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Review Article

'Aleppo Evil', 'White Leprosy', 'Busi Yasi': Biological and Clinical Aspects of Cutaneous Leishmaniasis with New Insights from Suriname

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Abstract

The parasitic disease Leishmaniasis in its various clinical manifestations is one of the most serious public health threats in many parts of the world, its global prevalence and yearly incidence rates exceeding 12 million and 2.5 million cases, respectively, and its burden estimated at 2.4 million disability-adjusted life years. For these reasons, the World Health Organization has classified Leishmaniasis as a category 1 disease *i.e.*, an emerging and/or uncontrolled disease. In the Republic of Suriname (South America), Cutaneous Leishmaniasis (CL) is so far the principal form of the disease. For a long time it was believed that *Leishmania (Viannia) guyanensis* was the only *Leishmania* species causing CL in the country. Furthermore, the vectors involved in its transmission were not exactly known, and animal reservoirs had not been identified. Lately, however, important new insights have been obtained in these fundamental biological aspects of CL in Suriname. This paper presents a few historical highlights of Leishmaniasis; addresses relevant epidemiological and etiological aspects of this disease; elaborates on the biology as well methods for diagnosis and available forms of treatment of this condition; then focuses on recent insights in these topics in Suriname; and concludes with possible future approaches on the treatment and diagnosis of CL in the country.

ABBREVIATIONS

CL: Cutaneous Leishmaniasis; MCL: Muco-Cutaneous Leishmaniasis; VL: Visceral Leishmaniasis; PI: Pentamidine Isethionate

INTRODUCTION

Leishmaniasis is a complex of parasitic diseases caused by kinetoplastid flagellates of the genus *Leishmania* (family Trypanosomatidae). This disease is endemic in eighty-eight countries throughout the world [1], and 350 million people are at risk to develop this disease [1]. Worldwide annual prevalence and incidence rates exceed 12 million and 2.5 million individuals, respectively [1], and the estimated disease burden is 2.4 million disability-adjusted life years [1]. However, these numbers may be higher as Leishmaniasis has only been documented in forty of the eighty-eight countries where it is present [2]. For these reasons, Leishmaniasis is classified as a WHO category 1 disease, *i.e.*, an emerging and/or uncontrolled disease, acknowledging it as a severely neglected condition in terms of scientific research, diagnostic methods, and treatment options [3,4].

Leishmaniasis is encountered in both the Old World - Southern Europe, Africa, the Middle-east, as well as Western and Central Asia - and the New World, *i.e.*, Southern USA to Northern Argentina [5,6]. The disease is particularly prevalent in poor populations in tropical and subtropical regions [7-9], as well as war-torn countries such as Afghanistan [10], Sudan [11], and Iraq [12,13]. However, over the past decade, Leishmaniasis has increasingly been reported in industrialized, non-endemic countries [8,14]. This is probably attributable to the movement of non-immunized refugees, military personnel, and tourists to endemic disaster areas, war zones, and holiday resorts [8,15]. In addition, new foci of Leishmaniasis are established by the

migration of infected people to regions where the disease is absent and the population has no immunity [8]. Immuno-compromised individuals such as those infected with HIV also contribute to the spread of Leishmaniasis [16].

Leishmania parasites comprise two subgenera, *Leishmania* and *Viannia* (Table 1). The subgenus *Leishmania* can be found in both the Old and the New World, while the subgenus *Viannia* is restricted to the New World [5]. These parasites are transmitted by the bite of infected female sand flies of the genera *Phlebotomus* in the Old World and *Lutzomyia* in the New World [17]. So far, thirty sand fly species have been identified as vectors of *Leishmania* parasites [17]. Some are involved in the zoonotic transmission cycle of Leishmaniasis - which includes animal reservoir hosts - while others are anthroponotic having man as their sole source of infection [18].

An infection with *Leishmania* parasites can develop into two major clinical manifestations, systemic Visceral Leishmaniasis (VL) and Cutaneous Leishmaniasis (CL) (Table 1). VL is the most severe form of the disease and develops following invasion of the parasites into the lymphatic tissues including bone marrow and other organs such as liver and spleen [19]. VL is lethal if left untreated, and is prevalent in seventy countries throughout the world with the majority of cases (about 300,000 per year) occurring in South-Central Asia, particularly India, Bangladesh, and Nepal [20].

