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Protozoa

Trypanosomatidae

Leishmania (Genus) and Leishmaniasis

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Phylum Euglenozoa

Class Kinetoplastea

Order Trypanosomatida

Family Trypanosomatidae

Genus *Leishmania*

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Chapter 12

Leishmania (Genus) and Leishmaniasis

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Introduction

Leishmaniasis comprises a group of diseases caused by protozoans of the genus *Leishmania* (Ross, 1903b; Gibson, 1983) that are transmitted by the bites of phlebotomine sand flies. Of the 53 *Leishmania* species described, approximately 20 are known to be human pathogens (Table 1) (Akhoundi et al., 2016). The clinical manifestations of *Leishmania* infections range from lesions of the skin and mucous membranes to lethality (Herwaldt, 1999). Cutaneous leishmaniasis (CL), the most common form of the disease, comes in many forms including localized cutaneous leishmaniasis (LCL), characterized by a single, self-healing ulcer, diffuse cutaneous leishmaniasis (DCL) that presents as non-ulcerating lesions that are widespread on the body, disseminated cutaneous leishmaniasis (DCL), characterized by more than 10 lesions of mixed-types, mucocutaneous leishmaniasis (MCL), associated with destruction of the nasopharyngeal mucus membranes, visceral leishmaniasis (VL) where there is no initial cutaneous pathology and parasites spread to the visceral organs, and a complication of VL termed post kala-azar dermal leishmaniasis that is characterized by a erythmatous maculopapular rash that can extend to the entire body.

Leishmania species cause morbidity and mortality throughout large areas of the Old and New World and leishmaniasis is considered an emerging disease with an annual incidence of 0.9–1.7 million cases (Alvar et al., 2012). Leishmaniasis is found on all continents except Australia and Antarctica and is endemic in 98 countries, with 350 million people at risk of infection and causing 20,000 to 40,000 deaths per year (Alvar et al., 2012). An increased prevalence of *Leishmania*-HIV co-infection is responsible for the recent emergence of leishmaniasis in the Western world (Alvar et al., 2012; Desjeux and Alvar, 2003). Morbidity and mortality caused by leishmaniasis amount to an estimated 2.4 million disability-adjusted life-years (DALYs) (Desjeux, 2004) and the disease has recently been declared by the World Health Organization (WHO) as a category I Neglected Tropical Disease (NTD).

Historical Evidence

Evidence of *Leishmania*-like organisms from the blood of reptiles exists in fossil ambers of an extinct sand fly from Burma estimated to be approximately 100 million-years-old (Poinar et al., 2004a; 2004b) and from 20–30-million-year-old ambers from the Dominican Republic, although the vertebrate host is unknown in this case (Poinar, 2008). Human lesions, similar to those known as an ailment termed Oriental Sore, were first described in tablets from the Assyrian King Ashurbanipal in the 7th century BCE (= before current era), however, the information is thought to be derived from texts dating as old as 1500–2500 BCE (Steverding, 2017). In addition, Ancient Egyptian medical reports from 1500 BCE describe a condition known as Nile Pimple that is thought to refer to cutaneous leishmaniasis (Maspero, 1910). Physical evidence of *Le*. *donovani* DNA has been documented in Egyptian mummies dating as far back as 2050–1650 BCE (Zink et al., 2006) and immunological technique have been used to demonstrate *Leishmania* in a Peruvian mummy from 800 BCE (Frias et al., 2013).

In the Middle Ages, Arabic scientists made many references to descriptions of lesions, reminiscent of cutaneous leishmaniasis; the first being in 930 from the Baghdad region in Iraq (Edrissian et al., 2016) and a dermal condition known as Balkh Sore from Afghanistan by the Persian philosopher and physician Avicenna (980–1037) (Severding, 2017). In the New World, disfiguring facial lesions are depicted on pre-Columbian ceramics from the 5th century (Tuon et al., 2008) and skulls dating back to the 11th century discovered in northern Chile have morphological and molecular evidence of leishmaniasis in the New World (Costa et al., 2009).

Table 1. Clinical and Epidemiological Characteristics of *Leishmania* Species

***Leishmania* species**	**Subgenus**	**Old World and/or New World**	**Proven vector species**	**Clinical manifestation**	**Primary reservoir hosts**	**Distribution**	**Estimated global incidence**
Le. donovani	*Leishmania*	OW	*P. alexandri* *P. argentipes* *P. martini* *P. orientalis*	VL, PKDL	Dogs, foxes, opossums, rodents	Central Africa, South Asia, Middle East, India, China	50,000–90,000 VL cases; unknown number of PKDL cases
Le. tropica	*Leishmania*	OW	*P. arabicus* *P. guggisbergi* *P. rossi* *P. saevus* *P. sergenti*	LCL, RCL, rarely VL	Rock hyraxes	Central Africa, North Africa, Middle East, Central Asia, India	200,000–400,000 CL; unknown number of viscerotropic or RCL
Le. aethiopica	*Leishmania*	OW	*P. longipes* *P. pedifer* *P. sergenti*	LCL, DCL, DsCL, MCL	Rock hyraxes	East Africa	20,000–40,000 CL; breakdown of LCL, DCL, DsCL, MCL unknown
Le. major	*Leishmania*	OW	*P. duboscqui* *P. papatas,* *P. salehi*	CL	Gerbils, other rodents	Central Africa, North Africa, Middle East, Central Asia	230,000–420,000 LCL
Le. infantum	*Leishmania*	OW/NW	*Lu. almerio,* *Lu. cruzi* *Lu. evansi* *Lu. longipalpis* *Lu. migonei* *P. ariasi* *P. balcanicus* *P. brevis* *P. chineesis* *P. kandelakii* *P. langeroni* *P. longiductus* *P. perfillewi* s.l. *P. perniciosus* *P. sichuanensis* *P. smimovi* *P. tobbi* *P. turanicus* *P. wui*	LCL, VL	Dogs	North Africa, Mediterranean basin, Middle East, Central Asia, North America, Central America, South America	6,200–12,000 VL in Old World; 4,500–6,800 VL in New World; Unknown number of CL cases
Le. mexicana	*Leishmania*	NW	*Lu. ayacuchensis* *Lu. olmeca olmeca,* *Lu. ovallesi* *Lu. anthaphora*	LCL, DCL, DsCL	Forest rodents	North America (including the United States), Central America, South America	†Included in the 187,200–300,000 total cases of New World CL
Le. amazonensis	*Leishmania*	NW	*Lu. faviscutellata* *Lu. longipalpis* *Lu. nuneztovari anglesi* *Lu. omeca novice* *Lu. olmeca reducta*	LCL, DCL, DsCL	Rain forest rodents, marsupials, foxes, bats	South America	†Included in the 187,200–300,000 total cases of New World CL
Le. venezuelensis	*Leishmania*	NW	*Lutzomyia* spp. implicated but not proven		Unknown	Northern South America	†Included in the 187,200–300,000 total cases of New World CL

Table 1. Clinical and Epidemiological Characteristics of *Leishmania* Species (continued)

***Leishmania* species**	**Subgenus**	**Old World and/or New World**	**Proven vector species**	**Clinical manifestation**	**Primary reservoir hosts**	**Distribution**	**Estimated global incidence**
Le. braziliensis	*Viannia*	NW	*Lu. carrerai* *Lu. complexa* *Lu. fischeri*	LCL, MCL, DCL, RCL	Opossums, rain forest rodents	Western Amazon basin, South America, Central America	†Included in the 187,200–300,000 total cases of New World CL
Le. guyanensis	*Viannia*	NW	*Lu. anduzei* *Lu. ayacuchensis* *Lu. shawi* *Lu. umbratilis,* *Lu. whitmani*	LCL, DsCl, MCL	Sloths	Northern South America	†Included in the 187,200–300,000 total cases of New World CL
Le. lainsoni	*Viannia*	NW	*Lu. nuneztovari anglesi* *Lu. ubiquitalis*	LCL	Forest rodents	Brazil, Bolivia, Peru	†Included in the 187,200–300,000 total cases of New World CL
Le. lindenbergi	*Viannia*	NW	*Lu. atunesi* implicated	LCL		Brazil	†Included in the 187,200–300,000 total cases of New World CL
Le. naiffi	*Viannia*	NW	*Lu. ayrozai* *Lu. squamiventris*	LCL	Armadillos, rodents	Brazil, French Guiana	†Included in the 187,200–300,000 total cases of New World CL
Le. panamensis	*Viannia*	NW	*Lu. gomezi* *Lu. harmanni* *Lu. panamensis* *Lu. trapidol* *Lu. yulli*	LCL, MCL	Sloths	Central America, South America	†Included in the 187,200–300,000 total cases of New World CL
Le. peruviana	*Viannia*	NW	*Lu. ayacuchensis* *Lu. peruensis*	LCL, MCL	Opossums, dogs	Peru, Bolivia	†Included in the 187,200–300,000 total cases of New World CL
Le. shawi	*Viannia*	NW	*Lu. whitmani*	LCL	Sloths, rodents	Brazil	†Included in the 187,200–300,000 total cases of New World CL
Le. martiniquensis	*Mundinia*	OW/NW	Unknown	LCL, VL	Horses, cattle	Martinique, Thailand, Central Europe, United States	Unknown
Le. orientalis	*Mundinia*	OW	Unknown	LCL	Unknown	Thailand	Unknown
Le. colombiensis	*Mundinia*	NW	*Lu. hartmanni*	LCL, VL	Sloths	Colombia	†Included in the 187,200–300,000 total cases of New World CL

VL = visceral leishmaniasis, PKDL = post kala-azar dermal leishmaniasis, DCL = diffuse cutaneous leishmaniasis, DsCL = disseminated cutaneous leishmanniasis, LCL = localized cutaneous leishmaniasis, MCL = mucocutaneous leishmaniasis, RCL = recidivans cutaneous leishmaniasis, *P.* = *Phlebotomus*, *Lu.* = *Lutzomyia*. † Accounting of CL cases in the New World is complex as there are multiple *Leishmania* species circulating in the same geographical area, variable clinical manifestations associated with each species and species identification is rarely reported. Table compiled from multiple authoritative sources.

In modern times (16th–19th century) there are many reports of cutaneous leishmaniasis (CL), generally conditions named for the location they were acquired (for example, Aleppo boil, Baghdad boil, Jericho boil); interestingly, many of these terms are still used today (Severding, 2017). The earliest report of a disease likely to be visceral leishmaniasis did not occur until the 19th century with a description of an outbreak of a disease in 1824–1827 that caused emaciation, enlarged spleens, acute anemia, intermittent fever, and a dried, scaly appearance of the skin (Gibson, 1983; Twining, 1827). The Hindi term kala-azar that roughly translates to black fever and referring to the grayish discoloration of the skin was coined late in the 19th century to describe VL (Steverding, 2014). In the 20th century the Scottish pathologist William Boog Leishmana discovered ovoid bodies from the spleen of a soldier who died from a disease of emaciation while serving at Dum Dum, a town in India, and termed the disease Dum Dum Fever (Leishman, 1903); at the same time Charles Donovan identified similar bodies from splenic aspirates of Indian patients (Donovan, 1903), but the 2 scientists could not agree if the parasites were trypanosomes or a new species. Ronald Ross, investigating kala-azar in India, found similar parasites from spleens of patients with chronic splenomegaly (Ross, 1903a) and settled the controversy, declaring the parasites a new species named *Leishmania donovani* (Ross, 1903b). Leishmaniasis was not reported in the new world until the 20th century; CL reported from Brazil in 1909, referred to as Baurú ulcers (Carini and Paranhos, 1909; Lindenberg, 1909), and VL, also from Brazil, in the 1930s (da Cunha et al., 1937).

Leishmaniasis continues to be a major global health threat and, although endemic in Europe, Africa, Asia, and America, 90% of cases occur in just 13 countries (Afghanistan, Algeria, Bangladesh, Bolivia, Brazil, Columbia, Ethiopia, India, Iran, Peru, South Sudan, Sudan, and Syria). The burden of leishmaniasis is largely underestimated, however, due to misdiagnosis and lacking surveillance systems. Human migration, political instability, climate change, and warfare is expanding *Leishmania*-endemic regions and increasing the propensity for epidemics worldwide. As an example, cutaneous leishmaniasis is currently spreading as refugees move from Syria through Turkey into Europe and other regions throughout the world (Hayani et al., 2015; Nimer, 2018). As a consequence, there is a significant risk that cutaneous leishmaniasis (CL) will reemerge in southern Europe, where the natural sand fly vectors for *Leishmania tropica* and *Le. major* are already endemic, and travelers, not only refugees, may be affected (Di Muccio et al., 2015).

Nomenclature and Morphology

Leishmania species are flagellated, single cell, protozoans in the order Kinetoplastida and the family Trypanosomatidae. As with other members of this group, *Leishmania* spp. are characterized by a unique mitochondrian that contains a kinetoplast at the base of the flagellum. The kinetoplast contains DNA (kDNA) that represents 10–20% of the total cellular DNA (Simpson, 1987) and is organized as an interlocked network containing dozens of maxi- and thousands of mini-circles. Mini-circles encode for guide RNAs (gRNAs) that function in a unique RNA editing mechanism (Read et al., 2016) and maxi-circles are analogous to mitochondrial DNA in other eukaryotes. Due to the high copy number of mini-circles, kDNA can be utilized for diagnostic purposes via polymerase chain reaction (PCR) (Van der Auwera and Dujardin, 2015; Galluzzi et al., 2018).

Historically, *Leishmania* species were classified based on the clinical symptoms they generated, those causing CL considered as *Le. tropica* and those causing VL as *Le. donovani*. As more parasites from around the world were examined and molecular techniques became available, it became clear that there were many different subgroups within the genus *Leishmania* (Lainson and Shaw, 1987). Today, at least 4 subgenera exist for the genus *Leishmania*: *Sauroleishmania, Leishmania, Mundinia,* and *Viannia* (Espinosa et al., 2018), the latter 3 subgenera contain species known to infect humans. Species of the subgenera *Sauroleishmania* infect lizards do not cause human disease. The vast majority of species that cause human disease belong to the *Leishmania* and *Viannia* subgenera; those belonging to *Leishmania* are found in both the Old and New World and those belonging to *Viannia* are exclusively found in the neotropics. These 2 subgenera can be distinguished by the location the parasites grow within the sand fly gut, *Leishmania* (*Leishmania*) spp. found anterior to the pylorus and *Leishmania* (*Viannia*) in the mid and hindgut.

Life Cycle

Leishmania are digenic parasites, completing their life cycle within 2 hosts. These parasites develop within the alimentary track of phlebotomine sand flies (order Diptera: family Psychodidae, subfamily Phlebotominae) and are transmitted to humans when female sand flies blood feed (Figure 1). Once deposited in the vertebrate host the parasites are quickly phagocytosed by cells of the mononuclear phagocyte system, where they establish their niche. *Leishmania* have 2 primary morphological forms, long (5–15 μm), extracellular promastigotes with long flagella in sand flies and small (3–5 μm), intracellular amastigotes with rudimentary flagella within vertebrate hosts.

