Summary

•In humans obligate intracellular parasite with replication in macrophages

• Cutaneous form: chronic painless ulcers or nodules, amastigotes in smear

• Visceral form: chronic fever, hepatosplenomegaly, pancytopenia, persistent inflammatory state. Lethal if not treated

•Diagnosis of kala azar: amastigotes in bone marrow and other sites, serology, antigen detection

•Mucocutaneous: chronic destructive lesions in mouth/nose, frequent clinical diagnosis

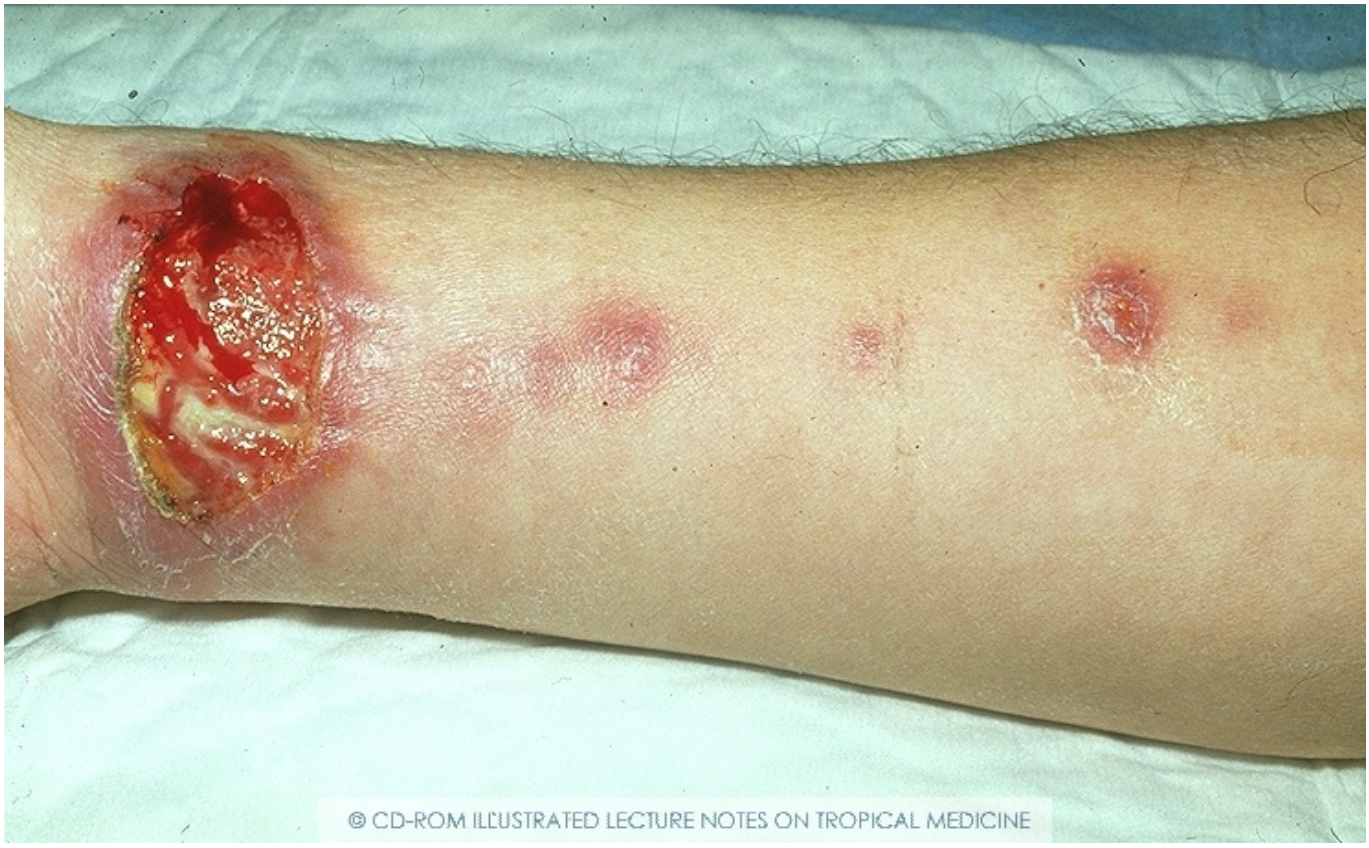
•Transmission via about 30 species of sandflies

