A Systematic Review of Research on Treatment & Prevention of the Neglected Tropical Disease Leishmaniasis

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A SYSTEMATIC REVIEW OF RESEARCH ON TREATMENT & PREVENTION OF
THE NEGLECTED TROPICAL DISEASE LEISHMANIASIS

A Thesis
Presented to the
Graduate School of
Clemson University

In Partial Fulfillment of the
Requirements for the Degree
Master of Science, Genetics.

by
Aryn Akerberg
May 2023

Accepted by:
Todd Lyda, Ph.D., Committee Chair
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ABSTRACT

Leishmaniasis is a life-threatening neglected tropical disease that is caused by the protozoa parasite, *Leishmania*. Over one million new human leishmaniasis cases occur each all over the world, affecting most the deeply impoverished regions of the world. The disease presents in three different forms: cutaneous leishmaniasis, mucocutaneous (mucosal) leishmaniasis, and visceral leishmaniasis. Symptoms can range from self-limiting lesion to more life-threatening conditions such as fever, kidney disease, and anemia. The *Leishmania* parasite is transmitted via the bite of the female phlebotomine sandfly and can infect many other mammals such as canines, rodents, bats, etc. The canine leishmaniasis epidemic is also important and has a crucial part in making progress in overall disease reduction. There is no current completely effective method of preventing or treating leishmaniasis. Vaccine research has been limited and has not yielded efficacious approaches up to this point. Additionally, current treatment methods are expensive, ineffective, and not easily accessible to most regions of infection. The *Leishmania* parasite is a complex pathogen with a capability to mutate and adapt as needed. New and creative treatments and preventatives are necessary to make progress in disease reduction. Parasite intracity and socioeconomic impact must be considered equally for research and development. Leishmaniasis will always be a difficult disease to control, but considerations of current failures can help push towards innovative solutions in the future.
ACKNOWLEDGEMENTS

I would like to thank Dr. Lyda for his support and guidance throughout this research process. Additionally, I would like to thank my committee and the entire genetics department for their commitment in the advancement of knowledge and their support always.
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CHAPTER ONE
INTRODUCTION

Leishmaniasis is a deleterious parasitic disease that currently burdens over 100 endemic countries but has no effective vaccine, prevention, or treatment due the complexity of interactions among the host, the parasite, and the vector. Leishmaniasis is caused by a protozoan parasite of the genus *Leishmania* which has over 20 pathogenic species (Akhoundi, 2017). Disease infection is transmitted by the female phlebotomine sandfly and currently has a transmission rate of roughly 1 million new cases each year (World Health Organization, 2023). Although the disease is prevalent in poverty stricken tropical regions, it burdens different populations all over the world. Because leishmaniasis is a vector-borne infectious disease caused by multiple *Leishmania* species, treatment and vaccine development are difficult (Neuber, 2008).

There are four manifestations of the disease, summarized within Table 1. Symptoms and clinical sings are inconsistent amongst patients and can range from a cough and fever to more serious conditions such as hepatomegaly, anemia, and thrombocytopenia. Cutaneous leishmaniasis is the most common, with symptoms such as skin lesions, skin ulcers, and enlarged lymph nodes (de Vries HJ, 2015). Mucocutaneous leishmaniasis primarily attacks the mucous membranes of the nose, mouth, and throat (Handler, 2015). Typically, destructive lesions will begin to appear in these places causing the airways to become damaged. Visceral leishmaniasis is the least common but also produces the highest percentage of mortalities (Bi, Kaiming, et al, 2018). If visceral leishmaniasis is left untreated, it is fatal 95% of the time, and even with treatment, is still
fatal 7% of the time. (World Health Organization, 2023). Visceral leishmaniasis can remain in an incubation period for up to six months, or longer due to the complex interaction between the host and the parasite. Even after clinical signs have resolved, the disease can relapse later on in life. Clinical manifestations of this disease include fever, weight loss, hepatosplenomegaly, and pancytopenia (Bi, Kaiming, et al, 2018). The last presentation of leishmaniasis is post kala-azar dermal leishmaniasis which is a complication from visceral leishmaniasis. This is a special presentation of the disease that will only occur after initial visceral leishmaniasis infection. The primary sign of this disease is a moderate to severe rash. This presentation usually appears 6 months to 1 year after visceral leishmaniasis infection (Pan American Health Organization).
Table 1.1 Types of Leishmaniasis (Abadías-Granado I, 2021; Mokni M, 2019; Sundar S, 2018).

<table>
<thead>
<tr>
<th>Disease Type</th>
<th>Symptoms</th>
<th>Geography</th>
<th>Treatment</th>
<th>Incubation Period</th>
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<td>Visceral Leishmaniasis</td>
<td>Fever, weight loss, enlargement of the spleen/liver, anemia</td>
<td>Brazil, East Africa, India, Brazil, China, Ethiopia, Eritrea, Kenya, Somalia, South Sudan, Sudan, Yemen</td>
<td>Liposomal amphotericin B, Pentavalent Antimonial Therapy, Paromomycin</td>
<td>3-8 months</td>
</tr>
<tr>
<td>Cutaneous Leishmaniasis</td>
<td>Skin lesions, ulcers</td>
<td>Americas, Mediterranean Basin, Middle East, Central Asia, Afghanistan, Algeria, Brazil, Colombia, Iraq, Libya, Pakistan, Peru, Syrian Arab Republic, Tunisia</td>
<td>Miltefosine, Amphotericin B deoxycholate, Pentamidine, Pentavalent Antimonial Therapy</td>
<td>Commonly 2-8 weeks (months-years in rare cases)</td>
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<td>Mucocutaneous Leishmaniasis</td>
<td>Destruction of mucous membranes of the nose, mouth, and throat</td>
<td>Bolivia, Brazil, Ethiopia, Peru</td>
<td>Amphotericin B deoxycholate, Pentavalent Antimonial Therapy</td>
<td>1-3 months (sometimes years after initial cutaneous ulcer has healed)</td>
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<td>Post-kala-azar Dermal Leishmaniasis</td>
<td>Macular, 3alagas, or nodular rash on the face, upper arms, trunks, and other parts of the body</td>
<td>East Africa, Indian Subcontinent</td>
<td>NA</td>
<td>6 months-1 year after VL infection</td>
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Historical Evidence

*Leishmania*-like parasites have been found associated with fossils suggesting the parasite has been interacting with the insect host for millennia. *Leishmania* was found to be present in two types of extinct sandflies *Palaeomyia burmitis* and *Lutzomyia adiketis*, both dating back to over 30 million years ago (Poinar Jr G, 2008). The geographical origin of *Leishmania* is still being debated with three primary theories at the forefront. First, the Palaearctic Hypothesis indicates that *Leishmania* originated in the Palearctic region including Europe, Asia, Arabia, Africa, the Sahara and the Palaeocene (Lysenko AJ., 1971). Second, the Neotropical Hypothesis indicates that *Leishmania* originated in the Neotropical region including Central America, the Caribbean, and South America (Lainson, 1987). Third, the Supercontinent Hypothesis indicates that *Leishmania* evolved as two separate species in two different regions, *Sauroleishmania* in Africa and *Viannia* in South America (Steverding, 2017). It is known that *Leishmania* evolved prior to the breakup of Pangea, geographical location that the parasite originated is still an ongoing debate. The above hypotheses are based on phylogenetic trees and historical evidence (Thomaz-Soccol, 1993).

Although *Leishmania* was not officially characterized in humans until the 1800s, there are multiple other potential accounts of this disease in history (Manson-Bahr, 1996). From early civilization through the Middle Ages, there are reports of skin disease resulting in sores, similar to the description of cutaneous leishmaniasis (Boelaert M, 2014). In the 16th century, the Spanish colonization of the Americas came with reports of a disfiguring disease similar to mucocutaneous leishmaniasis. In the 19th century, reports
of visceral leishmaniasis began to circulate (Manson-Bahr, 1996). William Twining, a military surgeon working in India in the 1800s published instances of patients who had clinical signs of enlarged spleens, acute anemia, and intermittent fever. Around this time the term kala-azar was popularized to refer to visceral leishmaniasis, the word meaning ‘black disease’ (Steverding, 2017).

The first to officially characterize leishmaniasis was William Boog Leishman, a Scottish pathologist who was serving in the Army in India (Leishman WB, 1903). Leishman found leishmaniasis bodies (Leishmania amastigotes) in the spleen of a soldier who died from splenomegaly (enlargement of the spleen). He then infected a rat with the leishmaniasis bodies which produced similar results, such as splenomegaly in the rat. He published his findings in 1903 and suggested that this new disease agent was a form of trypanosomiasis (Lainson R, 2010). At the end of 1904, Leishman’s original theory was disproved, and the bodies were determined to be a new protozoan by Charles Donovan. The name Leishmania donovani was adopted, named after Major Charles Donovan. Sandflies were proven as the vectors of transmission in the 1920s by Edmond Sergent and Etienne Sergent. The two scientists placed sandflies in the same space with human volunteers for extended periods of time and found that lesions developed into the classical lesions seen previously (Costa MA, 2009).

Leishmania primarily affects underserved and impoverished populations, so the economic burden is particularly intense on individuals in these regions. According to a study on Leishmania in Nepal, the cost of treating one case of visceral leishmaniasis, 425...
USD, was higher than the average income per household, 405 USD (Okwor, 2016). In order to pay for treatment, most individuals affected by this disease sacrifice their entire savings or had to take out a loan. Most populations affected by this disease do not have access to health insurance or government associated health care programs, causing the cost of this disease to be unbearable for most people. Leishmaniasis and poverty are linked in a vicious cycle as the impoverished are more likely to both get this disease and suffer greater morbidities. Therefore, leishmaniasis is considered a neglected tropical disease (Okwor I, 2016).

