Chapter

Cutaneous Leishmaniasis

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Abstract

Cutaneous leishmaniasis (CL) is a widespread parasitic infection caused by the Leishmania, which is carried by female sandflies. The symptoms include basic ulcer to lethal systemic disease *i.e.*, formation of widely dispersed skin lesions of diverse types. Almost 350 million individuals are at danger and the disease is endemic in more than 98 countries. There are globally 12 million cases, with 2–2.5 million new cases annually. Cutaneous leishmaniasis is considered as critically neglected disease by WHO. Earlier it was difficult to identify the infecting parasite, but modern DNA techniques make it quite simple to identify the Leishmania species, allowing quick treatment decisions. The quick identification of Leishmania is made possible using the PCR method. There is currently no vaccination to prevent leishmaniasis, and pharmacological treatment is frequently ineffectual. There is a need for broad and well-conducted investigations to help its control. Amphotericin B, pentamidine isethionate, paromomycin, and antifungals are some of the drugs recommended for treatment. By organising direct, in-person training, which is a crucial step in improving attitudes and preventative actions toward CL and its control in endemic areas, it is necessary to underline the significance and necessity of teaching this at-risk population.

Keywords: cutaneous leishmaniasis, protozoa, diagnosis, PCR, identification

1. Introduction

In 190 developing nations, leishmaniasis, an infectious illness brought on by protozoa of the genus Leishmania, continues to be a significant public health issue [1]. Leishmania parasite infection can result in one of three main clinical forms, depending on the species that caused the infection. *Leishmania infantum* and *L. chagasi* have been found to be identical by genotyping [2] *Leishmania chagasi* is considerate a subpopulation of *L. infantum* that arose from imported European strains [3–5], therefore they should be regarded as synonyms and are presents in both NW and OW. The first is localised cutaneous leishmaniasis (CL), which can cause a single or numerous skin ulcers as well as satellite lesions or nodular lymphangitis. CL with mucosal involvement is the third kind, systemic visceral leishmaniasis (VL), which affects internal organs such the liver, spleen, and bone marrow and is lethal if untreated. CL without mucosal involvement (CL) is the second kind [6].

Males are more prone than females to get this sickness [7]. The leishmaniasis disease is spread by phlebotomine flies, of the genera Phlebotomus in the ancient world and Lutzomyia in the new world. The vector of this disease belongs to the order Diptera, class insecta, Family: Psychodidae [8]. The sandfly has a 3 mm length and is known for its "hopping" flight. They feature fragile, long legs, dagger-shaped mouthparts, huge, dark eyes, long antennae, and downward-facing mouthparts [9].

By being bitten by female sandflies, 20 different Leishmania species cause his illness. The disease is spread by 30 different species of sandflies. Humans and non-wild or domesticated animals serve as their reservoir hosts. Female sandflies consume reservoir hosts and contract the disease [10]. Use of contaminated syringes from sick people is how this parasite is spread. *Leishmania chagasi, Leishmania infantum,* and *Leishmania donovani* are the common species [11]. Due to the shift of individuals toward urban areas during the past 20 years, there has been a potential increase in leishmaniasis cases [12]. Leishmaniasis spread among people living in non-endemic areas through travel.

Just seven nations—Algeria, Afghanistan, Brazil, Iran, Peru, Syria and Saudi Arabia, —represent 90% of all CL cases. CL is the most common clinical type of leishmaniasis worldwide [13]. The Old World species of Leishmania parasites, such as *L. infantum*, *L. tropica* and *L. major* (common in, the Middle East, the horn of Africa, the Mediterranean basin and the Indian subcontinent), and the New World species, such as *L. chagasi*, *L. mexicana*, *L. amazonensis, L. naiffi*, (endemic in Middle and South America). Self-limiting ulcers are frequently caused by Old World species, but American tegumentary leishmaniasis, which also causes MCL and disseminated cutaneous leishmaniasis (DCL), is typically caused by New World species [14].

Leishmaniasis patients have a wide immunological spectrum, from those with a robust T cell response, as shown by delayed-type hypersensitivity (DTH) and high levels of interferon γ (IFNγ), to those who lack a DTH response but still have high antibody levels. [11]. Others with a robust DTH have few parasites in their lesions because Leishmania spp. are killed by IFNγ -activated macrophages instead of being neutralised by antibodies, but people with merely a humoral response are unable to manage the parasite count [15, 16].