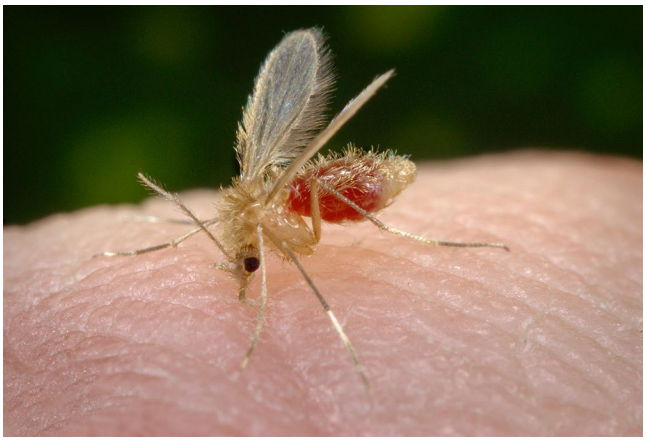

Figure 1. A *Phlebotomus papatasi* sand fly, which landed atop the skin surface of the photographer who had volunteered himself as host for this specimen's blood meal. The sand flies are members of the Dipteran family Psychodidae and the subfamily Phlebotominae. This specimen was still in the process of ingesting its blood meal, which is visible through its distended transparent abdomen. Source: United States Public Health Image Library, image 10275; J. Gathany, 2006. Informed consent granted by human subject: J. Gathany, 2006. Public domain.

Leishmania are obligate intracellular parasites that survive and multiply within the mature phagolysosome compartment of mononuclear phagocytes. Metacyclic promastigotes that enter the skin are quickly phagocytosed either directly by macrophages or dendritic cells or indirectly through apoptotic infected neutrophils that are rapidly recruited to the bite site (van Zandbergen et al., 2004). *Leishmania* do not actively invade host cells, but rely on the phagocytic capacity of these cells to gain entry. This process is receptor-mediated (Alexander and Russell, 1992; Chang and Dwyer, 1978), eventually resulting in the formation of a phagolysosome, and is known to induce host-cell signal transduction pathways (Guy and Belosevic, 1993). Many receptors are known to mediate entry of *Leishmania* parasites including, complement receptors 1 and 3, FcReceptors, mannose receptors, scavenger receptors, and fibronectin receptors (Ueno and Wilson, 2012). Recently, Toll-like receptors also have been implicated during *Leishmania* infection (Chauhan et al., 2017). These receptors are part of the innate system of pattern recognition, a system used by host cells to discriminate between infectious non-self and self. Upon interaction with ligand, these receptors initiate signal transduction pathways that ultimately lead to the modulation of phagocyte functions. The repetitive structure and glycan modifications associated with many *Leishmania* cell surface molecules serve as pathogen associated molecular patterns (PAMPs) that are recognized by the pattern recognition receptors (PRRs) (Podinovskaia and Descoteaux, 2015).

Once phagocytosed, *Leishmania* parasites are delivered to an intracellular vacuole termed a phagosome. Phagocytosis of a foreign body typically results in phagosome fusion with lysosomes allowing for degradation of phagosomal contents within 30 minutes. This *Leishmania* delays the phagosome maturation process, taking approximately 5 hours, the delay theoretically allowing enough time for the parasites to start differentiating toward the more resistant amastigote stage (Desjardins and Descoteaux, 1997; Scianimanico et al., 1999); full differentiation into amastigotes occurs between 24 and 48 hours post engulfment. *Leishmania* amastigotes multiply within the phagolysosome and eventually escape from the cell by a poorly defined mechanism and re-invade other phagocytes. Different species of *Leishmania* induce morphologically distinct phagolysosomes. *Leishmania*. *mexicana* resides in spacious vacuoles that contain many amastigotes, whereas *Le*. *major* and *Le*. *donovani* induce tight fitting phagosomes that contain only 1 amastigote (McConville et al., 2007).

When a sand fly ingests blood, it is retained within a peritrophic matrix in the abdominal midgut until digestion is completed. *Leishmania* amastigotes within infective blood transform into procyclic promastigotes within 12–18 hours within the peritrophic matrix. Procyclic promastigotes rapidly divide within the digesting blood meal and differentiate into longer, more motile nectomonads. When blood digestion is completed and excreted (~ 3–5 days), nectomonads are found in the gut attached to the epithelial cell microvilli via their flagella (Sacks and Kamhawi, 2001). Approximately 7 to 10 days following the initial blood meal the parasites transform into short, actively dividing forms called leptomonads and migrate to the thoracic midgut and stomodeal valve (an invagination of the foregut into the midgut). Leptomonads produce a substance, promastigote secretory gel that imbeds the parasites (Gossage et al., 2003). The end-point of parasite development in the sand fly is differentiation into the infectious stage, metacyclic promastigotes. These infectious forms are short, slender forms with a flagella twice as long as the body and are highly motile (Sacks and Perkins, 1984). Different than *Plasmodium* sporozoites that reside in mosquito salivary glands and are injected during blood-feeding, *Leishmania* metacyclic promastigotes do not invade the salivary glands and are regurgitated. The stomodeal valve is physically obstructed by the promastigote secretory gel, interfering with blood-feeding and leading to regurgitation and persistent but intermittent feeding (Rogers et al., 2004).

Clinical Manifestations

Leishmaniasis is a group of diseases characterized by a range of clinical symptoms that fall under 2 primary

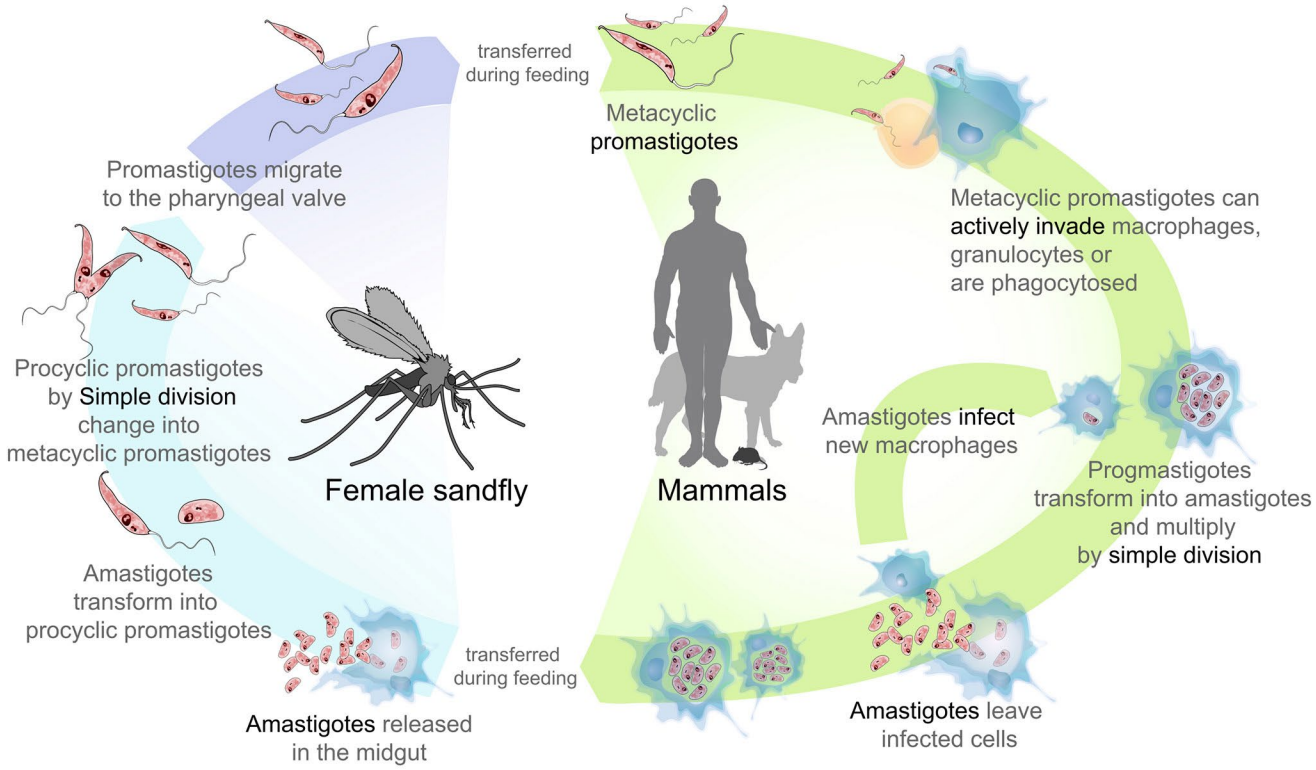

Figure 2. Life cycle of the parasites from the genus *Leishmania*. Source: Wikimedia Commons; Ruiz Villarreal, 2008. Public domain.

categories, **cutaneous leishmaniasis** (CL) and **visceral leishmaniasis** (VL). Although this is an ever-changing number as new species are described and taxonomic phylogenies revised, there currently are 22 *Leishmania* species known to be pathogenic to humans (Table 1; PAHO, 2024). The different pathological manifestations that present are primarily associated with the *Leishmania* spp. initiating the infection.

Cutaneous Leishmaniasis (CL)

The World Health Organization (WHO) estimates that 1.2 million annual cases of CL worldwide with 70–75% of the cases occurring in just 10 countries, Afghanistan, Algeria, Brazil, Colombia, Costa Rica, Ethiopia, Iran, North Sudan, Peru, and Syria. However, these statistics are largely underestimated due to misdiagnosis and lack of surveillance systems (see Figures 3 and 4).

There has been a recent increase in the incidence of CL in the United States due to several factors, including travel to endemic regions, immigration, and military operations. Twenty cases of CL were identified in United States military personnel deployed during the 1990–1991 Gulf War in the Middle East. More recently, CL has had a profound effect on United States troops; 1,287 deployed United States military personnel contracted leishmaniasis during campaigns in Iraq and Afghanistan during 2001–2006 and 522 cases in personnel who served in southwest and central Asia (Pavli and Maltezou, 2010). In addition, CL is found in the United States, being endemic in southern Texas and may be spreading north to Oklahoma (Clarke et al., 2013; Kipp and Hergert, 2019).

The most common form of this CL is **localized cutaneous leishmaniasis (LCL)**, characterized by localized, self-limiting cutaneous ulcers and powerful lifelong immunity upon healing. The lesions most often occur on exposed areas of skin where the sand fly vector can take a blood meal and begin as papules that eventually ulcerate. The patients are generally well and slight pain may or may not be associated with the lesions. Multiple lesions of this condition usually correspond to multiple sand fly bites. Although the lesions eventually heal (3–18 months), even without treatment, they are associated with substantial scarring and often social stigm (Bennis et al., 2018). Ancient civilizations noted that individuals who had healed from Oriental sores were protected from further disease (Steverding, 2017). Leishmanization, the practice of innoculating individuals with exudates from active lesions into the buttocks of young children, particularly girls, has been used in the Middle East and Central Asia for centuries to prevent the development of facial scars

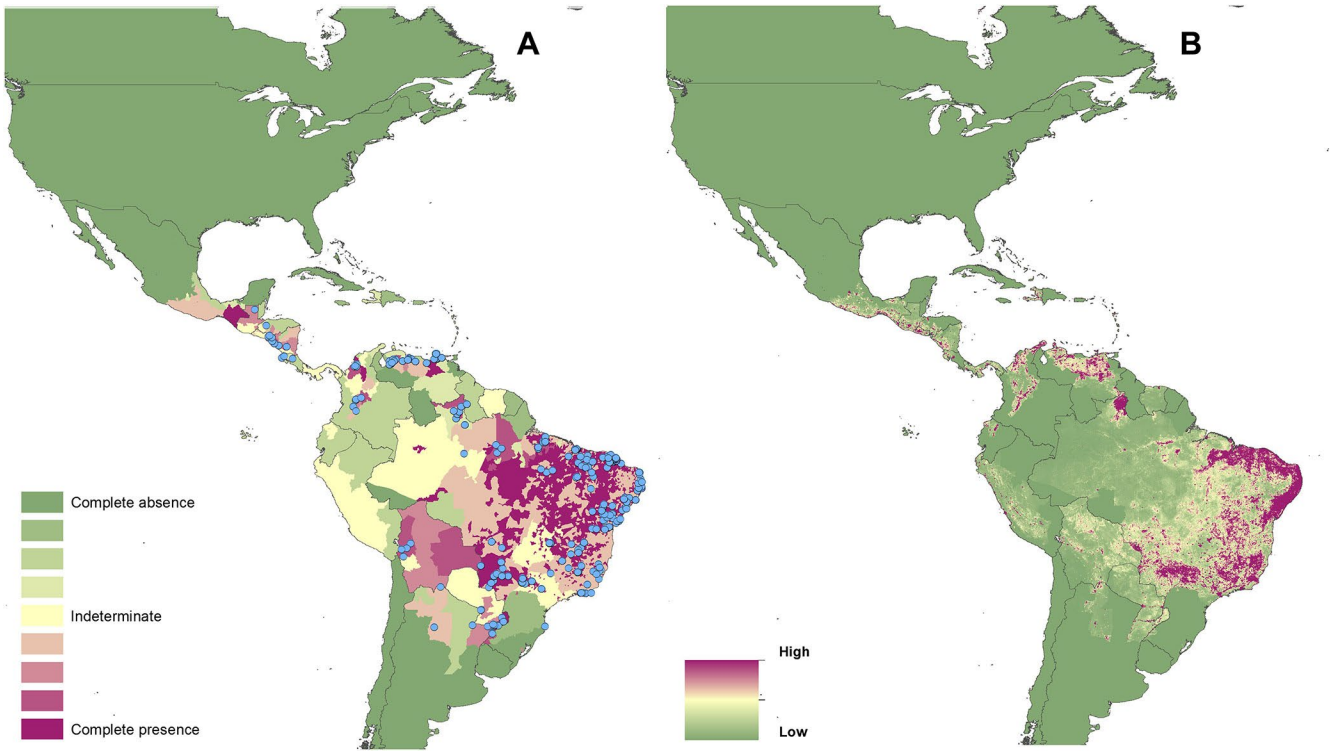

Figure 3. Reported and predicted distribution of cutaneous leishmaniasis in the New World. A) Evidence consensus for presence of the disease ranging from green (complete consensus on the absence: −100%) to purple (complete consensus on the presence of disease: +100%). The blue spots indicate occurrence points or centroids of occurrences within small polygons. B) Predicted risk of cutaneous leishmaniasis from green (low probability of presence) to purple (high probability of presence). Source: Pigott et al., 2014. License: CC0 1.0 Universal.

