immunity but a weak antibody response, although specific antibodies can be detected. The cell-mediated immunity is responsible for a marked delayed type hypersensitivity response to leishmanin in active and cured cases.

The sores are distributed on the exposed parts of the body, particularly on the face and extremities. The average number of sores is around two. Oriental sore is not associated with systemic manifestations although there may be enlargement of the draining lymph nodes. **In contrast to *L. major* infection, *L. tropica* causes dry lesions, more swollen and less necrotic. The exudate is less profuse and accumulates as a thick crust.**







Cutaneous lesions in cutaneous leishmaniasis.

Diagnosis

1. Microscopy

It is made by the microscopic examination of material obtained by puncture of the indurated edge of the sore and stained with Giemsa or Wright stain. The amastigote form of the parasite will be seen in large numbers inside the macrophages. Smears made by scraping the floor of the ulcer are often negative because amastigote-infected macrophages are destroyed in the presence of secondary bacterial infection. If smears are negative, a biopsy from the margin of the ulcer at times provides specific proof of infection.

2. Culture

Isolation of the promastigotes of *L. tropica* may be made from the aspirates of the ulcer by culture in the NNN medium.

The specimen for culture is obtained by injecting a little volume of sterile physiological saline in the indurated (stiff or solid) margin of the ulcer and then aspirating it. A few drops of the aspirate are then inoculated into each medium.

Leishmania major

Its mammalian hosts are the great gerbil (*Rhombomys opimus*) and fat sand rat (*Psammomys obesus*), and important sand fly vectors are *Phlebotomus papatasi*, *P. dubosqi* and *P. salehi*. The infection in humans occurs in epidemics in groups of people inhabited zoonotic foci. Transmission between humans without a mammalian reservoir host has not been well established. *L. major* causes **rural, zoonotic, cutaneous leishmaniasis** or **Oriental sore**. It causes a **wet lesion** which becomes necrotic and exudative, forming a loose crust above a granulomatous base that eventually produces the characteristic scar.

The lesions number may be more than 100. Lymphatic spread may occur in *L. major* infections, with subcutaneous nodules in a linear distribution. As in the case of *L. tropica*, spontaneous cure of infection usually results in a solid immunity and marked delayed type hypersensitivity response to intradermal inoculation of leishmanin. The method of diagnosis of *L. major* infection is similar to that caused by *L. tropica.*

Leishmania aethiopica

In man, it may cause **cutaneous leishmaniasis** and **diffuse cutaneous leishmaniasis (DCL)**. Its lesions are more swollen and less necrotic than those of *L. tropica*. They are frequently barely exudative, with gradual scaling or exfoliation of the dermis at the centre.

These lesions may last for years before healing. The DCL is a rare form of disease caused by *L. aethiopica*. The parasites are restricted to the skin but become widely distributed over much of the surface, in large, swollen plaques and nodules.

These lesions may last for years before healing. The DCL is a rare form of disease caused by *L. aethiopica*. The parasites are restricted to the skin but become widely distributed over much of the surface, in large, swollen plaques and nodules. However, the abundant parasites in the nodules provide easy distinction. In this condition, neither humoral nor cell-mediated immune responses are activated. It is difficult to treat and it may last for the rest of the life of the patient.

New world leishmaniasis

Leishmania braziliensis

It occurs in tropical South America and Central America respectively. They cause **mucocutaneous leishmaniasis** or **Espundia** which is similar to that of *L. donovani* and *L.tropica*, except that the amastigotes occur inside the macrophages of the skin and mucous membrane of the nose and buccal cavity but not in internal organs; and they are transmitted by **sand flies** of the genera ***Lutzomyia***.