In addition to varying clinical symptoms based on species, Leishmania species also differ in their sensitivity to treatment options [17]. Because of this, determining the species is crucial to how leishmaniasis will manifest clinically. The identification of the Leishmania parasite that causes the disease used to be difficult, in disparity to many other infectious diseases. With the development of new DNA techniques, Leishmania parasites can now be recognised quite easily. With the motto "Small bite, big threat," the World Health Organisation (WHO) highlighted the serious and growing risk of vector-borne diseases, particularly leishmaniasis, on World Health Day 2014 [18]. In order to improve vector management, diagnosis, and the treatment toolset to prevent additional incidence and morbidity, leishmaniasis is considered a category 1 emergent and uncontrolled sickness and requires more intensive study. The major subjects of this review are the diagnosis, management, prevention, and strategies for the management and control of CL caused by both Old World and New World species.

2. Sandfly and Leshmania life cycle

In the blood meal of several hematophagus arthropods, the eaten amastigotes (protist cell, non-motile) in the infected host change into promastigotes (external

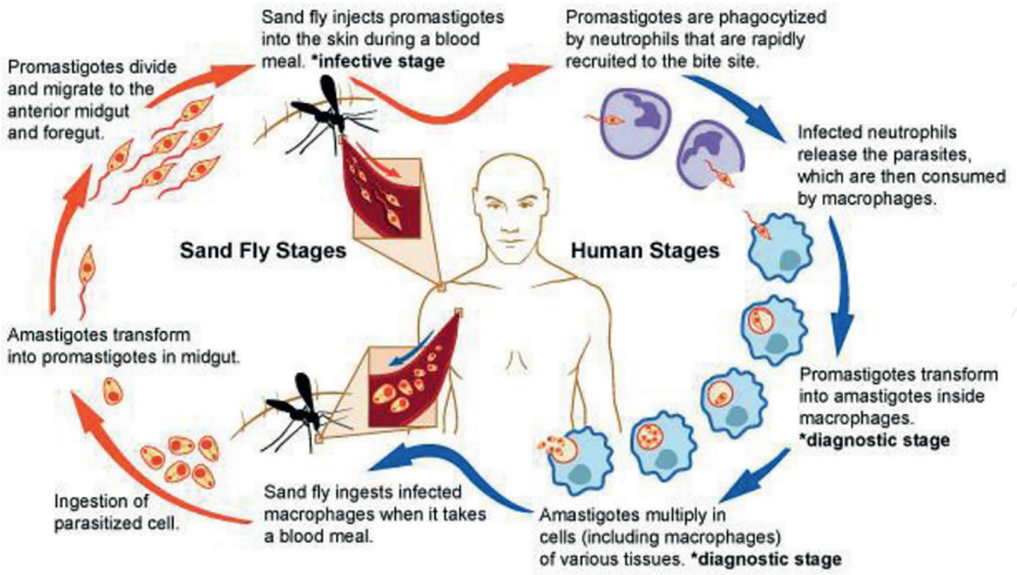

Figure 1.
Leishmaniasis' life cycle (http://www.niaid.nih.gov/topics/leishmaniasis/pages/lifecycle.aspx).

flagellum). When a host is inappropriate, parasites are discharged with the excrement [19]. When the blood meal is digested, trypanosomatids such as Leshmania species and others bind to the parasites on the midgut epithelium. The parasite is then kept in the gut and begins a stage differentiation. [20]. Promastigotes then proceed to Sandfly's foregut, which is covered in cuticles, of the sandfly. In the foregut some attach and some remain free for next transmission by bite [21] into the vertebrate host.

Promastigotes reproduce by binary fusion in the sandfly's digestive system. After seven days, promastigotes undergo metacyclogenesis and become contagious (metacyclic promastigotes). The sandfly will puncture the host's skin while feeding in order to inject saliva and metacyclic promastigotes into the host (**Figure 1**).

Metamorphose into the amastigote form within the host macrophage. They multiply inside the phagolysosome by binary fission until the cell ruptures and spreads the infection to additional phagocytic cells. This keeps the cycle going.

