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Review Article: Brief Insights on Leishmaniasis

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Abstract

In this review article, leishmaniasis is discussed briefly as a parasitic disease caused by different species of *Leishmania* genus that remains a serious global public health problem in many countries in Africa, Americas, and Asia. The disease is transmitted by sandflies and the endemic areas are mainly concentrated in the tropics where the arthropod vectors occur. As the Earth's climate continues to change, the number of sand flies is expected to increase, putting more people at risk of contracting the virus. Leishmaniasis can cause three types of leishmaniasis are cutaneous leishmaniasis (CL), mucosal leishmaniasis (MCL) and visceral leishmaniasis (VL). The outcome of the infection depends on the *Leishmania* parasite species and the host immune response. Regarding treatment, few treatment options are available in areas where epidemics are severe. In addition, all first- and second-line drugs currently used to treat leishmaniasis have toxicity, cost, and/or administration problems. Therefore, the potential emergence of widespread drug resistance indicates an urgent need to develop new and effective treatments for leishmaniasis. In conclusion, leishmaniasis epidemics has shown incidence in many areas in last decade, and new epidemics can happen at any time in conflict areas and neighboring regions where the disease was previously endemic.

Keywords: Parasitic disease, cutaneous leishmaniasis, mucosal leishmaniasis, visceral leishmaniasis, Iraq

Taxonomy

The genus *Leishmania* includes a large number of species, reflecting the ability of this group to support a variety of vertebrates and invertebrates that parasitize and cause a variety of clinical symptoms, particularly in humans (Rodriguez et al., 2018). The taxonomy within the genus *Leishmania* is still not fully understood, and debate continues over the definition of the genus (Cantanhêde et al., 2021). Current molecular data and phylogenetic analyzes support simplifying the classification of *Leishmania* by reducing the number of species (Figures 1, 2), (Bruschi and Gradoni, 2018; Schönian et al., 2018; Klatt et al., 2019).

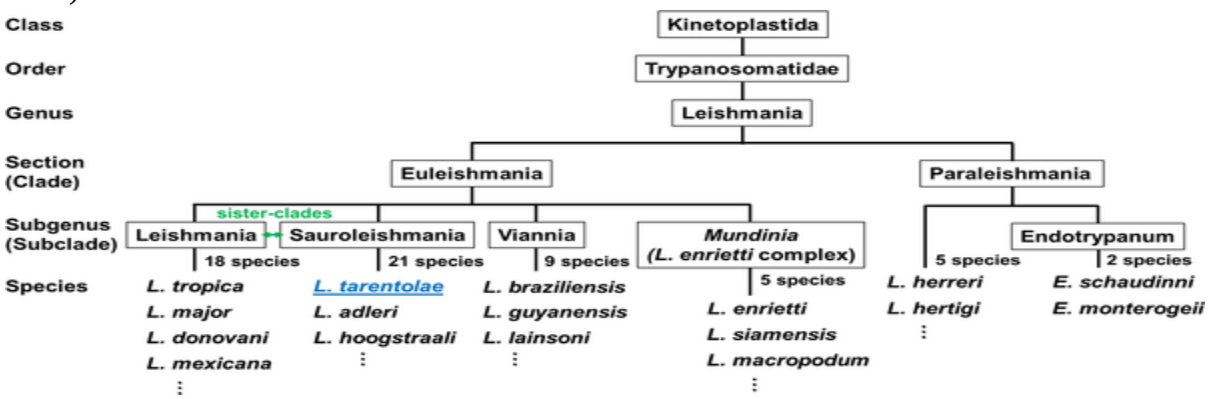

Figure (1): Scientific classification of *Leishmania* genus (Klatt et al., 2019)

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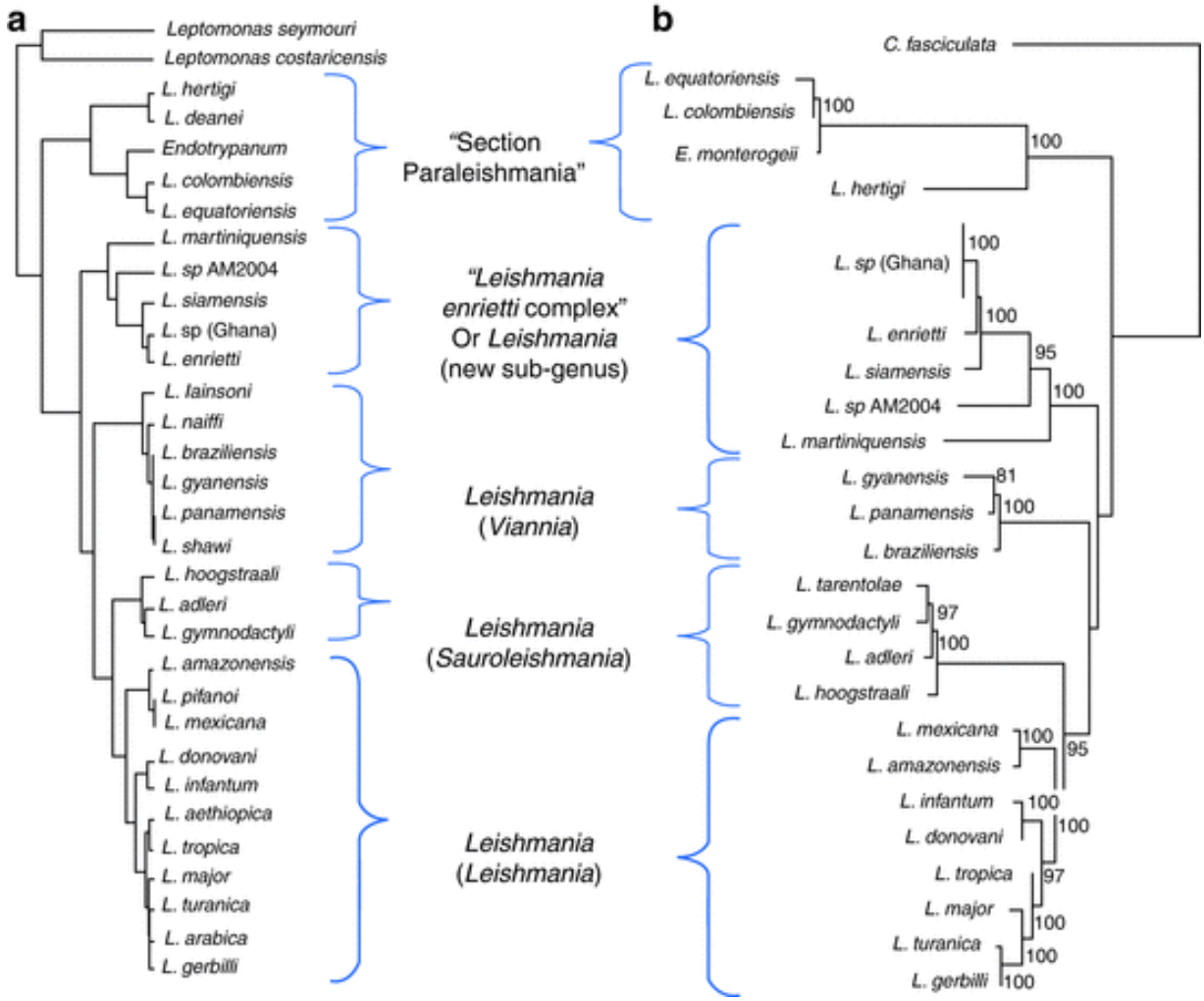

Figure (2): Different species and sub-species of Leishmania genus (Schönian et al., 2018)

Distribution and epidemiology

It is estimated that approximately one million people develop new infections each year, with millions more at risk on five continents and poses a risk of infection to 350 million people (Almeida-Souza et al., 2024). Approximately, 0.9-1.6 million new cases are reported annually and 12 million are infected worldwide. CL is more widespread than VL and is sold in more than 90 countries (Jain et al., 2023). Most cases occur in South and Central America and the Mediterranean region stretching from the Middle East to Central Asia. Less affected regions include South Asia and Sub-Saharan Africa (SSA), although CL and VL are endemic in SSA and East Africa (e.g. Ethiopia and Kenya), (Hailu et al., 2024). VL is mainly distributed in South Asia, Sub-Saharan Africa, South America and Central America. High-burden countries such as India, Bangladesh, Sudan, Ethiopia and Brazil account for 90% of VL patients, whereas Southern Europe, Central Asia and the Middle East have lower VL incidence (Figure 3), (Scarpini et al., 2022; Silva, 2022).