•Zoonotic transmission: animal reservoir (especially dogs and rodents)

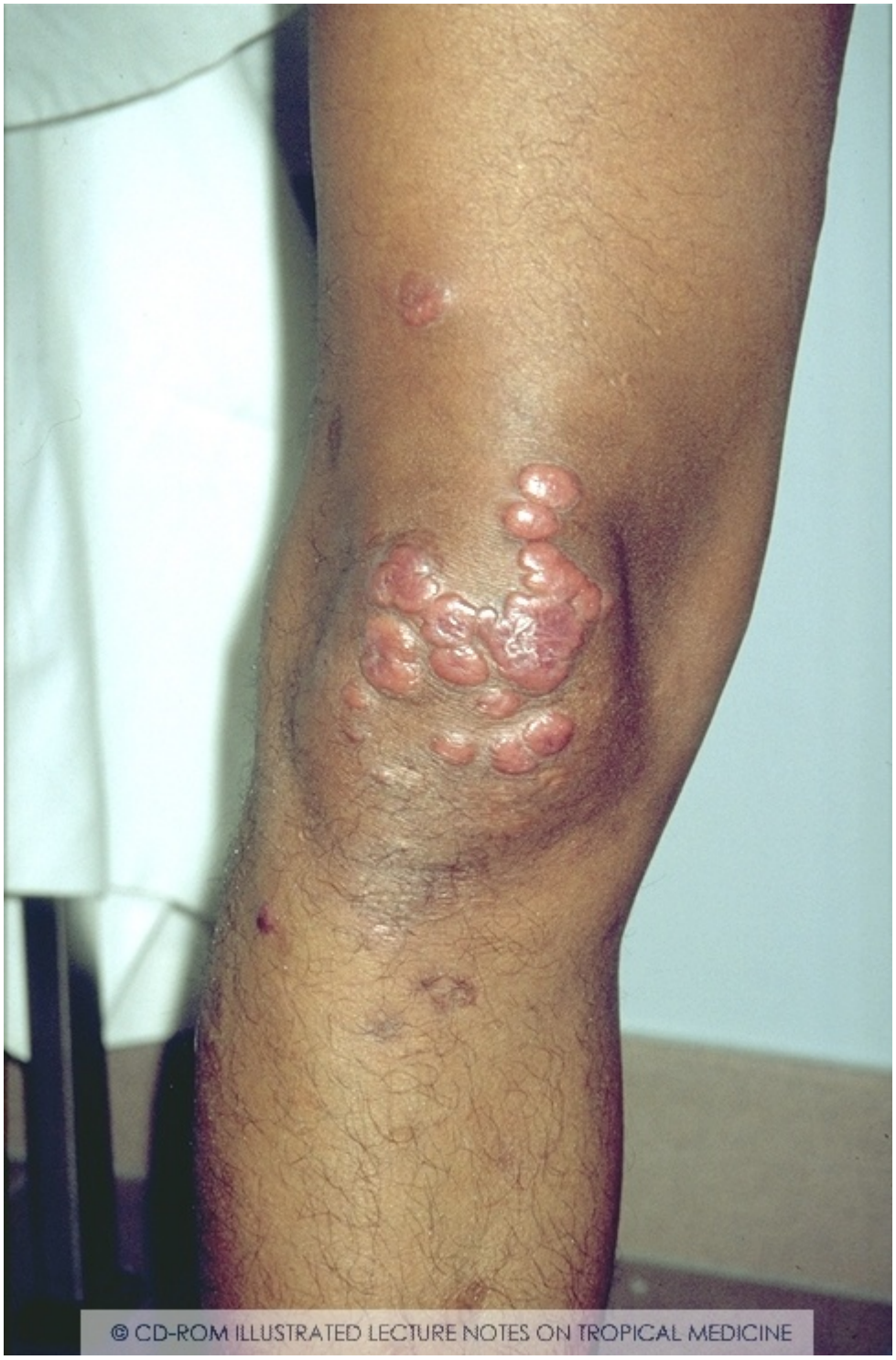
•Anthroponotic transmission: human reservoir, e.g. Indian kala azar and in cutaneous *L. tropica*

•Treatment with antimony derivatives, amphotericin B, miltefosin, pentamidine. Combination treatment increasingly in use.