Although less severe, CL can cause considerable mutilation and is the most common manifestation of Leishmaniasis. There are several forms of CL which can vary from chronic skin ulcers to erosive mucosal disease with progressive destruction of the nasopharynx and severe facial disfigurement [21]. CL is present in eighty-two countries throughout the world, and the global incidence is estimated at 1.5 to 2 million per year [1,8,14]. The main foci are Afghanistan, Syria, and Brazil [14]. The diverse clinical spectra of Leishmaniasis and its complex pathology are reflected by the fact that twenty of the thirty known *Leishmania* species are pathogenic to humans [22].

Table 1: Characteristics of *Leishmania* species found in humans according to their geographical distribution and clinical manifestation.

Subgenus	*L. (Leishmania)*	*L. (Leishmania)*	*L. (Viannia)*	*L. (Viannia)*
Old World	*L. donovani*	*L. major*		
	L. infantum	*L. tropica*		
		L. killicki[a]		
		L. aethiopica		
		L. infantum		
New World	*L. infantum*	*L. infantum*	*L. braziliensis*	*L. braziliensis*
		L. mexicana	*L. guyanensis*	*L. panamensis*
		L. pifanol[a]	*L. panamensis*	
		L. venezuelensis	*L. shawi*	
		L. garnhyami[a]	*L. naïffi*	
		L. amazonensis	*L. lainsoni*	
			L. lindenbergi	
			L. peruviana	
			L. colombiensis[b]	
Principal tourism	Viscerotropic	Dermotropic	Dermotropic	Mucotropic

[a]Species status is under discussion
[b]Taxonomic position is under discussion

Historical highlights

CL is also known as 'Aleppo boil' or 'Aleppo evil', 'Baghdad boil,' 'Bay sore,' 'Biskra button,' 'Chiclero ulcer,' 'Delhi boil,' 'Kandahar sore,' 'Lahore sore,' '*Leishmaniasis tropica*,' 'oriental sore,' 'Pian bois', 'white leprosy', and 'uta', and has extensively been documented. VL is known as 'kala-azar' ('black fever') and 'Dum Dum fever'. Many of these names refer to the location the disease had been contracted. For instance, Aleppo is located in Syria, Baghdad in Iraq, and Delhi, Lahore, and Dum Dum in India. Other names such as 'bouton d'un' or 'sore of one year' refer to the time required for the lesions to heal spontaneously. In Arabic countries of the Middle East, Leishmaniasis is known as 'al okht' - 'the little sister'- because "everybody has one".

In the Old World, the earliest description of Leishmaniasis dates back to around 2,000 BC, when it was presented in the Pharaoh's Papyrology as 'Nile pimple' [23]. The text book Exodus (9:10) of the Old Testament also mentions a condition reminiscent of CL: "*And they took ashes of the furnace, and stood before Pharaoh; and Moses sprinkled it up toward the heaven; and it became a boil breaking forth with blains upon man, and upon beast*" (King James Version). The Persian polymath Ibn Sina (also known as Avicenna; 981-1037 AD) provided one of the most detailed early clinical descriptions of 'the sore of Balkh', and mentioned that it might be caused by "*a wicked or vicious mosquito bite*" [23].

The first description of CL in the English language was from the Scottish physician Alexander Russell (1715-1768), who further recounted from his practice in Aleppo that the local people made a distinction between a 'male' type ('dry' lesions) and a 'female' type ('wet' lesions). These denominations were later recognized as early references to infections caused by *L. (L.) major* and *L. (L.) tropica*, respectively. The appearance and the ability of the lesions to heal spontaneously were the most important features that distinguished CL from other skin conditions.