3. Early defence against Leishmania

The several species of Leishmania that cause cutaneous leishmaniasis each have unique characteristics. However, the parasites do share a life cycle in which a sand fly transmits a promastigote, a flagellated form of the parasite, to mammalian hosts such as humans, dogs, and rodents [22]. The promastigotes enter various phagocytic cells after being introduced into the skin by a sand fly's bite. Promastigotes change into an amastigote, a circular, non-flagellated replicative form, inside the phagolysosome of macrophages. When sand flies consume amastigotes while feeding on a host, the amastigotes then change into promastigotes and reproduce within the sand fly, the life cycle is complete. However, during a natural infection, other elements found in the sand fly saliva are delivered in the skin that affects early immune responses. Most experimental infections involve injecting promastigotes into the skin with a needle [23]. The biological significance of studies looking at the early response to infection without taking into account the conditions present

during a natural infection, such as the inoculation site, the number of parasites, and the components present during the sand fly bite, must therefore be carefully interpreted [24, 25].

While neutrophils, dendritic cells (DCs), and monocytes that are recruited to the infection site also have a chance of contracting the parasite and play critical and distinctive roles in the establishment of the immune response to infection.

4. Causes of leishmaniasis

The most prevalent type of leishmaniasis that affects people is cutaneous leishmaniasis. It is a cutaneous condition spread by the bite of a phlebotomine sand fly and brought on by a single-celled parasite. Leishmania species that can lead to cutaneous leishmaniasis number around thirty.

5. Signs and symptoms (diagnosis)

5.1 Cutaneous leishmaniasis

Skin scrapings samples—often collected from the advantage of lesions—that have been microscopically examined are typically used to make the diagnosis. Although quick and inexpensive, this has a low sensitivity, especially in persistent lesions [26]. Although, bacteria and fungi present in the biopsy samples may contaminate the cultures of the lesions. Additionally, the needs for growth vary among species. Isoenzyme electrophoresis can be used to identify specific Leishmania species, although the procedure is time-consuming, expensive, and requires extensive parasite cultivation. Although PCR is preferable for a direct investigation of clinical material, monoclonal antibodies can also be employed to identify species in cultured strains. High specificity and sensitivity PCR is quick. Leishmania detection and genetic characterisation are also possible at the same time [27]. The PCR sensitivity for one investigation on American cutaneous leishmaniasis was 100% [28]. Due to the absence of considerable antibody generation in cutaneous leishmaniasis, antibody detection is not very sensitive.

Additionally, in cases of American cutaneous leishmaniasis, reports of cross-reactivity between leishmanial antigens and antibodies made by other kinetoplastids, such as *Trypanosoma cruzi*, have been made [29]. The Montenegro (leishmanin) skin test, which looks for a particular type of delayed-type hypersensitivity on the skin, is another method for diagnosing cutaneous leishmaniasis that is currently available. *L mexicana* antigen is injected intradermally while being watched for a native reaction [30]. This test's limitations include the difficulty to distinguish between an infection that is currently present and one that has already occurred, as well as instances of false positive results in cases of other skin conditions [31].

5.2 Mucocutaneous Leishmaniasis

Mucocutaneous leishmaniasis develops after the commencement of cutaneous leishmeniasis and is defined by the destruction of the pharyngeal, oral, and nasal canals. The occurrence of this disease is also significantly influenced by genetic factors. Although nasal inflammation and stuffiness are the main early signs of

mucocutaneous leishmaniasis, the septum may slowly perforate and ulcerate. The larynx, mouth, throat, soft palate, and face are all affected by the lesion [32]. Bacterial infections may not harm the bones, but untreated illness can cause diarrhoea, pneumonia, and tuberculosis [33]. Suffocation, lung diseases, and starvation are other factors that might lead to death (due to closure of laryngeal aperture) [34].

5.3 Post kala-azar dermal leishmaniasis

A recurrence of kala-azar known as post-kala-azar dermal leishmaniasis (PKDL) may show up on a person's skin months or even up to 20 years after being partially treated, left untreated, or even in those who were thought to have had adequate treatment [35, 36]. In up to 60% of instances that are being treated in Sudan, they can be proven. They appear as facial redness or hypopigmented skin lesions (such macules, papules, and nodules). Even though PKDL can be caused by any organism that causes kala-azar, it is frequently linked to *Leishmania donovani*, which causes various disease patterns in Sudan and India. Nodules from the African type frequently ulcerate as they advance, whereas nodules from the Indian variant frequently expand with time and create plaques but seldom do so. Involvement of the nerves is more common in the African variation than in the Indian subcontinent [37]. Histology shows a variety of chronic inflammatory cells; macrophage or epitheloid granuloma may be present [38]. The variation in parasite concentration throughout investigations may be due to the less sensitive diagnostic techniques utilised in previous entries.