General



Leishmania braziliensis ulcer on the wrist and spread via the lymphatics. Lesions occurred after a visit to rural Bolivia. Copyright ITM



Diffuse cutaneous leishmaniasis due to infection with *Leishmania aethiopica*. Copyright ITM

There are several species of *Leishmania* parasites and these can cause various clinical

conditions. They can be responsible for chronic ulcers and skin nodules. Sometimes both skin and mucosae are affected (mucocutaneous leishmaniasis). When deep organs are affected, the condition is called visceral leishmaniasis. The *Leishmania* species that cause these various clinical conditions always have the same morphology under the microscope. However, there are differences in parasite DNA, proteins, enzymes and mode of development in the insect vector, etc. *Leishmania* parasites can in turn be infected with a RNA virus (the "leishmania virus") though the significance of this is not yet known.

The classification, distribution and pathogenicity of the various *Leishmania* species is quite complicated. New data are regularly becoming available (for example, *L. tropica* was shown to be able to cause visceral leishmaniasis in rare cases). The whole taxonomy will probably change as more and more genetic information becomes available. A distinction is made between zymodemes (iso-enzyme patterns), schizodemes (kDNA analyses with restriction enzymes), serodemes (via reactions with monoclonal antibodies) and rapdemes (using PCR with random primers). Some 30 different *Leishmania* species have been described (10 in the Old World and 20 in the New World). Many of these can infect humans. The genus *Leishmania* is frequently subdivided into the subgenera *Leishmania* and *Viannia*.

There are substantial geographical genetic variations. Hence in the dry western part of *Peru* *L. peruviana* causes the disease "uta", an ulcerative form without mucocutaneous lesions. This organism contains less DNA in some of the chromosomes than the virulent L. braziliensis, the pathogen causing Espundia, a disease which occurs in the forests on the other side of the Andes in Eastern Peru. One of the differences is the number of copies of the leishmanolysin gene, which codes for an important surface antigen (gp63). This zinc protease has a role in adhesion to macrophages and survival in the phagolysosome. It is regarded as an important virulence factor. *L. braziliensis* contains more leishmanolysin genes than *L. peruviana*. The protein is being studied as, among other things, the basis for an experimental vaccine.

Classification

There is still no generally accepted internationally agreed definitive taxonomy. The following table can serve for orientation:

Leishmania species			
New World			
L. (Viannia) braziliensis	LCL, mucosal	zoonotic	Latin America
L. (Viannia) panamensis	LCL, mucosal	zoonotic	Northern South America and southern Central America
L. (Viannia) peruviana	LCL	zoonotic	Peru
L. (Viannia) guyanensis	LCL	zoonotic	South America
L. (Viannia) lainsoni	LCL	zoonotic	South America
L. (Viannia) columbiensis	LCL	zoonotic	Northern South America
L. (Leishmania) amazonensis	LCL, DCL	zoonotic	South America
L. (Leishmania) mexicana	LCL, DCL	zoonotic	Central America, Mexico
L. (Leishmania) pifanoi	LCL	zoonotic	South America
L. (Leishmania) venezuelensis	LCL	zoonotic	Northern South America
L. (Leishmania) garnhami	LCL	zoonotic	South America
Old World			
L. (Leishmania) aethiopica	LCL, DCL	zoonotic	Ethiopia, Kenya
L. (Leishmania) killicki	LCL	zoonotic	North Africa
L. (Leishmania) major	LCL	zoonotic	North and East Africa, Middle East, Central Asia
L. (Leishmania) tropica	LCL	anthroponotic	North Africa, Middle East, Central Asia
L. (Leishmania) donovani	LCL, visceral	anthroponotic	Central Asia, Africa
Old and New World			
L. (Leishmania) infantum	LCL, visceral	zoonotic	South Europe, North Africa, Central and South America

Legend: LCL : localised cutaneous leishmaniasis ; DCL : diffuse cutaneous leishmaniasis

Visceral leishmaniasis is mainly caused by the *Leishmania donovani* complex. There are several species in this complex:

1. *Leishmania donovani* (India, Pakistan, sub-Saharan Africa, East Africa)

2. *Leishmania infantum* (Mediterranean Basin, Middle East)

3. *Leishmania chagasi* (South America = *Leishmania infantum*)

4. *Leishmania archibaldi* (Africa) – of unclear importance

In the Old World skin lesions are mainly due to:

1. *L. tropica* (Mediterranean basin, Middle East). Frequently dry lesions

2. *L. major* (Middle East, sub-Saharan Africa). Frequently moist lesions

3. *L. aethiopica* (Ethiopia, Kenya). Sometimes also affects mucosa

4. *L. killicki* (North Africa) – of lesser importance

In addition, *L infantum* and *L donovani* (more exceptionally) can also cause skin lesions.