In 1885, Surgeon Major David Douglas Cunningham of the British Indian Army identified the parasite that caused 'Delhi boil'. Four years later, the Russian army physician Peter Borovsky reported that the parasites that caused Leishmaniasis were protozoa. Earlier, in 1903, the American physician James Horner Wright had described the protozoa in a sore of an Armenian child. This was the first detailed description of the causative organism of Leishmaniasis. He called it *Helcosoma tropicum,* but the German parasitologist Max Lühe changed this in 1906 to *Leishmania tropica*. Also in the year 1903, the Scottish pathologist and British Army medical officer William Boog Leishman and Colonel Charles Donovan of the Indian Medical Service, working separately in India, described similar parasites in spleen smears from patients with 'Dum Dum fever' and 'kala-azar'. These were later named *Leishmania donovani* by the British medical doctor Ronald Ross.

The transmission of Leishmaniasis by sand flies was separately demonstrated by the physician Edmund Sergent and his colleagues of the Pasteur Institute Algeria in 1921, and the scientist Saul Adler from the University of Jerusalem in 1940. However, already in 1908, the biologist and bacteriologist Charles Nicolle had discovered that *Leishmania* parasites were the causative agents of both CL and VL. He and his colleagues also identified the dog as a reservoir for a *Leishmania* species now known as *L. (L.) infantum* after this parasite was first observed in children.

Pre-Inca pottery discovered in Ecuador and Peru and depicting skin lesions and deformed faces reminiscent of CL, dates the existence of the disease in the New World back to the first century AD. The different clinical forms were referred to as 'uta' and 'espundia', respectively, and corresponded with the expressions 'valley sickness', 'Andean sickness', and 'white leprosy' in fifteenth- and sixteenth-century texts from the Incas and the Spanish colonists that referred to Leishmaniasis.

The first clinical description of CL in South America was in 1895 and from the dermatologist Juliano Moreira who linked it to 'Biskra's nodule' [24]. However, the etiological cause remained unknown until 1909, when the dermatologist Adolpho Lindenberg identified 'Leishman-Donovan bodies' in skin lesions of patients with 'Bauru ulcer' in São Paulo, Brazil. The Brazilian parasitologist Gaspar Viannia described in 1911 a new species of *Leishmania* and named it *Leishmania braziliensis* [25].

In 1922, just after the experiments of Dr. Sergent became known, Henrique Aragão from the Instituto Oswaldo Cruz in Rio de Janeiro, Brazil, inoculated the nose of a dog with pulverized infected phlebotomine sand flies. This produced an ulcer containing *Leishmania* parasites at the site of the inoculation, proving that sand flies also transmitted the parasites in the New World [26]. Subsequent studies performed between 1934 and 1946 led to the identification of various other *Leishmania* species in the New World causing the wide spectrum of clinical symptoms and manifestation known today [27].

Biological aspects

Life cycle: *Leishmania* parasites are heteroxenous, *i.e.*, they require more than one host to complete their life cycle (Figure 1). This begins as flagellated first-stage procyclic promastigotes in the gut of a female where they multiply to end as metacyclic promastigotes [17,27-29]. The latter migrate to the proboscis of the sand fly and can be inoculated into a mammalian host during the fly's next blood meal [17,27-29]. Next, they enter into or are phagocytosed by the host's tissue macrophages in which they survive using a variety of sophisticated defense mechanisms [30]. They then multiply and develop into non-flagellated amastigotes [17,27-29], leave the infected cell, spread throughout the reticulo-endothelial system, and invade other macrophages of the host [27-29,31]. The parasite's life cycle is completed when an infected host is bitten by a sand fly which takes up amastigotes from the skin of the host [32]. The conversion of the amastigotes into promastigotes is triggered by the decrease in temperature and the increase in pH when they move from the host to the sand fly's midgut [32]. The development into the vector takes five to seven days [33].

The subgenera *Leishmania* and *Viannia* can be distinguished from each other on the basis of their position in the vector's intestine during their development. The subgenus *Leishmania* develops in the sand fly's midgut before moving to the esophagus while the subgenus *Viannia* develops first in the hindgut [30]. However, the end result is similar. Clinical disease becomes apparent within weeks to months after infection, depending on the (sub-) species of parasite and the host's immune status [27-29].