6. Clinical features

6.1 Scratches

An infection brought on by a sandfly bite can either remain asymptomatic or show up as an expanding, ulcerating papule after an incubation period of 1 to 12 weeks. An average scratch or lesions resembles an ulcer that is not painful and has a raised, indurated edge and a necrotic base that is frequently coated in an adherent crust of dried exudate. Most patients have 1 or 2 lesions, typically on exposed areas that range in diameter from 0.5 to 3 cm [39]. However, there is a great deal of variation: whereas some lesions develop sporotrichoid nodular lymphangitis, others do not ulcerate. Pain can be brought on by typical secondary bacterial infections. Atrophic scars are left behind after the majority of lesions heal over several months to years. *L. tropica* lesions typically take longer to heal—about 10 months—than those caused by *L. major* or *L. mexicana*, and *L. brazilinesis* lesions typically last considerably longer [40, 41]. Partial resistance to reinfection results from natural resolution [42].

In a study of 475 cases of *L. major* in Saudi Arabia, the parasite was found in 50–80% of smears (depending on the researcher and the technique employed), 70% of skin biopsies, and only 50% of cultures. In 10–20% of cases, even after combining all three procedures, the parasite remained undetectable. In New World illness, the issue is frequently worse, especially in lesions older than six months [43].

6.2 Alternative methods

Alternative methods for obtaining tissue for diagnosis include needle aspirates and slit skin smears. A needle aspirate is obtained using a 2 ml leur lock syringe, a 20 gauge needle, and 0.3 ml 0.9% saline. The needle is inserted through healthy skin and 0.1 ml is injected into the margin of the lesion. The tiny tissue fragments are aspirated after being severed from the needle track's edge while the needle is being rotated back and forth and suctioned. To create smears and inoculate cultures, use the aspirate. Slit-skin smears are made by pinching the edge of the lesion between the thumb and fingers, making a 1 mm deep slit with a scalpel, and then scraping the cut edge [44].

Because antibodies frequently go undetected or are present in low, serology is ineffective for treating cutaneous diseases [45]. Similar to the tuberculin test, the Leishmanin skin test identifies cell-mediated immunity; it turns positive after the scratches start to crust and stays that way for ever. It is unable to differentiate between current and previous infections [46].

7. Morphology

A papule that resembles an insect bite marks the beginning of the lesion. The papule persists and gradually grows in size rather than regressing. The nodular, nodulo-ulcerative, and ulcerative forms are three common morphological presentations in the following stage. The latter features a flat foundation with marginal rolls that are either small or significant. If it is a secondary infection, the base is made up of granulation tissue and may be covered in pus.

Satellite papules, which are tiny (2–4 mm) papules along the perimeter of the lesion, are another crucial aspect of morphology. Skin crease orientation is visible in the lesions. Multiple lesions frequently cluster together; the sand fly bites the same spot repeatedly, delivering parasites with each bite.

Special clinical features of *L. major* infection are: [47].

1. Diffuse thickening without ulceration (erysipeloid form).

2. Leishmanial cheilitis.

3. Chiclero's ulcer; this is an ulcer on the pinna of the ear.

4. Mycetoma-like lesions.

5. Leishmanial dactylitis.

6. Cutaneous leishmaniasis of the nose.

7. Sporotrichoid cutaneous leishmaniasis: the infection spreads along the lymphatics as in sporotrichosis.

Leishmaniasis recidivans, also known as lupoid leishmaniasis, is a unique clinical form of *L. tropica* infection; in this situation, the sore appears to heal but recurs along

the edge of the lesion, a process that may last for many years and may be disfiguring. Like cutaneous tuberculosis, it (lupus vulgaris). Typically, there are few parasites, and the LST is quite positive.