(Steverding, 2017). Today, leishmanization is still the only effective leishmaniasis vaccine that leads to lifelong protection (Nagill and Kaur, 2011).

Diffuse cutaneous leishmaniasis (DCL) is less common and characterized by multiple slow growing, non-tender nodules that disseminate all over the body. The nodules do not ulcerate and typically contain large numbers of parasites. DCL is a chronic disease that can persist for 20 years or more (Hashiguchi et al., 2016) and is often misdiagnosed as lepromatous leprosy. The condition is thought to reflect an underlying lack of a cellular immune response as evidenced by poor ability to respond to *Leishmania* antigen (Scott and Novais, 2016).

Disseminated cutaneous leishmaniasis (DsCL) is a non-chronic condition where there are multiple (≥ 10) lesions of different types, often ulcerating on more than 2 parts of the body. Patients are strongly positive for the Leishmanin skin test, indicating strong cellular immunity (Hashiguchi et al., 2016).

Recidivans cutaneous leishmaniasis (RCL) is a reactivation after a lesion is healed, usually within 2 years. Reactivated lesions typically encircle the previous scar and can be difficult to treat (Gitari et al., 2018). This condition is primarily associated with *Leishmania tropica* infection.

Mucocutaneous leishmaniasis (MCL) occurs after an initial local cutaneous lesion has healed. The parasites disseminate to the nasopharyngeal mucus membranes. The disease is characterized by destruction of the nasal septum, lips, palate, and sometimes larynx. Patients with MCL are at risk of death due to aspiration pneumonia.

Leishmania major.

Cutaneous leishmaniasis in the Old World accounts for the majority of global CL incidence in the world. The majority of these cases are caused by either *Leishmania major* or *Le. tropica* (Table 1). *Leishmania major* is endemic in the Middle East and North, West, and East Africa, and Central Asia. Confirmed vector species include *Phlebotomus duboscqi* in western and eastern Africa, *P. papatasi* and *P. bergeroti* in the Middle East, North Africa, and Europe, and *P. salehi* in India, Iran, and Pakistan. *Leishmania major* is a zoonosis with several rodent species implicated as reservoirs, differing geographically.

Leishmania major infection predominately manifests as LCL with lesions starting as a small papule, occasionally with nodules, at the site of the sand fly bite. Lesions generally

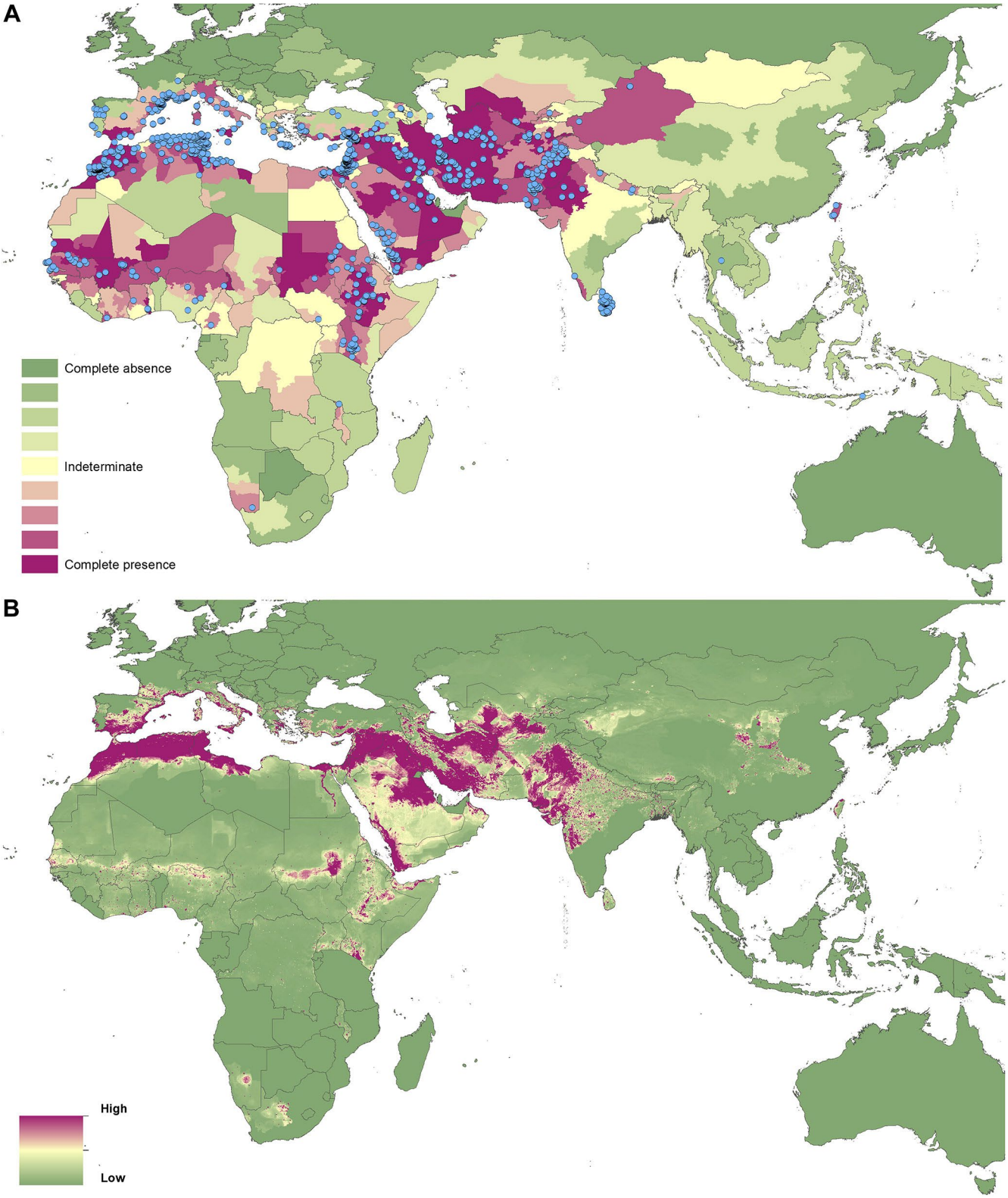

Figure 4. Reported and predicted distribution of cutaneous leishmaniasis in the Old World. A) Evidence consensus for presence of the disease ranging from green (complete consensus on the absence: −100%) to purple (complete consensus on the presence of disease: +100%). The blue spots indicate occurrence points or centroids of occurrences within small polygons. B) Predicted risk of cutaneous leishmaniasis from green (low probability of presence) to purple (high probability of presence). Source: Pigott et al., 2014. License: CC0 1.0 Universal.

occur on the head, neck, or extremities where sand flies have access to the skin. The incubation period is generally 2–4 weeks but may range from days to years (Goto and Lindoso, 2010). The papules enlarge and develop into large painless ulcers with a raised darker pigmented border (Darmstadt et al., 1993; Morris-Jones and Weber, 2004). The lesions are typically wet and associated with severe inflammation (Burza et al., 2018). Rarely is there more than 1 lesion present and self-healing usually occurs within 1 year (Melby et al., 1992); however, there is severe scarring due to the necrosis and inflammation associated with the lesions (Burza et al., 2018). Those that heal become immune to further infection; this natural occurring immunity provides the rational for vaccine development.

Leishmania tropica.

The geographic range of *Leishmania tropica* includes Central and North Africa, the Middle East, and Central Asia. *Le. tropica* infection has generally thought of as an anthroponotic infection, however, in some locations it appears to be a zoonosis (Kamhawi et al., 1995; Saliba et al., 1997; Talmi-Frank et al., 2010), with the rock hyrax as a reservoir host (Talmi-Frank et al., 2010). Vector species include *Phlebotomus guggisbergi*, *P. rossi*, *P. saevus*, and *P. arabicus*, and *P. sergenti*, with the latter 2 being the most common.

Similar to *Leishmania major* infections, the majority (76%) of lesions occur on the head and neck, followed by 30–36% on the extremities and trunk (Solomon et al., 2014). Most patients present with 1 LCL lesion and 95% have fewer than 3 lesions (Solomon et al, 2014). Lesions due to *Le. tropica* infection typically take longer to heal than those caused by *Le. major*, with the majority healing within 2 years (Handler et al., 2015). The lesions ulcerate but are dry in nature (Burza et al., 2018). In some cases, lesions can develop into hyperkeratotic plaques that resemble large warts (Burza et al., 2018). Individuals that have healed from a previous cutaneous ulceration due to *Le. tropica* can relapse, causing RCL (Burza et al., 2018). This chronic relapsing form of the disease generally begins with nodules, with lesions forming at the periphery of the old lesion scar.

Of the 32 cases of leishmaniasis identified in United States soldiers during the 1990–1991 Desert Storm Campaign, 12 were characterized as viscerotropic caused by *Leishmania tropica* (Hyams et al, 1995). Viscerotropic leishmaniasis is a syndrome where parasites spread to visceral organs and is associated with a prolonged systemic illness that includes fever, malaise, abdominal pain, and intermittent diarrhea but does not progress to fatal VL (Magill et al., 1993).

Most recently, the civil war in Syria has been associated with a large epidemic of *Leishmania tropica* CL (Rehman et al., 2018). Before the onset of the civil war, WHO statistics indicate approximately 14,000 new CL cases per year in Syria with incidence increasing to 27,825 in 2010 (Rehman et al. 2018) and to 89,357 in 2019 (WHO, 2024). The governmental surveillance system for leishmaniasis has lost access to some provinces in Syria so only sparse reliable data have been published since 2011. A recent humanitarian organization reported nearly 65,000 cases from a few provinces in northern Syria, with the majority associated with *Le. tropica* infection, although *Le. major, Le. infantum,* and *Le. donovani* are also present (Rehman et al, 2018). Syrian refugees are migrating to nearby areas where suitable vectors are endemic, expanding the epidemic.

Leishmania aethiopica.

Leishmania aethiopica is a zoonotic disease, primarily occurring in the highland areas of Ethiopia with the rock hyrax as a reservoir. The annual burden of CL in Ethiopia is approximately 20,000–40,000 cases per year (Alvar et al., 2012) with 99% with *Le. aethiopica* as the etiological agent (van Griensven et al., 2016). While LCL is the most frequent manifestation, clinical symptoms are diverse with MCL and DCL also being relatively common (Padovese et al., 2009). The reasons for the diverse symptomology are unclear and may involve many factors, including the high level of genetic polymorphism exhibited by this species (Pratlong et al., 2009).

Leishmania aethiopica LCL lesions are slower to develop, typically do not ulcerate, and are more chronic compared to other LCL lesions, requiring 2–5 years to heal (Handler et al., 2015). Both MCL and DCL caused by *Le. aethiopica* are reportedly less responsive to common antileishmanial drugs (Padovese et al., 2009). Treatment for DCL has been notoriously difficult and even if lesions regress, relapse is common upon cessation of chemotherapy (van Griensven et al., 2016).

Leishmania mexicana.

Cutaneous leishmaniasis epidemiology in the Americas is extremely complex with multiple *Leishmania* species with overlapping geographical distributions, a variety of sand fly vectors, and many different reservoir hosts. Due to this complexity and that reporting structures do not require species identification, the exact incident levels of each species is difficult to discern. *Leishmania mexicana* is endemic in North America, Central America, and South America and is the species endemic to the United States.

Forest rodents serve as reservoir hosts in most of the Americas, with 3 species of woodrats serving as reservoir hosts in the United States, including *Neotoma micropus, N. albigula,* and *N. floridana*. Several confirmed vectors have

been identified (Table 1); *Lutzomyia anthaphora* is the only confirmed vector in the United States (Endris et al., 1987; McHugh et al., 1993).

Leishmania mexicana lesions resemble those caused by *Le. major* being generally less severe and healing quickly. However, they can be slow to develop, sometimes taking up to 6 months, and can persist for 20 years (Handler et al., 2015). Lesions occur roughly 50% of the time on the ear, a manifestation referred to as chiclero's ulcer; the term is used because LCL on the ear is common among men that visit the forests to collect chicle (natural form of gum). DCL rarely presents with infection with *Le. mexicana*.

Leishmania braziliensis.

Leishmania braziliensis, endemic in South America and Central America (Grimaldi et al., 1987) is known in some places locally as espundia. Infection with *Le. braziliensis* results in severe cutaneous lesions and is associated with satellite subcutaneous nodules and lymph node involvement (Melby et al. 1992). LCL lesions caused by *Le. braziliensis* generally ulcerate and may heal within 6 months; however, 2–5% of cases develop into MCL and these require treatment (Burza et al., 2018). Although several species have been implicated in MCL, *Le. braziliensis* accounts for the majority of cases in the New World (Strazzulla et al., 2013).

MCL usually presents after the healing of a primary skin lesion but can begin to develop prior to lesion resolution (Daneshbod et al., 2011). Mucosal involvement normally appears within 2 years of LCL but has been reported to take up to 30 years (Samady et al., 1996). Although general subsequent mucosal involvement generally occurs in < 5% of cases, it may be as high as 20% in certain regions (David et al., 1993).

It is unknown why some patients are more susceptible to MCL. *Leishmania* RNA virus (LRV)-1 was identified in both *Le. braziliensis* and *Le. guyanensis*, both associated with MCL, leading to the hypothesis that MCL is actually virally mediated (Scheffter et al., 1995). It is hypothesized that virally infected *Leishmania* are recognized by host PRR that induce killing of the parasite and allowing dispersal of the virus. This dispersal in turn triggers a metastatic hyperinflammatory reaction, resulting in tissue damage (Weinkopff et al., 2013; Zangger et al., 2013).

Additional New World *Leishmania* Species.

Leishmania amazonensis is restricted to South America and is associated with LCL, DCL, and DsCL. *Leishmania peruviana* causes LCL, a disease known as uta in preschool age children in the Peruvian Andes (Davies et al., 1997). Ulcera de Bejuco is CL caused by *Le. panamensis*, characterized by shallow ulcers that metastasize along lymphatic vessels. There is no spontaneous healing of the lesions and 2–5% develop MCL (Koff and Rosen, 1994). *Leishmania guyanensis* infection also is associated with multiple ulcers that can spread along the lymphatics and is known as pianbois. These lesions generally require treatment and often reoccur (Burza et al., 2018).

Visceral Leishmaniasis

Visceral leishmaniasis (VL) affects the spleen, liver, bone-marrow, and other visceral organs. There is no cutaneous pathology associated with initial presentation and clinical manifestations include persistent fever, hepatosplenomegaly, and weight loss. The disease can be either acute or gradual and is generally fatal within 2 years without treatment as a result of secondary bacterial infections or severe anemia. Acute malnutrition and high parasite burdens are present in young children with VL (Harhay et al., 2011). In the Indian subcontinent, hyperpigmentation of the skin is associated with VL, so is often referred as kala-azar, meaning black fever. Although endemic in 97 countries and territories, nearly 90% of the global burden of VL occurs in just 6 countries: Brazil, Ethiopia, India, Somalia, South Sudan, and Sudan (WHO, 2017).