Vectors: As mentioned earlier, female phlebotomine sand flies (Diptera: Psychodidae) are the vectors of Leishmaniasis [17]. The name 'sand fly' refers to the generally pale, sandy color of the insect, but this can range from almost white to almost black. Adults are around 3.5 mm in body length (about one-third to half the size of a mosquito), very hairy, and hold their wings in a characteristic V shape over their backs at rest (Figure 2); [34]. During the day, sand flies hide in dark and wet places such as cracks and rocks, walls, as well as tree trunks and stumps from ground level up to the forest canopy. The hematophageous females are particularly active at dusk. However, several species also bite during the day when disturbed. These are probably the main species that infect individuals in the forest [17,35].

The classification of phlebotomine sand flies is still controversial and far from definitive [34]. However, the position of the Old World species is less disputed than that of the New World species and generally accepted [4]. The sand fly genera in the Old World include *Phlebotomus, Sergentomyia*, and *Chinius*, the former of which harbors the proven or suspected vectors of Leishmaniasis [34]. The sand flies of the New World are still classified according to the system proposed by Lewis [36] but with revisions added by Young and Duncan [37] who recognized the genera *Lutzomyia, Brumptomyia*, and *Warileya*. Only the genus *Lutzomyia* harbours proven or probable vectors of *Leishmania* [37].

It is still not certain whether all suspected sand flies are also true vectors, or whether some merely function as incidental hosts. To implicate any sand fly species as a vector, it must be demonstrated that the insect and the parasite share the same habitat, that the parasite completes its life cycle in the sand fly, and that the parasite is transmitted by the bite of the sand fly [35]. Thus, there is a very specific relationship between parasite and vector, and a particular *Leishmania* species is usually transmitted by only one or two specific sand fly species.

Reservoirs: Mammals that most commonly function as reservoir hosts for *Leishmania* species are domestic and wild animals such as dogs and several types of rodents [35,38-46] as well as humans [10,47,48]. A wild animal can only be implicated as a reservoir if it has a role in maintaining the parasite population in nature and sand fly feeding is demonstrated [4]. An important feature of infection in wild animals is their non-pathogenicity: infected wild mammals have usually no clinical symptoms but can maintain the infection for a long period [4].

Most parasites are able to infect more than one mammalian species [35]. However, only a few of them serve as principal reservoirs; the remainder are likely incidental hosts which probably do not play an important role in the transmission cycle