**Current Efforts to Combat Leishmaniasis**

There is currently no effective method of prevention of leishmaniasis. According to the World Health Organization, Leishmaniasis is listed as one of the neglected tropical diseases for which development of new treatments is a priority (World Health Organization, 2023). Current treatments are either expensive, ineffective, or a combination of both. No vaccine against any form of the disease is approved for use in humans (Palumbo, 2009). Drugs and treatments that used to be effective are no longer due to increased drug resistant strains (Palumbo, 2009). Socioeconomic factors also play a significant role in the burden of this disease (Wijerathna, 2020). Housing, hygiene, nutrition, and economic status all contribute to the likelihood of an individual being infected with this disease and affect a person’s ability to fully recover from infection (Palumbo, 2009). There are potentially unknown treatments that could be very effective but continued research in this area is limited (World Health Organization, 2023).
Preventative measures for Leishmaniasis can be categorized into three broad categories: vector reservoir control, animal reservoir control, and human reservoir control. Vector control relates to different levels of environmental management, with the most popular being the use of insecticides (Pathirage, 2019). In most insecticide studies, there have been divided results on whether insecticides are effective enough to justify potential environmental damages. There are also few studies directly studying human infection rates after insecticide intervention; most studies assess to reduction of sandfly population (Piscopo TV, 2007). Insecticides have proven useful for short-term delay but are not necessarily ideal as a long-term option as it has been shown that the sandflies will evolve to become resistant to such treatment (Montoya, 2021). Animal reservoir control mainly focuses on canine leishmaniasis control, as they are the most frequently infected animals. Canine control is achieved using insecticides, vaccines, and culling (González, 2015). Again, the limited studies on the direct effects of human disease prevention with these methods have made the effectiveness of this method inconclusive. Human prevention would ideally include a vaccine, but this has not yet been developed. However, other methods include treated bed nets, health education, and animal/vector control. A summary of current vaccine efforts is summarized in Table 2 which will be explored further in Chapter IV (De Brito, 2020).
References


CHAPTER TWO
LEISHMANIASIS BASICS

Geographic Distribution

There are more than 53 known species of the *Leishmania* parasite but only 20 that infect humans (WHO, 2023). Species variation is common even within the same geographic region which has relevance to the difficulty in developing effective treatments and vaccines (Herrera, 2020). The 20 infectious species are as follows: *L. aethiopica, L. amazonensis, L. braziliensis, L. colombiensis, L. donovani, L. guyanensis, ‘Ghana strain’, L. infantum, L. lainsoni, L. lindenbergi, L. major, L. martiniquensis, L. 11alagasy, L. naiffi, L. panamensis, L. peruviana, L. ‘siamensis’, L. shawi, L. tropica, L. venezuelensis and, L. waltoni* (M, Downing 2017). Different species of *Leishmania* are correlated with the different types of leishmaniasis: visceral, cutaneous, and mucosal leishmaniasis. A summary of these species and their correlated disease presentations and geographical distributions are summarized in Table 2.1.
Cutaneous leishmaniasis, the most common presentation of the disease, is many regions of the world (WHO, 2023). However, 90% of all cases are found in Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia, and Syria (WHO, 2023). The entire list of cutaneous species can be found in Table 2.1 which can be further divided into two categories, Old World species and New World species. Old World species include *L. major*, *L. infantum*, and *L. tropica*. New world species include *L. amazonensis*, *L. chagasi*, *L. 12alagasy*, *L. naiffi*, *L. braziliensis*, and *L. guyanensis* (de Vries, 2105). The difference in Old World species and New World species is the difference in correlating clinical signs regarding cutaneous leishmaniasis. However, few studies are confirmed on these differences. Typically, Old World species cause self-healing, minor ulcers whereas
New World species can cause a special variation of more severe lesions called American tegumentary leishmaniasis (Adriano, 2013).

Mucosal leishmaniasis is believed to have derived from infection of New World species such as *L. braziliensis, L. panamensis, L. amazonensis, L. infantum*, and *L. guyanensis*. It is most commonly found in South America but is also found less commonly throughout the world such as Asia, Europe, and Africa with varying symptomatic presentations (WHO, 2023). However, mucosal leishmania is unusual because it can also develop from subsequent visceral and cutaneous leishmaniasis species (Pace, 2014). Like cutaneous leishmaniasis, mucosal species can also cause a presentation of American tegumentary leishmaniasis (Strazzulla, 2013).

Visceral leishmaniasis is primarily caused by *Leishmania donovani* and *Leishmania infantum*. *L. infantum* is primarily found in the Mediterranean basin, China, the Middle East, and South America and infection is primarily in dogs. *L. donovani* is primarily found in eastern Africa, Bangladesh, India, and Nepal (Alvar J, 2006). Overall, visceral leishmaniasis is primarily found in the Indian subcontinent and eastern Africa (Alvar J., 2006).
Table 2.1 *Leishmania* infectious species (Adrian, 2017; Berman, 2012; Van Henten, 2019; Dahroug, 2011).

<table>
<thead>
<tr>
<th>Species Name</th>
<th>Disease Presentation</th>
<th>Geographical Distribution</th>
<th>Old vs New World</th>
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<tr>
<td><em>Leishmania aethiopica</em></td>
<td>Cutaneous</td>
<td>Ethiopia</td>
<td>Old World</td>
</tr>
<tr>
<td><em>Leishmania brasiliensis</em></td>
<td>Visceral, Cutaneous, &amp; Mucosal</td>
<td>Southern US, Mexico, &amp; Central/South America</td>
<td>New World</td>
</tr>
<tr>
<td><em>Leishmania chagasi</em></td>
<td>Visceral</td>
<td>Brazil</td>
<td>Old World</td>
</tr>
<tr>
<td><em>Leishmania donovani</em></td>
<td>Visceral</td>
<td>Africa, China, India, Nepal, southern Europe, Russia, &amp; South America</td>
<td>Old world (except Europe)</td>
</tr>
<tr>
<td><em>Leishmania infantum</em></td>
<td>Visceral</td>
<td>East Africa, Indian, &amp; Brazil</td>
<td>New World</td>
</tr>
<tr>
<td><em>Leishmania major</em></td>
<td>Cutaneous</td>
<td>Northern Africa, the Middle East, Northwestern China, &amp; Northwestern India</td>
<td>Old World</td>
</tr>
<tr>
<td><em>Leishmania 14alagasy</em></td>
<td>Cutaneous</td>
<td>Mexico &amp; Central America</td>
<td>New World</td>
</tr>
<tr>
<td><em>Leishmania peruviana</em></td>
<td>Visceral, Cutaneous, &amp; Mucosal</td>
<td>Andean valleys of Peru</td>
<td>Old World</td>
</tr>
<tr>
<td><em>Leishmania tropica</em></td>
<td>Cutaneous</td>
<td>Middle East, North Africa, &amp; southeastern Europe</td>
<td>Old World</td>
</tr>
<tr>
<td><em>Leishmania venezualensis</em></td>
<td>Cutaneous</td>
<td>Venezuela</td>
<td>Old World</td>
</tr>
<tr>
<td><em>Leishmania Viannia brasiliensis</em></td>
<td>Visceral, Cutaneous, &amp; Mucosal</td>
<td>Southern Mexico to northern Argentina</td>
<td>New World</td>
</tr>
</tbody>
</table>
b. Parasite Life Cycle

The *Leishmania* parasite is transmitted via the bite of the phlebotomine female sandfly, so it has a digenetic life cycle including the mammalian host and the insect vector (CDC, 2023). The sandfly stages begin when the sandfly takes a blood meal from an infected host (human, canine, or other animal reservoirs) and ingests a macrophage with *Leishmania* amastigotes. The amastigotes turn into amastigotes once taken up from the macrophage (Handman E., 2002). The developmental sequence of the five major promastigote forms, seen in figure 2.2, are as follows: procyclic promastigotes, nectomonad promastigotes, leptomonad promastigotes, haptomonad promastigotes and metacyclic promastigotes (Hayes, 2014). The exact position of haptomonad promastigotes in the developmental sequence is uncertain. (Sunter 2017; Dostálová, 2012).

<table>
<thead>
<tr>
<th><em>Leishmania</em></th>
<th>Visceral, Cutaneous, &amp; Mucosal</th>
<th>North of Amazon Basin</th>
<th>New World</th>
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<tr>
<td><em>Viannia guyayensis</em></td>
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<tr>
<td><em>Leishmania</em></td>
<td>Visceral, Cutaneous, &amp; Mucosal</td>
<td>Colombia &amp; Central America</td>
<td>New World</td>
</tr>
<tr>
<td><em>Viannia panamensis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Leishmania amazonensis</em></td>
<td>Cutaneous</td>
<td>South America</td>
<td>New World</td>
</tr>
</tbody>
</table>
a) comparison between amastigote & promastigote forms
b) developmental sequence of the five major promastigote forms
K = kinetoplast; N = nucleus; F = flagellum. (Reproduced with permission from Sunter 2017)
The *Leishmania* cell cycle, includes the following phases: G1, S (DNA synthesis), G2, M (mitosis), and C (cytokinesis). G0 refers to nonreplicative metacyclic promastigotes which are not committed to replication at all. G1, the longest stage, includes the increase in transcription and protein synthesis. DNA replication begins during the S phase. The amount of replication origins on chromosomes for *Leishmania* is still unknown as there has been debate amongst differing studies (Efstathiou, 2021). G2 phase includes duplication of centrioles and cytoplasmic organelles, resulting in increased cell volume and size (Kimblin, 2008). M phase refers to the stage where mitosis is performed, but chromosomes are not condensed into 30-nm fibers. *Leishmania* parasites lack the N-terminal portion and globular domain of histone H1, making it unable to dismantle their nuclear envelope (Dostálová, 2012). Between mitosis and cytokinesis, the cell undergoes remodeling to increase in length and width. Cytokinesis then signals the end of the cell division cycle (Hecker, 1985).

Infection of a mammalian host begins when a sandfly takes a blood meal, and metacyclic promastigotes are regurgitated into the site of the bite (Sunter, 2017). From the site of the bite, macrophages are recruited for healing which cause the promastigotes to be phagocytized by the macrophages (Sunter, 2017). Within the macrophages, promastigotes differentiate into amastigotes which are able to multiply and infect different tissues throughout the host. A complete life cycle of *Leishmania* transmission from sandfly to human is illustrated in Image 2.3 (CDC, 2023).
Clinical Presentation

Cutaneous Leishmaniasis

Cutaneous leishmaniasis, the most common presentation of the disease, has an estimated one million new cases per year (WHO, 2023). It is characterized by mild to severe skin lesions on the extremities and face (WHO, 2023). The infected host’s immune system has a curious role in the how and where the infection presents. Unusual presentations of CL can be lupoid features on the face (erythematous infiltrated plaque),
lip fissures, or psoriasiform plaques on the nose (Gupta L.K., 2017). After the bite of the sandfly, the incubation period for *Leishmania* can vary depending on the species but commonly is between 2-8 weeks (PAHO/WHO, 2023). Disease presentation typically begins as a red, 19alagas lesion which grows over time eventually forming an ulcer (Grevelink, 1996). Lesion severity and average time to heal is incredibly variable and depends on the host’s immune system, treatment options, *Leishmania* species, and the host environment. Some ulcers are minor, taking only weeks to heal on their own (Piscopo, 2007). However, it is common for ulcers to take months to heal and results in severe scarring, causing lifelong disfiguration. Figure 2.4 illustrates an example of the healing sequence for cutaneous leishmaniasis (Afghan, 2011).