In (mainly South and Central) America skin lesions are caused by the *L. mexicana* and *L. braziliensis* complex. These complexes are subdivided into species:

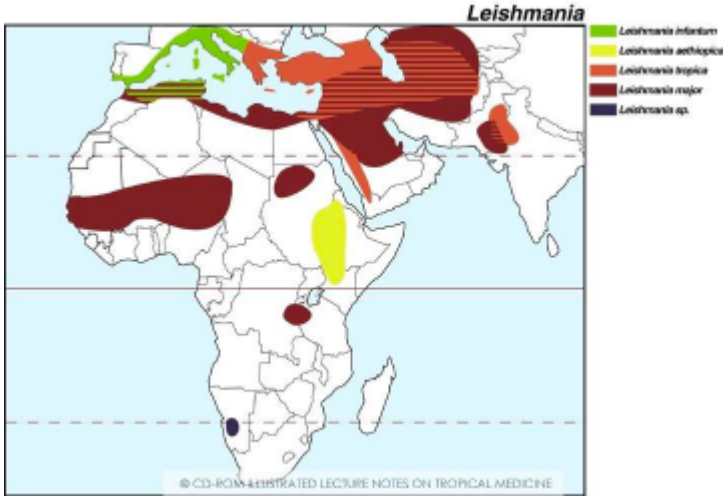
1. *L. mexicana* complex: *L. mexicana, L. venezuelensis, L. amazonensis*

2. *L. braziliensis* complex: *L. braziliensis, L. panamensis, L. guyanensis, L. peruviana*

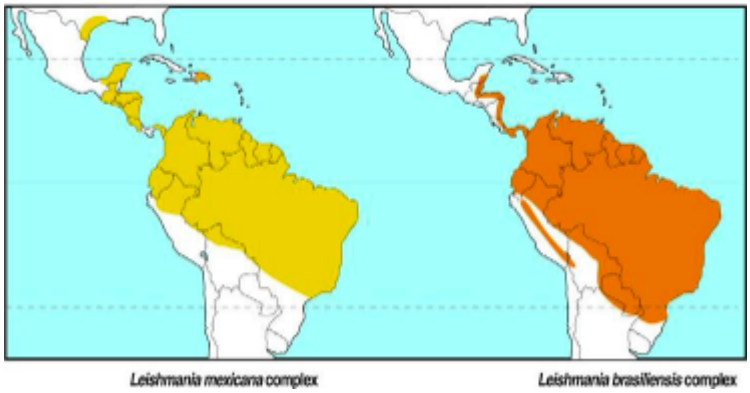
Mucosal lesions are common in infections with *L. braziliensis*. One should always keep in mind that the clinical lesions of leishmaniasis are a consequence of the parasite species on the one hand and of the immunological resistance and reaction of the patient on the other.

Infections occur very rarely with other *Leishmania* species: *L.(Viannia) naiff, L. (Viannia) shawi.*

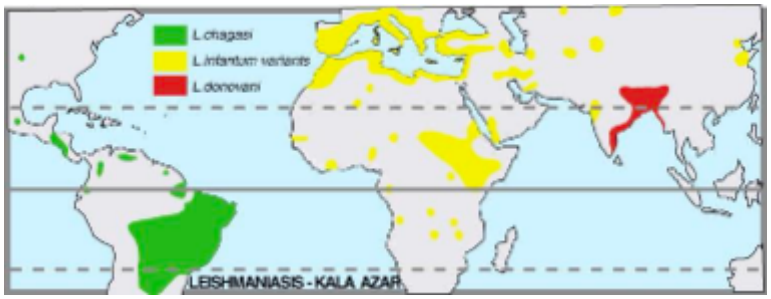
Distribution



Map Leishmania infantum, L. aethiopica, L. tropica, L. major. Adapted from Colour Atlas



Map *Leishmania mexicana* and *L.braziliensis*. Adapted from Colour Atlas.



Map of the areas endemic for *Leishmania chagasi, L. infantum, L. donovani,* pathogens leading to kala azar. Adapted from Colour Atlas

Mucocutaneous leishmaniasis occurs in Central and South America and occasionally in East Africa.