8. Infections in animals

8.1 Clinical signs

8.1.1 Dog

Wild dogs and domestic dogs are the main reservoirs of zoonotic visceral leishmaniasis caused by *L. infantum* in the Mediterranean area, Middle East, Asian countries, and Latin America. Dogs serving as the main reservoir of visceral leishmaniasis have made research into the immune response and searching for Leishmania antigens linked to protective cellular immunity in canine visceral leishmaniasis more attractive. Recent studies have shed fresh light on the genetic underpinnings, pathophysiology, immunology, and epidemiology of canine leishmaniasis. These new discoveries have improved understanding of the condition and aided in the creation of novel diagnostic techniques and infection-control strategies, such as dog collars impregnated with insecticide, new medications, and second-generation vaccines [48, 49].

Other infected dogs may remain asymptomatic or display one or more minor illness, which is known as oligosymptomatic infection [50]. Some infected dogs may experience symptoms that result in death. The distinctive histological feature in the skin, liver, and spleen is a granulomatous inflammatory response connected to Leishmania amastigotes within macrophages [51].

The clinical indications of canine visceral leishmaniasis were used to categorise a group of mixed-breed dogs with spontaneous Leishmania infections as symptomatic or asymptomatic [52].

Domestic dogs in Latin America have been observed to naturally contract *L. braziliensis, L. peruviana, L. panamensis, Leptodactylus colombiensis, and L. mexicana* [48]. There is currently no conclusive proof that dogs serve as reservoir hosts for the domestic spread of CL. [53, 54]. The majority of research focuses on determining the prevalence of CL in dogs, but little is known about the parasitic and immunological aspects of the infection.

8.1.2 Cats

Leishmaniasis can occur in cats, but most infected cats are believed to remain asymptomatic. Most frequently, lesions of the skin or mucosa are described, either with or without visceral symptoms. Visceral symptoms, however, might appear without cutaneous involvement. Skin lesions can be found anywhere on a cat, although most frequently appear on the lips, nose, ears, eyelids, and paws. The most frequent lesions encountered are localised papules, nodules and chronic crusted or ulcerated lesions; regional lymphadenopathy may also be present. Rare reports of alopecia, scales, and hemorrhagic pustules or nodules have been made. Initial lesions are frequently single, although they can sometimes be numerous and occasionally spread.

There have been reports of oral, nasal, and in certain cases other mucous membranes (such the anal mucosa) being involved. Some cats can have ocular symptoms, including unilateral or bilateral uveitis, conjunctivitis, and blepharitis (which can

develop into panophthalmitis). Fever, hepatomegaly, jaundice, vomiting, diarrhoea, lymphadenopathy, dyspnea, nasal discharge, anaemia, and leukopenia are just a few of the visceral abnormalities and symptoms that have been observed in cats [55].

8.1.3 Equidae

Skin lesions can occasionally appear on horses, mules, and donkeys, especially on the head, neck, legs, and inguinal or axillary regions. Solitary or numerous papules or nodules, which frequently have ulcers, are the most typical lesions. Additionally, widespread skin illness has been documented. Although visceral leishmaniasis in horses has not been documented, parasites and *L. braziliensis* nucleic acids have been found in the blood and bone marrow of other animals in South America [55].

8.2 Other domestic animals

Rarely have medical instances in cattle or other small ruminants been reported. Only skin lesions, occasionally accompanied by lymphadenopathy, were noted in goat, sheep and cattle. In Germany, a pregnant cow with *Lechytia martiniquensis* infection had several ulcerative or plaque-like skin lesions on various body parts. After giving birth, it fully recovered. The only clinical symptom in experimentally infected sheep was a fever. Pigs with an experimental infection exhibited no symptoms.

Rarely, *L. enriettii* skin lesions, frequently on the ear, have been found in naturally infected guinea pigs. The earliest lesions in experimentally infected animals start out as redness and swelling but quickly progress into sizable, ulcerated lumps that resemble tumours. While some investigations discovered secondary lesions at other locations, including as the skin, lip, and genitalia, others claimed the lesions did not spread. Additionally, parasites were found in various internal organs. Spontaneous healing has been documented in certain studies but not others. Hamsters formed non-ulcerated nodules that went away on their own after contracting *L. enriettii* infection in an experiment.