![Figure 2.4 Stages of Cutaneous Leishmaniasis](Reproduced with permission from Leishmaniasis Images | DermNet, 2023).

**Mucosal Leishmaniasis**

Mucosal leishmaniasis is a presentation of the disease that mainly affects the mucosal tissues, typically the nostril walls, the larynx, and the oral cavity, however, infection can be present throughout the body. Consistent with cutaneous leishmaniasis, symptoms can vary depending on species genetics, host genetics, and environmental
factors. Mucosal leishmaniasis clinical signs begin similar to cutaneous leishmaniasis, originating with small ulcers and lesions on the body (Strazzulla, 2013). Mucosal leishmaniasis can develop months to years after original cutaneous infection. Clinical signs begin as oral and pharyngeal abnormalities such as congestion or bleeding and lymph node enlargement (CDC). Mucosal leishmaniasis has a higher incidence rate than visceral leishmaniasis but is still not as frequent as cutaneous (CDC). It is a more severe and threatening form of the disease as it jeopardizes important areas such as the face and airway. The reasons why mucosal leishmaniasis specifically attacks the mucosal linings are poorly understood but it is assumed to have correlation to parasite characteristics (de Oliveira, 2012).

Mucosal leishmaniasis in the pharynx is a common foci for infection. Symptoms begin as mild to severe edema in the uvula, tonsillar pillars, and the posterior wall of the pharynx (Jirmanus L, 2012). Progression involves increase of granulation tissue and involvement of the Wladeyer’s lymphatic ring (Di Lella F, 2006). Mucosal leishmaniasis in the larynx is the third most common foci for infection (Di Lella F, 2006). Symptoms begin with increase in granulous tissue and increased inflammation throughout. A common symptom with laryngeal infection is alteration in an individual’s voice, illustrating damage to the vocal cords. Involvement of the ears is not a common characteristic of mucosal leishmaniasis occurring at a low incidence (Di Lella F, 2006). Similarly, involvement of the mouth is uncommon but is a threatening potential complication. The conditions described above with mucosal leishmaniasis are most times life threatening or life altering conditions for patients. If the disease does begin to heal,
severe disfigurement and scarring is present for the rest of a person’s life. Images of different effects of mucosal leishmaniasis can be seen in Figure 2.5. (Bowles, 2015, Miranda 2007).

Figure 2.5 Stages of mucosal leishmaniasis (Reproduced with permission from Bowles, 2015; Miranda, 2007). 1) skin lesion on the extremities; 2) lesions on the tongue; 3) severe, late stage mucosal leishmaniasis involving a majority of the face.

**Visceral Leishmaniasis**

The last presentation of leishmaniasis is visceral leishmaniasis, the least common presentation but the deadliest of the three (Pasha, 2022). Transmission is the same as mucosal and cutaneous but differs in that it can also be transmitted via blood transfusion,
intravenous drug use, organ transplants, and other accidents that pass bloodborne pathogens (Pasha, 2022). Infection usually begins when amastigotes invade host tissues, mostly the spleen, bone marrow, liver, and lymph nodes. Incubation period ranges from 2-6 months but can be longer/shorter depending on the patient (WHO, 2023). Visceral leishmaniasis is sometimes mild without many prevalent clinical signs, but it also has the ability to present in a much more life-threatening form (Torres-Guerrero, 2017). Signs include fever, weight loss, hepatosplenomegaly, pancytopenia, anemia, and skin rash. As visceral leishmaniasis attacks important organs in the body, cases without treatment are typically fatal (van Griensven, 2019).
Figure 2.6 Hepatosplenomegaly seen in a patient with severe visceral leishmaniasis (Reproduced with permission from PAHO/WHO, 2012)
Laboratory Diagnosis

Cutaneous leishmaniasis

Cutaneous leishmaniasis leads to ulcers and lesions that are similar to many other diseases. Therefore, efficient, and accurate laboratory diagnosis is crucial for disease treatment. Diagnostic methods include parasitological examination, molecular testing, or clinical examination.

The *Leishmania* intradermal skin test is a former diagnostic method with a sensitivity rate of 86-100% (Pagheh, 2014). This test is performed by injecting *Leishmania* antigen into the forearm in order to evoke immune response from previously infected individuals. Positive diagnosis is based on hypersensitivity skin reactions. Skin reactions to LST ≥ 5 mm is positive and <5 mm is negative (Goto, 2010). Unfortunately, this test is not able to differentiate between past and present infections. Frequently, individuals with healed leishmaniasis will test positive for life with LST. Additionally, the *Leishmania* antigen needed for this test is not being actively manufactured, making this test not currently an accessible option for most countries (de vries, 2015). Another less commonly used laboratory test for cutaneous leishmaniasis is serological tests. These tests are not utilized as frequently due to the low humoral immune response caused from leishmaniasis infection (de vries, 2015). There are a few immunological clinical trials but none that have succeeded so far (Carstens-Kass, 2021).

Direct parasite diagnosis is currently the gold standard for diagnostic methods (Hosseinzadeh, 2012). Histopathologic examination for cutaneous leishmaniasis is done via an in vitro parasite culture with materials from lesions. Diagnosis is characterized by
visualization and confirmation of amastigotes, that are round or oval bodies 2–4 μm in diameter with distinctive nuclei and kinetoplasts. Visualization of amastigotes under microscopy are seen in Figure 2.7. The edge of ulcers and lesions are the most accurate samples for testing. Disadvantages of this diagnostic method include limitations to determining what species of *Leishmania* is present. However, visualization is extremely accurate and non-invasive for the patient. Species determination is an important tool for patient treatment and preventative development and isoenzyme analysis has been an avenue to accomplish this (Sousa, 2014). Isoenzyme analysis is a method that analyzes electrophoretic banding patterns to study species differences of intracellular enzymes. This process has been used to differentiate between *Leishmania* species and to develop phylogenetic trees (Sousa, 2014). This process can be time consuming and expensive, so it is not an accessible method for most. A more sustainable option for species differentiation is PCR testing (de Vries, 2015).
Although laboratory testing is the ideal option for diagnostics, it is not available in most places where leishmaniasis is common. Leishmaniasis is typically prevalent in impoverished areas where medical resources are limited. So most commonly, patient diagnosis is reliant on symptoms, patient history, and assessed risk factors. It is crucial for disease control progression that affordable and reliable diagnostics become available for cutaneous leishmaniasis, specifically to have a test that also differentiates species. Without further development and research in this area, treatments and preventatives will also fail to
advance. Risk factors and socioeconomic factors will be discussed in later sections of this review.

**Mucosal Leishmaniasis**

Diagnostic methods for mucosal leishmaniasis are comparable to cutaneous leishmaniasis. The reliable standard for diagnosis is the visualization of amastigotes in mucosal lesions. However, accuracy of histological methods is apparently dependent on *Leishmania* species. For example, *L. infantum* is able to be easily identified with this method using the Giemsa or Hematoxylin-eosin stains. Comparably, the strain *L. braziliensis* is only effectively diagnosed half of the time using this method. This difference is understood to be due to the number of amastigote bodies in lesions. *L. infantum* has a higher number of bodies present in infection when compared to *L. braziliensis*. This distinction with mucosal leishmaniasis that is not known to be true for other presentations of leishmaniasis and might shed light on the reason why different mucosal species of *leishmania* cause varying symptoms with ranging severity (Bowles, 2015).

Immunohistochemical identification can provide information regarding leishmania species by identifying specific enzymes using zymodemes. However, this method is not used frequently anymore due to the increase of PCR methods. PCR methods are efficient and effective methods that work for both amastigotes or promastigote identification. Additionally, it is between 97-97% accurate (Wu 2020). However, PCR methods fall short due to cost and accessibility. The Montenegro Skin Test is also used for mucosal leishmaniasis, but again is not able to detect the difference between previous and new infections. Lastly, serological tests for mucosal leishmaniasis have proved more useful
than cutaneous. Antigens can be detected using ELISA and immunoblot. However, accuracy is lower for these tests to due cross reactions of antigens of other trypanosomes. All the above-mentioned tests are helpful tools in leishmania diagnosis but not necessarily in species identification, a useful tool for effective patient treatment. Similar, to cutaneous disease, mucosal leishmaniasis is lacking affordable and reliable diagnostic methods at this time (Bowels, 2015).

**Visceral Leishmaniasis**

Like other Leishmania presentations, direct visualization via microscopy is the current gold standard for diagnosis. Unlike cutaneous or mucosal leishmania, visceral leishmaniasis diagnosis must involve visualization of invasive samples such as spleen, bone marrow, lymph node aspirates, or liver biopsy (Herwaldt BL, 1999). Bone marrow aspiration is most performed for sample collection but occasionally spleen aspiration. Spleen aspirates rate around 93-99% effective whereas bone marrow rates around 53-86%. Diagnosis of visceral leishmaniasis is more difficult than mucosal or cutaneous. Noninvasive samples of peripheral blood, buffy coat, and peripheral blood mononuclear cells have been evaluated but with low success. Serologic methods are again available for visceral leishmaniasis but are not used often. They are more commonly used in high income countries with stable medical establishments (Gari-Toussaint M, 1994). The gold standard for visceral leishmaniasis diagnosis continues to be direct parasite diagnosis. However, antibody-based diagnosis such as rK39 strip test and the direct agglutination test (DAT) can be affordable options for endemic countries (Chappuis F, 2006). They
sometimes produce false positives in healthy individuals, but false negatives are not common.