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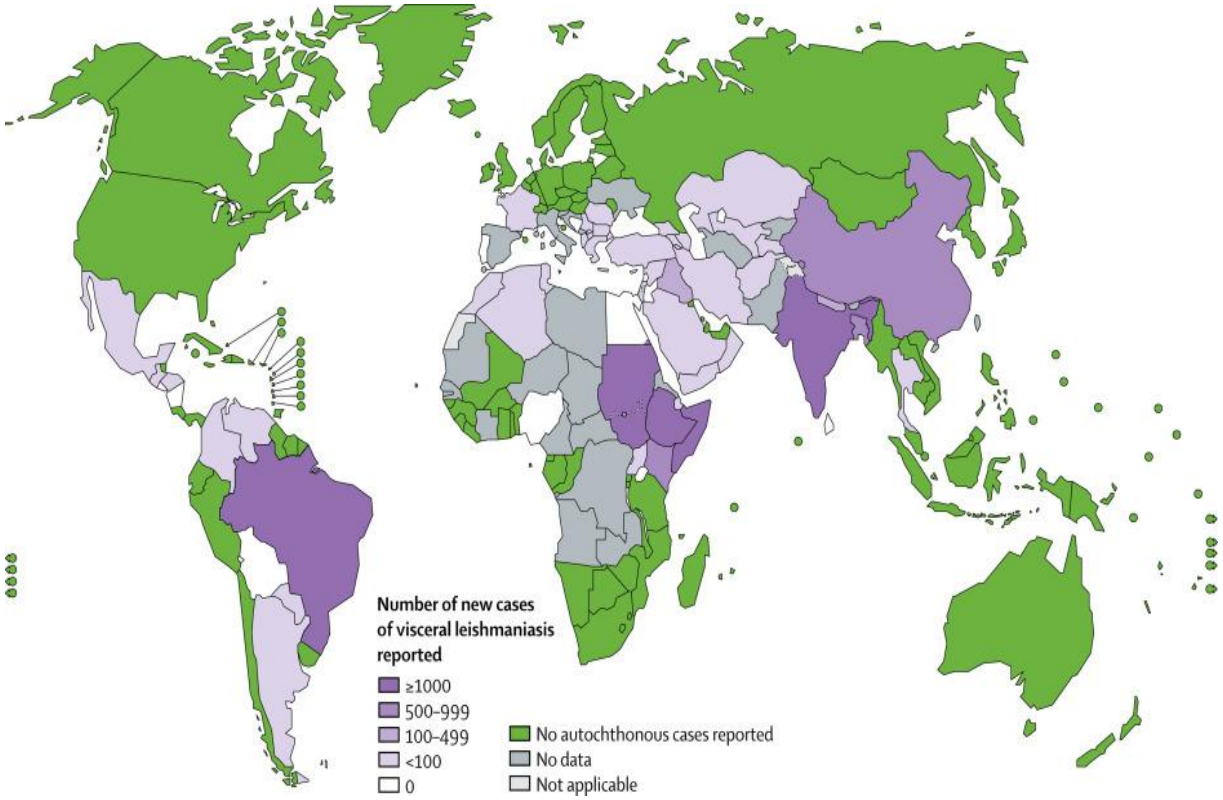

Figure (3): Global distribution and epidemiology of leishmaniasis (Silva, 2022)

Life Cycle

Leishmania species live as intracellular parasites (amastigotes) in the macrophages of vertebrates such as mammals and as extracellular parasites (promastigotes) in the gut of insect vectors (Cavalcante-Costa et al., 2022). These insects suck the blood of their vertebrate hosts and prostate when biting the skin. Parasites are recognized and absorbed by surface receptors on macrophages and dendritic cells (Pimenta et al., 2018). This finding strongly suggests the importance of macrophage entry mechanisms, with polymorphonuclear leukocytes being the first phagocytes detected in the host. Parasites promote apoptosis of infected neutrophils and are captured by macrophages. Within the host cell, the parasite migrates to the phagosome, differentiates into a flagellate, and grows rapidly through binary division. When densely populated macrophages burst, flagellates are released and spreading throughout the host (Martínez-López et al., 2018; Kupani et al., 2021). After bloodsucking by insects, amastigotes transform into promastigotes that survived for 4-7 days in digestive tract of insects and developed then to infected stage. When the sandfly bites the host's skin again, the parasite is released back into the host's bloodstream, ending the cycle (Figure 4), (Pimenta et al., 2018; Carreira et al., 2021).

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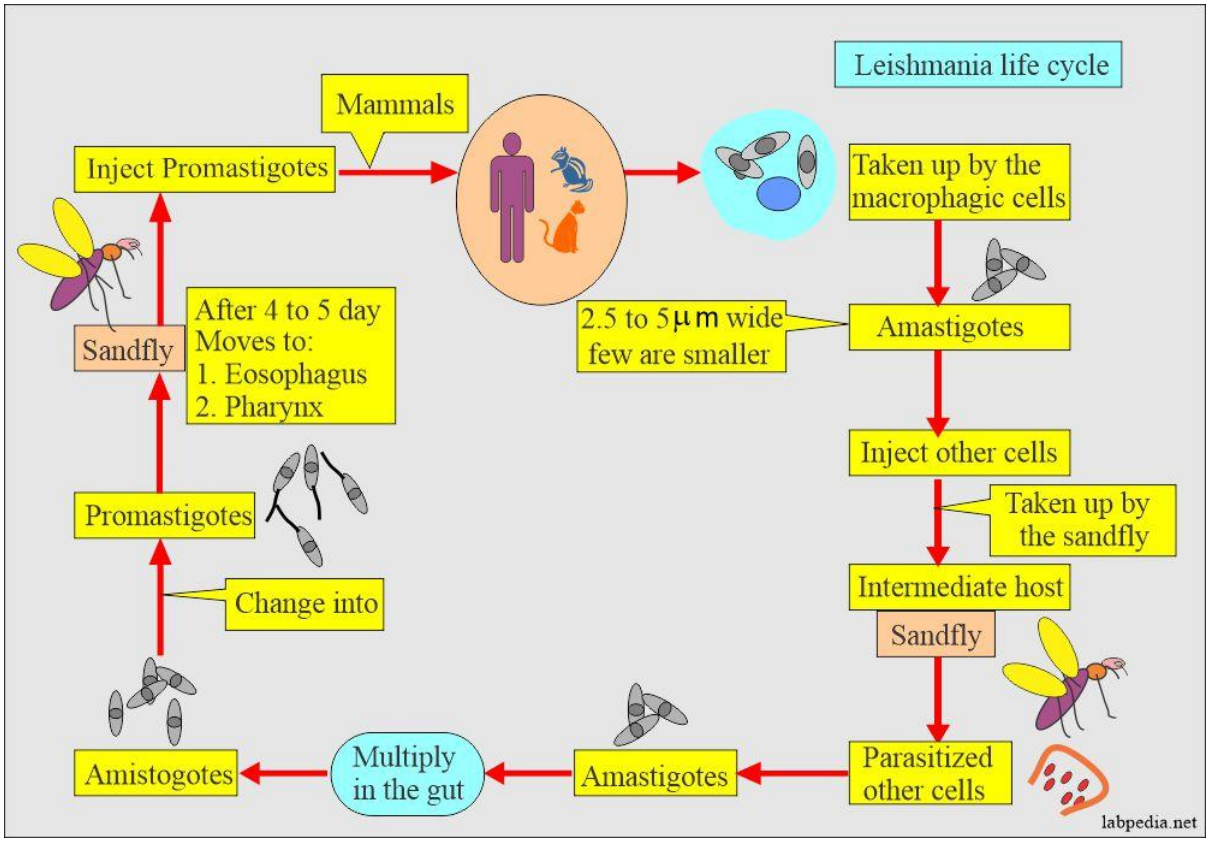

Figure (4): Life cycle of *Leishmania* spp.

Vector

Both males and females sandflies are feed on plants, but females also need a blood meal before they can lay eggs (Cecílio et al., 2022). Reptiles, amphibians, birds and mammals are possible hosts, and the feeding habits vary with sandfly species, and the vampire host species is an important factor in the spread of leishmaniasis (Edman and Spielman, 2020; Mendoza-Roldan et al., 2021). About 800 species of sandflies have been described and divided into five generally recognized genera; in the Old World the genera *Phlebotomus* and *Sergentomia*; while in the New World, *Lutzomya*, *Brumptomya* and Varillia (Figure 5), (Akhoundi et al., 2016).