Post-kala-azar dermal leishmaniasis (PKDL) is a late manifestation of VL caused by *Leishmania donovani* following treatment. PKDL presents as a hypopigmented macular or erythematous maculopapular rash on the face that can, in some instances, extend to the entire body. In PKDL, the parasites seem to persist in the skin after treatment. The syndrome can be mistaken for lepromatous leprosy but can be distinguished by the preservation of sensation (Burza et al., 2018).

Figure 5. Distribution of hunt clubs with confirmed cases of visceral leishmaniasis, United States and Canada. States in which hunt clubs or kennels had ≥ 1 dog infected with *Leishmania infantum* are shaded. *Leishmania*-positive foxhounds were also found in Nova Scotia and Ontario, Canada. Source: Duprey et al., 2006. Public domain.

Leishmania donovani.

The WHO estimates that over 70% of global VL caused by *Leishmania donovani* cases occurs in the Indian subcontinent and eastern Africa (WHO, 2016). Currently, East Africa has the highest burden of VL due to ongoing success with elimination efforts in Southeast Asia (Alves et al., 2018). VL caused by *Le. donovani* is considered anthroponotic because humans are the primary reservoir, although domestic dogs have been implicated as a possible minor reservoir host (Jambulingam et al., 2017).

The primary sand fly vector for VL in India and Bangladesh is *Phlebotomus arentipes*, and *P. orientalis* and *P. martini* for East Africa. In India the disease is associated with poor housing conditions where houses typically are made of mud walls and livestock and humans live under the same roof, creating an excellent ecological niche for the vector. VL in East Africa occurs primarily in arid and semi-arid lowland areas and is associated with migrant agricultural workers that typically sleep outdoors (Argaw et al., 2013).

Asymptomatic *Leishmania donovani* infections are common in endemic areas with seroprevalance in healthy individuals ranging between 7–63% in India (Srivastava et al., 2013) and 7–46% in Ethiopia (Ayehu et al., 2018; Abbasi et al., 2013). The underlying mechanisms that lead to clinical disease are not elucidated although malnutrition is thought to play a role and immunosuppression, particularly HIV coinfection in Ethiopia, is a major contributor. Commonly the incubation period is between 2 and 6 months and between 2–23% of asymptomatic individuals will present with VL symptoms within a year (Burza et al., 2018). VL caused by *Le. donovani* is almost always fatal unless treated and viable parasites can persist even after successful treatment, reactivating to cause disease if the individual becomes immunosuppressed (Diro et al., 2015).

Even without immunosuppression, PKDL can develop after apparently successful treatment. PKDL occurs in 25–50% of treated patients in Sudan within 6 months but is less common (5–10%) and occurs much longer after treatment (2–3 years) in India (Zijlstra et al., 2003). Interestingly, 5% of Indian PKDL patients report no previous VL episode (Zijlstra et al., 2016). In Asia, 90% of PKDL cases are of the macular type and African PKDL cases are primarily papular rash (Burza et al., 2018). Up to 85% of PKDL cases in East Africa are self-curing within 12 months and primarily pose aesthetic problems, although a small number will develop severely debilitating forms (Zijlstra et al., 2016). Important, however, is that PKDL lesions remain infectious for sand flies, serving as a reservoir of infection (Molina et al., 2017).

Leishmania infantum.

Leishmania infantum has a wide geographic distribution being endemic in the Americas, North Africa, the Mediterranean Basin, the Middle East, and Central Asia. Over 90% of VL cases due to *Le. infantum* occur in Brazil. VL was first discovered in the new world in 1937 and the parasite isolates were thought to be a new species and named *Le. chagasi* (Da Cunha et al., 1937). The following year, the discoverers realized that the parasites behaved like *Le. infantum* and concluded that the parasites that cause VL in the New World was identical to *Le. infantum* (Da Cunha, 1938), however the name *Le. chagasi* continued to be utilized in the literature. Modern molecular tools also are not able to distinguish *Le. infantum* from *Le. chagasi* (Mauricio et al., 1999), leading to the general agreement that the isolates from different geographical and host origins are, indeed, the same species. However, minor phenotypic and genotypic differences have led some authors to separate them into 2 species or, alternatively, 2 subspecies named *Le. infantum infantum* and *Le. i. chagasi* (Lainson and Rangel, 2005).

Infection with *Leishmania infantum* is primarily zoonotic, with the domestic dog as the reservoir host. Transmission occurs through the bite of sand flies of the genus *Lutyzomia* in the New World and *Phlebotomus* in the Old World. VL in Brazil used to be primarily restricted to rural areas in northeastern Brazil (De Melo and Fortaleza, 2013). Deforestation and associated changes in ecological habitats for the vector, urbanization, human migration, and the spread of HIV are changing the epidemiological profile to include urban epicenters of disease and a southward expansion (Arias et al., 1996). This changing epidemiological picture complicates prevention as measures directed at controlling the disease through vector control (insecticide spraying, use of repellents, or environmental management) or through management of canine leishmaniasis (dog culling or vaccination) are more difficult in urban settings (De Melo and Fortaleza, 2013). Moreover, control methods directed at controlling canine leishmaniasis have had varied outcomes (Romero and Boelaert, 2010).

As with *Leishmania donovani,* asymptomatic infections are common (9–24%) with individuals infected with *Le. infantum*. Conversely, there is little PKDL associated with *Le. infantum* infection except in cases of immunosuppression (Stark et al., 2006). *Leishmania infantum* can also cause LCL associated with single nodules and minimal inflammation that self-heal and induce immunity (Burza et al., 2018).

Visceral leishmaniasis (VL) caused by *Leishmania infantum* in the Old World continues to be primarily a rural disease, however a recent outbreak in Spain occurred in an urban area and was linked to a wild hare reservoir host (Arce et

al., 2013). Direct transmission without a sand fly vector also has been documented in Spain between intravenous drug users co-infected with HIV through needle sharing (Alvar et al., 1997).

During 2001–2016, 25 VL diagnoses were reported from United States soldiers deployed in the Middle East (Stahlman et al., 2017). Over the past decade, it has begun to be appreciated that asymptomatic VL is common in endemic regions, however the role of asymptomatic individuals in disease transmission and how many may progress to fulminant VL is less clear. Recently, higher risk United States military personnel were assessed 11 years after deployment in the Iraq War (2002–2011) to determine the rate of asymptomatic individuals; nearly 20% of these individuals were positive for *Leishmania infantum* infection (Modý et al., 2019). The risk of reactivation to VL for United States military veterans or to blood safety in the United States blood supply remains to be determined.

Canine Leishmaniasis

Zoonotic leishmaniasis can be found in all forms: Visceral, cutaneous, and mucocutaneous. However, canines act as the major reservoir of infection for *Leishmania infantum* which makes zoonotic visceral leishmaniasis (ZVL) the most pervasive of all forms of zoonotic leishmaniasis diseases (Gramiccia and Gradoni, 2005). Canine leishmaniasis (CanL) traditionally affects dogs in the same geographic regions as human visceral leishmaniasis such as the Middle East, South Asia, Central America, South America, North Africa, and East Africa (Tuon et al., 2008). Although dog ownership is not strictly necessary to place a person at higher risk of infection with leishmaniasis, regions with higher rates of CanL observe higher rates of human disease as well. In recent years, cases of CanL have been seen in non-endemic areas (Duprey et al., 2006).

Figure 6. Canine visceral leishmaniasis. Source: Dantas-Torres, 2008. License: CC BY 2.0.

In the United States, it was previously thought that CanL cases seen were strictly due to foreign travel. However, in the late 1990s, infections began to appear in the foxhound kennels (over 40% presented with disease) where foxhounds had no history of travel. A survey of the United States and Canada in the early 2000s found that 18 states and 2 provinces were enzootic for canine leishmaniasis (Duprey et al., 2006; see Figure 5). With no seropositive cases of *Leishmania* found in wild canines or in humans with close contact to the kenneled dogs, dog-to-dog transmission was considered to be the main route of infection. Risk factors associated with dog-to-dog transmission were thought to be the large number of animals housed together at a time, travel of foxhounds internationally and across state lines for breeding and club practices, and the inherent nature of the breed (Duprey et al., 2006).

Transmission.

As with human infections, *Leishmania* transmission to dogs principally occurs through the bite of phlebotomine sand flies through most of the world. Old World transmission occurs through the bite of *Phlebotomus* spp. sand flies, and *Lutzomyia* spp. sand flies in the New World (Killick-Kendrick, 1999). The sand fly vector appears to have preferential feeding on short-haired canines as higher rates of infection have been observed in short hair breeds (Dantas-Torres, 2008; França-Silva et al., 2003). Dogs receive bites in hairless areas such as the ear pinna, nose, and inguinal and perianal areas (Alvar et al., 2004). Although any dog is at risk for infection, dog breeds such as cocker spaniel, boxer, rottweiler, and German shepherd appear to be the most susceptible to infection (Killick-Kendrick, 1999). Thus far, very few breeds, like the Ibizian Hound, have shown resistance to developing clinical signs (Solano-Gallego et al., 2009).

Experimental studies have demonstrated that phlebotomine sand flies from non-endemic areas may become infected with *Leishmania* after feeding on an infected animal (Travi et al., 2002; van Griensven and Diro, 2019; Aronson and Joya, 2019). PCR analysis can deliver results with a quicker turnaround time (under 24 hours) and is able to detect low levels of parasitemia compared to the lengthier traditional methods

of diagnosis (Srivastava et al., 2011; Aronson et al., 2017; Akhoundi et al., 2017). However, PCR-based assays can still be disadvantageous for many endemic regions as the techniques are generally more expensive and complex than microscopy, require well trained staff, and require more financial resources to maintain equipment and purchase reagents (Burza et al., 2018; Vink et al., 2018).

Loop mediated isothermal amplification (LAMP) assays may be the answer to keeping all the advantages of molecular techniques while also reducing the associated disadvantages. In LAMP assays, there is no longer a need for expensive equipment as DNA amplification occurs at a constant temperature (~ 60 °C), reagents are lyophilized, and the product is easily visualized with simple methods (Mukhtar et al., 2018). Currently, the Loopamp *Leishmania* Detection Kit™ (Eiken Chemical Company, Japan) is available on the market and has shown promise for point-of-care testing with multiple sample types for several *Leishmania* species and assessment of cure for VL and PKDL (Vink et al., 2018; Mukhtar et al., 2018; Verma et al., 2017).

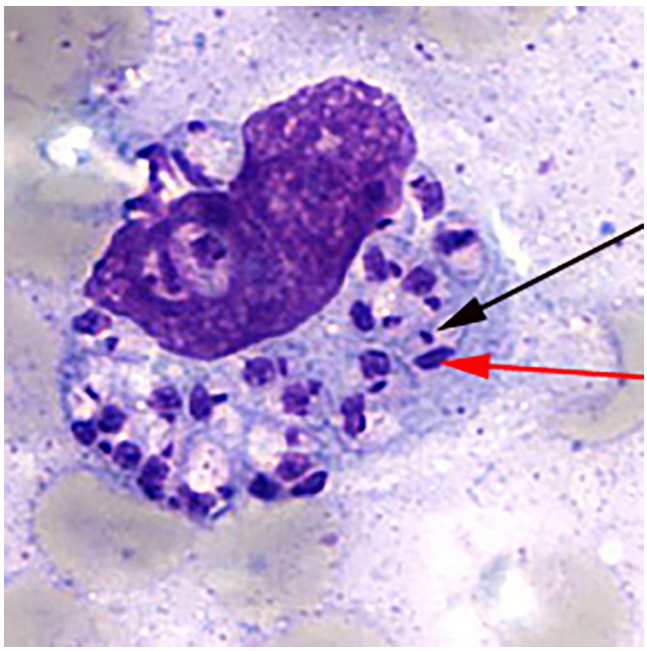

Figure 7. Light-microscopic examination of a stained bone marrow specimen from a patient with visceral leishmaniasis—showing a macrophage (a special type of white blood cell) containing multiple *Leishmania* **amastigotes** (the tissue stage of the parasite). Note that each amastigote has a **nucleus** (red arrow) and a rod-shaped **kinetoplast** (black arrow). Visualization of the kinetoplast is important for diagnostic purposes, to be confident the patient has leishmaniasis. Source: United States Centers for Disease Control and Prevention, DPDx. Public domain.

Treatment

Visceral Leishmaniasis

Many of the treatments for VL are toxic and/or expensive. The use of the different drugs, dosage, and treatment regimens differs depending on the *Leishmania* species initiating the infection, the geographical location, associated drug resistance in the region, and HIV co-infection. For current guidelines consult Aronson et al. (2017) and Burza et al. (2018).

The heavy metal antimony was first introduced as a therapy for leishmaniasis in the 1940s and for decades pentavalent antimonials were used as a monotherapy. Currently there are 2 antimony formulations in use: Sodium stibogluconate marketed as Pentostam® from Glaxo-Smith Kline and megalumine antimoniate marketed as Glocontime® from Rhone-Poulenc. These drugs have poor oral bioavailability so are delivered either by intramuscular injection or intravenously at a dose of 20 mg/kg per day for 16–28 days. The treatment is painful to administer and there are many adverse symptoms associated with the treatment, including pancreatitis, hepatotoxicity, cardiotoxicity, and induction of arrhythmias (Sundar and Chakravarty, 2010). Drug resistance is emerging to sodium stibogluconate on the Indian subcontinent so is no longer recommended as a therapy in this area (WHO, 2010; Sundar et al., 2000).

Miltefosine (hexadecylphosphocholine) was introduced as a chemotherapeutic to treat visceral leishmaniasis in 2002 as a result of a special public-private program initiated by the WHO with the pharmaceutical company Asta Medica to repurpose the anti-cancer compound (Sunyoto et al., 2018). Miltefosine is a broad spectrum anti-microbial, originally developed as an anti-cancer agent in the 1980s but adverse events in several phase I and II clinical studies resulted in the discontinuation of the drug's development as an oral anti-cancer drug (Planting et al., 1993; Verweij et al., 1993). As the only oral drug available to treat leishmaniasis, its introduction was seen as a breakthrough treatment. Unfortunately, this compound, marketed as Impavido® never reached its potential in controlling leishmaniasis due to gastrointestinal side effects, emergence of drug resistance, and cost (Dorlo et al., 2014; Ostyn et al., 2014; Rijal et al., 2013; Sundar et al., 2012). An economic analysis concluded that to be an effective public health tool, the drug should cost no more than US$ 50–60 per treatment (den Boer et al., 2011); currently the price for the public or non-for-profit sector in developing countries is US$ 117–160 and set at a market price of US$ 33,000–51,000 in the United States (Sunyoto et al., 2018).