Figure 2.8 (Reproduced with permission from van Griensven, 2019): *Leishmania* amastigotes found in spleen tissue of a visceral leishmania patient. Red arrows show the kinetoplast & black arrows show the nucleus.
References


CHAPTER THREE
VECTOR, HOST & PATHOGEN

*Leishmania Parasite*

Structure & function

*Leishmania* has two predominant forms, the amastigote in the mammalian host and the promastigote in the sand fly (Clos, 2022). There is not a great difference among internal organelle structures between the two, they both have the same organelles which function relatively the same (Kima, 2007). Diagrams with listed organelles can be found in Figure 3.3. The cell shape in both the amastigote and promastigote are conserved by cross-linked subpellicular corset microtubules (Aleman C, 1969). The kinetoplast, a mass of concentrated mitochondrial DNA, is found in an important organelle connected to the basal body, which from the flagellum extends (Yilmaz, 2022). The kinetoplast sits at the base of the flagellar pocket, an important morphological detail. The flagellar pocket allows a necessary interface between the parasite and the host which allows for pathogenicity. (Ogbadoyi, 2003). The essential aspect of defining cellular morphology is the analysis of the flagellum, kinetoplast, and the flagellar pocket and their relation to each other. The *Leishmania* parasite has a specifically conserved cell shape, flagellum length, and kinetoplast/nucleus position as they are all crucial to the parasite’s survivability (Hayes P, 2014).
**Genome plasticity**

The genome diversity amongst *Leishmania* species is high amongst species. Consequently, treatment and vaccine development has proven to be difficult. Different species of *Leishmania* parasites have variation in chromosome number and gene content (Ivens AC, 2006). The *Leishmania* genome it is 32 Mb and has over 8,300 coding genes (Ivens AC, 2005). Genes across species are relatively conserved with only a few species-specific genes (Peacock, 2007). The *Leishmania* parasite lacks transcriptional control, so it uses a few unique adaptive mechanisms to be able to control gene expression depending on the environmental conditions (Ubeda, 2014). For example, mRNA stability and translation rates are usually regulated by the 3’ untranslated regions of the parasite (Boucher N, 2002). To overcome drugs and to facilitate resistance, *Leishmania* is able to utilize DNA variation methods like aneuploidy, gene amplification, and gene deletion (Muller M, 2010).

Cellular aneuploidy refers to the occurrence of differing numbers of chromosomes, either by deletion or addition (Mannaert, 2012). Typically, aneuploidy results in cell death or in harmful phenotypes (Peacock, 2007). However, the *Leishmania* parasite can utilize aneuploidy to its advantage (Sterkers, 2012). *Leishmania* can amplify or delete smaller specific regions of DNA (Downing T, 2011). Because the genome has several repeated DNA sequences (RSs), nearly all of the genome is able to be rearranged to generate new chromosomal elements (Mannaert, 2012). Therefore, the *Leishmania* parasite can amplify or rearrange regions under drug or environmental pressures, making it extremely adaptable. Additionally, single-nucleotide polymorphisms (SNPs) and small
nucleotide insertions/deletions also add to potential genome plasticity (Ubeda JM, 2014). Understanding the variable genome of *Leishmania* is crucial in understanding why treatment is so difficult.

**Anti-Immune System Strategies**

The *Leishmania* parasite does not evade the immune system but rather take advantage of innate immune cells and modulating their phenotype and function (Leprohon, 2014). Immediate steps after initial infection are critical for successful, long-term infection (Basmaciyan, 2019). The pro-inflammatory properties of the sandfly saliva play a crucial role in phagocyte chemoattraction; therefore, promoting parasite uptake via phagocytosis (Basmaciyan, 2019). The lipophosphoglycan (LPG) coat protects the parasite from degradation and allows for successful differentiation into the intracellular form, the amastigote (Bichiou, 2015). A summary of the immune cell processes is included in figure 3.1.
The interaction between the macrophage and *Leishmania* parasites have an important interaction as it determines the ability of the infection to become long-lastin (Bichiou, 2015). While macrophages are usually used to destroy harmful invaders to the body, the parasite can overcome this challenge and utilize the immune cell to its advantage. The macrophage typically uses reactive oxygen species (ROS) and reactive nitrogen species (RNS) to destroy engulfed pathogens. However, the *Leishmania* parasite is equipped to avoid these processes, the first line of defense being the LPG coat (Bichiou,
Second, the parasites can induce the macrophage to produce arginase, which provides key nutrients for the pathogen as well as diminishes the production of the parasitotoxic nitrogen (Liu, 2012). Third, the leishmanial metalloprotease gp63 is able to interfere with macrophage signaling pathways leading to ROS and RNS reduction (Liu, 2012). The amount that *Leishmania* is able to inhibit macrophage attacks is unique to the parasite species, a potential implication for why visceral leishmaniasis is much more severe than the other forms of the disease (Liu, 2012).

Figure 3.3. Macrophage & *Leishmania* interaction (Liu, 2012).
Leishmania parasites also have a unique interaction with neutrophils. Neutrophils are rapidly recruited at the site of the infection to produce neutrophil extracellular traps (NETS) to inhibit parasite infection. NETs are networks of extracellular fibers composed of DNA and neutrophils which are able to trap pathogens. Again, the ability of the neutrophil NETS to be effective against the Leishmania is species-dependent. Neutrophils can kill L. amazonensis, but other species of Leishmania are able to benefit from the presence of the NETS. L. donovani and L. infantum are completely resistant to the NETS and utilize their features to be a long-lasting disease (Semini, 2017). L. Mexicana is able to utilize the NETS to create more severe cutaneous lesions. The exact interactions between neutrophils and the parasite are unknown but this immune roadblock is no problem for most species of Leishmania (Semini, 2017). The third oddity to discuss shifts from the immune system interactions to the parasite cell structure. Leishmania parasites have a unique flagellum that is crucial to their ability to transition from promastigote to amastigote, key steps in the life cycle. Flagellum function includes motility, attachment within the sand fly gut, and potential sensory functions. Therefore, it is critical for infection transmission (Goto, 2023). Procyclic promastigotes have long, motile flagellums but metacyclic promastigotes have an even longer flagellum with a shortened cell body to increase speed and motility. However, when promastigotes invade macrophages and enter the amastigote phase there is a dramatic shift in cell body shape (Goto 202). The flagellum shrinks so that it is barely emergent from the flagellar pocket and the cell body becomes short and round. The amastigote flagellum is not motile but it still has an important function in signaling and parasite nutrition. The differences between the two stages can be seen in figure 3.3. Most parasites are unable to
transition from promastigote to amastigote flagellum so easily (Goto, 2023). Typically, parasite amastigotes lose flagellum function completely. However, this oddity is a crucial aspect of the infectious nature of *Leishmania* (Loría-Cervera, 2017).
Figure 3.3. The difference between the promastigote & amastigote stages (Reproduced with permission from Landfear, 2022). a) microscopy pictures of Leishmania procyclic/metacyclic promastigote vs amastigote. b) comparison between promastigote & amastigote forms with organelle details.
Sandfly

Difference in Fly Vector Species

The phlebotomine sandfly is an important aspect of the leishmaniasis disease as they are the main vector of the *Leishmania* parasite species. There are ~1000 sandfly species but only around 100 that can transmit the disease to humans (Chagas, 2018). Like parasite classification, the sandfly is categorized by Old World and New World species. Old World species include three genera: *Phlebotomus*, *Sergentomyia*, and *Chinius* which originate in the palaearctic region, the afrotropical region, the alagasy region, the oriental region, and the Australian region. The New World sandflies include the following genera: *Lutzomyia*, *Warileya*, and *Brumptomyia* which originate in the Nearctic and Neotropical regions (Akhoundi, 2016). However, the *Leishmania* vectors in Central and South America belong to the *Lutzomyia* species or the *Phlebotomus* species in Africa, Asia, and Europe (Figure 3.4). The most common *Leishmania* species are transmitted by select sandfly species, despite the great diversity between both parasite and vector species (Bates, 2008).
Figure 3.4 Geographical distribution of sandfly species (Reproduced with permission from Cecílio, P., 2022)
Structure & Function of the Sandfly

The female phlebotomine sandfly (Figure 3.5) is the vector of the Leishmania parasite and is a crucial aspect of understanding the leishmaniasis disease. The Leishmania parasite begins developing as a promastigote in the lumen of the sandfly’s alimentary tract by attaching to the wall of the canal. In the midgut of the sandfly, promastigotes attach via their flagella. Additionally, the hindgut is lined with cuticle which serves as a developmental destination for some Leishmania species. While the parasite is in the sandfly, it undergoes developmental advancements that are crucial to the infectivity in the host, therefore, the sandfly and Leishmania parasite relationship is important (Ramalho-Ortigao, 2010).

The infection of sandflies with the Leishmania parasite begins when amastigotes are ingested by the fly during a blood meal. It takes ~ 4-5 days for the parasite to develop from an amastigote to infective metacyclics. The sandfly attempts to eliminate parasites in the midgut via digestive proteases, but parasites are able to protect themselves via the phosphoglycan containing molecules which are surface bound to the amastigotes (Pitaluga AN, 2009). Additionally, the surface LPG coat is also able to protect against proteases. The sandfly also attempts to inhibit the parasite via the peritrophic matrix. This aspect is supposed to stop the parasite from leaving the endoperitrophic space which the parasite is able to via the use of chitinases. Chitinases are also crucial for the Leishmania migration to the thoracic midgut area of the stomodeal valve. The chitinases attack the lining of the valve, making it no longer functional (Schlein Y, 1998). The morphology
and intricacies of the parasite and sandfly relationship provide yet another crucial point for research and treatment/vaccine development.

Figure 3.5 The morphology of the sandfly (Reproduced with permission from “Researching the Enemy”).

Canine Host

Clinical Presentation

Although leishmaniasis finds a home in several mammalian hosts, the canine host is a common reservoir. Canine leishmaniasis is similar to human leishmaniasis in a way that it can be just as severe and deadly (Maia, 2018). Control and prevention of canine leishmaniasis is an important aspect of control and prevention programs for human
leishmaniasis, as dogs can serve as a key reservoir. Canine leishmaniasis is mainly caused by the *Leishmania infantum*, which causes visceral canine leishmaniasis (REF). Canine Leishmaniasis is currently endemic to 70 countries but continues to increase yearly, spreading to new countries. Clinical signs are variable but can include skin lesions, weight loss, lymphadenopathy, splenomegaly, anemia, thrombocytopenia, and renal disease (Peterson, 2009). Survival rate with canine leishmaniasis is diverse as the leishmaniasis ranges from mild presentation to life-threatening disease, represented in Figure 3.6 (Solano-Gallego, 2011). Life expectancy of dogs with leishmaniasis is difficult to assess due to the variable nature of the disease (Pereira, 2020). Cutaneous canine leishmaniasis is also a possible presentation of the disease. Cutaneous is usually non-life threatening but painful and uncomfortable for the animal (Solano-Gallego, 2011).
Figure 3.6 Cutaneous leishmaniasis in canines (Reproduce dwith permission from Solano-Gallego, 2011). Diffuse lesions on the extremities with mucosal involvement seen in picture B & D.