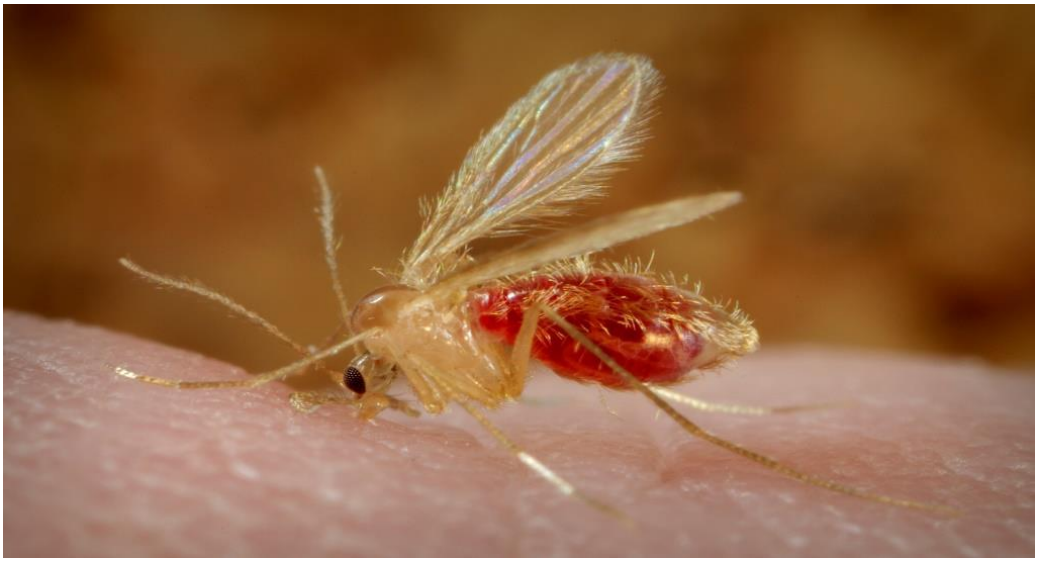

Figure (5): Sand fly, the vector host of *Leishmania*

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Reservoir

Many *Leishmania* species are zoonotic, with various reservoir mammal species maintaining *Leishmania* in the wild for long periods. Depending on the outbreak, the host may be a wild or domesticated mammal, or in some cases even a human (Kushwaha et al., 2022). Most reservoir hosts are well adapted to *Leishmania* and cause only mild infections that can last for years. Dogs are an important exception, as they often suffer from fatal systemic diseases (Baneth et al., 2021). Reserves are present in seven different orders of mammals. Mosquitoes, hyrax, marsupials, and edentulous animals are reservoirs of zoonotic cutaneous leishmaniasis in the wild. Humans are considered to be reservoirs of *L. donovani* and *L. tropicalis*. Gerbils (*Rhombomys opimus*) are the main gerbil hosts in the arid regions of Central Asia (Shaw et al., 2023; Yurchenko et al., 2023).

Types

1. CL

It's initially manifests itself as frequent insect bites. When a sand fly bites, a small wound is formed on the skin. It heals on its own after a few months, but the scar remains (Ahmad et al., 2022). During wound healing, leukocyte migration occurs, relaxation of the affected area, necrosis of the affected area, and healing granuloma formation (Salavastru et al., 2019). Among the *L. major* infections, ischial ulcers are the most common clinical form and occur more frequently in the lower extremities. The skin changes usually become necrotic rapidly, are often accompanied by multiple eczema and severe infections, and heal slowly (Bhatia et al., 2024). Several diseased individuals could experience spontaneous remission throughout 14-240 days. *Leishmania monocytogenes* can also present as skin abscesses, lupus, and sporozoites (Oliveira-Ribeiro et al., 2017). Topical papules, cholesteatoma, or malignant cholesteatoma usually occur on the face and other exposed sites (SCASSO et al., 2018). The systemic phenotype is called relapsing leishmaniasis (LR) and presents as new lesions around the old tropical leishmaniasis rash, whereas plaque leishmaniasis of the lupus type is primarily associated with tropical leishmaniasis (Mueller et al., 2009; Gurel et al., 2020).

2. DCL

It's progressively chronic form of polyparasitism which caused by a *Leishmania*-specific allergic disease and occurs in the form of skin lesions that are not leprosy-like skin lesions. The main pathogenic species include *L. Ethiopia* as well as the *L. Mexicana* (Kaushal, 2016; Silveira, 2019).

3. MCL

It results in damage to the nasal and pharyngeal cavities, causing facial disfigurement, disfigurement and lifelong pain. There are occasional reports of MCL from the Sudan and other parts of the Old World (Crovetto-Martínez et al., 2015). However, classic Brazilian MCL infections are limited, and sometimes even after complete healing of the initial lesion; metastatic lesions in the cheek and nasal mucosa appear years later (Volpedo et al., 2021). MCL typically exists as a zoonotic disease and its life cycle is mediated by the forest mosquito *Lutzomyia* (Hashiguchi et al., 2018).

4. VL or Kala-azar (KA)

It is the most severe, feared and devastating form of the disease, which is also known as kalaazar, burdwan fever, dum dum fever and state disease (Rana and Sivaperumal, 2018). This parasite causes a variety of clinical signs and in severe cases can progressively cause the

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lethal form that manifested by persistent high temperature, splenic enlargement, hepatic enlargement, significant emaciation, severe anemia, pancreatitis, and hyperglycemia (Zijlstra and El-Hassan, 2001). This parasite attacks and increases the number of macrophages (free mononuclear phagocytes), affecting endothelial reticular systems (Singh et al., 2018). VL is usually caused by *L. donovani* (Chang et al., 2017).