8.3 Captive wild species and wild animals

The few known cases of leishmaniasis in wild or captive canids have resembled leishmaniasis in canines. Some nonhuman primates have been found to have visceral involvement with nonspecific symptoms (such pale mucous membranes and weight loss). A lion displayed clinical symptoms of colitis, including bloody diarrhoea, epistaxis, weight loss, and footpad sores.

L. martiniquensis-infected captive Australian marsupials have experienced skin lesions that include elevated, crusty or ulcerative pale nodules as well as localised to converging patches of thickened skin. Some rats infected with the *L. mexicana* complex in the wild have been reported to have skin lesions. These lesions are described as ulcers or swollen bumps with thinning hair. The tail base was said to be where they appeared most frequently, but they might also appear on the ears or toes. Numerous species have been known to have subclinical infections [55].

9. Treatment

Luckily, there are some rules to follow. A wait-and-see strategy for spontaneous cure may be acceptable because most lesions heal quickly without therapy, especially for patients who live in endemic areas because spontaneous healing is linked to the

development of protective immunity. It is recommended to actively treat lesions that are multiple or persistent, have associated lymphangitis, are on cosmetically or functionally significant places like the hands or face, have several lesions. Patients with early, non-inflamed lesions should have local therapy; patients with many lesions or more complex lesions should receive systemic therapy.

9.1 Sodium stibogluconate and Meglumine antimoniate

Patients with cutaneous leishmaniasis can receive an intralesional infiltration of 1–5 ml (100 mg/ml) sodium stibogluconate (SSG) on alternate days for three days once a month, and in the majority of instances, this causes full healing by the end of the second month [56]. For numerous and larger lesions, a higher dose and more than three regimens were required. In addition to intralesional SSG in several lesions, Sharma et al. employed intramuscular SSG (800 mg/day) [56]. Meglumine antimoniate, a substitute medication that is the top drug of choice in Ecuador [57]. In Nepal, sodium antimony gluconate (20 mg/Kg/day) is administered intramuscularly to PKDL patients for duration of 30 to 72 days [58].

Drug resistance is a problem, and cases of visceral leishmaniasis with SSG resistance were reported in Nepal [59, 60]. So far, this issue was not reported from Nepal in cutaneous leishmaniasis. It is unknown that what is the most effective treatment for American utaneous and mucocutaneous leishmaniasis (ACML)? Since the 1940s, pentavalent antimonial medications have been utilised, such as sodium stibogluconate (SSG) and meglumine antimonate (Glucantime, MA), but they are costly, poisonous, and uncomfortable [61]. It is advised to identify the precise species of Leishmania before beginning treatment because medications that are effective for one species of Leishmania may not be effective for another. Sadly, leishmaniasis is an orphan disease in affluent countries, and nearly all of the available treatments are poisonous and have serious adverse effects [61].

Compared to parenteral therapy, this results in higher local concentrations and fewer systemic side effects. 5–10 1–5 ml antimony infiltrations are performed two–three times per week. Make sure the injection is in the lesion and not in the tissues beneath the skin. It might be really painful. There is growing evidence that it is useful in treating *L. tropica* infections, as well as CL brought on by *L. major* [62].

10. Physical methods

Patients of all ages have received treatment for cutaneous leishmaniasis using a variety of physical techniques, such as surgical excision, cauterization, cryotherapy, and the use of local heat.

10.1 Cryotherapy

Cryotherapy involves applying liquid nitrogen repeatedly to a lesion up to 2 mm outside the lesion margin using a cotton-tipped applicator or a cotton swab with moderate pressure. Each application's freezing time is 15–20 seconds. The process is carried out two or three times with brief breaks, taking between 30 and 120 seconds in total. The whitening of the skin at 2–3 mm outside the lesion's edges indicates proper treatment [63–66]. The typical post-freeze pattern includes blistering of the lesion for two to three days, crusting, mild oedema and the development of an eschar [63–66].

11. Treatment in animals

Animals rarely receive topical treatments, but radio-frequency-induced heat therapy was effective in treating two dogs with several localised mucocutaneous lesions on the snout. In certain animals, such as some cats and some horses, cutaneous lesions did not recur following surgical resection; but, in other instances, surgical resection alone was ineffective.