Injectable paromomycin was introduced to combat leishmaniasis in 2006 and is used in combination treatment regimens with both pentavalent antimonials and miltefosine (Burza et al., 2018). The low cost of this drug (~ US$ 10–15)

is of great benefit to the public health sector. Amphotericin B deoxycholate, an anti-fungal drug, and its liposomal formulation are also used as monotherapy and in combination with other drugs.

Cutaneous Leishmaniasis

Most CL lesions will spontaneously heal within 2–18 months (David and Craft, 2009). For LCL caused by *Leishmania major* and *Le. mexicana* where 70% and 88%, respectively, of cases heal within 4 months, no treatment may be warranted. Treatment may be conducted to accelerate healing to reduce scarring, to reduce the risk of dissemination, or if the lesions are on the face or joints (Hodiamont et al., 2014; Weina et al., 2004). The Infectious Diseases Society of America and American Society of Tropical Medicine and Hygiene Clinical Practice Guidelines (Aronson et al., 2017) recommend local/topical therapy for non-healing simple lesions and systemic therapy for complex (MCL, RCL, DCL, DsCL, multiple lesions, lesions on face) and suggest that if resolution is apparent, management can occur without treatment if the patient concurs.

Treatment of CL has traditionally been administered by intralesional injection of the aforementioned drugs or topical application of antimicrobials such as paromomycin with or without methylbenzethonium chloride or gentamycin. Cryotherapy, a stimulus that decreases the lesion tissue temperature and results in cryonecrosis, has also been utilized (López-Carvajal et al., 2016). Recently, thermotherapy has been reintroduced as a therapy as amastigotes are heat sensitive and devices that deliver the radiofrequencies that deliver a temperature of 40–42 °C are relatively inexpensive (Burza et al., 2018). Systemic treatment is generally reserved for immunocompromised patients, individuals with multiple or refractory lesions, or patients at a risk for developing MCL (Burza et al., 2018; Aronson et al., 2017).

Table 2: Clinical Staging of Canine Leishmaniasis, Therapy and Prognosis

Clinical stage	Serology[a]	Clinical signs	Laboratory tests	Therapy	Prognosis
Stage I mild disease	Negative to low antibody levels	Mild clinical signs such as peripheral lymphadenopathy or papular dermatitis	Usually no clinicophathological abnormalities observed; normal renal profile	Allopurinol alone or with meglumine animoniate or miltefosine	Good
Stage II moderate disease	Low to high[b] antibody levels	Stage I signs plus diffuse or symmetrical cutaneous lesions such as exfoliative dermatitis/ onychogryphosis ulcerations (planum nasale, footpads, boy prominences, mucocutaneous junctions), anorexia, weight loss, fever, and epistaxis	Clinicopathological abnormalities such as mild non-regenerative anemia, hypergammaglobulinemia, hypoalbuminemia, serum hyperviscosity syndrome; normal renal profile [blood creatinine levels < 1.4 mg/dl] and non-proteinuric [urine protein to creatinine ratio (UPC) < 0.5] or UPC = 0.5–1	Allopurinol with meglumine animoniate or miltefosine	Good to guarded
Stage III severe disease	Medium to high antibody levels	Stage I & II signs plus may present vasculitis, arthritis, uveitis, or glomerulonephritis	Clinicopathological abnormalities from Stage II and chronic kidney disease (CKD) with UPC>1 or creatinine 1.4–2.8 mg/dl	Allopurinol with meglumine animoniate or miltefosine and follow guidelines for CKD	Guarded to poor
Stage IV extreme disease	Medium to high antibody levels	Stage I, II, & III signs plus pulmonary thromboembolism or nephrotic syndrome and end stage renal disease	Clinicopathological abnormalities from Stage II and creatinine 2–5 mg/dl or > 5 mg/dl. Nephrotic syndrome (UPC > 5)	Allopurinol alone and follow guidelines for CKD	Poor

[a] Dogs with negative to low antibody levels are confirmed as infected with additional diagnostic techniques. [b]High antibody levels are a conclusive diagnosis and are detected through immunofluorescence antibody test (IFAT) or enzyme-linked immunosorbent assay (ELISA). Table modified from Solano-Gallego et al., 2017.

Animal Models and Immunology

Susceptibility and resistance to *Leishmania* infection are regulated by genetic determinants and animal models have been instrumental in deciphering these mechanisms (Blackwell et al., 2009). In addition, many immunological aspects of the disease have been elucidated through the use of animal models, including mice, hamsters, dogs, and non-human primates (Loría-Cervera and Andrade-Narváez, 2014).

Mouse Model

Due to the existence of multiple strains of inbred mice, the simplicity of maintaining large numbers, and the vast number of reagents available for murine systems, experimental leishmaniasis in mice has been the primary animal model utilized for leishmaniasis research. For VL in mice, infection is primarily introduced via intravenous injection of large numbers of parasites. Two primary genetic loci, *Slc11a1* (also known as *Lsh/Bcg/XXX*) and *H2* [Major Histocompatibility Complex (MHC)], are associated with resistance to *Leishmania donovani* in mice. *Slc*11, the gene that encodes the transporter Nramp is responsible for resistance to *Le. donovani*, *Mycobacteria*, and *Salmonella* (Bellamy, 1999). In mouse strains that express the wild-type Nramp (for example, CBA mice), *Le*. *donovani* proliferation in the liver is inhibited. In strains that express a mutant Nramp (for example, BALB/c and C57Bl/6), parasite growth is unrestrained (Bellamy, 1999). The resistance mechanism only manifests in the initial stages of infection and MHC alleles that ultimately dictate adaptive immune responses can override Nramp susceptibility by inducing cure associated with reduced parasitic load in the liver. The non-cure mice progress to a chronic phase without clearing of the parasites.

The immune response to experimental VL and ultimate outcome of infection depends on the organ (liver or spleen) that is being assessed, the inoculation route and dose, and the parasite genotype (Loría-Cervera and Andrade-Narváez, 2014). Importantly, mice do not present the pathological features of human disease so are best used to determine infection susceptibility or resistance, rather than for assessing disease.

Rodents are a natural host for many cutaneous leishmaniasis causing species and so provide a good model for elucidating both immunological and genetic mechanisms of infection and pathology. Experimental infections with *Leishmania major* in particular have been instrumental in dissecting the immunological mechanisms of resistance (primarily C57Bl/6 strain) and susceptibility (primarily BALB/c strain) to infection and disease.

Resolution of cutaneous lesions in C57Bl/6 mice has been associated with a T-helper 1 (Th1) adaptive immune response that involves the production of interferon-gamma (IFN-γ) by CD4+ T-helper cells and stimulation of nitric oxide that is involved in destruction of amastigotes (Sacks and Noben-Trauth, 2002). Susceptibility in Balb/c mice correlates with a T-helper 2 (Th2) response characterized by the production of interleukin-4, interleukin-10, and transforming growth factor-beta (TGF-β) (Sacks and Noben-Trauth, 2002). The susceptible mice develop non-healing lesions and progressive disease, but not the clinical manifestations associated with VL. Genetic mapping assessing healing and non-healing phenotypes of progeny from crosses between resistant and susceptible mice revealed multiple genetic loci that influence both immune responses and wound healing (Sakthianandeswaren et al., 2009).

There are profound differences in the mechanisms that mediate susceptibility and resistance to infection and pathology associated with different *Leishmania* species. For example, C57Bl/6 and C3H mice that heal *Le. major* infections develop chronic disease when infected with either *Le*. *mexicana* or *Le*. *amazonensis* (Loría-Cervera and Andrade-Narváez, 2014). Chronic lesions due to *Le*. *amazonensis* are not dependent on a Th2 phenotype (Afonso and Scott, 1993) and parasite burden and pathology is exacerbated by Th1 cells (Soong et al., 1997). On the other hand, the non-healing phenotype of *Le*. *mexicana* infections in C57Bl/6 mice is associated with a Th2 response (Satoskar et al., 1995). There is no mouse model that recapitulates MCL caused by any *Leishmania* species so this system has had limited utility in understanding the pathology associated with MCL.

Hamster Model

The Syrian golden hamster (*Mesocricetus auratus*) is considered the best experimental model to study visceralizing *Leishmania* species (*Le*. *donovani* and *Le*. *infantum*) because this species is highly susceptible and reproduces the clinical pathology associated with visceral disease in humans (Melby et al., 2001). However, the dearth of immunological reagents (for example, antibodies for cell markers and cytokines) has hindered full understanding of the mechanisms of immunity.

Most studies in hamsters involve injection of large numbers of parasites via intravenous, intracardial or intraperitoneal injection that does not mimic the natural route of infection. Progressive disease involves uncontrolled parasite replication in the liver, spleen, and bone marrow despite the induction of T-helper 1 cytokines. Failure to control VL is associated with an immune suppression response associated with the production of TGF-β that triggers lymphocyte apoptosis (Banerjee et al., 2011), a lack of nitric oxide, the cytotoxin known to be required for killing of parasites (Pérez et al., 2006) and an inability of macrophages to process and present antigens to T-cells (Rodrigues et al., 1992).

Dog Model

Wild canines and domestic dogs serve as the primary reservoirs of zoonotic *Leishmania infantum* infection so the use of dogs as a model has recently gained momentum both to understand human VL and to identify mechanisms to treat canine VL. The primary mechanisms of protective immunity to *Le. infantum* in dogs is the activation of macrophages by a Th1 immune response (Vouldoukis et al., 1996). Canine VL is a multisystemic disease with variable clinical manifestations. Studies indicate a mixed cytokine response in CanL (Loría-Cervera and Andrade-Narváez, 2014). However, studies on experimentally infected dogs have shown that asymptomatic or resistant dogs produce a cell-mediated immune response to parasite antigens and more T-helper 1 associated cytokines than symptomatic dogs (Pinelli et al., 1994). The continued use of the dog as a model to study VL has the possibility of better understanding this complicated disease (see Table 2).

Non-Human Primate Models

Non-human primates are valuable models of human disease because of their similarities to human physiology and immunity. However, they are expensive and difficult to obtain and maintain, thus limited studies employ this model.

The Asian rhesus macaque (*Macaca mulatta*) is quite susceptible to *Leishmania* infection and the progression of CL and immune responses mimic CL in humans (Loría-Cervera and Andrade-Narváez, 2014). New World owl monkeys (*Aotus trivirgatus*) develop VL when infected with *Le. donovani* and exhibit weight loss, anemia, and hepatosplenomegaly similar to humans (Broderson et al., 1986). In contrast, squirrel monkeys (*Saimiri sciureus*) develop VL when infected with *Le. donovani* but recover and are resistant to reinfection (Dennis et al., 1986). Both symptomatic and asymptomatic *Le. donovani* infections are detectable in vervet monkeys (*Chlorocebus pygerythrus*) (see Gicheru et al., 1995) and Indian langurs have been used in vaccination studies (Misra et al., 2001).

Although the development of a non-human primate model that mimics human VL would certainly help elucidate the mechanisms of pathogenesis and immunity in humans, due to financial and ethical reasons, these models are typically only used when another model is not sufficient to answer a particular research question or as a final test for vaccines and drugs developed using other animal models.

Sand Flies

There has been partial success utilizing animal models for vaccine development against leishmaniasis; however, there still has been no licensure of an efficacious vaccine for humans. This general failure has led scientists to posit that the animal models should more closely mimic a natural infection (Reed et al., 2016). Sand flies are not simply syringes that inoculate parasites; rather they are a sort of pharmacy, capable of dispensing a plethora of pharmacologically active compounds into the skin of their hosts. These inoculated molecules have profound effects on the host immune system, exhibiting anti-haemostatic, anti-inflammatory, and immunosuppressive activities that facilitate blood feeding, while enhancing the establishment of the pathogens they transmit (McDowell, 2015). Moreover, *Leishmania*-infected sand flies also deposit a parasite-derived molecule, promastigote secretory gel (PSG), that accelerates wound healing while simultaneously enhancing survival and growth of *Leishmania* parasites (Giraud et al., 2018). To mimic a natural route of infection, many studies now incorporate sand fly saliva in the challenge inoculum and it has been posited that utilization of sand flies to inoculate parasites in the laboratory setting would advance vaccine development (Reed et al., 2016).

Inoculation of *Leishmania* parasites in the presence of sand fly saliva leads to enhancement of disease in animal models (Belkaid et al., 1998; Bezerra and Teixeira, 2001; Norsworthy et al., 2004; Samuelson et al., 1991; Theodos et al., 1991; Titus and Ribeiro, 1988). On the other hand, sand fly saliva has powerful immunogenic molecules that elicit strong hypersensitivity reactions in individuals that are repeatedly bitten. Repeated exposure to sand fly bites causes a delayed-type hypersensitivity (DTH) response recognized by local human inhabitants as a painful skin disease called harara (Adler and Theodor, 1957). Elicitation of this response has been suggested to be an evolutionary advantage for sand flies, by increasing blood-flow at the bite site and, therefore, decreasing the amount of time it takes for a sand fly to take a full blood meal (Belkaid et al., 2000). Although advantageous for sand flies, the DTH elicited by repeated exposure to sand fly bites (Kamhawi et al., 2000) or salivary gland homogenate (Belkaid et al., 1998) inhibits *Leishmania* infection in animal models. Thus, the inflammatory processes induced by the bite influence immunity to *Leishmania* parasites that are co-delivered with salivary components, effectively limit the ability of the parasite to cause devastating disease.

The prevalence of *Leishmania* infected sand flies in the field is quite low. For example, assessment of the sand fly vectors in specific areas in Iraq revealed that the highest infection rate was 2.3% (Aronson et al., 2003). Individuals in these areas can receive from 100 to 1,000 bites in a single night; therefore, the amount of sand fly saliva that is injected far outweighs any *Leishmania* antigen that may be inoculated, suggesting that sand fly saliva could be utilized as a part of a multi-component anti-leishmanial vaccine (Reed et al., 2016).