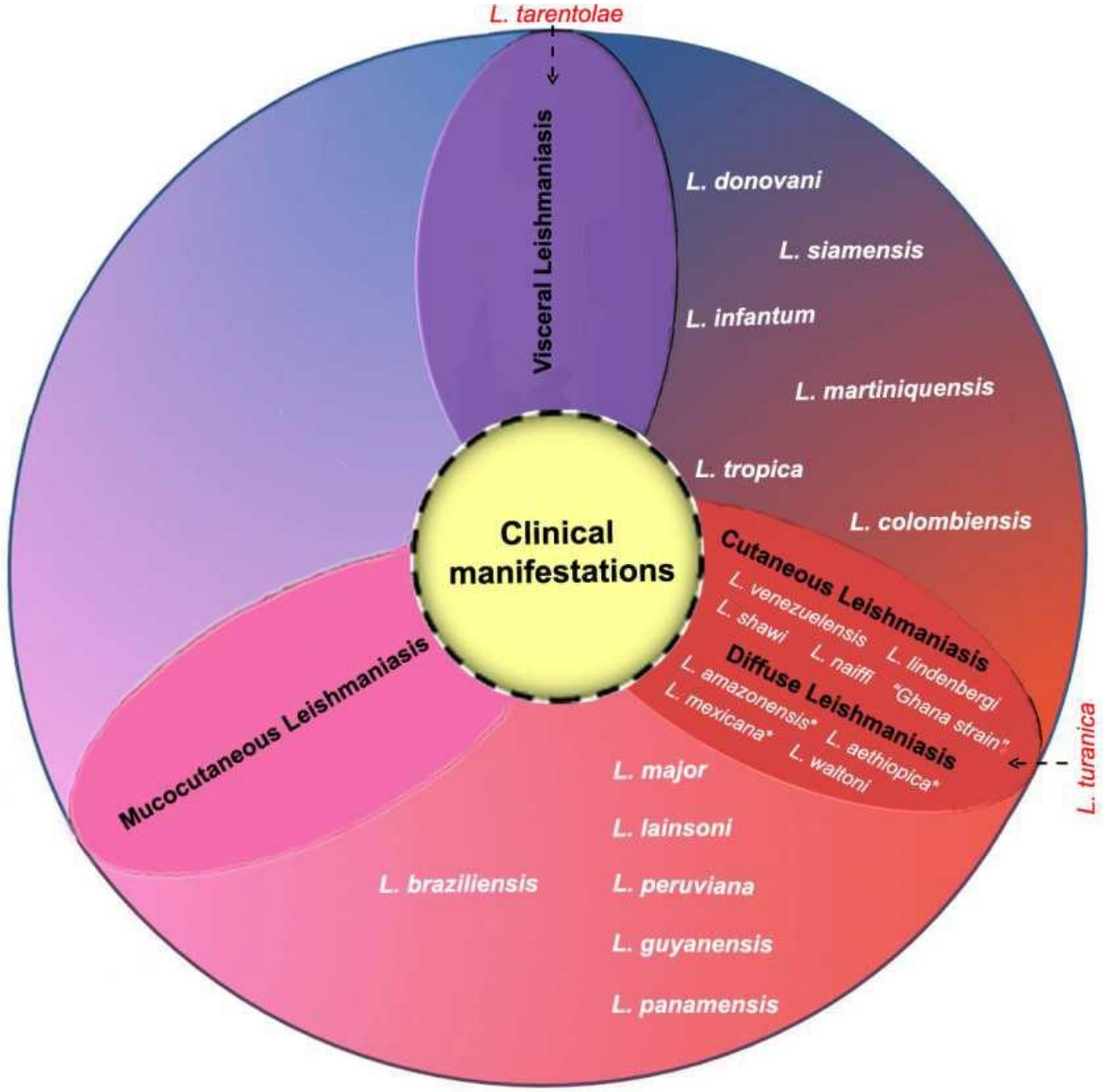

Figure (6): Clinical manifestations of different types of leishmaniasis

Immune response

Leishmania-specific antibody levels were high and immune response was strong. Affected individuals produce different immunoglobulins that observed at early and late stages of leishmaniasis (Magalhães et al., 2021). Elevated IgG1 levels have been observed in asymptomatic carriers; whereas elevated IgG2 levels have been observed in symptomatic infections (Figure 7), (Elmahallawy et al., 2021; Ibarra-Meneses et al., 2022).

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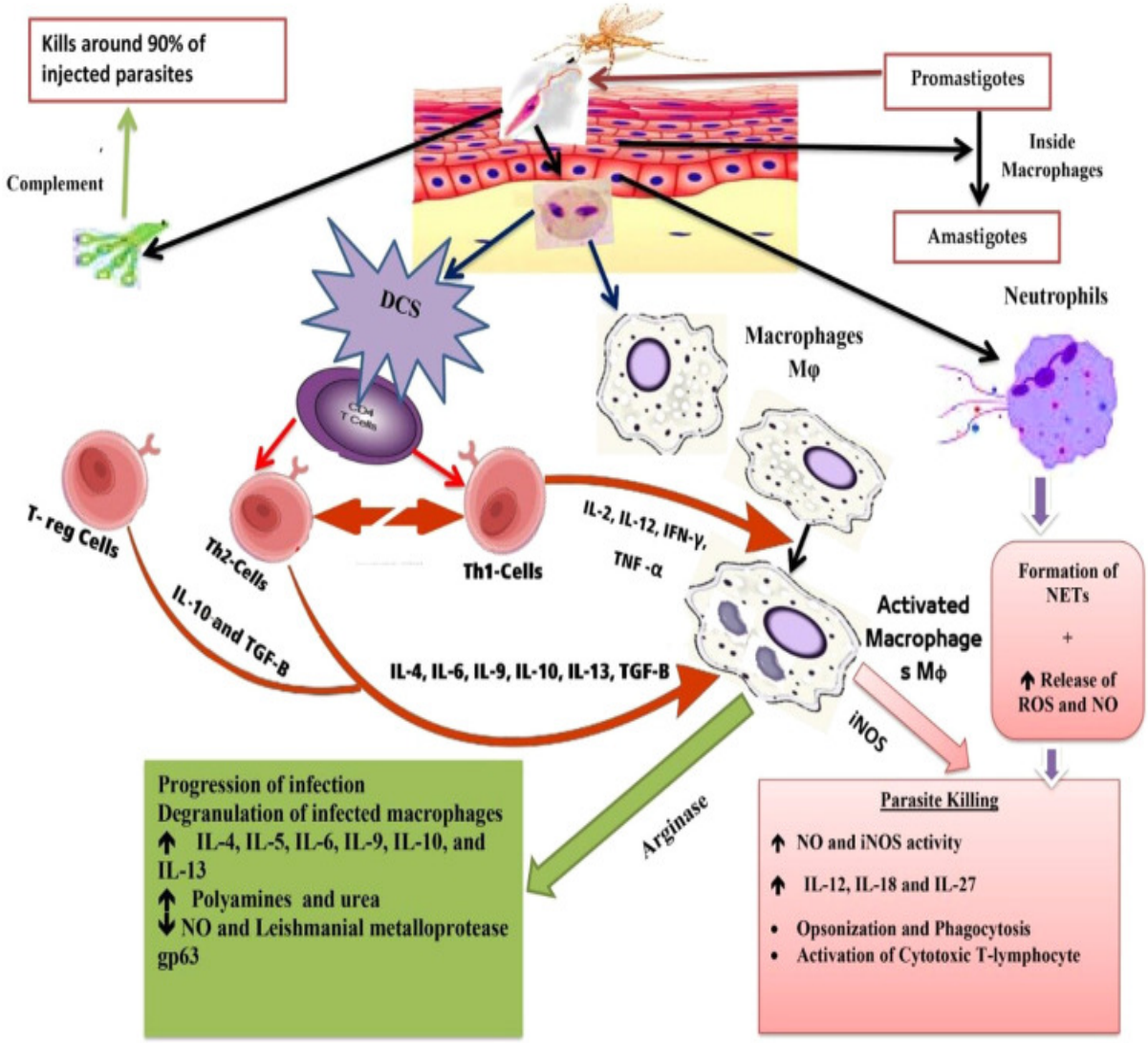

Figure (7): Host immune response against leishmaniasis (Elmahallawy et al., 2021)

Diagnostic methods

The diagnosis of leishmaniasis remains difficult because large variation in apparently clinical symptoms (Figure 8). Therefore, various confirmatory tests have been developed and used to confirm the disease, monitor treatment response, prevent introduction the parasite to regions and prevent spread by asymptomatic carriers (Rezvan and Hamoon Navard, 2017; De Brito et al., 2020).

1. Direct methods

1.1. Microscopic examination

Direct parasite testing is almost definitive diagnostic method for detection of infection by the microscopic examination of different tissues (Srividya et al., 2012). The sensitivity of spleen examination is 93.1–98.7%, whereas the sensitivity of bone marrow and lymph node absorption is lower (Kumar et al., 2020). The main disadvantages of this approach are that specimen collection (especially spleen aspiration) is painful and might be fatal (Srivastava et al., 2011).

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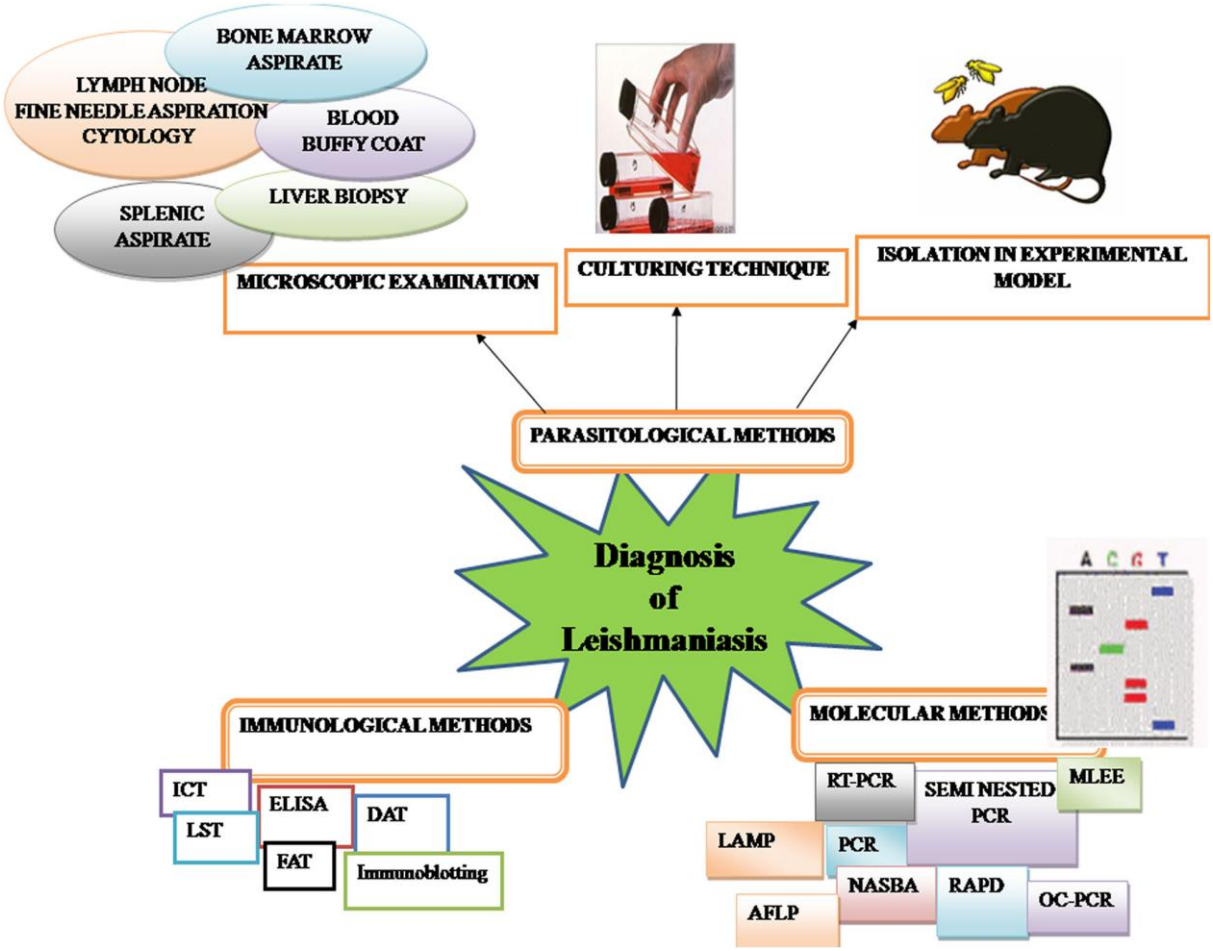