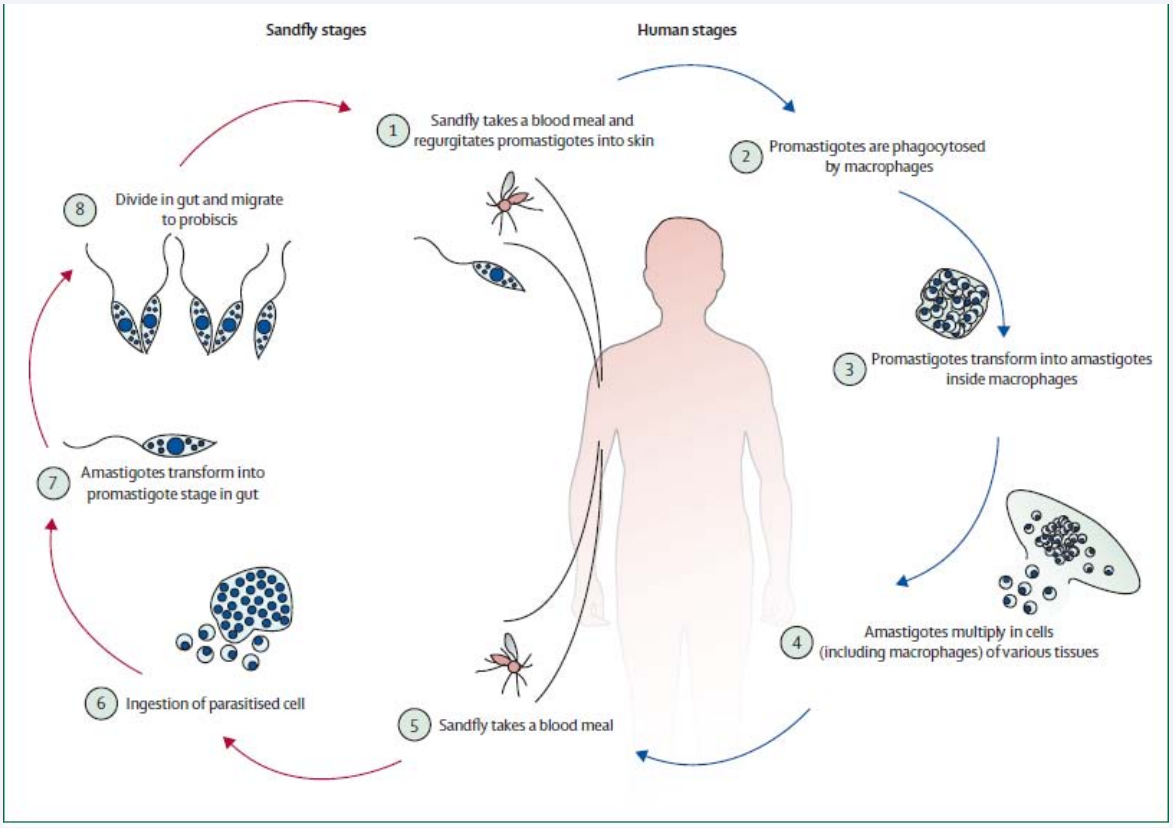

Figure 1 Life cycle of *Leishmania* parasites (Reithinger *et al*, 2007).

Figure 2 Feeding female sand fly (*Phlebotomus papatasi*) with characteristic hairy body and V-shaped angle of the wings (Maroli et al., 2013).

[35]. As reported by Shaw [49], a primary reservoir refers to a mammalian host of a parasite that is responsible for infection in the wild. The 'genuine' mammalian host is thus a key factor in the epidemiology of Leishmaniasis.

Clinical aspects

Manifestations: The most prominent aspects of CL are the extensive ulceration and the disfiguring scar formation [50]. Lesions are often painless, enlarge slowly, and ultimately ulcerate at their center to produce volcano-shaped 'wet'lesions. The course of the disease is strongly determined by the infecting *Leishmania* species. In the Old World, CL is caused by *L. (L.) tropica*, *L. (L.) infantum*, *L. (L.) major*, *L. (L.) aethiopica*, and *L. (L.) donovani* [4,15]. The New World species as well as *L. (L.) major* and *L. (L.) tropica* characteristically cause ulcerated lesions (Figure 3); [4]. Alternatively, the lesion may not ulcerate but remain as a smooth nodule or may become hyperkeratotic. These cases are referred to as 'dry' lesions. Nodular lesions are common for infections with *L (L.) aethiopica*, while complex infections and hyperkeratonic lesions are typical for those with *L. (L.) tropica* (Figure 3); [31]. The diameter of the lesions varies between 5 and 10 cm [31]. *L. (L.) aethiopica* can also cause diffuse CL which

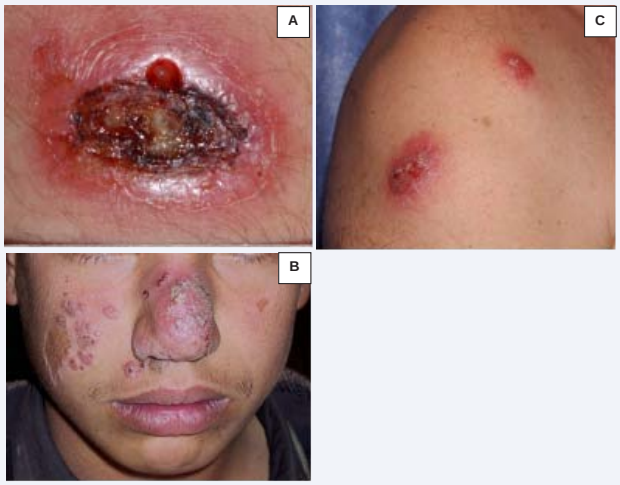

Figure 3 Lesions associated with CL. (A) ulcerated lesion (volcano-shaped); (B) hyperkeratotic nodular lesions; (C) smooth nodular lesions (Bailey and Lockwood, 2007).

is characterized by widely disseminated cutaneous macules, papules, nodules, or plaques, or by diffuse infiltration of the skin [4]. Lymphatic spread and lymph gland involvement preceding the development of the lesion are also common [15]. These dreadful clinical manifestations make CL one of the most serious skin diseases, particularly in developing countries [51].