11.1 Vector control

Theoretically, infection might be controlled by preventing transmission by the sandfly vector. Depending on the species, some sandflies are endophagic and eat indoors, while others are exophagic and eat outdoors. Examples of tactics include deterrents, particularly pyrethroids, and insecticides like DDT. The majority of the times, sandflies are still quite sensitive to insecticides, despite reports of DDT resistance. Spraying has been shown effective at the local level, but it is unclear what effect blanket spraying would have on the sandfly population, and these programmes are challenging to maintain. Bednets offer defence against species that consume living things [67] with pyrethroid-impregnated nets providing extra protection dropping biting rates by up to 64–100% [68]. The success of bednets as a long-term control method depends on routine, replacing damaged nets, re-impregnation and dissemination to rural areas. The ability of bednets to stop the spread of malaria to children has raised incentive to improve this method of malaria control, including research into bednets treated with long-lasting insecticides [68].

11.2 Animal reservoirs

Leishmaniasis is a zoonotic disease that is widespread throughout the world with significant reservoirs of infection in sylvatic and domestic animals. The dog population has been targeted throughout the Mediterranean basin and Brazil. The use of dog collars impregnated with delta methrin has proven to be the most successful tactic, offering dogs up to 86% protection during high transmission seasons [69]. Dog killing has also been used, and modelling from a Brazilian study suggests that in areas with low endemicity, both dog killing and dog collars should have a greater proportional impact. However, as transmission rates rise, the relative benefit of dog collars decreases [70]. However, the effectiveness of vector and animal reservoir control programmes has been diminished due to decreased pesticide use and rising prevalence among urban populations. Given the wide range of epidemiological situations, the multiplicity of factors that affect disease transmission, and the continuous understanding regarding the biology of the parasite, its vector, and its reservoir hosts, disease management has thus proven to be extremely difficult to achieve. To avoid these problems, a vaccine for humans or dogs is a preferable option.

11.3 Vaccines

A plausible basis for vaccine development can be found in the discovery that strong immunity against re-infection accompanies spontaneous or medication-induced recovery from CL or VL. This finding gave rise to the long-standing technique of "leishmanization," or the use of live parasites retrieved from skin lesions to cause lesions in desired body regions in order to prevent sickness on re-infection.

Such a custom has been practised for at least 2000 years. Between 1982 and 1986, Iran had about 1.2 million patients receive this live vaccination [71]. 93% of people who had skin lesions after receiving the vaccination had a positive leishman-delayed hypersensitivity skin test, which is a good field predictor of population immunity. Skin lesions occurred in almost 50% of vaccination recipients. Additionally, a considerable decrease in disease incidence was seen, going from 14% in the group that had not gotten the immunisation to 2.5% in the group that had. Although it has not always produced cross-species protection, the use of heterologous organisms with lower pathogenicity as vaccines against a more virulent species is justified by the greater extent of immunological cross-reactivity between species at the humoral and cellular levels. Leishmanization is no longer used due to the risk of localised sickness and HIV-related spread, as well as the impossibility of delivering new cultures of a live vaccination in the field. A different approach with attenuated organisms enables exposure to a considerably wider spectrum of antigens than is possible with more complex subunit vaccines, resulting in the establishment of an immune response that is most similar to that of a natural infection. However, even using naturally pathogenic organisms in such a way to treat human or experimental murine leishmaniasis [72], exposed organisms [73] or genetically manipulated organisms [74], there has been little achievement. Similar to this, dead vaccinations have low immunogenicity and effectiveness even when supplemented with adjuvants, such as bacille Calmette-Guérin BCG or alum [75]. Interestingly, BCG alone caused a positive leishmanin skin test in some people, most likely because Mycobacteria and Leishmania have antigenic cross-reactivity. While the effectiveness of the single-dose *L. major* vaccination combined with BCG in dogs was around 70% [76]. It has showed promise as a possible vaccination policy against a natural *L. infantum* infection to administer to dogs naturally excreted secretory antigens extracted from culture supernatant of *L. infantum* promastigotes [77]. TSA, LeIF, and LmSTI1 are three recombinant leishmanial antigens that have shown immunogenicity in canine [78]. Recent developments in the creation of a vaccination that prevents transmission are hopeful given the significance of dogs as virus reservoirs. 92–97% protection against zoonotic visceral leishmaniasis was produced by the *Labrus donovani* fucose-mannose ligand (FML) antigen in combination with saponin (FML vaccination and Leishmune), with considerable protection shown out to 12 months [79]. Investigating the use of specific compounds as human vaccinations is an alternative strategy. Animal models for a gp63 peptide vaccination were successful [80]. The failure to adequately elicit cellular immunity, a necessary component for the management of intracellular infections, has, nevertheless, contributed to the generally poor effectiveness of these vaccines in humans.