Diagnostic methods for visceral canine leishmaniasis are an important aspect of disease control. Molecular diagnosis of the disease is the current gold standard for testing and can detect between .001 and .01 parasite presence. Parasite target molecular tests involve the identification of parasite features such as the DNA minicircles from the *Leishmania* kinetoplast, glycoproteins, and RNA. Sample source testing utilizes bone marrow and lymph node samples to look for parasite presence. Less invasive methods include swabbing of the conjunctive and oral mucosa (Solano-Gallego, 2009). POC molecular tests involve amplifying DNA to test for parasite presence. Serological
diagnosis involves the test of the presence of antibodies. The direct agglutination test (DAT) was the first serological test designed for canine leishmaniasis (Paltrinieri, 2016). It utilizes the agglutination of Coomassie-stained Leishmania promastigotes (Paltrinieri, 2016). It is a reliable test and low cost so has been used consistently for the past few decades (Sousa, 2011). The immunofluorescence antibody test (IFAT) is the most reliable test with a sensitivity of almost 100% in symptomatic canines. However, it also has cross reactivity with other trypanosomes (Paltrinieri, 2016). Flow cytometry quantifies antibodies against Leishmania surface antigens. Similarly, this method has high sensitivity and is a good diagnostic tool to be used in the coming years (Silvestre, 2008).
Prevention & Treatment

Prevention of canine leishmaniasis is focused on sandfly repellent in the form of spot treatment or collar form to prevent the sandfly bite, similar to how tick repellent works. There are a few different types of insecticide repellents, synthetic pyrethroids,
permethrin, or deltamethrin, with varying effectiveness (Solano-Gallego, 2011). Long-
acting insecticide collars and treatment are effective at preventing *Leishmania* infection
in dogs who live in endemic areas. However, they are only effective if the regimen is
accurately followed (Noli, 2014). Collars and treatments need to be reapplied continually
to maintain protection against sandfly bite. Additionally, these options are viable for pet
dogs but are not useful when addressing infected stray dogs, which are a significant
portion of the infected population. Preventing disease in stray animals is very difficult
and mostly relies on other environmental treatments, such as the regular administration of
insecticides in sandfly heavy areas. Genetic resistance to insecticide dog collars and
treatments have not been specifically studied but resistance to both methods is presumed
possible as the *Leishmania* parasite is prone to genetic plasticity (Rossi, 2008).

Unlike human leishmaniasis, canine leishmaniasis does have 4 approved vaccines,
summarized in Table 3.1. First generation leishmaniasis vaccines utilize killed/attenuated
parasites but have not at all been commercially approved by the WHO (Montoya, 2021).
Second generation vaccines are based on fractionated ancients and recombinant proteins,
which have had much greater success (Velez, 2020). Leishmune® was the first approved
second-generation vaccine in 2004 (Velez, 2020). It is formulated with a fucose mannose
ligand from the parasite promastigotes and a saponin adjuvant (Gradoni, 2015). However,
it has been discontinued due to lack of effectiveness. Trial III phases showed limited side
effects and successful reduction in disease (between 60-80%). Studies were generally
positive regarding the possible prognosis of the Leishmune vaccine but nevertheless
research was discontinued. Leish-Tec® is another second-generation vaccine that protects
against *L. donovani* using a recombinant protein A2 from parasite amastigotes with saponin as the vaccine adjuvant (Gradoni, 2015). Trials have shown to induce immunity with minimal side effects (Toepp, 2018). A recent study on Leish-Tec® in 2017 in Brazil showed significant presence of anti-A2-specific IgG antibodies. Additionally, the incidence of infected vaccinated dogs was 27% while unvaccinated was 42%. Therefore, it was concluded that Leish-Tec® is still a viable canine leishmaniasis vaccine to be used in combination with other methods (Grimaldi, 2017). CaniLeish®, released in 2011, is formulated from proteins of *L. infantum* promastigotes with an adjuvant of Quilaja saponaria saponin (QA-21). The vaccine has shown to be tolerated well with few side effects and has shown to reduce infectious burden, so is currently still used (European Medicines Agency, 2016). The last clinically approved vaccine is LetiFend®, was first approved in 2016. It is a recombinant vaccine using a chimerical protein made up of flour proteins from *L. infantum*. The proteins are ribosomal proteins LiP2a, LiP2b, and LiP0 as well as histone H2A. Side effects are minimal and non-life threatening. It has been found to have a 72% efficacy at preventing canine leishmaniasis (Veterinary Medicines Division, 2016). Clinically approved vaccines are summarized in Table 3.1.
### Table 3.1 Canine vaccine summary

<table>
<thead>
<tr>
<th>Vaccine Name</th>
<th>Species</th>
<th>Outcome</th>
<th>Vaccine efficacy</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leishmune</td>
<td><em>L. donovani</em></td>
<td>Protection against canine leishmaniasis</td>
<td>76-80%</td>
<td>17, 18</td>
</tr>
<tr>
<td>Leish-Tec</td>
<td><em>L. donovani</em> <em>L. infantum</em></td>
<td>Some cases of protection; some infection breakthrough</td>
<td>58-70%</td>
<td>19, 20, 14</td>
</tr>
<tr>
<td>CaniLeish®</td>
<td><em>L. infantum</em></td>
<td>Protection against canine leishmaniasis</td>
<td>68-92%</td>
<td>19, 21</td>
</tr>
<tr>
<td>LetiFend®</td>
<td><em>L. infantum</em></td>
<td>Protection against canine leishmaniasis</td>
<td>72%</td>
<td>16, 22</td>
</tr>
</tbody>
</table>

A complete cure for canine leishmaniasis has not yet been discovered so treatment is aimed at reduction of parasitic load, symptomatic control, and decrease transmission rate. Allopurinol is a treatment method used in many places that has had moderate success (Ahuja, 2012). It has low toxicity and can improve clinical signs quickly (NOLI et al., 2005). Allopurinol is a hypoxanthine analogue that can disrupt purine metabolism. Resistance has not yet been reported but is possible by the nature of the *Leishmania* genome (Torres, 2011). Antimonial therapy in canines is like that in humans and uses meglumine antimoniate and sodium stibogluconate. These drugs are toxic but are still used predominantly in the Mediterranean region (Morales-Yuste, 2022). Resistance to antimonials in canine leishmaniasis is known and will likely increase if the drugs are continued to be used (Ahuja, 2012). Miltefosine is an oral antileishmanial drug that is
used in canines with severe leishmaniasis, usually with kidney damage. It has been successful in reducing clinical symptoms, but resistance has been described (Gonçalves, 2021). Paromomycin is an aminoglycoside antibiotic (type of bactericidal antibiotics for infections caused by Gram-negative pathogens) that is used for canine leishmaniasis. The mechanism of paromomycin likely includes blocking protein synthesis and it has shown to be useful when used in combination with allopurinol (Hirokawa, 2007, Athanasiou LV, 2013). Amphotericin B, commonly used for fungal infections, causes membrane instability in the *Leishmania* cell leading to cell death. It is not commonly used in canine leishmaniasis but has the possibility to be used in the future (Hernández, 2015).
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CHAPTER FOUR

DISEASE CONTROL & TREATMENT IN HUMANS

Prevention

Prevention of leishmaniasis can be categorized into three broad categories. Vector control, animal control, and human control (Figure 4.1). Vector control relates to different levels of environmental management, with the most popular being the use of insecticides. Insecticides can be used to spray the outside of residential areas as well as directly on termite mounds/animal burrows. In most insecticide studies, there have been divided results on whether insecticides are actually effective enough to justify potential environmental damages. There are also few studies directly studying human infection rates after insecticide intervention, most studies just relate to reduction of sandfly population. Insecticides are a good option for short-term delay but not ideal as a long-term option as it is possible that the sandflies will evolve to become resistant to such treatment. One study done by Davies et al. in 2003 studies specifically the results from using insecticides on the inside of households. The results showed a significant reduction of infection rates between the village with consistently sprayed houses and the control village. Limitations of this study include length and pre-trial comparison data. The study was only two years, so it is difficult to know how this method would sustain over multiple years. Additionally, this study also does not include pre-study infection data so there is no way to compare findings to original conditions. (Davies, 2000). Other than insecticides, vector control involves housing initiatives such as plastering of walls and increased access of reliable housing for residents in disease-stricken areas. Such initiatives are
beneficial for not only the improvement of leishmaniasis rates but many other diseases. Similarly, increased disease prevention education is necessary for the advancement of overall health as a society. However, there are no studies that analyze housing initiatives and education as producing leishmaniasis reduction. Therefore, no direct conclusions can be drawn.

Figure 4.1 Leishmaniasis prevention in humans, vectors, and animal population
Animal reservoir control mainly includes the use of insecticides, vaccines, and animal control/animal elimination, most often directed towards canines as they are the most frequently infected animals with leishmaniasis and have a large correlation with increased human transmission. Again, the limited studies on the direct effects of human disease prevention with these methods have made the effectiveness of this method inconclusive. Animal elimination involves specifically reducing the amount of a specific animal in a certain area. Dietze et al. (1997) studied the direct impacts of canine reduction in Brazil on human infection rates. The study involved two different valleys in Brazil, one with animal control and the other without. Results after 12 months showed no significant reduction in human leishmaniasis infection in humans (Dietze, 1997). Animal insecticides can range from spot treatments to long-term insecticide canine collars. Gavgani et al. studied the effects of insecticide collars used on domestic dogs in Iran on the rates of pediatric visceral leishmaniasis. The results of this study showed significant reduction in pediatric infection rates after one year (Gavgani, 2002). These studies indicate potential correlation for reservoir control, an area of continued consideration for prevention research and disease control (Stockdale, 2013). However, animal culling has not experienced great long-term success as control for other infectious diseases (Degeling, 2016). At best, it has been a temporary solution to reduce infection in a region for a minimal amount of time, the rate of newly infected canines can outpace dog culling efforts. In places where canine culling is common, such as Brazil, the overall seropositivity rates of both domestic dogs and stray dogs has not decreased over the years (Sousa-Paula LC, 2019). It has been speculated that culling could be beneficial for
leishmaniasis control if combined with other interventions, but the practice remains controversial and is met with resistance in most regions (Degeling, 2016). Prevention of human disease from dog reservoirs would ideally include both a human vaccine, but this has not yet been developed. However, other methods that are used include insecticide-treated bed nets, untreated bed nets, and insecticide barriers (clothing, curtains, soap, etc). Bed nets, both untreated and treated, have had a significant history in protecting against other infectious diseases, such as malaria (Goodman, 1999). Review studies of bed nets and leishmaniasis have shown they are positively correlated with decreasing the overall exposure to sandfly population. However, they are not necessarily correlated with decrease in overall human leishmaniasis incidence in an area. The issue with bed nets is that they may not be used correctly, and they only protect against sandfly bites at night (Montenegro-Quiñonez, 2022). However, they are a low-cost option that can be easily accessible to most populations, so they are a potential solution to use in combination with other methods. Likewise, insecticide impregnated fabrics have also been shown to reduce leishmaniasis. Additionally, clothing of all types can act as a barrier for leishmaniasis prevention (Stockdale, 2013). The CDC and the WHO recommend covering exposed skin whenever possible when frequenting *Leishmania* prevalent areas (CDC).