The muco-cutaneous form of Leishmaniasis (MCL) partially or totally destructs the mucous membranes of nose, mouth, and throat cavities as well as surrounding tissues, which leads to even more severe mutilation and stigmatization of patients [1,19]. MCL is a known risk of the subgenus *Viannia*. In South America, this form is mainly caused by *L. (V.) braziliensis* and *L. (V.) panamanensis*, but cases of MCL caused by *L. (V.) guyanensis* have also been documented [4]. Notably, between 1 and 10% of patients infected with these *Leishmania* species may develop this form of the disease [52].

In general, lesions caused by CL heal spontaneously, but this process takes months to years depending on the parasite species. For instance, lesions caused by *L. (L.) major* and *L. (L.) mexicana* heal within three to nine months while those caused by *L. (L.) tropica*, *L. (L.) braziliensis*, and *L. (L.) panamanensis* take six to fifteen months to heal [4,21].

Diagnosis: Early diagnosis of CL is essential to decide on the proper treatment to prevent extensive scarring and severe facial disfigurement. It is important to establish the correct diagnosis, because other diseases with clinical spectra comparable to that of CL - such as leprosy, skin cancers, and tuberculosis - are common in endemic countries [15,21].

Particularly in these regions, the diagnosis of CL is often based on clinical symptoms and history of exposure, together with microscopic visualization of amastigotes in Giemsa-stained skin smears, aspirates, and/or cultures. Although microscopy has a relatively high specificity, the sensitivity is only between 54 and 77% [21]. Culture of parasites can provide important information about the identity of the infecting species [15], but this technique requires expertise and is time-consuming and expensive, while contamination is a constant risk that can affect 30% of the samples [15,53].

Serology-based methods are also parasite-specific but rather difficult because antibodies and antigens are often undetectable or present at low titres [53]. The Montenegro skin test detects *Leishmania*-specific cell-mediated immunity, in particular the delayed-type hypersensitivity to anti-*Leishmania* antigens [52]. However, this method cannot distinguish between past and present infections [52].

Molecular techniques are increasingly used for the diagnosis of CL. Multilocus enzyme electrophoresis is the current golden standard for the identification of *Leishmania* to species and sub-species levels [54]. This method differentiates parasite species in patient material on the basis of their isoenzyme profiles as revealed by electrophoretic analysis [54]. Polymerase chain reaction (PCR)-based approaches for the diagnosis of CL directly identify *Leishmania* species in clinical samples. Their specificity is 100%, and their sensitivity is 20 to 30 and 55 to 70% higher for localized CL and MCL, respectively, when compared to microscopy [15,53,55].

Relatively recent PCR-based methods for the identification of *Leishmania* species are PCR-restriction fragment length polymorphism (RFLP) and quantitative real-time PCR (qPCR) amplification. The former technique combines amplification of specific targets with the digestion of the PCR products with specific enzymes [56]. The latter involves the detection of a specific target sequence by a fluorogenic probe such as Taqman or fluorescent resonance energy transfer [57]. This method is also used for treatment evaluation and prognosis of CL since it allows the continuous monitoring of the amplified PCR products [57].

Treatment: The options available for the treatment of CL are intravenous or intralesional administration of pentavalent antimoniate compounds such as sodium stibogluconate (Pentostam®) and meglumine antimoniate (Glucantime®) which are considered first-line therapies [4,15]. Other options are pentamidine isethionate (PI), amphotericin B, miltefosine, a variety of antifungal agents, and/or heat- or cryotherapy [15]. So far, evidence-based data to select a particular treatment in a particular case are insufficient. For this reason, therapy of Leishmaniasis is based on the infecting *Leishmania* species guided by expert opinions.