Visceral leishmaniasis occurs from western China to the Mediterranean Basin, East Africa and Central and South America. It is very rare in Africa south of the equator. The majority of cases occur in 6 countries: Bangladesh, Nepal, India, Ethiopia, Sudan and Brazil

The cutaneous form is seen from India to the Mediterranean Basin, the northern half of the African continent and in Central and South America.

Leishmaniasis does not occur in Northern Europe, Canada, Uruguay, Chile, South Africa, Australia and Oceania. While Southeast Asia was thought to be leishmania free, an increasing number of visceral leishmaniasis cases have been reported from Thailand more recently.

For additional information and geographical risk in Europe; see www.leishrisk.net

Vector

The parasite is transmitted by the bite of infected female sandflies: *Phlebotomus* in the Old World and Lutzomyia in Central and South America. These genera, together with the blood-sucking genus *Sergentomyia* [little significance for man, as they suck blood from reptiles], belong to the Psychodidae family. Morphologically they very closely resemble each other. The name “sandfly” can be confusing as this name is sometimes used for other species as well. Sandflies are vectors of leishmaniasis, pappataci virus (an arbovirus) and *Bartonella* bacteria.

Sandfly. *Lutzomyia* and *Phlebotomus* species are vectors of leishmaniasis in the New, resp. Old World. Photo Cochabamba, Bolivia

Only some 10% of the approximately 600 known species of sandflies are vectors, and only 30 of these are important. A fly remains infected for life. In endemic areas, a minority of sandflies are infected usually below one per cent.

The female insects need blood in order to lay their eggs. Most species bite at night and at dusk. There are exceptions to this, such as Lutzomyia wellcomei, the main vector of L. braziliensis, which bites mainly during daytime. They can suck blood both from animals (cats, dogs, various rodents, cattle, birds and lizards, etc.) and man. They are small, soundlessly flying insects (approximately 2 mm in length). Because of these small dimensions they can get through standard mosquito nets. Impregnation with permetrine (cf. malaria) can help. Because of the very short mouthparts of the insects, they cannot bite through clothing. They are poor flyers. They will usually fly quite low and will remain in the vicinity of their breeding ground. They will also not fly when there is any wind. This knowledge can be exploited by having a fan or ventilator on at night in the bedroom to prevent sandflies from flying. They require high humidity and temperature for breeding, although they can be observed in dry regions provided there are sites with a favourable local microclimate (crevices, termite mounds, caves, hollows and holes in tree roots, etc) where 15 to 80 tiny eggs can be laid. The larvae cannot survive drying out. They will feed on organic waste and then pupate. Sandflies reproduce optimally at 23-28°C and at a relative humidity of 70-100%. Temperatures below 10°C or above 40°C are unfavourable for their survival. Measures used to control adult sandflies include the use of insecticides for residual spraying of dwellings and animal shelters, space-spraying, insecticide-treated nets, impregnated dog-collars and personal protection through application of repellents/insecticides to skin or fabrics. Bednets will be most useful in areas with peridomestic vectors (e.g. P*. argentipes* in India) whereas in areas where the vector bites in the field (e.g. *P. martini* in Kenya and Uganda) this can be expected to be less effective. Because the breeding-sites of sandflies are generally unknown, control measures that act specifically against immature are not feasible. Reports of insecticide-resistance refer to only three sandfly species (*P. papatasi, P. argentipes and S. shorttii*) against DDT in one country (India), although there are reports of DDT-tolerance in several countries.

Pathophysiology

An important aspect of the immune system is the balance between two arms of the T-helper response. Broadly speaking, the T-helper1 (Th1) response is tailored to intracellular pathogens, such as viruses and some bacteria and parasites. Because these organisms live inside cells, they are not accessible to antibodies. The Th1 response therefore stimulates other defence mechanisms such as macrophages. The T-helper2 (Th2) system, by contrast,

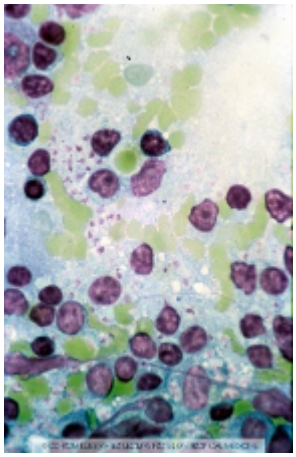
promotes a vigorous antibody response. The two arms are antagonistic, so a strong Th1 response means a weak Th2 response and vice versa. In leishmaniasis, where the parasites are intracellular, a strong Th1 response will kill the parasite and a strong Th2 response will lead to uncontrolled disease.