**Vaccines**

The most effective form of leishmaniasis prevention would potentially be a vaccine option, for both humans and canines. An effective vaccine would be one that
would be both safe and accessible to the general population. However, a safe vaccine against human leishmaniasis has yet to be developed. In this section, I will discuss the major approaches to vaccine development in human leishmaniasis.

Pathogen-based vaccines

Pathogen-based vaccines utilize a live version of a pathogen to generate disease immunity. One of the first types of leishmania vaccines to be utilized was referred to as leishmanization. Simply, leishmanization is the injection of live leishmania parasites into the skin. Injection would occur primarily on non-vital areas of the body, such as the extremities, to avoid infection at a later time in vital areas of the body. The hope was that a minor, controlled infection would provide immunity against subsequent and possibly more major infections. This method has shown promising results in some cases but is no longer a viable option due to the unpredictable nature of leishmaniasis disease progression in humans (Duthie, 2022). For example, concerns that the vaccination would result in the emergence of a mutated (and thereby potentially more pathogenic) strains of *Leishmania* limit enthusiasm for this approach. Frequently, even after leishmaniasis lesions are healed, the parasite still exists within the body. Therefore, infection could be recurrent (Kedzierski L, 2010). This especially poses a major threat for the immunocompromised, including the elderly, the sick, etc. Leishmanization is illustrated in Figure 4.2 (PachecoFernandez, 2021). From 1964 to 1986, leishmanization was performed using *L. major* on over 160,000 people in Iran. Overall, the incidence rate in the Iran area fell in 1983 and 1984 and for the vaccinated who did become infected, lesions were smaller and healed quicker (Duthie, 2022). Leishmanization has promising
results but would need to be developed to be a safer and more consistent option (Nadim, 1997).

Live attenuated vaccines use a weakened form of the parasite to induce an immune response, like the process of leishmanization but with potentially less severe side effects. Attenuation *leishmania* parasites fall into two broad categories, undefined or defined. Undefined attenuation via genetic modification implies that the specific genetic modification is not always known. These modified parasites can be generated by long term *in vitro* culture (spontaneous emergence of attenuation) or through chemical mutagenesis. Defined attenuation involves the targeting of specific genes that are involved in virulence. Defined attenuation is the preferred method as the plasticity of the *leishmania* genome will sometimes lead to reversion back to virulence when using
undefined attenuation. Examples of both undefined and defined attenuation can be found in Table 4.1. While live attenuated vaccines offer hope for leishmaniasis prevention, there are still a few potential drawbacks. Whenever live vaccines are under consideration, a major concern is reversion to virulence (Zabala-Peñafiel, 2020). Additionally, there is concern that modified parasite species could recombine with a virulent parasite to give rise to more pathogenic progeny (Akopyants, 2009). Live attenuated vaccines may also be a difficult option due to access and sustainability of working with live parasites. Similarly, the financial cost to develop such research is significant. Therefore, making live attenuated vaccines may not be a sustainable option for developing countries (Gillespie, 2016).

Killed parasite vaccines use dead whole-parasites to induce immunity against leishmaniasis. Killed parasites are treated and killed in a controlled setting via autoclave, heat therapy, cycles of freezing and thawing, or by formaldehyde (T. Kobets, 2011). The major appeal of such methods is the safety and cost effectiveness. However, these types of vaccines are typically less effective when compared to live vaccines (Bruhn KW, 2012). Killing the *leishmania* parasite causes key proteins related to immunogenicity to be lost. Additionally, when using a killed parasite vaccine, the life cycle of the *leishmania* parasite is no longer captured, a potentially crucial aspect to invoking immune response (Selvapandiyan, 2009). Studies have shown that killed parasite vaccines fail to induce long-term immunity, a potential limitation (Okwor, 2009, Armijos, 1998).

A potential category of pathogen-based vaccines are those that utilize *leishmania* proteins to induce immunity. In this section, I will highlight a few of the most promising
peptide vaccines. First, the glycoprotein 63 (gp63), also known as leishmanolysin, is a metalloprotease found on the surface of the *Leishmania* parasite. This is the most abundant promastigote protein, and its expression is upregulated in amastigotes. The function of the protein is to enhance the ability to adhere to macrophages. Therefore, interruption of gp63 function in *Leishmania* parasites could hinder the ability of parasites to survive and reproduce in the host (Mercado-Camargo, 2020). Results using the protein as a vaccine have been inconclusive, with the reasons for the failure of some experiments and success of others being unclear but likely due to the adaptive nature of the disease, *Leishmania* parasite is able to adapt to these vaccination attempts.

The LACK protein is an antigen found in *L. major* that has linked functions to signal transduction, RNA processing, and cell cycle control (Kelly, 2003). Studies have shown the LACK antigen induces partial immunity to parasites (Gurunathan, 1997). The promastigote surface antigen (PSA) is another major membrane bound protein found in *Leishmania*. The primary function of this category of antigen is parasite/host interactions. Studies using this antigen have shown success by producing immunity (Handman, 1995). There are multiple other potential targets on the *Leishmania* parasite for protein vaccine development creating great opportunities for continued research in this area. Lastly, DNA vaccines are being considered as potential candidates. This category of vaccines involves the use of a specific DNA sequence typically encoding an antigen to invoke immune response (WHO, 2023). The targets of DNA *Leishmania* vaccines are similar to the protein vaccines discussed above. However, instead of injecting proteins directly, the DNA for genes encoding for those proteins is delivered. Generally, the DNA vaccines
provide longer lasting immunity when compared to protein vaccines. As referenced in Table 1, DNA cocktail vaccines have an even greater ability to control parasite burden (Sukhbir, 2016).

**Sandfly saliva based vaccines**

The second potential option for vaccine candidates are those targeting the sandfly vector, specifically, sandfly saliva proteins. By targeting saliva proteins, the vaccine would likely disrupt transmission. Currently, there are three hopeful candidates for salivary protein vaccination. First, PdSP15 is a salivary binding protein from the sandfly species *P. duboscqi* which is a vector of *L. major* in Sub-saharan Africa (Abdeladhim M, 2014). Second, LJM19 is a salivary protein whose function is currently unknown. Third, LJL143 is a salivary protein with anti-coagulant activity. Both of these are of the sand fly family *Lutzomyia longipalpis* which is a vector of *L. infantum* in Latin America (Gomes R, 2008). Studies have shown that sandfly-based vaccines are able to induce a TH1 delayed type hypersensitivity response. Trials have successfully induced immunity in canines, but salivary vaccines have not yet been explored in humans (Collin N, 2009).
Figure 4.3 Sandfly vaccine illustration (Reproduced with permission from Gomes R, 2008).
<table>
<thead>
<tr>
<th>Type of Vaccine</th>
<th>Example</th>
<th>Target Host</th>
<th>Parasite Species</th>
<th>Method</th>
<th>Outcome</th>
<th>Trial Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live pathogen based</td>
<td>Leishmanization</td>
<td>Humans</td>
<td><em>L. Major</em></td>
<td>Injection of live parasite into the skin</td>
<td>Significant protection increase; unpredictable side effects -&gt; not viable</td>
<td>2</td>
</tr>
<tr>
<td>Live Attenuated Parasites</td>
<td>BALB/c undefined modification</td>
<td>Mice</td>
<td><em>L. Major</em></td>
<td>Chemical mutagenesis of <em>L. Major</em> &amp; subsequent injection of parasite</td>
<td>Decreased lesion size</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>BALB/c undefined modification</td>
<td>Mice</td>
<td><em>L. Mexiana</em></td>
<td>Gentamicin Pressure of <em>L. Mexicana</em></td>
<td>Protection &amp; no lesions</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>BALB/c defined modification</td>
<td>Mice</td>
<td><em>L. Major</em></td>
<td>Knockout of the paraflagellar rod-2 gene &amp; subsequent infection</td>
<td>Decreased lesion size; non-motile <em>leishmania</em> parasites</td>
<td>5</td>
</tr>
<tr>
<td>Vaccination Type</td>
<td>Species</td>
<td>Host</td>
<td>Vaccine Constituents</td>
<td>Outcome</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>-------------------------------------</td>
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<td>----------------------------------------------------------</td>
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<td></td>
</tr>
<tr>
<td>Whole-killed parasites</td>
<td>C57BL/6 Mice</td>
<td>Mice</td>
<td>Injection of killed parasite into the footpad or rump</td>
<td>Resolved primary infection but loss of sustained infection induced immunity</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Killed promastigote vaccine cocktail + Calmette-Guérin adjuvant</td>
<td>Human</td>
<td>Human</td>
<td>Injection of killed promastigotes</td>
<td>72.9% protection after 12 months but 0% protection after 24 months</td>
<td>12</td>
<td></td>
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<tr>
<td>Protein based</td>
<td>Gp63, leishmanolysin</td>
<td>mice</td>
<td>T cell epitope containing gp63 injected subcutaneously</td>
<td>Protection and decreased lesion size</td>
<td>14</td>
<td></td>
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<tr>
<td>LACK antigen</td>
<td>mice</td>
<td>L. Major</td>
<td>Injection of LACK antigen &amp; subsequently infected with leishmania infection</td>
<td>Protection against cutaneous leishmaniasis achieved</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Promastigote Surface Antigen-2</td>
<td>mice</td>
<td>L. Major</td>
<td>Intraperitoneal vaccination with PSA-2 with corynebacterium parvum as adjuvant</td>
<td>Complete protection; no lesion development</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>Gp63 &amp; Hsp70 cocktail vaccine</td>
<td>mice</td>
<td><em>L. donovani</em></td>
<td>Immunized subcutaneously twice/every three weeks with T cell epitopes of gp63 &amp; Hsp70 individually &amp; in combination</td>
<td>Reduction in splenic &amp; hepatic parasite burden; greater reduction for cocktail vaccine.</td>
<td>20</td>
</tr>
<tr>
<td>---</td>
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</tr>
<tr>
<td>Sandfly Saliva</td>
<td>PdSP15</td>
<td>Rodents</td>
<td>NA</td>
<td>NA</td>
<td>7/10 subjects showed significant reduction in disease (CL)</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>LJM19</td>
<td>Hamster</td>
<td>NA</td>
<td>Intradermal inoculation</td>
<td>Reduced parasite load and reduced symptomatic presentation of VL</td>
<td>22</td>
</tr>
</tbody>
</table>
Treatment

Treatment development for leishmaniasis has proven to be difficult for the same reason as vaccine development. The extreme genome plasticity of *Leishmania* has made medication resistance common (Sinha R, 2018). Additionally, the variety of *Leishmania* species has made it challenging to develop treatment that is widely applicable. In this section, I will be addressing past and current leishmaniasis treatment options and benefits/drawbacks of each.