Literature Cited

Abbasi, I., S. Aramin, A. Hailu, W. Shiferaw, et al. 2013. Evaluation of PCR procedures for detecting and quantifying *Leishmania donovani* DNA in large numbers of dried human blood samples from a visceral leishmaniasis focus in Northern Ethiopia. BMC Infectious Diseases 13: 153. doi: 10.1186/1471-2334-13-153

Adler, S., and O. Theodor. 1957. Transmission of disease agents by phlebotomine sandflies. Annual Review of Entomology 2: 203–236. doi: 10.1146/annurev.en.02.010157.001223

Afonso, L. C., and P. Scott. 1993. Immune responses associated with susceptibility of C57BL/10 mice to *Leishmania amazonensis*. Infection and Immunity 61: 2,952–2,959. doi: 10.1128/iai.61.7.2952-2959.1993

Akhoundi, M., T. Downing, J. Votýpka, K. Kuhls, et al. 2017. *Leishmania* infections: Molecular targets and diagnosis. Molecular Aspects of Medicine 57: 1–29. doi: 10.1016/j.mam.2016.11.012

Akhoundi, M., K. Kuhls, A. Cannet, J. Votýpka, et al. 2016. A historical overview of the classification, evolution, and dispersion of *Leishmania* parasites and sandflies. PLoS Neglected Tropical Diseases 10: e0004349. doi: 10.1371/journal.pntd.0004349

Alexander, J., and D. G. Russell. 1992. The interaction of *Leishmania* species with macrophages. Advances in Parasitology 31: 175–254. doi: 10.1016/S0065-308X(08)60022-6

Ali, N., and S. Hussain. 2014. *Leishmania donovani* bodies in bone marrow [clinical image]. Clinical Case Reports 2: 238–239. doi: 10.1002/ccr3.97

Alvar, J., C. Cañavate, B. Gutiérrez-Solar, M. Jiménez, et al. 1997. *Leishmania* and human immunodeficiency virus coinfection: The first 10 years. Clincal Microbiology Reviews 10: 298–319. doi: 10.1128/CMR.10.2.298

Alvar, J., C. Cañavate, R. Molina, J. Moreno, et al. 2004. Canine leishmaniasis. Advances in Parasitology 57: 1–88. doi: 10.1016/S0065-308X(04)57001-X

Alvar, J., I. D. Vélez, C. Bern, M. Herrero, et al. 2012. Leishmaniasis worldwide and global estimates of its incidence. PLoS One 7: e35671. doi: 10.1371/journal.pone.0035671

Alves, F., G. Bilbe, S. Blesson, V. Goyal, et al. 2018. Recent development of visceral leishmaniasis treatments: Successes, pitfalls, and perspectives. Clincal Microbiology Reviews 31: e00048-18. doi: 10.1128/CMR.00048-18

Arce, A., A. Estirado, M. Ordobas, S. Sevilla, et al. 2013. Re-emergence of leishmaniasis in Spain: Community outbreak in Madrid, Spain, 2009 to 2012. Euro Surveillance : Bulletin Européen sur les maladies transmissibles 18: 20546. doi: 10.2807/1560-7917.es2013.18.30.20546

Argaw, D., A. Mulugeta, M. Herrero, N. Nombela, et al. 2013. Risk factors for visceral leishmaniasis among residents and migrants in Kafta-Humera, Ethiopia. PLoS Neglected Tropical Diseases 7: e2543. doi: 10.1371/journal.pntd.0002543

Arias, J. R., P. S. Monteiro, and F. Zicker. 1996. The reemergence of visceral leishmaniasis in Brazil. Emerging Infectious Diseases 2: 145–146. doi: 10.3201/eid0202.960213

Aronson, N. E., and C. A. Joya. 2019. Cutaneous leishmaniasis: Updates in diagnosis and management. Infectious Disease Clinics of North America 33: 101–117. doi: 10.1016/j.idc.2018.10.004

Aronson, N. E., R. Coleman, P. Coyne, E. Rowton, et al. 2003. Cutaneous leishmaniasis in U.S. military personnel, southwest/central Asia, 2002–2003. Mortality and Morbidity Weekly Report 52: 1,009–1,012.

Aronson, N. E., B. L. Herwaldt, M. Libman, R. Pearson, et al. 2017. Diagnosis and treatment of leishmaniasis: Clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). American Journal of Tropical Medicine and Hygiene 96: 24–45. doi: 10.4269/ajtmh.16-84256

Ayehu, A., Y. Aschale, W. Lemma, A. Alebel, et al. 2018. Seroprevalence of asymptomatic *Leishmania donovani* among laborers and associated risk factors in agricultural camps of West Armachiho District, Northwest Ethiopia: A cross-sectional study. Journal of Parasitology Research 2018: 5751743. doi: 10.1155/2018/5751743

Banerjee, R., S. Kumar, A. Sen, A. Mookerjee, et al. 2011. TGF-β-regulated tyrosine phosphatases induce lymphocyte apoptosis in *Leishmania donovani*-infected hamsters. Immunology and Cell Biology 89: 466–474. doi: 10.1038/icb.2010.108

Belkaid, Y., S. Kamhawi, G. Modi, J. Valenzuela, et al. 1998. Development of a natural model of cutaneous leishmaniasis: Powerful effects of vector saliva and saliva preexposure on the long-term outcome of *Leishmania major* infection in the mouse ear dermis. Journal of Experimental Medicine 188: 1,941–1,953. doi: 10.1084/jem.188.10.1941

Belkaid, Y., J. G. Valenzuela, S. Kamhawi, E. Rowton, et al. 2000. Delayed-type hypersensitivity to *Phlebotomus papatasi* sand fly bite: An adaptive response induced by the fly? Proceedings of the National Academy of Sciences of the United States of America 97: 6,704–6,709. doi: 10.1073/pnas.97.12.6704

Bellamy, R. 1999. The natural resistance-associated macrophage protein and susceptibility to intracellular pathogens. Microbes and Infection 1: 23–27. doi: 10.1016/s1286-4579(99)80010-0

Bennis, I., V. De Brouwere, Z. Belrhiti, H. Sahibi, et al. 2018. Psychosocial burden of localised cutaneous leishmaniasis: A scoping review. BMC Public Health 18: 358. doi: 10.1186/s12889-018-5260-9. doi: 10.1186/s12889-018-5260-9

Bezerra, H. S., and M. J. Teixeira. 2001. Effect of *Lutzomyia whitmani* (Diptera: Psychodidae) salivary gland lysates on *Leishmania* (*Viannia*) *braziliensis* infection in BALB/c mice.

Memórias do Instituto Oswaldo Cruz 96: 349–351. doi: 10.1590/s0074-02762001000300011

Blackwell, J. M., M. Fakiola, M. E. Ibrahim, S. E. Jamieson, et al. 2009. Genetics and visceral leishmaniasis: Of mice and man. Parasite Immunology 31: 254–266. doi: 10.1111/j.1365-3024.2009.01102.x

Broderson, J. R., W. L. Chapman, Jr., and W. L. Hanson. 1986. Experimental visceral leishmaniasis in the owl monkey. Veterinary Pathology 23: 293–302. doi: 10.1177/030098588602300310

Burza, S., S. L. Croft, and M. Boelaert. 2018. Leishmaniasis. Lancet 392: 951–970. doi: 10.1016/S0140-6736(18)31204-2

Carini, A., and U. Paranhos. 1909. Identification de L'"Ulcera de Bauru" avec le bouton d'Orient. Bulletin de la Société de pathologie exotique 1909: 255–256.

Chang, K.-P., and D. M. Dwyer. 1978. *Leishmania donovani*. Hamster macrophage interactions in vitro: Cell entry, intracellular survival, and multiplication of amastigotes. Journal of Experimental Medicine 147: 515–530. doi: 10.1084/jem.147.2.515

Chauhan, P., D. Shukla, D. Chattopadhyay, and B. Saha. 2017. Redundant and regulatory roles for Toll-like receptors in *Leishmania* infection. Clinical and Experimental Immunology 190: 167–186. doi: 10.1111/cei.13014

Clarke, C. F., K. K. Bradley, J. H. Wright, and J. Glowicz. 2013. Emergence of autochthonous cutaneous leishmaniasis in northeastern Texas and southeastern Oklahoma [Case report]. American Journal of Tropical Medicine and Hygiene 88: 157–161. doi: 10.4269/ajtmh.2012.11-0717

Costa, M. A., C. Matheson, L. Iachetta, A. Llagostera, et al. 2009. Ancient leishmaniasis in a highland desert of northern Chile. PLoS One 4: e6983. doi: 10.1371/journal.pone.0006983

Daneshbod, Y., A. Oryan, M. Davarmanesh, S. Shirian, et al. 2011. Clinical, histopathologic, and cytologic diagnosis of mucosal leishmaniasis and literature review. Archives of Pathology and Laboratory Medicine 135: 478–482. doi: 10.1043/2010-0069-OA.1

Dantas-Torres, F. 2008. Canine vector-borne diseases in Brazil. Parasites and Vectors 1: 25. doi: 10.1186/1756-3305-1-25

Darmstadt, G. L., A. T. Lane, and W. W. Tunnessen, Jr. 1993. Picture of the month, cutaneous leishmaniasis. American Journal of Diseases of Children 147: 1,339–1,340. doi: 10.1001/archpedi.1993.02160360081025

David, C. V., and N. Craft. 2009. Cutaneous and mucocutaneous leishmaniasis. Dermatologic Therapy 22: 491–502. doi: 10.1111/j.1529-8019.2009.01272.x

David, C., L. Dimier-David, F. Vargas, M. Torrez, et al. 1993. Fifteen years of cutaneous and mucocutaneous leishmaniasis in Bolivia: A retrospective study. Transactions of the Royal Society of Tropical Medicine and Hygiene 87: 7–9. doi: 10.1016/0035-9203(93)90398-a

Davies, C. R., E. A. Llanos-Cuentas, P. Campos, J. Monge, et al. 1997. Cutaneous leishmaniasis in the Peruvian Andes: Factors associated with variability in clinical symptoms, response to treatment, and parasite isolation rate. Clinical Infectious Diseases 25: 302–310. doi: 10.1086/514535

De Melo, E. C., and C. M. Fortaleza. 2013. Challenges in the therapy of visceral leishmaniasis in Brazil: A public health perspective. Journal of Tropical Medicine 2013: 319234. doi: 10.1155/2013/319234

den Boer, M., D. Argaw, J. Jannin, and J. Alvar. 2011. Leishmaniasis impact and treatment access. Clinical Microbiology and Infection 17: 1,471–1,477. doi: 10.1111/j.1469-0691.2011.03635.x

Dennis, V. A., R. Lujan, W. L. Chapman, Jr., and W. L. Hanson. 1986. *Leishmania donovani*: Cellular and humoral immune responses after primary and challenge infections in squirrel monkeys, *Saimiri sciureus*. Experimental Parasitology 61: 319–334. doi: 10.1016/0014-4894(86)90187-6

Desjardins, M., and A. Descoteaux. 1997. Inhibition of phagolysosomal biogenesis by the *Leishmania* lipophosphoglycan. Journal of Experimental Medicine 185: 2,061–2,068. doi: 10.1084/jem.185.12.2061

Desjeux, P. 2004. Leishmaniasis: Current situation and new perspectives. Comparative Immunology, Microbiology and Infectious Diseases 27: 305–318. doi: 10.1016/j.cimid.2004.03.004

Desjeux, P., and J. Alvar. 2003. *Leishmania*/HIV co-infections: Epidemiology in Europe. Annals of Tropical Medicine and Parasitology 97 (Supplement 1): 3–15. doi: 10.1179/000349803225002499

Di Muccio, T., A. Scalone, L. Gradoni, M. Marangi, et al. 2015. Epidemiology of imported leishmaniasis in Italy: Implications for a European endemic country. PLoS One 10: e0129418. doi: 10.1371/journal.pone.0129418

Diro, E., J. van Griensven, R. Mohammed, R. Colebunders, et al. 2015. Atypical manifestations of visceral leishmaniasis in patients with HIV in north Ethiopia: A gap in guidelines for the management of opportunistic infections in resource poor settings. Lancet, Infectious Diseases 15: 122–129. doi: 10.1016/S1473-3099(14)70833-3

Donovan, C. 1903. On the possibility of the occurrence of trypanosomiasis in India. British Medical Journal 2: 2–79.

Dorlo, T. P., S. Rijal, B. Ostyn, P. J. de Vries, et al. 2014. Failure of miltefosine in visceral leishmaniasis is associated with low drug exposure. Journal of Infectious Diseases 210: 146–153. doi: 10.1093/infdis/jiu039

Duprey, Z. H., F. J. Steurer, J. A. Rooney, L. V. Kirchhoff, et al. 2006. Canine visceral leishmaniasis, United States and Canada, 2000–2003. Emerging Infectious Diseases 12: 440–446. doi: 10.3201/eid1205.050811

Edrissian, G., M. B. Rokni, M. Mohebali, M. Nateghpour, et al. 2016. History of medical parasitology and parasitic infections in Iran. Archives of Iranian Medicine 19: 601–607. doi: 0161908/AIM.0014

Endris, R. G., D. G. Young, and P. V. Perkins. 1987. Experimental transmission of *Leishmania mexicana* by a North American sand fly, *Lutzomyia anthophora* (Diptera: Psychodidae).

Journal of Medical Entomology 24: 243–247. doi: 10.1093/jmedent/24.2.243

Espinosa, O. A., M. G. Serrano, E. P. Camargo, M. M. G. Teixeira, et al. 2018. An appraisal of the taxonomy and nomenclature of trypanosomatids presently classified as *Leishmania* and *Endotrypanum*. Parasitology 145: 430–442. doi: 10.1017/S0031182016002092

França-Silva, J. C., R. T. da Costa, A. M. Siqueira, G. L. L. Machado-Coelho, et al. 2003. Epidemiology of canine visceral leishmaniosis in the endemic area of Montes Claros Municipality, Minas Gerais State, Brazil. Veterinary Parasitology 111: 161–173. doi: 10.1016/s0304-4017(02)00351-5

Frias, L., D. Leles, and A. Araújo. 2013. Studies on protozoa in ancient remains: A review. Memórias do Instituto Oswaldo Cruz 108: 1–12. doi: 10.1590/S0074-02762013000100001

Galluzzi, L., M. Ceccarelli, A. Diotallevi, M. Menotta, et al. 2018. Real-time PCR applications for diagnosis of leishmaniasis. Parasites and Vectors 11: 273. doi: 10.1186/s13071-018-2859-8

Gibson, M. E. 1983. The identification of kala-azar and the discovery of *Leishmania donovani*. Medical History 27: 203–213. doi: 10.1017/s0025727300042691

Gicheru, M. M., J. O. Olobo, T. M. Kariuki, and C. Adhiambo. 1995. Visceral leishmaniasis in vervet monkeys: Immunological responses during asymptomatic infections. Scandinavian Journal of Immunology 41: 202–208. doi: 10.1111/j.1365-3083.1995.tb03554.x

Giraud, E., T. Lestinova, T. Derrick, O. Martin, et al. 2018. *Leishmania* proteophosphoglycans regurgitated from infected sand flies accelerate dermal wound repair and exacerbate leishmaniasis via insulin-like growth factor 1-dependent signalling. PLoS Pathogens 14: e1006794. doi: 10.1371/journal.ppat.1006794

Gitari, J. W., S. M. Nzou, F. Wamunyokoli, E. Kinyeru, et al. 2018. Leishmaniasis recidivans by *Leishmania tropica* in Central Rift Valley Region in Kenya. International Journal of Infectious Diseases 74: 109–116. doi: 10.1016/j.ijid.2018.07.008

Gossage, S. M., M. E. Rogers, and P. A. Bates. 2003. Two separate growth phases during the development of *Leishmania* in sand flies: implications for understanding the life cycle. International Journal for Parasitology 33: 1,027–1,034. doi: 10.1016/S0020-7519(03)00142-5