Figure (8): Various techniques applied in leishmania's diagnosis (Thakur et al., 2020)

1.2. Culture

Two-phase media is used when it is necessary to convert the amastigote strain to the promastigote strain. The optimum temperature for incubating cultures is 22 to 28°C, and cultures were assessed weekly. Culture is reliable, but is having many advantages and may be contaminated with bacteria, yeast, or other fungal species (Nayak et al., 2018; Reimão et al., 2020a).

2. Indirect methods

2.1. Molecular diagnosis

2.1.1. Traditional polymerase chain reaction (PCR)

This assay has achieved great attention because it is highly sensitive and specific effective method for diagnosing and controlling of blood parasites post therapy (Castelli et al., 2021). PCR should also be used in asymptomatic patients because PCR has been shown to be useful for confirming asymptomatic infections (Molina et al., 2020). PCR involves an addition of complementary bases to DNA and the use of specific primers to amplify and detect specific regions of the genome (Akhoundi et al., 2017). It can be used to detect *Leishmania* DNA in samples based on use of different target sequences of kinetoplast DNA (kDNA) that contain a large number of specific DNA sequences in the form of large and small loops (Doğantürk et al., 2022). Each parasite contains more than 10,000 copies of kinetoplast DNA, but in comparison, *Leishmania* parasites contain 40 to 200 rRNA gene copies. kDNA amplification is efficient target for PCR detection (León et al., 2017). The copy number of the biological

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material, the choice of primer, the targeting method used for DNA extraction and the PCR protocol are major factors (Gharban, 2024).

2.1.2. Real-time PCR (qRT-PCR)

It is an advanced molecular technique to detect the parasite as well as to genetic characterization and enumeration it (Galluzzi et al., 2018). Unlike traditional PCR, qRT-PCR has high sensitivity, low risk of contamination, and assay time of less than one hour. In this method, the oligonucleotide probe is labeled with a fluorophore and *Leishmania* DNA can be detected based on the emitted fluorescence (Moreira et al., 2018; Merdekios et al., 2021).

2.2. Serology

Serological techniques of leishmaniasis infection in humans and dogs are widespread and widely applied such as enzyme-linked immunosorbent assay (ELISA), indirect fluorescent antibody test (IFAT), rapid adhesion screening test (FAST), and direct agglutination test (DAT), (Thakur et al., 2020). These methods have been characterized by their highly degrees of specificity and sensitivity in detection of specific antibodies developed against the parasite at the earlier and late stage of disease (Kumar et al., 2020). However, there are some drawbacks like cross-reactions that occur with other protozoa, particularly *Trypanosoma cruzi*, *Ehrlichia caninum* or other *Leishmania* species in Central and North America, potentially leading to false-positive results. This limitation leads to possible limitations in the use of recombinant peptides. Therefore, serologic testing is not an appropriate method to diagnose or monitor recurrence after treatment (Gondim et al., 2017; Marlais et al., 2018; Thakur et al., 2020).

2.2.1. DAT

It's clinically accurate, reliable, noninvasive, and simple tool. In the direct validation assay, frozen or suspended promastigotes are used as antigens. The sample is incubated with the antigen in a microtiter plate (Mohebali et al., 2020).

2.2.2. IFAT

This method uses the fluorescence microscopy to detect antibody-antigen interactions and for determining *Leishmania* antibody titers in extensive screening of infected patients (Yentur Doni et al., 2023). IFAT has a high specificity but it expensive laboratory equipment, and serial dilution of serum (Mniouil et al., 2018).

2.2.3. ELISA

Protein-based ELISA assays have improved sensitivity and specificity as well as accuracy. Recombinant RK39 protein is a powerful and specific diagnostic marker (Figueiredo et al., 2021; Gharban, 2024). This antigen expressed in amastigote kinesin domain of *Leishmania chagas*. The sensitivity of protein is 100% and the specificity is 96% (Santos et al., 2018). The antigen was recognized by a unique immunoglobulin detected in the sera of infected patients. The assay uses special proteins that need to serum to perform (Siqueira et al., 2021).

2.2.4. Flow cytometry

This method can be used to quantify, examine, and separate thousands of particles suspended in a liquid stream (Adan et al., 2017). Live parasites and amastigote parasites can be used in FC. Viable amastigotes detected in mammals have seen as benefit targets than promastigotes for diagnosing infected individuals (Ribeiro et al., 2020). This method was used in the

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promastigote assay to detect the presence of Fc-AFPA IgG antibodies. Fc-Flow cytometry has a sensitivity of 95% and a specificity of 100% (Silva et al., 2019).

2.2.5. Nanodiagnostics

These techniques improve sensitivity and rapid detection while reducing costs. Sensitivity requires that the particle interact with the molecule of interest. The first step in most experiments with nanoparticles is to bind the nanoparticle probe for biomolecular material to generate special signals that calculated as biomolecular signatures. Since most biological structures are nanometer-sized, nanodiagnostics offer potential for growth (Gedda et al., 2021; Vega et al., 2023). The nanoshell consists of gold nanoparticles, and small DNA and protein fragments can be recognized and labeled by antibodies (Singh et al., 2019). Currently, nanotechnology plays great roles in developments of diagnosis at various fields and in future therapy (Mandal, 2023).

Treatment

1. Pentavalent Antimonials

Sodium Antimony Gluconate and Meglumine Antimonate has been the first-line of therapy. It inhibits the biosynthesis of macromolecules in amastigates, and can be used topically or systemically by injection. Serious adverse reactions such as hepatotoxicity and cardiac toxicity have been reported and require patient monitoring (Oliveira et al., 2011; Berbert et al., 2018).

2. Amphotericin B

Amphotercin B (AmB) is a polypeptide antibiotic commonly used to treat VL but also effective in CL. The mechanism of action of AmB is the formation of transmembrane channels to modulate the permeability of the parasite's plasma membrane. This disrupts the flow of ions in the parasite and causes its death. This occurs through preferential binding to sterols in the extracellular membrane (Chattopadhyay and Jafurulla, 2011; Sundarn and Singh, 2016).

3. Miltefosine

Miltefosine is an exceptional remedy for leishmaniasis. Because of its teratogenic potential, women of childbearing potential should use contraception during treatment. The drug remains in the body for a long time (half-life: 6 to 8 days). Prolonged treatment may reduce patient compliance and tolerance development (Dorlo et al., 2012; Reimão et al., 2020b).

4. Paromomycin Sulphate

This drug causes translational errors that affect the fidelity of *Leishmania* protein synthesis. Increased RNA misinterpretation can lead to protein defects that affect parasite survival (Matos et al., 2020). In addition, changes in lipid metabolism, effects on parasite membrane flux and changes in the adsorption properties of the parasite were observed, leading to growth arrest (Oliveira et al., 2021).