In the early 1900s, one of the first leishmaniasis treatments was developed, pentavalent antimonial. Urea stibamine was one of the first drugs developed to treat Indian kala-azar in the 1920s, followed by synthesis of antimony gluconate in the 1930s. There are currently two antimonials that are being used in the clinical setting, meglumine antimoniate (Glucatime) and sodium stibogluconate (Pentostam). However, the pathway of action of the antimonials is still not understood but it is theorized that the inhibition of SH dependent enzymes is likely important. These drug choices have low efficacy for a few reasons. First, administration of the drug is difficult to maintain. To be effective, these drugs must be taken daily for three weeks. This is a difficult regimen to guarantee in impoverished countries with low access to medical care. Second, the side effects associated with antimonials are sometimes severe. Side effects include intramuscular pain, nausea, vomiting, weakness, myalgia, diarrhea, rashes, hepatotoxicity, and cardiotoxicity (Frézard, 2009). These drugs are toxic, which is an area especially of concern for immunocompromised individuals such as the elderly, HIV/AIDS patients,
transplant recipients, pregnant individuals, etc. Additionally, high rates of drug resistance have been apparent throughout endemic regions (Haldar, 2011). For example, in 2014 around 70% of visceral leishmaniasis cases were resistant to antimonials. Resistance is correlated to parasite thiol metabolism. The thiol molecule increases oxidative stress in the macrophage inhibiting pentavalent reduction into trivalent form (REFS). Additionally, host factors are correlated with resistance. Host factor examples include inhibition of drug activation, gene amplification, reduced drug concentration inside the parasite, and decreased drug uptake (Mohapatra, 2014). For these reasons, antimonials are not the ideal option for leishmaniasis treatment and are not correlated with a high level of efficacy.

Liposomal amphotericin B is a treatment option developed in the last two decades for treatment of both visceral and cutaneous leishmaniasis (Sundar, 2010). Amphotericin B is able to limit parasite burden by binding to ergosterol, the major sterol component of the Leishmania parasite membrane (Sannigrahi, 2019). By binding to ergosterol, it compromises parasite membrane stability, resulting in leakage of cytoplasmic contents and cell death. This drug is administered intravenously daily for ~15-45 days. The length of therapy is related to the type of leishmaniasis as well as the causative species of parasite. Due to the mechanism of action, resistance to amphotericin B is not of great concern (Sundar, 2010) because it primarily affects ergosterol. However, it is an expensive drug and causes a range of side effects including nausea, vomiting, rigors, fever, hypertension/hypotension, and hypoxia. Nephrotoxicity is a very common long-term complication with amphotericin B and is a main reason why this drug is not an ideal option for treatment. Nephrotoxicity is a dangerous condition that involves hypokalemia (low potassium levels), hypomagnesemia (low serum magnesium levels), metabolic acidemia
(buildup of acid due to kidney disease), and polyuria (production of large amounts of dilute urine) (Laniado-Laborín, 2009).
Paromomycin is a relatively new broad-spectrum antibiotic treatment for leishmaniasis that was introduced in 2006. The mechanism of action is unknown, but it is theorized that it also affects membrane fluidity and interferes with mitochondrial membrane potential (REF), possibly as a result of blocking protein synthesis by binding to 16S ribosomal RNA. This has been a promising treatment option due to its relatively low cost, high efficacy, and reduced side effects and has been developed in capsule, injection, and topical form. Relative to other treatment options, paromomycin is the most cost-effective method on the market currently (REF), costing ~$2 US dollars to treat one patient. This drug is often used in combination with miltefosine for treatment of visceral leishmaniasis (Wiwanitkit, 2012).
Pentamidine is traditionally a less common option for leishmaniasis treatment due to high levels of toxicity and low efficacy. The mechanism of action of this drug is assumed to be the inhibition of the mitochondrial topoisomerase II, which is crucial for the basic functioning of the cell (REF). Increased resistance has caused pentamidine to become less effective over the years (Tiwari, 2019). Furthermore, there are concerns that pentamidine could cause irreversible damage to an individual, the main reason why it is not currently recommended as a treatment option. Around 50% of all pentamidine users experience some kind of adverse reaction to the medication. Common side effects include hypotension, hypoglycemia, nephrotoxicity, hepatotoxicity, neutropenia, spontaneous pneumothorax, and pancreatitis. Drug induced toxicity is typically relatively reversible but pentamidine has been known to cause long-term damage to organs (LiverTox, 2012).

Miltefosine is a moderately safe option for leishmaniasis treatment and has cure rates up to 82% for *L. panamensis* and other species. The mechanism of action of this drug is proposed to be inhibition of the cytochrome c oxidase causing decreased oxygen consumption and decreased ATP levels resulting in apoptosis cell death (Pinto-Martinez, 2019). Mainly used for cutaneous leishmaniasis, it is administered orally based on the patient's body weight twice a day. Side effects are the most limited out of all the leishmaniasis medications and include nausea and vomiting. It is often used in combination with paromomycin for the best results (Mayo Clinic, 2023). This medication cannot be used during pregnancy as it has been known to cause fetal harm, however, that is one of the few adverse side effects of this treatment. Overall, this drug is effective; however, it is currently only in use pertaining to cutaneous leishmaniasis. Unfortunately,
the cost of miltefosine is unsustainably high. For a 28-day regimen, it costs around 57,000 USD (Shahriar, 2020).

Imiquimod is a topical treatment used to treat cutaneous leishmaniasis. The mechanism of action is stimulating “interferon -γ secretion by CD4 T helper-1 lymphocytes by activating macrophages to destroy amastigotes”. This treatment has proven to be incredibly useful in quickening cutaneous leishmaniasis healing and reducing the amount of scarring. This is a fantastic option to use in combination with other treatment methods (Fuentes-Nava, 2021). Other external treatments for cutaneous leishmaniasis include cryotherapy and heat therapy. Cryotherapy involves applying liquid nitrogen to lesions to reduce scarring. Opposingly, heat therapy involves heating lesions to 50 degrees Celsius to quicken healing. Both methods have shown promising results but are typically used in combination with other treatment methods (McGwire, 2014).

Figure 4.5 Healing results from imiquimod (Reproduced with permission Marti-Marti, 2021).
Table 4.2 Summary of current leishmaniasis treatment methods

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism</th>
<th>Pros</th>
<th>Cons</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentavalent Antimonials</td>
<td>Inhibit DNA topoisomerase, glycolytic enzymes &amp; fatty acid betaoxidation</td>
<td>Low cost; effective</td>
<td>High levels of toxicity; high levels of resistant strain development</td>
<td>31, 32, 33</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>Binds to ergosterol to weaken cell membrane -&gt; cell death</td>
<td>High cure rates</td>
<td>Expensive; low compliance with patient medication regimen</td>
<td>43, 35</td>
</tr>
<tr>
<td>Paromomycin</td>
<td>Inhibits protein synthesis by binding to 16s ribosomal RNA</td>
<td>Cost effective</td>
<td>Low effectivity</td>
<td>36</td>
</tr>
<tr>
<td>Pentamidine</td>
<td>Inhibition of topoisomerase II</td>
<td>Short treatment course</td>
<td>High toxicity. Increased resistant strains. Low efficacy</td>
<td>37, 38</td>
</tr>
<tr>
<td>Miltefosine</td>
<td>Inhibition of cytochrome c oxidase -&gt; reduced oxygen/ATP</td>
<td>Minimal side effects; easy administration</td>
<td>Cannot be used during pregnancy</td>
<td>39, 40</td>
</tr>
</tbody>
</table>


References


CHAPTER FIVE
SOCIOECONOMIC IMPLICATIONS

Risk Factors & Morbidities

Socioeconomic inequality

Although Leishmaniasis can infect any individual where infected sand flies exist, there are risk factors that increase likelihood. Outside of medicinal prevention and treatment, understanding risk factors is an important aspect of reducing disease incidence worldwide. First, traveling to endemic areas increases the likelihood of transmission. Tourists and travelers are 3-10 times more likely to be infected with leishmaniasis when compared to the local population. Due to the neglected nature of the disease, travelers are likely not aware of the risk of infection. If they are aware of the risk, travelers also frequently dismiss the severity of the disease. Data on travelers and leishmaniasis is limited because it is not reported often and is frequently misdiagnosed (Alcais, et al 1997). Canine transportation and importation poses a risk at expanding leishmaniasis to new countries. Global warming has allowed it so that sandfly populations can now exist in the Southern United States and Canada, making it an easy target for leishmaniasis transmission in places of dog importation. However, these things are not tightly regulated, and the disease will continue to expand to new areas each year (Duprey ZH, 2006).

Second, social conditions are a huge aspect to consider when analyzing leishmaniasis infection rates. Poor living conditions are associated with high disease
incidence as it is easier for a sandfly to invade a person's living area in this manner. Cracked, broken, and run-down housing conditions make an easy target for sandfly invasion. Additionally, living in regions with animals such as dogs, pigs, and rodents increases likelihood of transmission as these are potential disease reservoirs (Stauch, 2011). Environments with low socioeconomic status are associated with living near unsanitary water sources, overpopulated living conditions, and undisposed waste. These are all increased factors of high transmission as it is an ideal environment for sandflies to feed. The most impoverished areas and populations are highly susceptible to leishmaniasis as poverty directly affects and influences housing status and living conditions (Boelaert, 2009).