Goto, H., and J. A. Lindoso. 2010. Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis. Expert Review of Anti-Infective Therapy 8: 419–433. doi: 10.1586/eri.10.19

Gramiccia, M., and L. Gradoni. 2005. The current status of zoonotic leishmaniases and approaches to disease control. International Journal for Parasitology 35: 1,169–1,180. doi: 10.1016/j.ijpara.2005.07.001

Grimaldi, Jr., G., Jr., J. R. David, and D. McMahon-Pratt. 1987. Identification and distribution of New World *Leishmania* species characterized by serodeme analysis using monoclonal antibodies. American Journal of Tropical Medicine and Hygiene 36: 270–287. doi: 10.4269/ajtmh.1987.36.270

Guy, R. A., and M. Belosevic. 1993. Comparison of receptors required for entry of *Leishmania major* amastigotes into macrophages. Infection and Immunity 61: 1,553–1,558. doi: 10.4269/ajtmh.1987.36.270

Handler, M. Z., P. A. Patel, R. Kapila, Y. Al-Qubati, et al. 2015. Cutaneous and mucocutaneous leishmaniasis: Clinical perspectives. Journal of the American Academy of Dermatology 73: 897–908; quiz 909–910. doi: 10.1016/j.jaad.2014.08.051

Harhay, M. O., P. L. Olliaro, M. Vaillant, F. Chappuis, et al. 2011. Who is a typical patient with visceral leishmaniasis? Characterizing the demographic and nutritional profile of patients in Brazil, East Africa, and South Asia. American Journal of Tropical Medicine and Hygiene 84: 543–550. doi: 10.4269/ajtmh.2011.10-0321

Hashiguchi, Y., E. L. Gomez, H. Kato, L. R. Martin, et al. 2016. Diffuse and disseminated cutaneous leishmaniasis: Clinical cases experienced in Ecuador and a brief review. Tropical Medicine and Health 44: 2. doi: 10.1186/s41182-016-0002-0

Hayani, K., A. Dandashli, and E. Weisshaar. 2015. Cutaneous leishmaniasis in Syria: Clinical features, current status and the effects of war. Acta Dermato-Venereologica 95: 62–66. doi: 10.2340/00015555-1988

Herwaldt, B. L. 1999. Leishmaniasis. Lancet 354: 1,191–1,199. doi: 10.1016/S0140-6736(98)10178-2

Hodiamont, C. J., P. A. Kager, A. Bart, H. J. de Vries, et al. 2014. Species-directed therapy for leishmaniasis in returning travellers: A comprehensive guide. PLoS Neglected Tropical Diseases 8: e2832. doi: 10.1371/journal.pntd.0002832

Hyams, K. C., K. Hanson, F. S. Wignail, J. Escamilla, et al. 1995. The impact of infectious diseases on the health of U. S. troops deployed to the Persian Gulf during operations Desert Shield and Desert Storm. Clinical Infectious Diseases 20: 1,497–1,504. doi: 10.1093/clinids/20.6.1497

Jambulingam, P., N. Pradeep Kumar, S. Nandakumar, K. P. Paily, et al. 2017. Domestic dogs as reservoir hosts for *Leishmania donovani* in the southernmost Western Ghats in India. Acta Tropica 171: 64–67. doi: 10.1016/j.actatropica.2017.03.006

Kamhawi, S., S. K. Abdel-Hafez, and A. Arbagi. 1995. A new focus of cutaneous leishmaniasis caused by *Leishmania tropica* in northern Jordan. Transactions of the Royal Society of Tropical Medicine and Hygiene 89: 255–257. doi: 10.1016/0035-9203(95)90526-x

Kamhawi, S., Y. Balkaid, G. Modi, E. Rowton, et al. 2000. Protection against cutaneous leishmaniasis resulting from bites of uninfected sand flies. Science 290: 1,351–1,354. doi: 10.1126/science.290.5495.1351

Killick-Kendrick, R. 1999. The biology and control of phlebotomine sand flies. Clinics in Dermatology 17: 279–289. doi: 10.1016/s0738-081x(99)00046-2

Kipp, E. J., and M. Hergert. 2019. Endemic human cutaneous leishmaniasis incidence in the United States. JAMA Dermatology 155: 259–260. doi: 10.1001/jamadermatol.2018.4951

Koff, A. B., and T. Rosen. 1994. Treatment of cutaneous leishmaniasis. Journal of the American Academy of Dermatology 31(5 Part 1): 693–708; quiz 708–710. doi: 10.1016/s0190-9622(94)70229-2

Lainson, R., and E. F. Rangel. 2005. *Lutzomyia longipalpis* and the eco-epidemiology of American visceral leishmaniasis, with particular reference to Brazil: A review. Memórias do Instituto Oswaldo Cruz 100: 811–827. doi: 10.1590/s0074-02762005000800001

Lainson, R., and J. J. Shaw. 1987. Evolution, classification, and geographical distribution. *In* W. Peters and R. Killick-Kendrick, eds. The Leishmaniases in Biology and Medicine, Volume I: Biology and Epidemiology. Academic Press, London, United Kingdom, p. 1–120.

Leishman, W. B. 1903. On the possibility of the occurrence of trypanosomiasis in India. British Medical Journal 2: 1,252–1,254.

Lindenberg, A. 1909. L'Ulcere de Bauru ou le bouton d'Orient au Bresil. Bulletin de la Société de pathologie exotique 1909: 252–254.

López-Carvajal, L., J. A. Cardona-Arias, M. I. Zapata-Cardona, V. Sánchez-Giraldo, et al. 2016. Efficacy of cryotherapy for the treatment of cutaneous leishmaniasis: Meta-analyses of clinical trials. BMC Infectious Diseases 16: 360. doi: 10.1186/s12879-016-1663-3

Loría-Cervera, E. N., and F. J. Andrade-Narváez. 2014. Animal models for the study of leishmaniasis immunology. Revista do Instituto de Medicina Tropical de São Paulo 56: 1–11. doi: 10.1590/S0036-46652014000100001

Magill, A. J., M. Grögl, R. A. Gasser, Jr., W. Sun, et al. 1993. Visceral infection caused by *Leishmania tropica* in veterans of Operation Desert Storm. New England Journal of Medicine 328: 1,383–1,387. doi: 10.1056/NEJM199305133281904

Maspero, G. 1910. The Dawn of Civilization: Egypt and Chaldaea, 5th edition. Society for the Promotion of Christian Knowledge, London, United Kingdom.

Mauricio, I. L., M. K. Howard, J. R. Stothard, and M. A. Miles. 1999. Genomic diversity in the *Leishmania donovani* complex. Parasitology 119: 237–246. doi: 10.1017/s0031182099004710

McConville, M. J., D. de Souza, E. Saunders, V. A. Likic, et al. 2007. Living in a phagolysosome; metabolism of *Leishmania* amastigotes. Trends in Parasitology 23: 368–375. doi: 10.1016/j.pt.2007.06.009

McDowell, M. A. 2015. Vector-transmitted disease vaccines: Targeting salivary proteins in transmission (SPIT). Trends in Parasitology 31: 363–372. doi: 10.1016/j.pt.2015.04.011

McHugh, C. P., M. Grogl, and R. D. Kreutzer. 1993. Isolation of *Leishmania mexicana* (Kinetoplastida: Trypanosomatidae) from *Lutzomyia anthophora* (Diptera: Psychodidae) collected in Texas. Journal of Medical Entomology 30: 631–633. doi: 10.1093/jmedent/30.3.631

Melby, P. C., B. Chandrasekar, W. Zhao, and J. E. Coe. 2001. The hamster as a model of human visceral leishmaniasis: Progressive disease and impaired generation of nitric oxide in the face of a prominent Th1-like cytokine response. Journal of Immunology 166: 1,912–1,920. doi: 10.4049/jimmunol.166.3.1912

Melby, P. C. R. D. Kreutzer, D. McMahon-Pratt, A. A. Gam, et al. 1992. Cutaneous leishmaniasis: Review of 59 cases seen at the National Institutes of Health. Clinical Infectious Diseases 15: 924–937. doi: 10.1093/clind/15.6.924

Misra, A., A. Dube, B. Srivastava, P. Sharma, et al. 2001. Successful vaccination against *Leishmania donovani* infection in Indian langur using alum-precipitated autoclaved *Leishmania major* with BCG. Vaccine 19: 3,485–3,492. doi: 10.1016/s0264-410x(01)00058-5

Modý, R. M., I. Lakhal-Naouar, J. E. Sherwood, N. L. Koles, et al. 2019. Asymptomatic visceral *Leishmania infantum* infection in U.S. soldiers deployed to Iraq. Clinical Infectious Diseases 68: 2,036–2,044. doi: 10.1093/cid/ciy811

Molina, R., D. Ghosh, E. Carillo, S. Monserrat, et al. 2017. Infectivity of post-kala-azar dermal leishmaniasis patients to sand flies: Revisiting a proof of concept in the context of the kala-azar elimination program in the Indian subcontinent. Clinical Infectious Diseases 65: 150–153. doi: 10.1093/cid/cix245.

Morris-Jones, S., and M. Weber. 2004. Medical mystery: Painless ulcers: The answer. New England Journal of Medicine 350: 2,313–2,314; discussion 2,313–2,314. doi: 10.1056/NEJMc045252

Mukhtar, M., S. S. Ali, S. A. Boshara, A. Albertini, et al. 2018. Sensitive and less invasive confirmatory diagnosis of visceral leishmaniasis in Sudan using loop-mediated isothermal amplification (LAMP). PLoS Neglected Tropical Diseases 12: e0006264. doi: 10.1371/journal.pntd.0006264

Nagill, R., and S. Kaur. 2011. Vaccine candidates for leishmaniasis: A review. International Immunopharmacology 11: 1,464–1,488. doi: 10.1016/j.jim.2015.03.017

Nimer, N. A. 2018. A review on emerging and reemerging of infectious diseases in Jordan: The aftermath of the Syrian crises. Canadian Journal of Infectious Diseases and Medical Microbiology 2018: 8679174. doi: 10.1155/2018/8679174

Norsworthy, N. B., J. Sun, D. Elnaiem, G. Lanzaro, et al. 2004. Sand fly saliva enhances *Leishmania amazonensis* infection by modulating interleukin-10 production. Infection and Immunity 72: 1,240–1,247. doi: 10.1128/IAI.72.3.1240-1247.2004

Ostyn, B., E. Hasker, T. P. Dorlo, S. Rijal, et al. 2014. Failure of miltefosine treatment for visceral leishmaniasis in children and men in south-east Asia. PLoS One 9: e100220. doi: 10.1371/journal.pone.0100220

Padovese, V., M. Terranova, L. Toma, G. A. Barnabas, et al. 2009. Cutaneous and mucocutaneous leishmaniasis in Tigray, northern Ethiopia: Clinical aspects and therapeutic concerns. Transactions of the Royal Society of Tropical Medicine and Hygiene 103: 707–711. doi: 10.1016/j.trstmh.2009.02.023

Pavli, A., and H. C. Maltezou. 2010. Leishmaniasis, an emerging infection in travelers. International Journal of Infectious Diseases 14: e1032–e1039. doi: 10.1016/j.ijid.2010.06.019

Pérez, L. E., B. Chandrasekar, O. A. Saldarriaga, W. Zhao, et al. 2006. Reduced nitric oxide synthase 2 (NOS2) promoter activity in the Syrian hamster renders the animal functionally deficient in NOS2 activity and unable to control an intracellular pathogen. Journal of Immunology 176: 5,519–5,528. doi: 10.4049/jimmunol.176.9.5519

Piggot, D. M., S. Bhatt, N. Golding, K. A. Duda, et al. 2014. Global distribution maps of the leishmaniases. eLife 3: e02851. doi: 10.7554/eLife.02851.

Pinelli, E., C. J. Boog, V. P. Rutten, B. van Dijk, et al. 1994. A canine CD8+ cytotoxic T-cell line specific for *Leishmania infantum*-infected macrophages. Tissue Antigens 43: 189–192. doi: 10.1111/j.1399-0039.1994.tb02321.x

Planting, A. S., G. Stoter, and J. Verweij. 1993. Phase II study of daily oral miltefosine (hexadecylphosphocholine) in advanced colorectal cancer. European Journal of Cancer 29A: 518–519. doi: 10.1016/s0959-8049(05)80142-x

Podinovskaia, M., and A. Descoteaux. 2015. *Leishmania* and the macrophage: A multifaceted interaction. Future Microbiology 10: 111–129. doi: 10.1111/cei.13014

Poinar, Jr., G., 2008. *Lutzomyia adiketis* sp. n. (Diptera: Phlebotomidae), a vector of sp. n. (Kinetoplastida: Trypanosomatidae) in Dominican amber. Parasites and Vectors 1: 22. doi: 10.1186/1756-3305-1-22

Poinar, Jr., G., and R. Poinar. 2004a. Evidence of vector-borne disease of Early Cretaceous reptiles. Vector-Borne Zoonotic Diseases 4: 281–284. doi: 10.1089/vbz.2004.4.281

Poinar, Jr., G., and R. Poinar. 2004b. *Paleoleishmania proterus* n. gen., n. sp., (Trypanosomatidae: Kinetoplastida) from Cretaceous Burmese amber. Protist 155: 305–310. doi: 10.1078/1434461041844259

Pratlong, F., J. Dereure, C. Ravel, P. Lami, et al. 2009. Geographical distribution and epidemiological features of Old World cutaneous leishmaniasis foci, based on the isoenzyme analysis of 1048 strains. Tropical Medicine and International Health 14: 1,071–1,085. doi: 10.1111/j.1365-3156.2009.02336.x

Read, L. K., J. Lukes, and H. Hashimi. 2016. Trypanosome RNA editing: The complexity of getting U in and taking U out. Wiley Interdisciplinary Reviews. RNA 7: 33–51. doi: 10.1002/wrna.1313

Reed, S. G., R. N. Coler, D. Mondal, S. Kamhawi, et al. 2016. *Leishmania* vaccine development: Exploiting the host-vector-parasite interface. Expert Review of Vaccines 15: 81–90. doi: 10.1586/14760584.2016

Rehman, K., J. Walochnik, J. Mischlinger, B. Alassil, et al. 2018. Leishmaniasis in northern Syria during Civil War. Emerging Infectious Diseases 24: 1,973–1,981. doi: 10.3201/eid2411.172146

Rijal, S., B. Ostyn, S. Uranw, K. Rai, et al. 2013. Increasing failure of miltefosine in the treatment of kala-azar in Nepal and the potential role of parasite drug resistance, reinfection, or noncompliance. Clinical Infectious Diseases 56: 1,530–1,538. doi: 10.1093/cid/cit102

Rodrigues, Jr., V., J. S. Da Silva, and A. Campos-Neto. 1992. Selective inability of spleen antigen presenting cells from *Leishmania donovani* infected hamsters to mediate specific T cell proliferation to parasite antigens. Parasite Immunology 14: 49–58. doi: 10.1111/j.1365-3024.1992.tb00005.x

Rogers, M. E., T. Ilg, A. N. Nikolaev, M. A. Ferguson, et al. 2004. Transmission of cutaneous leishmaniasis by sand flies is enhanced by regurgitation of fPPG. Nature 430: 463–467. doi: 10.1038/nature02675

Romero, G. A., and M. Boelaert. 2010. Control of visceral leishmaniasis in Latin America: A systematic review. PLoS Neglected Tropical Diseases 4: e584. doi: 10.1371/journal.pntd.0000584

Ross, R. 1903a. Further notes of Leishman's bodies. British Medical Journal 2: 1,401.