5. Cryo-and Thermotherapy

These assays use temperatures to kill parasites and reduce host damage by the radio frequency generators with intralesional injection of antimony compounds (Sundar et al., 2024).

6. Immunotherapy

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Studies have shown that immunotherapy can be used as an adjunct to chemotherapy in the treatment of CL. Unlike chemotherapy, immunotherapy kills the parasite by stimulating the host immune response (Akbari et al., 2021). In an otitis model, the application of immunotherapy directly to existing lesions significantly reduced disease severity as indicated by the size of the ear lesions and ulceration. Topical administration of immunotherapy reduces disease severity in an otitis model (Inêz-Ferreira et al., 2017).

Control strategies

Spraying household insecticide residues was once an important practice, but it is now used less frequently. Different types of insecticide can be applied in control programs and being effective against sandflies (Roatt et al., 2020).

References

[1] Adan, A., Alizada, G., Kiraz, Y., Baran, Y., and Nalbant, A. (2017). Flow cytometry: basic principles and applications. *Critical reviews in biotechnology*, *37*(2), 163-176.

[2] Ahmad, S., Obaid, M. K., Taimur, M., Shaheen, H., Khan, S. N., Niaz, S., and Haleem, S. (2022). Knowledge, attitude, and practices towards cutaneous leishmaniasis in referral cases with cutaneous lesions: A cross-sectional survey in remote districts of southern Khyber Pakhtunkhwa, Pakistan. *Plos one*, *17*(5), e0268801.

[3] Akbari, M., Oryan, A., and Hatam, G. (2021). Immunotherapy in treatment of leishmaniasis. *Immunology Letters*, *233*, 80-86.

[4] Akhoundi, M., Kuhls, K., Cannet, A., Votýpka, J., Marty, P., Delaunay, P., and Sereno, D. (2016). A historical overview of the classification, evolution, and dispersion of Leishmania parasites and sandflies. *PLoS neglected tropical diseases*, *10*(3), e0004349.

[5] Akhoundi, M., Downing, T., Votýpka, J., Kuhls, K., Lukeš, J., Cannet, A., and Sereno, D. (2017). Leishmania infections: Molecular targets and diagnosis. *Molecular aspects of medicine*, *57*, 1-29.

[6] Almeida-Souza, F., Calabrese, K. D. S., Abreu-Silva, A. L., and Cardoso, F. (Eds.). (2024). *Leishmania Parasites: Epidemiology, Immunopathology and Hosts*. BoD–Books on Demand.

[7] Baneth, G., Petersen, C., Solano-Gallego, L., and Sykes, J. E. (2021). Leishmaniosis. In *Greene's Infectious Diseases of the Dog and Cat* (pp. 1179-1202). WB Saunders.

[8] Berbert, T. R. N., Mello, T. F. P. D., Wolf Nassif, P., Mota, C. A., Silveira, A. V., Duarte, G. C., and Silveira, T. G. V. (2018). Pentavalent antimonials combined with other therapeutic alternatives for the treatment of cutaneous and mucocutaneous leishmaniasis: A systematic review. *Dermatology research and practice*, *2018*(1), 9014726.

[9] Bhatia, M., Chaudhary, J., Jain, A., and Dhingra, A. (2024). Current Therapeutic Strategies for Buruli Ulcer. *Emerging Approaches to Tackle Neglected Diseases: From Molecule to End Product*, 166.

[10] Bruschi, F., and Gradoni, L. (Eds.). (2018). *The leishmaniases: old neglected tropical diseases* (Vol. 1). Cham, Switzerland: Springer.

[11] Cantanhêde, L. M., Mata-Somarribas, C., Chourabi, K., Pereira da Silva, G., Dias das Chagas, B., de Oliveira R. Pereira, L., and Cupolillo, E. (2021). The maze pathway of

coevolution: a critical review over the Leishmania and its endosymbiotic history. *Genes*, *12*(5), 657.

[12] Carreira, J. C. A., and da Silva, A. V. M. (2021). The Role of Neutrophils in the Interaction with Leishmania: Far beyond a Simple Trojan Horse?. *Open Journal of Animal Sciences*, *11*(3), 399-421.

[13] Castelli, G., Bruno, F., Reale, S., Catanzaro, S., Valenza, V., and Vitale, F. (2021). Molecular diagnosis of leishmaniasis: quantification of parasite load by a real-time PCR assay with high sensitivity. *Pathogens*, *10*(7), 865.

[14] Cavalcante-Costa, V. S., Queiroz-Oliveira, T., Horta, M. F., and Castro-Gomes, T. (2022). Leishmania and Their Vertebrate Host Cells. In *Lifecycles of Pathogenic Protists in Humans* (pp. 143-165). Cham: Springer International Publishing.

[15] Cecílio, P., Cordeiro-da-Silva, A., and Oliveira, F. (2022). Sand flies: Basic information on the vectors of leishmaniasis and their interactions with Leishmania parasites. *Communications biology*, *5*(1), 305.

[16] Chang, K. P., Kolli, B. K., and Collaborators. (2017). Overview of leishmaniasis with special emphasis on kala-azar in South Asia. *Neglected Tropical Diseases-South Asia*, 1-63.

[17] Chattopadhyay, A., and Jafurulla, M. (2011). A novel mechanism for an old drug: amphotericin B in the treatment of visceral leishmaniasis. *Biochemical and biophysical research communications*, *416*(1-2), 7-12.

[18] Crovetto-Martínez, R., Aguirre-Urizar, J. M., Orte-Aldea, C., Araluce-Iturbe, I., Whyte-Orozco, J., and Crovetto-De la Torre, M. A. (2015). Mucocutaneous leishmaniasis must be included in the differential diagnosis of midline destructive disease: two case reports. *Oral surgery, oral medicine, oral pathology and oral radiology*, *119*(1), e20-e26.

[19] De Brito, R. C. F., Aguiar-Soares, R. D. D. O., Cardoso, J. M. D. O., Coura-Vital, W., Roatt, B. M., and Reis, A. B. (2020). Recent advances and new strategies in Leishmaniasis diagnosis. *Applied Microbiology and Biotechnology*, *104*, 8105-8116.

[20] Doğantürk, Y. E., Kuşkucu, M. A., Hökelek, M., and Bahar-Tokman, H. (2022). Development of real-time PCR-based system that detects kinetoplast DNA for diagnosis of Leishmania infections. *Cerrahpaşa Med J*, *46*(2), 138-43.

[21] Dorlo, T. P., Balasegaram, M., Beijnen, J. H., and de Vries, P. J. (2012). Miltefosine: a review of its pharmacology and therapeutic efficacy in the treatment of leishmaniasis. *Journal of Antimicrobial Chemotherapy*, *67*(11), 2576-2597.

[22] Edman, J. D., and Spielman, A. (2020). Blood-feeding by vectors: physiology, ecology, behavior, and vertebrate defense. In *The Arboviruses:* (pp. 153-190). CRC Press.

[23] Elmahallawy, E. K., Alkhaldi, A. A., and Saleh, A. A. (2021). Host immune response against leishmaniasis and parasite persistence strategies: A review and assessment of recent research. *Biomedicine and Pharmacotherapy*, *139*, 111671.

[24] Figueiredo, M. M., Dos Santos, A. R., Godoi, L. C., de Castro, N. S., de Andrade, B. C., Sergio, S. A., and Fernandes, A. P. (2021). Improved Performance of ELISA and Immunochromatographic Tests Using a New Chimeric A2- Based Protein for Human Visceral Leishmaniasis Diagnosis. *Journal of Immunology Research*, *2021*(1), 5568077.

[25] Galluzzi, L., Ceccarelli, M., Diotallevi, A., Menotta, M., and Magnani, M. (2018). Real-time PCR applications for diagnosis of leishmaniasis. *Parasites and vectors*, *11*, 1-13.

[26] Gedda, M. R., Madhukar, P., Shukla, A., Mudavath, S. L., Srivastava, O. N., Singh, O. P., and Sundar, S. (2021). Nanodiagnostics in leishmaniasis: A new frontiers for early elimination. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, *13*(2), e1675.

[27] Gharban, H. A. (2024). Article Review: Skin Diseases in Dromedary Camels. *Journal La Lifesci*, *5*(3), 206-217.