11.4 DNA vaccines

The creation of DNA vaccines has provided an innovative solution to this issue. According to Wolff et al., intramuscular injection of plasmid DNA expressing a variety of reporter genes could cause muscle cells to produce proteins [81]. This study provides a solid basis for the concept that pure recombinant nucleic acids can be delivered in vivo to regulate protein production. Later studies found that DNA vaccines could defend mice from CL [82]. The ability to retain information over the long term is one of the main determinants of a vaccine's effectiveness. DNA encoding leishmanial antigen LACK is better to leishmanial protein and IL-12 protein immunisation for maintaining antigen-specific Th1 responses that can restrict *L. major* infection [83]. Antigen persistence and IL-12 activation by CpG motifs are two factors that explain

why DNA vaccination is more effective than protein and adjuvant. In an experiment with leishmaniasis, a study finding that anti-IL-10R antibody resulted to sterile cure but loss of immunity against re-infection showed that parasite persistence was essential for the preservation of immunity [84]. However, DNA vaccines can nonetheless induce long-lasting immunity in the absence of noticeable antigen. This is done either by allowing undetectable antigen to remain, potentially in follicular DCs, or by inducing antigen-independent immune responses [85]. According to studies, memory T cells are diverse, with one fraction (central memory T cells) migrating through lymph nodes and another migrating to tissues and producing effector cytokines [86]. Recent research shows that even in the absence of parasites, central memory T cells can mediate long-term memory [87]. Finding the pathways that cause central memory T cells to be induced would therefore be a significant problem for DNA vaccines. The potential for improving DNA vaccine immunogenicity has been investigated in a number of ways. By acting as TLR ligands, novel adjuvants including CpG motifs and monophosphoryl lipid A release IL-12 and encourage a Th1 response. Heterologous primeboost immunisation has been used as another method to improve human responses to vaccination. Various combinations have been studied, but priming with DNA and boosting with MVA have received the most attention [88]. This work demonstrates that IL-10 plays a crucial role in the immune response by showing that the efficacy of the vaccine against these antigens in the BALB/c model was dictated by IL-10 from regulatory T cells. The most frequently sampled gene among expressed sequence tags from cDNA libraries of *L. major* was TryP, according to early investigations [89], in vulnerable BALB/c mice as a reproducibly protective antigen against infection. LACK, *L. major* stress-inducible protein 1, Leishmania elongation factor, and HASPB1, a stage-specific hydrophilic acylated surface protein in mice, are other antigens that are effective in mice and non-human primates. In order to immunise against the saliva-containing *L. major* challenge, the use of salivary antigens in plasmid DNA has also been examined [90]. The 8500 discovered genes now that *L. major* Friedlin's genome sequence is complete serve as a source of potential vaccine candidates [91]. There is no presumed requirement that the target antigen be a surface molecule because the parasite is inside, which significantly expands the pool of potential vaccine candidates. One such thorough vaccine screening of 100 different amastigote-expressed Leishmania genes was done in a BALB/c mouse model challenged with the *L. major* LV39 substrain using DNA vaccination [90]. A heterologous prime-boost strategy for immunising against experimental visceral leishmaniasis in dogs was successful using DNA/recombinant cysteine proteinases type I and type II [92]. We are eagerly awaiting the outcomes of additional dog experiments as well as a human study of a DNA vaccination.

12. Conclusion

Renewing hope that control is possible is the international acknowledgement of this disease's significance, coordinated by WHO programmes, particularly in India, where 70% of the world's VL burden is found. A better coordination of control programmes should be possible thanks to new techniques for early case detection. Only when the local infrastructure is developed to support the provision of healthcare will more extensive control be possible in the world's less developed areas. This attempt is hampered by the introduction of HIV, drug-resistant strains, and changes in the epidemiology of the vector. The development of new techniques and, eventually, a protective vaccine will require a sustained worldwide effort and the requisite funding.

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Conflict of interest

The authors declare no conflict of interest.

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