Ross, R. 1903b. Note on the bodies recently described by Leishman and Donovan. British Medical Journal 2: 1,261–1,262. doi: 10.1136/bmj.2.2237.1261

Sacks, D., and S. Kamhawi. 2001. Molecular aspects of parasite-vector and vector-host interactions in leishmaniasis. Annual Review of Microbiology 55: 453–483. doi: 10.1146/annurev.micro.55.1.453

Sacks, D., and N. Noben-Trauth. 2002. The immunology of susceptibility and resistance to *Leishmania major* in mice. Nature Reviews, Immunology 2: 845–858. doi: 10.1038/nri933

Sacks, D. L., and P. V. Perkins. 1984. Identification of an infective stage of *Leishmania* promastigotes. Science 223: 1,417–1,419. doi: 10.1126/science.6701528

Sakthianandeswaren, A., S. J. Foote, and E. Handman. 2009. The role of host genetics in leishmaniasis. Trends in Parasitology 25: 383–391. doi: 10.1016/j.pt.2009.05.004

Saliba, E. K., N. Saleh, O. Y. Oumeish, S. Khoury, et al. 1997. The endemicity of *Leishmania tropica* (zymodeme MON-137) in the Eira-Yarqa area of Salt District, Jordan. Annals of Tropical Medicine and Parasitology 91: 453–459.

Samady, J. A., C. K. Janniger, and R. A. Schwartz. 1996. Cutaneous and mucocutaneous leishmaniasis. Cutis 57: 13–20.

Samuelson, J., E. Lerner, R. Tesh, and R. G. Titus. 1991. A mouse model of *Leishmania braziliensis braziliensis* infection produced by coinjection with sand fly saliva. Journal of Experimental Medicine 173: 49–54. doi: 10.1084/jem.173.1.49

Satoskar, A., H. Bluethmann, and J. Alexander. 1995. Disruption of the murine interleukin-4 gene inhibits disease progression during *Leishmania mexicana* infection but does not increase control of *Leishmania donovani* infection. Infection and Immunity 63: 4,894–4,899. doi: 10.1128/iai.63.12.4894-4899.1995

Scheffter, S. M., R. T. Ro, I. K. Chung, and J. L. Patterson. 1995. The complete sequence of *Leishmania* RNA virus LRV2-1, a virus of an Old World parasite strain. Virology 212: 84–90. doi: 10.1006/viro.1995.1456

Scianimanico, S., M. Desrosiers, J. F. Dermine, S. Mèresse, et al. 1999. Impaired recruitment of the small GTPase rab7 correlates with the inhibition of phagosome maturation by *Leishmania donovani* promastigotes. Cellular Microbiology 1: 19–32. doi: 10.1046/j.1462-5822.1999.00002.x

Scott, P., and F. O. Novais. 2016. Cutaneous leishmaniasis: Immune responses in protection and pathogenesis. Nature Reviews, Immunology 16: 581–592. doi: 10.1038/nri.2016.72

Simpson, L. 1987. The mitochondrial genome of kinetoplastid protozoa: Genomic organization, transcription, replication, and evolution. Annual Review of Microbiology 41: 363–382. doi: 10.1146/annurev.mi.41.100187.002051

Solano-Gallego, L., L. Cardoso, M. Grazia Pennisi, C. Petersen, et al., 2017. Diagnostic challenges in the era of canine *Leishmania infantum* vaccines. Trends in Parasitology 33: 706–717. doi: 10.1016/j.pt.2017.06.004

Solano-Gallego, L., A. Koutinas, G. Miró, L. Cardoso, et al. 2009. Directions for the diagnosis, clinical staging, treatment and prevention of canine leishmaniasis. Veterinary Parasitology 165: 1–18. doi: 10.1016/j.vetpar.2009.05.022

Solomon, M., E. Schwartz, F. Pavlotsky, N. Sakka, et al. 2014. *Leishmania tropica* in children: A retrospective study. Journal of the American Academy of Dermatology 71: 271–277. doi: 10.1016/j.jaad.2013.12.047

Soong, L., C. H. Chang, J. Sun, B. J. Longley, Jr., et al. 1997. Role of CD4+ T cells in pathogenesis associated with *Leishmania amazonensis* infection. Journal of Immunology 158: 5,374–5,383.

Srivastava, P., A. Dayama, S. Mehrotra, and S. Sundar. 2011. Diagnosis of visceral leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene 105: 1–6. doi: 10.1186/s13071-017-1969-z

Srivastava, P., K. Gidwani, A. Picado, G. Van der Auwera, et al. 2013. Molecular and serological markers of *Leishmania donovani* infection in healthy individuals from endemic areas of Bihar, India. Tropical Medicine and International Health 18: 548–554. doi: 10.1111/tmi.12085

Stahlman, S., V. F. Williams, and S. B. Taubman. 2017. Incident diagnoses of leishmaniasis, active and reserve components, U. S. Armed Forces, 2001–2016. MSMR 24: 2–7.

Stark, D., S. Pett, D. Marriott, and J. Harkness. 2006. Post-kala-azar dermal leishmaniasis due to *Leishmania infantum* in a human immunodeficiency virus type 1-infected patient. Journal of Clinical Microbiology 44: 1,178–1,180. doi: 10.1128/JCM.44.3.1178-1180.2006

Steverding, D. 2017.The history of leishmaniasis. Parasites and Vectors 10: 82. doi: 10.1186/s13071-017-2028-5

Strazzulla, A., S. Cocuzza, M. R. Pinzone, M. C. Postorino, et al. 2013. Mucosal leishmaniasis: An underestimated presentation of a neglected disease. BioMed Research International 2013: 805108. doi: 10.1155/2013/805108

Sundar, S., and J. Chakravarty. 2010. Antimony toxicity. International Journal of Environmental Research and Public Health 7: 4,267–4,277. doi: 10.3390/ijerph7124267

Sundar, S., D. K. More, M. K. Singh, V. P. Singh, et al. 2000. Failure of pentavalent antimony in visceral leishmaniasis in India: Report from the center of the Indian epidemic. Clinical Infectious Diseases 31: 1,104–1,107. doi: 10.1086/318121

Sundar, S., A. Singh, M. Rai, V. K. Prajapati, et al. 2012. Efficacy of miltefosine in the treatment of visceral leishmaniasis in India after a decade of use. Clinical Infectious Diseases 55: 543–550. doi: 10.1093/cid/cis474

Sunyoto, T., J. Potet, and M. Boelaert. 2018. Why miltefosine—a life-saving drug for leishmaniasis—is unavailable to people who need it the most. BMJ Global Health 3: e000709. doi: 10.1136/bmjgh-2018-000709

Talmi-Frank, D. C. L. Jaffe, A. Nassereddin, A. Warburg, et al. 2010. *Leishmania tropica* in rock hyraxes (*Procavia capensis*) in a focus of human cutaneous leishmaniasis. American Journal of Tropical Medicine and Hygiene 82: 814–818. doi: 10.4269/ajtmh.2010.09-0513

Theodos, C. M., J. M. Ribeiro, and R. G. Titus. 1991. Analysis of enhancing effect of sand fly saliva on *Leishmania* infection in mice. Infection and Immunity 59: 1,592–1,598. doi: 10.1128/iai.59.5.1592-1598.1991

Titus, R. G., and J. M. Ribeiro. 1988. Salivary gland lysates from the sand fly *Lutzomyia longipalpis* enhance *Leishmania* infectivity. Science 239: 1,306–1,308. doi: 10.1126/science.3344436

Travi, B. L., C. Ferro, H. Candfena, J. Montoya-Lerma, et al. 2002. Canine visceral leishmaniasis: Dog infectivity to sand flies from non-endemic areas. Research in Veterinary Science 72: 83–86. doi: 10.1053/rvsc.2001.0527

Tuon, F. F., V. A. Neto, and V. S. Amato. 2008. *Leishmania*: Origin, evolution and future since the Precambrian. FEMS Immunology and Medical Microbiology 54: 158–166. doi: 10.1111/j.1574-695X.2008.00455.x

Twining, W. 1827. Observations on diseases of the spleen particularly on the vascular engorgement of that organ common in Bengal. Transactions of the Medical and Physical Society of Bengal 1827: 351–412.

Ueno, N., and M. E. Wilson. 2012. Receptor-mediated phagocytosis of *Leishmania*: Implications for intracellular survival. Trends in Parasitology 28: 335–344. doi: 10.1016/j.pt.2012.05.002

Van der Auwera, G. and J. C. Dujardin. 2015. Species typing in dermal leishmaniasis. Clincal Microbiology Reviews 28: 265–294. doi: 10.1128/CMR.00104-14

van Griensven, J., and E. Diro. 2019. Visceral leishmaniasis: Recent advances in diagnostics and treatment regimens. Infectious Disease Clinics of North America 33: 79–99. doi: 10.1016/j.idc.2018.10.005

van Griensven, J., E. Gadisa, A. Aseffa, A. Hailu, et al. 2016. Treatment of cutaneous leishmaniasis caused by *Leishmania aethiopica*: A systematic review. PLoS Neglected Tropical Diseases 10: e0004495. doi: 10.1371/journal.pntd.0004495

van Zandbergen, G., M. Klinger, A. Mueller, S. Dannenberg, et al. 2004. Cutting edge: Neutrophil granulocyte serves as a vector for *Leishmania* entry into macrophages. Journal of Immunology 173: 6,521–6,525. doi: 10.4049/jimmunol.173.11.6521

Verma, S., R. Singh, V. Sharma, R. A. Bumb, et al. 2017. Development of a rapid loop-mediated isothermal amplification assay for diagnosis and assessment of cure of *Leishmania* infection. BMC Infectious Diseases 17: 223. doi: 10.1186/s12879-017-2318-8

Verweij, J., D. Gandia, A. S. Planting, G. Stoter, et al. 1993. Phase II study of oral miltefosine in patients with squamous cell head and neck cancer. European Journal of Cancer 29A: 778–779. doi: 10.1016/s0959-8049(05)80369-7

Vink, M. M. T., S. M. Nahzat, H. Rahimi, C. Buhler, et al. 2018. Evaluation of point-of-care tests for cutaneous leishmaniasis diagnosis in Kabul, Afghanistan. EBioMedicine 37: 453–460. doi: 10.1016/j.ebiom.2018.10.063

Vouldoukis, I., J. C. Drapier, A. K. Nüssler, Y. Tselentis, et al. 1996. Canine visceral leishmaniasis: Successful chemotherapy induces macrophage antileishmanial activity via the L-arginine nitric oxide pathway. Antimicrobial Agents and Chemotherapy 40: 253–256. doi: 10.1128/AAC.40.1.253

Weina, P. J., R. C. Neafie, G. Wortmann, M. Polhemus, et al. 2004. Old world leishmaniasis: An emerging infection among deployed US military and civilian workers. Clinical Infectious Diseases 39: 1,674–1,680. doi: 10.1086/425747

Weinkopff, T., A. Mariotto, G. Simon, Y. Hauyon-La Torre, et al. 2013. Role of Toll-like receptor 9 signaling in experimental *Leishmania braziliensis* infection. Infection and Immunity 81: 1,575–1,584. doi: 10.1128/IAI.01401-12

WHO (World Health Organization). 2017. Accelerated Plan for Kala-Azar Elimination 2017. World Health Organization, Geneva, Switzerland.

WHO (World Health Organization). 2010. Control of leishmaniasis: Report of a meeting of the WHO Expert Commitee on the Control of Leishmaniases. WHO Technical Report Series 9492010.

WHO (World Health Organization). 2016. Leishmaniasis in high-burden countries: An epidemiologial update based on data reported in 2014. Weekly Epidemiological Record 91: 285–296.

WHO (World health Organization). 2024. WHO in Syria: Leishmaniasis. https://www.emro.who.int/syria/priority-areas/leishmaniasis.html

Zangger, H., C. Ronet, C. Desponds, F. M. Kuhlmann, et al. 2013. Detection of *Leishmania* RNA virus in *Leishmania* parasites. PLoS Neglected Tropical Diseases 7: e2006. doi: 10.1371/journal.pntd.0002006

Zijlstra, E. E. 2016. The immunology of post-kala-azar dermal leishmaniasis (PKDL). Parasites and Vectors 9: 464. doi: 10.1186/s13071-016-1721-0

Zijlstra, E. E., A. M. Musa, E. A. Khalil, I. M. el-Hassan, et al. 2003. Post-kala-azar dermal leishmaniasis. Lancet, Infectious Diseases 3: 87–98. doi: 10.1016/s1473-3099(03)00517-6

Zink, A. R., M. Spigelman, B. Schraut, A.G. Nerlich, et al. 2006. Leishmaniasis in ancient Egypt and Upper Nubia. Emerging Infectious Diseases 12: 1,616–1,617. doi: 10.3201/eid1210.06016

Supplemental Reading

Ferreira, M. G., K. R. Fattori, F. Souza, and V. M. Lima. 2009. Potential role for dog fleas in the cycle of *Leishmania* spp. Veterinary Parasitology 165: 150–154. doi: 10.1016/j.vetpar.2009.06.026

García, L. S. 2007. Leishmaniasis. *In* L. S. García, ed. Diagnostic Medical Parasitology, 5th edition. ASM Press, Washington, DC, United States, p. 190–217. doi: 10.1128/9781555816018.ch8

Lindoso, J. A. L., C. H. V. Moreira, M. A. Cunha, and I. T. Queiroz. 2018. Visceral leishmaniasis and HIV coinfection: Current perspectives. HIV/AIDS (Auckland, New Zealand) 10: 193–201. doi: 10.2147/HIV.S143929

Pigott, D. M., S. Bhatt, N. Golding, K. A. Duda, et al. 2014. Global distribution maps of the leishmaniases. eLife 3: e02851. doi: 10.7554/eLife.o2851

van Henten, S., W. Adriaensen, H. Fikre, H. Akuffo, et al. 2018. Cutaneous leishmaniasis due to Leishmania aethiopica. eClinicalMedicine 6: P69-81. doi: 10.1016/j.eclinm.2018.12.009

WHO (World Health Organization). 2023. Leishmaniasis. World Health Organization, Geneva, Switzerland.