[28] Gondim, L. F., Mineo, J. R., and Schares, G. (2017). Importance of serological cross-reactivity among Toxoplasma gondii, Hammondia spp., Neospora spp., Sarcocystis spp. and Besnoitia besnoiti. *Parasitology*, *144*(7), 851-868.

[29] Gurel, M. S., Tekin, B., and Uzun, S. (2020). Cutaneous leishmaniasis: A great imitator. *Clinics in dermatology*, *38*(2), 140-151.

[30] Hailu, A., Lado, M., Ali, A. A. B., Musa, A. M., and Mwiti, D. (2024). Leishmaniasis in Sub-Saharan Africa. In *Neglected Tropical Diseases-Sub-Saharan Africa* (pp. 99-135). Cham: Springer International Publishing.

[31] Hashiguchi, Y., Cáceres, A. G., Velez, L. N., Villegas, N. V., Hashiguchi, K., Mimori, T., and Kato, H. (2018). Andean cutaneous leishmaniasis (Andean-CL, uta) in Peru and Ecuador: the vector Lutzomyia sand flies and reservoir mammals. *Acta tropica*, *178*, 264-275.

[32] Ibarra-Meneses, A. V., Corbeil, A., Wagner, V., Onwuchekwa, C., and Fernandez-Prada, C. (2022). Identification of asymptomatic Leishmania infections: a scoping review. *Parasites and vectors*, *15*, 1-22.

[33] Inêz-Ferreira, T. M., Mendoza, L., Vilela, R., de Andrade, H. M., Haddad, J. P., Nunes, F. F. C., and Gondim, I. M. (2017). A Leishmania murine model to evaluate the immunomodulatory properties. Heliyon, 3 (2017), 1-17.

[34] Jain, A. S., Shah, H. M., Joshi, S. V., and Kharkar, P. S. (2023). Drugs for giardiasis, trichomoniasis, and leishmaniasis. In *Medicinal Chemistry of Chemotherapeutic Agents* (pp. 431-460). Academic Press.

[35] Kaushal, H. (2016). *Studies on Immunological Responses to Parasite Surface Antigen as a Potential Vaccine Candidate against Human Leishmaniasis and Estimation of Asymptomatic Leishmania Infection in the Endemic Areas* (Doctoral dissertation, BITS, Pilani).

[36] Klatt, S., Simpson, L., Maslov, D. A., and Konthur, Z. (2019). Leishmania tarentolae: Taxonomic classification and its application as a promising biotechnological expression host. *PLoS neglected tropical diseases*, *13*(7), e0007424.

[37] Kumar, A., Pandey, S. C., and Samant, M. (2020). A spotlight on the diagnostic methods of a fatal disease Visceral Leishmaniasis. *Parasite immunology*, *42*(10), e12727.

[38] Kupani, M., Pandey, R. K., and Mehrotra, S. (2021). Neutrophils and Visceral Leishmaniasis: Impact on innate immune response and cross- talks with macrophages and dendritic cells. *Journal of Cellular Physiology*, *236*(4), 2255-2267.

[39] Kushwaha, A. K., Scorza, B. M., Singh, O. P., Rowton, E., Lawyer, P., Sundar, S., and Petersen, C. A. (2022). Domestic mammals as reservoirs for Leishmania donovani on the Indian subcontinent: Possibility and consequences on elimination. *Transboundary and emerging diseases*, *69*(2), 268-277.

[40] León, C. M., Muñoz, M., Hernández, C., Ayala, M. S., Flórez, C., Teherán, A., and Ramírez, J. D. (2017). Analytical performance of four polymerase chain reaction (PCR) and real time PCR (qPCR) assays for the detection of six Leishmania species DNA in Colombia. *Frontiers in microbiology*, *8*, 1907.

[41] Magalhães, A., Carvalho, L. P., Costa, R., Pita, M. S., Cardoso, T. M., Machado, P. R., and Carvalho, A. M. (2021). Anti-Leishmania IgG is a marker of disseminated leishmaniasis caused by Leishmania braziliensis. *International Journal of Infectious Diseases*, *106*, 83-90.

[42] Mandal, A. K. (2023). Gold nanoparticles as theranostic delivery system in combating various diseases. *Book: Anatolyivna TV, Editor." Research advances in Microbiology and Biotechnology*, *6*, 100-128.

[43] Marlais, T., Bhattacharyya, T., Singh, O. P., Mertens, P., Gilleman, Q., Thunissen, C., and Miles, M. A. (2018). Visceral leishmaniasis IgG1 rapid monitoring of cure vs. relapse, and potential for diagnosis of post kala-azar dermal leishmaniasis. *Frontiers in cellular and infection microbiology*, *8*, 427.

[44] Martínez-López, M., Soto, M., Iborra, S., and Sancho, D. (2018). Leishmania hijacks myeloid cells for immune escape. *Frontiers in microbiology*, *9*, 883.

[45] Matos, A. P. S., Viçosa, A. L., Ré, M. I., Ricci-Júnior, E., and Holandino, C. (2020). A review of current treatments strategies based on paromomycin for leishmaniasis. *Journal of Drug Delivery Science and Technology*, *57*, 101664.

[46] Mendoza-Roldan, J. A., Mendoza-Roldan, M. A., and Otranto, D. (2021). Reptile vector-borne diseases of zoonotic concern. *International Journal for Parasitology: Parasites and Wildlife*, *15*, 132-142.

[47] Merdekios, B., Pareyn, M., Tadesse, D., Eligo, N., Kassa, M., Jacobs, B. K., and Cnops, L. (2021). Evaluation of conventional and four real-time PCR methods for the detection of Leishmania on field-collected samples in Ethiopia. *PLoS neglected tropical diseases*, *15*(1), e0008903.

[48] Mniouil, M., Fellah, H., Amarir, F., Sadak, A., Et-Touys, A., Bakri, Y., and Sebti, F. (2018). Comparative evaluation of immunochromatographic dipstick test (ICT) rk39, soluble antigen ELISA and IFAT for the sero-diagnosis of visceral leishmaniasis in Morocco. *Acta tropica*, *182*, 185-189.

[49] Mohebali, M., Keshavarz, H., Shirmohammad, S., Akhoundi, B., Borjian, A., Hassanpour, G., and Mahmoudi, S. (2020). The diagnostic accuracy of direct agglutination test for serodiagnosis of human visceral leishmaniasis: a systematic review with meta-analysis. *BMC Infectious Diseases*, *20*, 1-12.

[50] Molina, R., Jimenez, M., García-Martínez, J., San Martín, J. V., Carrillo, E., Sanchez, C., and Alvar, J. (2020). Role of asymptomatic and symptomatic humans as reservoirs of visceral leishmaniasis in a Mediterranean context. *PLoS Neglected Tropical Diseases*, *14*(4), e0008253.

[51] Moreira, O. C., Yadon, Z. E., and Cupolillo, E. (2018). The applicability of real-time PCR in the diagnostic of cutaneous leishmaniasis and parasite quantification for clinical management: current status and perspectives. *Acta tropica*, *184*, 29-37.

[52] Mueller, M. C., Fleischmann, E., Grunke, M., Schewe, S., Bogner, J. R., and Löscher, T. (2009). Case report: relapsing cutaneous leishmaniasis in a patient with ankylosing spondylitis treated with infliximab. *American Journal of Tropical Medicine and Hygiene*, *81*(1), 52.

[53] Nayak, A., Akpunarlieva, S., Barrett, M., and Burchmore, R. (2018). A defined medium for Leishmania culture allows definition of essential amino acids. *Experimental parasitology*, *185*, 39-52.

[54] Oliveira, L. F., Schubach, A. O., Martins, M. M., Passos, S. L., Oliveira, R. V., Marzochi, M. C., and Andrade, C. A. (2011). Systematic review of the adverse effects of cutaneous leishmaniasis treatment in the New World. *Acta tropica*, *118*(2), 87-96.

[55] Oliveira, S. S., Ferreira, C. S., Branquinha, M. H., Santos, A. L., Chaud, M. V., Jain, S., and Severino, P. (2021). Overcoming multi- resistant leishmania treatment by nanoencapsulation of potent antimicrobials. *Journal of Chemical Technology and Biotechnology*, *96*(8), 2123-2140.

[56] Oliveira-Ribeiro, C., Pimentel, M. I. F., Oliveira, R. D. V. C., Fagundes, A., Madeira, M. D. F., Mello, C. X., and Conceição-Silva, F. (2017). Clinical and laboratory profiles of patients with early spontaneous healing in cutaneous localized leishmaniasis: a historical cohort study. *BMC infectious diseases*, *17*, 1-8.