Third, environmental factors play a huge role in assessing leishmaniasis risk as it directly relates to the sandfly life cycle. Sandflies thrive in forested, humid, and warm environments. However, as both the sandfly and *Leishmania* species continue to adapt, it is becoming more common that sandflies are able to thrive in domestic areas (Walsh, 1993). Deforestation has been shown to reduce leishmaniasis in some areas but also can have an impact on the environment in other harmful ways. Those living or working in heavily forested areas are at a potentially greater risk of infection (Yazdanpanah, 2013). Socioeconomic inequality is not only associated with risk factors but also greater long-term effects after infection. The cost of leishmanial treatment is extremely high so it affects those of a lower economic status at a greater impact. A study was conducted in 2006 in Nepal to understand the economic impact of leishmanial treatment. It was found that treatment for visceral leishmaniasis costs around 425 USD per year which is 20 USD greater than the average household income. Updated studies have not been conducted, but
the cost of treatment has only risen. Most families and households are fiscally unable to support the cost of treatment. Even if treatment is paid for by healthcare systems, income is lost by inability to work, still pushing individuals and households deeper into poverty. Because the cost of treatment is so high, it is common that individuals in impoverished countries never seek out treatment. Therefore, worsening their condition and increasing the likelihood of severe symptoms or even death. Leishmaniasis plays a vicious part in worsening the cycle of poverty. Infection of leishmaniasis can cause a household to worsen their economic status sometimes resulting in loss of shelter, food resources, etc.

**Gender inequality**

Gender inequality also plays an underlying but prominent role in the social implications of leishmaniasis. Looking at leishmania infection statistics, it appears that men are infected with leishmaniasis at a higher rate than women (Okwa, 2007). However, when taking a deeper look, it is apparent that women are not infected at a lower rate but just receiving care and reporting infection at a lower rate. Infected women with leishmaniasis go under the radar. In countries where leishmaniasis continues to be prominent, women are not typically employed outside of the household. Therefore, they themselves do not have the capacity to fund or even receive transport to medical facilities (Vlassoff, 2007). Furthermore, perception and acceptance of women in these countries is also low. Many times, women are expected to remain quiet about health issues and push through symptoms. Therefore, women are 80% less likely to seek treatment than men (Velez, 2001). In general, women of these regions are more likely to downplay their overall health as they have accepted from culture their less important status. Furthermore, even female
children are less likely to receive care than male children. Frequently, female children are taken for treatment at a very late stage of leishmaniasis, causing the scarring to be even greater. Illustrations can be seen in Image 5.1. Gender inequality offers a huge roadblock when thinking about increasing overall care for leishmaniasis. Outside of the box thinking is required to reach all infected individuals and to truly make a difference in disease burden (Okwor, 2016).

Additionally, women in impoverished regions experience greater levels of appearance-related stigma when compared to men. Women’s inherent worth and overall treatment is directly related to their outside appearance. Therefore, significant scarring has a huge impact on stigmatization (Dahal, 2021). Stigmatization affects women's relationships, social activities, work capacity, and marriage relationships. Risk of divorce and abandonment is great when considering the effects of long-term disfigurement in women with leishmaniasis. Even though leishmaniasis is not able to be spread from person to person, it is common to be ostracized for contracting the disease. Outcast women in impoverished countries struggle to succeed economically or socially. Although men in these regions also experience psychological effects from leishmaniasis scarring, the overall impact of stigma on men is less. Men can provide for themselves, and their treatment is not typically tied to their outside appearance. Any person with leishmaniasis is going to struggle with the long-term effects. However, women in these regions are statistically experiencing greater morbidities and long-term social/economic effects in comparison to men (Al-Kamel, 2016).
Current Initiatives & US Implications for the future

Common misunderstandings about neglected tropical diseases (NTDs) is that they are considered neglected because they are not important, and they do not affect a large number of people. However, neglected status from the World Health Organization indicates that there are no major global efforts to solve or better the current standing of the disease (WHO, *Neglected Tropical Diseases*). Figure 5.2 summarizes some of the current NTDs from the WHO that have both serious YLDs (years lived with a disability) and YLLs (years of life lost). If the top NTD killers were compared to Ebola virus, it would be seen that they all killed more people annually than Ebola. Leishmaniasis ranks at the top
with 52,000 reported deaths in 2010. The World Health Organization has developed a road map at overcoming neglected tropical diseases, but progress has not been made specifically concerning leishmaniasis (WHO, Ending The Neglect To Attain The Sustainable Development Goals: A Road Map For Neglected Tropical Diseases 2021–2030.). Based on death statistics, it is clear that leishmaniasis prevention and treatment research has ample relevance.

Table 5.1 Death comparison by disease (Álvarez-Hernández DA, 2020).

<table>
<thead>
<tr>
<th>Neglected Tropical Disease</th>
<th>Deaths</th>
<th>Year of statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leishmaniasis</strong></td>
<td>52,000</td>
<td>2010</td>
</tr>
<tr>
<td>Rabies</td>
<td>27,000</td>
<td>2010</td>
</tr>
<tr>
<td>Dengue fever</td>
<td>15,000</td>
<td>2010</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>12,000</td>
<td>2010</td>
</tr>
<tr>
<td>Chagas disease</td>
<td>10,000</td>
<td>2010</td>
</tr>
<tr>
<td>African trypanosomiasis</td>
<td>9,000</td>
<td>2010</td>
</tr>
<tr>
<td><strong>Ebola</strong></td>
<td>7,000</td>
<td>2014</td>
</tr>
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</table>
Unfortunately, solving the leishmaniasis crisis is incredibly intricate and involves the analysis of a few opposing forces. Being a NTD, leishmaniasis does not have committed funding or specific research efforts from large corporations. Additionally, leishmaniasis is nearly an impossible disease to eliminate due to its ability to have many different zoonotic reservoirs. Some reservoirs are apparent and relatively easy to access such as humans and canines. However, there are additional reservoirs such as rodents, bats,
opossums, marsupials, etc. that are difficult to control (Olliaro, 2017). Therefore, making it nearly impossible to ever eliminate leishmaniasis completely from all potential reservoirs. Furthermore, outside factors such as poverty, malnutrition, and climate change make it challenging to stay a few steps ahead of the disease. As this disease continues to grow and spread across continents, challenges will only worsen, making it imperative that committed research and engagement from the government is apparent in the next few years (Kamhawi, 2017).

Figure 5.3 Aspects of leishmaniasis control

Although leishmaniasis has predominantly been in areas outside of the United States, it is gradually moving into the southern United States and will continue to do so
with globalization and climate change. There have been reported cases throughout Texas and Oklahoma in the past decade. *Leishmania mexicana*, causing cutaneous leishmaniasis, is endemic to Texas with around two dozen reported cases per year. However, a cross sectional study found that only 20% of all cases were being reported to the government (Kipp E.J., 2020). These statistics are just referring to human leishmaniasis. Canine leishmaniasis is spreading at a rapid rate in the United States, illustrated by Figure 5.4 (Gin, 2021). As climate change continues, the geographic distribution of the sand fly population will continue to move northward in the United States. It will facilitate migration, lengthen the time that sandflies are seasonally active, and increase overall insect population. According to a study conducted in 2010, human exposure in the United States will double by 2080 (González C., 2010). Leishmaniasis will continue to spread throughout the United States and beyond, so it is imperative that new solutions are developed in the immediate future to curb the spread of this deadly disease.
Figure 5.4 Canines with leishmaniasis throughout the US (Reproduced with permission from Gin, 2021)
References


Discussion of current failings & future directions

Leishmaniasis is a deadly parasitic disease that has had limited success in progress of disease reduction. Current failings can be placed into two categories of roadblocks. First, leishmaniasis progress is failing due to lack of advancement regarding socioeconomic factors. Leishmaniasis remains endemic in regions of intense poverty which creates several confounding factors such as malnutrition, lack of access to medical care, poor hygiene practice, and insufficient housing. Therefore, leishmaniasis prevention and treatment research must consider these difficulties as priority. Socioeconomic initiatives have to be implemented at a large scale in order for any efforts to be successful. This is going to require coordinated funding and planning from both local governments as well as international organizations such as the World Health Organization. Leishmaniasis is not the only neglected tropical disease to suffer from confounding socioeconomic factors as this is true for every single one. Therefore, efforts to reduce such outside impact would not only improve leishmaniasis outcomes but also every other neglected tropical disease endemic to similar impoverished regions.

Second, leishmaniasis progress is failing due to scientific roadblocks. Leishmanial diagnostic methods have proven to be inaccurate and cumbersome for both patient and medical provider. More accessible/reliable diagnostic methods are needed to quicken the diagnostic process of leishmaniasis. Additionally, increase in clinical
familiarity would also improve the time it takes for individuals to even receive treatment. Leishmaniasis is quickly spreading to new regions of the world and is officially classified as endemic to the United States. Clinical familiarity in all regions of the world is necessary to improve overall outcomes. According to the World Health Organization’s standards for neglected tropical disease classification, leishmaniasis no longer fits into this category. Changing the classification of this disease would increase clinical awareness and decrease likelihood of delayed diagnosis or complete misdiagnosis. It is difficult to make assessments on appropriate funding and research allocation with comprehensive statistics and reporting of the disease. Therefore, mandatory reporting of positive diagnosis should be required from both the US federal government as well as the World Health Organization. Reporting is currently only required in the state of Texas, but it is apparent that this is not capturing the full scope of infection in the US. Mandatory reporting would feed scientific efforts and allow more pointed and specific research to be done.

Current treatment regimens are expensive, toxic, and ineffective in treating leishmaniasis. Additionally, prophylactic vaccines have not made much progress in recent years, apart from canine vaccines. Currently, efforts have been focused on the human immune system and how it responds to the *Leishmania* parasite invasion. However, it has been shown that the *Leishmania* parasite is incredibly complex with great ability for genomic plasticity. Therefore, more specific efforts regarding how the *Leishmania* parasite is functioning within the host is crucial to advancement. Combination, live-attenuated vaccines have promising implications if targeted using
parasite pathogenesis. Furthermore, the genomic implications of leishmaniasis prevention and treatment are not to be overlooked. Comprehensive studies are needed to analyze the genomic differences between species and how they can adapt and overcome current antileishmanial methods.

**Conclusions**

The problem of the leishmaniasis disease is great and it involves numerous confounding factors. However, continued progress in leishmaniasis prevention and treatment is of the utmost importance. This disease is deadly and debilitating to individuals all around the world and will continue to affect new regions every year. Considering both socioeconomic factors and parasite immunology holds a promising future for the field of leishmanial prevention and treatment.