However, most available forms of treatment are invasive, have a long duration, and are associated with significant toxicity and side-effects [15]. These factors not only reduce patient compliance but also affect treatment outcome considerably. Poor treatment compliance is also an important cause of the emergence of drug resistance [2], along with co-infection with HIV [16]. Not surprisingly, 7 to 39% of patients in, for instance, parts of South America fail to respond to first-line treatment [58]. For all these reasons, many research efforts are dedicated to the evaluation of alternative dosage schedules and modes of delivery.

Developments in Suriname

The Republic of Suriname has a surface area of 163,820 km^2 and is situated on the north-east coast of South America,

bordering the Atlantic Ocean to the north, French Guiana to the east, Brazil to the south, and Guyana to the west (Figure 4). CL is the most common form of Leishmaniasis in Suriname where it is endemic and known as 'bosyaws', 'busiyasi' ('bos' and 'busi' referring to the country's forested interior, and 'yaws' to a chronic disfiguring and debilitating infectious disease), and 'dala soro' (referring to the dollar-shaped lesions) [59,60]; 'azo', 'tatay yasa', and 'kaasa' [60], meaning 'a sore that never dies', 'caused by a poisonous liana', and 'a dangerous disease that does not heal fast', respectively, in Surinamese tribal languages [60]; and, with the more recent advent of Brazilian gold miners, 'ferida brava' (ugly wound), 'leisho seco' (referring to the 'dry' form of CL), and 'leisho chorao' (denominating the 'wet', 'crying' form of CL) [60]. The disease mainly affects the relatively poor populations in the interior, individuals involved in gold mining, bauxite mining, and logging, as well as eco-tourists and recreational fishers and hunters [61]. CL occurs predominantly in the rainy seasons between May and August and November and March [59,61,62].

The first cases of CL in Suriname were reported in 1911 [59]. The incidence is roughly 5.45 per 1,000 for the hinterland, and 0.68 per 1,000 for the entire country [61,62], but no reliable epidemiological data are available. This is due, among others, to the lack of information about risk groups such as infected Brazilian gold miners who work (illegally) in the hinterland, and infected tribal people who treat themselves. Nevertheless, CL has become an increasing public health problem in the country.

Leishmania species: L. (V.) guyanensisis was long considered the principal species causing CL in Suriname [61,64]. However, in the past decades, a number of patients have presented with clinical forms of the disease that behaved differently from that associated with this *Leishmania* species [62,63]. In addition, the (illegal) Brazilian workforce in the hinterland might have introduced other *Leishmania* species such as *L. (V.) braziliensis* in Suriname [35,65,66]. Surinamese health authorities were very concerned about this possibility, as this parasite causes MCL for which there is no adequate treatment and expertise in the country.

Figure 4 Map of Suriname, and location of Suriname in South America (insert).

The presumption that there were other *Leishmania* species present in Suriname [63] was confirmed in the past decade by PCR-based methods identifying a few cases of Leishmaniasis caused by *L. (V.) lainsoni*, *L. (L.) amazonensis* [65,67], and *L. (V.) naiffi* [70], but also by *L. (V.) braziliensis* [67]. The latter study showed furthermore that almost 90% of the *Leishmania* infections in Suriname were attributable to *L. (V.) guyanensis*, but that approximately one of every ten was caused by *L. (L.) amazonensis* and *L. (V.) braziliensis*. These findings indicate that *L. (V.) guyanensis* is still the main species causing CL in Suriname, but emphasize the need to implement proper measures for diagnosing and treating cases of MCL that may emerge in the country.

Vectors: Although CL was known to be zoonotic in Suriname [35], very little was known about the vectors involved in its transmission. Suspected vectors were female phlebotomine sandflies (Diptera: Psychodidae) belonging to the species *Lutzomyia umbratilis*, *Lu. flaviscutellata*, and *Lu. whitmani* [5,35,69-71]. The former is the principal vector of *L. (V.) guyanensis* [35], but it was uncertain whether the other sand fly vectors also transmit Leishmaniasis in Suriname [35,72]. However, *Lu. flaviscutellata* and *Lu. whitmani* are proven vectors of *L. (L.) amazonensis* [17] which, as mentioned above, has also been detected in Suriname [63].