[57] Pimenta, P. F., de Freitas, V. C., Monteiro, C. C., Pires, A. C. M., and Secundino, N. F. C. (2018). Biology of the Leishmania− sand fly interaction. *Brazilian sand flies: biology, taxonomy, medical importance and control*, 319-339.

[58] Rana, S., and Sivaperumal, R. (2018). Virtual screening and molecular docking of Anti-Antileishmanial for selected pharmacophore for visceral Leishmaniasis. *Journal of Drug Delivery and Therapeutics*, *8*(6-s), 230-235.

[59] Reimão, J. Q., Coser, E. M., Lee, M. R., and Coelho, A. C. (2020a). Laboratory diagnosis of cutaneous and visceral leishmaniasis: current and future methods. *Microorganisms*, *8*(11), 1632.

[60] Reimão, J. Q., Pita Pedro, D. P., and Coelho, A. C. (2020b). The preclinical discovery and development of oral miltefosine for the treatment of visceral leishmaniasis: a case history. *Expert Opinion on Drug Discovery*, *15*(6), 647-658.

[61] Rezvan, H., and Hamoon Navard, S. (2017). An overview on Leishmania diagnosis. *Journal of Medical Microbiology and Infectious Diseases*, *5*(1), 1-11.

[62] Ribeiro, J. M., Bandeira, C. C., de Faria, B. G., Alves, M. L., Vieira, F. O., Giunchetti, R. C., and Souza-Fagundes, E. M. (2020). An ex vivo multiparametric flow cytometry assay using human whole blood to simultaneously measure cytotoxicity and leishmanicidal activities. *Experimental Parasitology*, *216*, 107940.

[63] Roatt, B. M., de Oliveira Cardoso, J. M., De Brito, R. C. F., Coura-Vital, W., de Oliveira Aguiar-Soares, R. D., and Reis, A. B. (2020). Recent advances and new strategies on leishmaniasis treatment. *Applied Microbiology and Biotechnology*, *104*, 8965-8977.

[64] Rodriguez, A. E., Estévez, J. O., Nevot, M. C., Barrios, A., and Florin-Christensen, M. (2018). Leishmania. *Parasitic protozoa of farm animals and pets*, 289-311.

[65] Salavastru, C. M., Sendrea, A. M., Cretu, S., and Tiplica, G. S. (2019). Infections of the Skin. *Advances in Integrative Dermatology*, 231-256.

[66] Santos, A. R. R. D., Serufo, Â. V., Figueiredo, M. M., Godoi, L. C., Vitório, J. G., Marcelino, A. P., and Fernandes, A. P. (2019). Evaluation of three recombinant proteins for the development of ELISA and immunochromatographic tests for visceral leishmaniasis serodiagnosis. *Memórias do Instituto Oswaldo Cruz*, *114*, e180405.

[67] Scarpini, S., Dondi, A., Totaro, C., Biagi, C., Melchionda, F., Zama, D., and Lanari, M. (2022). Visceral leishmaniasis: Epidemiology, diagnosis, and treatment regimens in different geographical areas with a focus on pediatrics. *Microorganisms*, *10*(10), 1887.

[68] SCASSO, F., FERRARI, G., DE VINCENTIIS, G. C., AROSIO, A., BOTTERO, S., CARRETTI, M., and VIAGGI, B. (2018). 105th Congress of the Italian Society of Otorhinolaryngology Head and Neck Surgery Official report: Emerging and re-emerging infectious disease in otorhinolaryngology. *Acta Otorhinolaryngologica Italica*, *38*(2 Suppl 1), S1.

[69] Schönian, G., Lukeš, J., Stark, O., and Cotton, J. A. (2018). *Molecular evolution and phylogeny of Leishmania* (pp. 19-57). Springer International Publishing.

[70] Shaw, J. J., Marinho-Júnior, J. F., Courtenay, O., and Brandão-Filho, S. P. (2023). Assessing reservoir host status in leishmaniasis with special reference to the infectiousness of Leishmania (Viannia) braziliensis infections in wild rodents. *Revista da Sociedade Brasileira de Medicina Tropical*, *56*, e0503-2023.

[71] Silva, E. D., Oliveira, B. C., Oliveira, A. P., Santos, W. J., Diniz, G. T., de Melo Neto, O. P., and Pereira, V. R. (2019). Performance evaluation of anti-fixed Leishmania infantum promastigotes immunoglobulin G (IgG) detected by flow cytometry as a diagnostic tool for visceral Leishmaniasis. *Journal of immunological methods*, *469*, 18-25.

[72] Silva, G. L. A. D. (2022). *Characterisation of the role of the ATR kinase homolog in L. major response to replication stress* (Doctoral dissertation, Universidade de São Paulo).

[73] Silveira, F. T. (2019). What makes mucosal and anergic diffuse cutaneous leishmaniases so clinically and immunopathogically different? A review in Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *113*(9), 505-516.

[74] Singh, A. K., Das, V. N., Amit, A., Dikhit, M. R., Mahantesh, V., Singh, S. K., and Bimal, S. (2018). Cytokines and chemokines differentially regulate innate immune cell trafficking during post kala- azar dermal leishmaniasis. *Journal of cellular biochemistry*, *119*(9), 7406-7418.

[75] Singh, O. P., Gedda, M. R., Mudavath, S. L., Srivastava, O. N., and Sundar, S. (2019). Envisioning the innovations in nanomedicine to combat visceral leishmaniasis: for future theranostic application. *Nanomedicine*, *14*(14), 1911-1927.

[76] Siqueira, W. F., Viana, A. G., Reis Cunha, J. L., Rosa, L. M., Bueno, L. L., Bartholomeu, D. C., and Fujiwara, R. T. (2021). The increased presence of repetitive motifs in the KDDR-plus recombinant protein, a kinesin-derived antigen from Leishmania infantum, improves the diagnostic performance of serological tests for

human and canine visceral leishmaniasis. *PLoS Neglected Tropical Diseases*, *15*(9), e0009759.

[77] Srivastava, P., Dayama, A., Mehrotra, S., and Sundar, S. (2011). Diagnosis of visceral leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *105*(1), 1-6.

[78] Srividya, G., Kulshrestha, A., Singh, R., and Salotra, P. (2012). Diagnosis of visceral leishmaniasis: developments over the last decade. *Parasitology research*, *110*, 1065-1078.

[79] Sundar, S., and Singh, A. (2016). Recent developments and future prospects in the treatment of visceral leishmaniasis. *Therapeutic advances in infectious disease*, *3*(3-4), 98-109.

[80] Sundar, S., Singh, J., Singh, V. K., Agrawal, N., and Kumar, R. (2024). Current and emerging therapies for the treatment of leishmaniasis. *Expert Opinion on Orphan Drugs*, *12*(1), 19-32.

[81] Thakur, S., Joshi, J., and Kaur, S. (2020). Leishmaniasis diagnosis: an update on the use of parasitological, immunological and molecular methods. *Journal of Parasitic Diseases*, *44*, 253-272.

[82] Vega, D. M., Di Meglio, M., del Valle Alonso, S., Alvira, F., and Montanari, J. (2023). Nanomaterials for diagnosis, treatment, and prevention of human cutaneous leishmaniasis: A review. *OpenNano*, *12*, 100158.

[83] Volpedo, G., Pacheco-Fernandez, T., Holcomb, E. A., Cipriano, N., Cox, B., and Satoskar, A. R. (2021). Mechanisms of immunopathogenesis in cutaneous leishmaniasis and post kala-azar dermal leishmaniasis (PKDL). *Frontiers in cellular and infection microbiology*, *11*, 685296.

[84] Yentur Doni, N., Bertani, P. J., Volpedo, G., Saljoughian, N., Varikuti, S., Matlashewski, G., and Satoskar, A. R. (2023). Development of a novel immunoFET technology- based POC assay for detection of Leishmania donovani and Leishmania major. *Parasite Immunology*, *45*(7), e12984.

[85] Yurchenko, V., Chistyakov, D. S., Akhmadishina, L. V., Lukashev, A. N., Sádlová, J., and Strelkova, M. V. (2023). Revisiting epidemiology of leishmaniasis in Central Asia: lessons learnt. *Parasitology*, *150*(2), 129-136.

[86] Zijlstra, E. E., and El-Hassan, A. M. (2001). Leishmaniasis in Sudan. 3. Visceral leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *95*(Supplement_1), S27-S58.