A gel produced by the *Leishmania* parasite in the gut of the sandfly prevents the insect from feeding properly. This causes more effort to feed, providing more chances for transmission of the parasite. The gel is injected into the human with the parasite and increases the severity of the infection. The crucial molecule in the gel, called filamentous proteophosphoglycan, interferes with the human immune system. The gel pushes the immune response to the non-protective T-h2 arm. The parasite thus manipulates the sandfly to make it feed more and then manipulates the host's immune system so that it can spread unchecked.

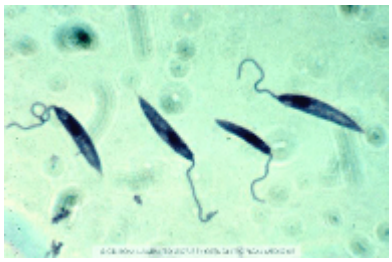
Sandfly saliva is important for the establishment of infection and disease pathogenesis. The sandfly saliva contains the vasodilator maxadilan. Saliva proteins seem to influence the immune response, resulting in a shift from Th1 to Th2 response. It is possible that the age-related decrease of susceptibility to leishmaniasis is due to anti-sandfly saliva antibodies.

Life cycle, Leishmania sp.

The parasite's life cycle is quite simple. When an infected sandfly bites, the parasite (as a promastigote) is injected directly into the skin. This unicellular parasite then penetrates the cells of the reticuloendothelial system (macrophages), where it multiplies in the form of amastigotes (the nonflagellate form) ("a" = without; "mastix" = whip). It is this form that can be seen in a skin biopsy or bone marrow aspirate. Multiplication results in bursting of the host cell, whereupon other cells become infected.



Leishmania amastigotes. This is the form present in human tissue. Copyright ITM



Leishmania promastigotes. The parasite has this morphology when residing in the sandfly vector. Copyright ITM

When another sandfly later bites, these infected cells can be ingested. The parasite is then still located in infected macrophages. The blood meal in the stomach is completely surrounded by a peritrophic membrane. The parasite transforms into a different form (promastigote with flagellum) in the insect and then multiplies. After 2-3 days the peritrophic membrane is digested and the parasites are released into the lumen of the stomach and intestine. They then attach to the microvilli of the intestine by means of their flagellae. They produce an enzyme, chitinase which damages the chitin coating of oesophageal-gastric junction, so that the valve between stomach and oesophagus no longer functions adequately and leaks resulting in a backflow of parasites to the mouthparts. The parasites accumulate 7 to 10 days later in the insect's proboscis and can be injected when the insect bites its next victim. The

insect is infectious 7-10 days after an infected meal and has to survive for this time in order

to be transmitted. Haemoglobin degradation products inhibit the secretion of chitinase and/or inhibit the enzyme itself making backflow of parasites to the mouthparts more difficult. Certain plant sugars do not have this effect. The insects also feed on plant juices. A balance between plant and animal feeding is required for successful transmission. A botanical description of the vector's environment (biotope) can be important in scientific studies.

Kala azar can be transmitted in other ways, but these are exceptional, namely shared use of needles among intravenous drug users or infected blood transfusion. Very rare cases of congenital kala azar infection have been reported.

Historical note, discovery of the parasite

The search for the origin of kala azar initially proceeded with great difficulty. Many hypotheses were investigated: for example, hookworm infection (ancylostomiasis) or malaria were thought to be responsible for the clinical condition. In 1900 an Irish soldier developed kala azar, after a stay in Dum Dum, near Calcutta, India. He died in England. The Scottish physician Dr. William Boog Leishman, later Director-General of the medical service of the British Army, carried out the autopsy. In spleen tissue he discovered small particles within the macrophages. He suspected that these were a sort of partly digested trypanosomes. A previously used name for visceral leishmaniasis was "Dum Dum fever" and refers to this historical event. The Irish physician Dr. Charles Donovan investigated splenic aspirates (needle biopsies of the spleen) from kala azar patients and confirmed Leishman's discovery. The tiny particles were called Leishman-Donovan bodies.