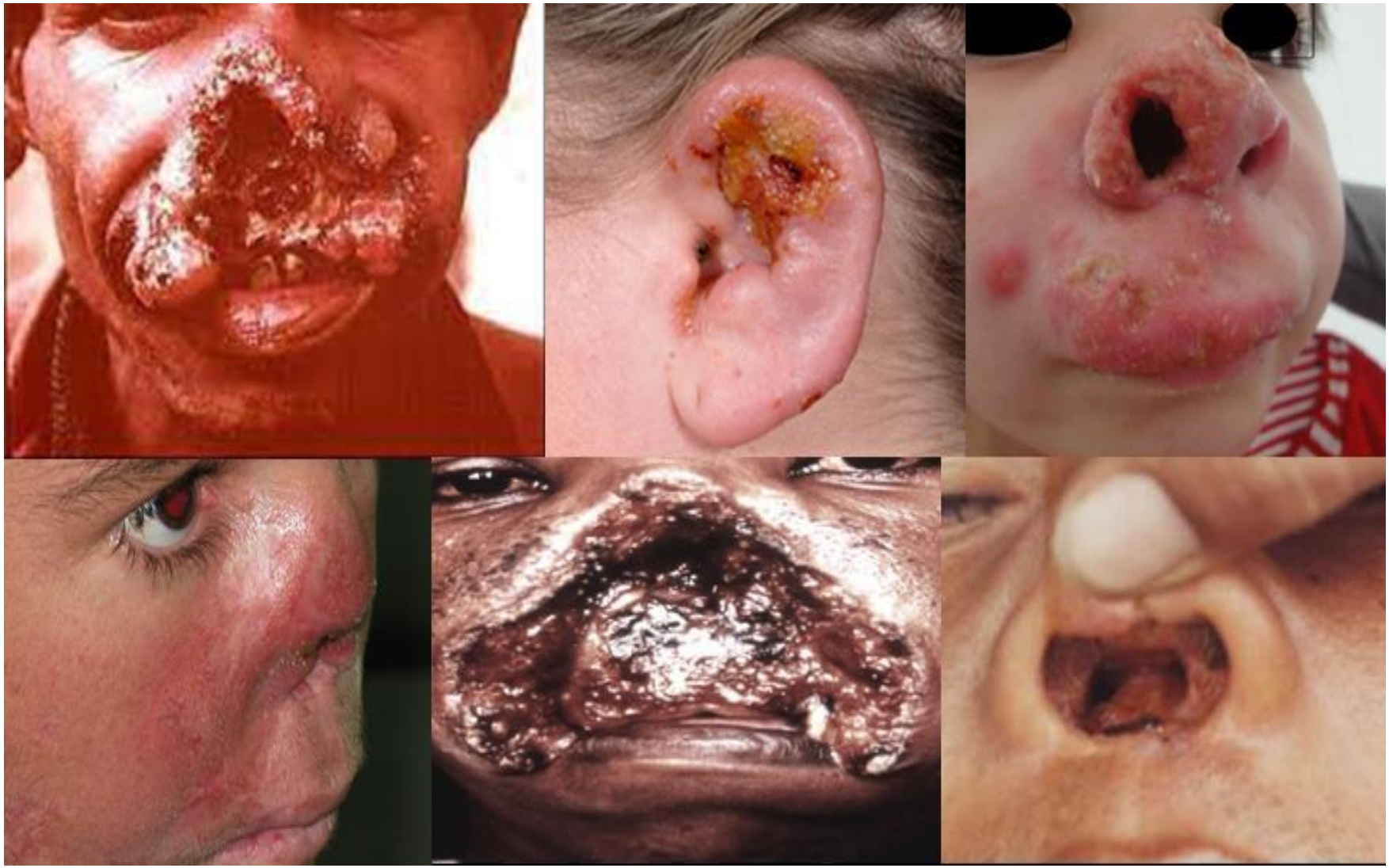
Their intracellular parasites (amastigote form) occur inside the macrophages of the skin and mucous membrane of the nose and buccal cavity. They do not occur either in the internal organs or in the peripheral blood. These parasites are morphologically and culturally indistinguishable from other species of *Leishmania*.

Espundia is zoonoses, the causative parasites are primarily of wild animals. When the various sand fly vectors feed on humans these parasites may be transmitted. In this unnatural host, they usually provoke an intense reaction and the eventual development of a skin lesion at the site of the bite; after about 7-10 days a tiny papule appears.

In most cases, it ulcerates, producing craterlike lesions with inflamed and elevated border. The lesion may be single or multiple, it is due to the infected macrophages transporting the parasite to other parts of the body thus establishing a secondary lesion.

L. braziliensis tends to produce such metastatic lesions in the nasal, pharyngeal and laryngeal mucosae. These lesions may appear within a few months of the original skin lesion, or years later when the patient appears to have been cured of his initial infection.

The pathology of the skin lesion is similar to that of *L. tropica*. Histological examination of the skin and mucous lesion shows infiltration of lymphocytes, plasma cells and large mononuclear cells and necrosis of tissues. The amastigote form is found in large numbers inside the monocytes at the periphery of the lesion. The diagnosis can be done as in cutaneous leishmaniasis.



Treatment of Old and New World leishmaniasis

The classic therapy for all cases of *Leishmania* is pentavalent antimony (sodium antimony gluconate, sodium stibogluconate). As an alternative, liposomal amphotericin B is a highly effective agent for visceral leishmaniasis, and it is currently the drug of choice for antimony resistant disease.