With the aim to establish which sand fly vector(s) may be responsible for the transmission of Leishmaniasis in Suriname, sand flies were captured in several hot spots in the hinterland, and female specimens were assessed for the presence of *Leishmania* species using qPCR. *Leishmania* species were identified using mini-exon PCR-RFLP. The study revealed thirty-four different species including four new records for Suriname (*Lu. aragaoi*, *Lu. damascenoi*, *Lu. ayrozai*, and *Lu. sordellii* [73]), as well as six proven vectors for Leishmaniasis (*Lu. squamiventrissensu lato*, *Lu. umbratilis*, *Lu. flaviscutellata*, *Lu. whitmani*, *Lu. ayrozai*, and *Lu. ubiquitalis* [73]). The latter sand fly species are proven vectors for *L. (V.) naiffi*, *L. (V.) guyanensis*, *L. (L.) amazonensis*, *L. (V.) braziliensis*, and *L. (V.) lainsoni* [26,34,35,71,74,75], corroborating the presence in Suriname of *Leishmania* species other than *L. (V.) guyanensis* including one associated with MCL.

Reservoirs: The animal reservoir for CL had also not unambiguously been identified in Suriname [35,61]. Candidates were a variety of wild animals [38-42,44,61] but also domestic animals such as cats [46], horses [46], and dogs [35,43]. Notably, evidence from the past decade had implicated the dog as a possible reservoir of Leishmaniasis in other South-American countries such as Columbia, Brazil, and Peru [76,77].

Focusing on the latter possibility, blood was collected from dogs from the animal shelter in Suriname's capital city Paramaribo, dogs from locations around Paramaribo, dogs brought to hunting trips in the hinterland, and dogs from foci of CL in the interior of Suriname. Assessing *Leishmania*-specific antibodies and *Leishmania*-specific DNA in blood samples of the animals using the direct agglutination test (DAT) [78,79] and qPCR [58], respectively, five dogs appeared sero-positive for canine Leishmaniasis, and *Leishmania* DNA was detected in one dog, albeit at relatively low quantities [79]. Thus, although not proven conclusively, the dog may well represent a reservoir for the leishmania parasites in Suriname.

Diagnosis: So far, the diagnosis of CL in Suriname - as in most endemic countries - is based on the clinical symptoms, the history of exposure, and microscopic examination of Giemsa-stained smears from biopsies. However, the sensitivity of such methods is less than 77% [80]. The leishmanin skin test, parasite culturing, and serology have a relatively low sensitivity and a variable specificity and are cumbersome [21,80], representing no valid alternatives.

On the other hand, molecular characterization of *Leishmania* parasites by PCR-based methods is the most sensitive single diagnostic test for CL [56,80-82]. This was confirmed by a recent study from our group [83,84] showing that the infecting parasites (*L. (V.) guyanensis*, *L. (V.) braziliensis*, *L. (L.) amazonensis*) could accurately be identified by real-time quantitative PCR of skin biopsies of patients with CL. This study showed, furthermore, that patient response to PI can be better anticipated based on the parasite load on 6 weeks after the treatment rather than on parasite load before treatment [83,84]. Due to the relatively high costs, however, this method is not routinely used in Suriname for diagnosing CL.

Treatment: PI given intramuscularly at the dose of three injections of 4 mg/kg on days 1, 4, and 7 is the first-line drug and the only treatment option for CL caused by *L. (V.) guyanensis* in Suriname [64]. However, not all patients respond adequately to this treatment (unpublished observations). This may be attributable to the presence of unresponsive strains of *L. (V.) guyanensis* and/or *Leishmania* species other than *L. (V.) guyanensis* in the country [57,63,68]. However, as many patients from the interior treat themselves in the forest with PI without medical supervision [85], the unsatisfactory results may also be due to poor compliance and incomplete therapy [86]. Obviously, this may result in sub-therapeutic drug blood levels contributing to the emergence of drug resistance [2,87] and therapy failure [4,88,89].

These considerations prompted the implementation of the clinical trial 'Pentamidine for Cutaneous Leishmaniasis in Suriname' (the PELESU study), a non-inferiority study aimed at the evaluation of, among others, the efficacy, toxicity, and cost-effectiveness of the standard 7-day regimen with respect to an experimental 3-day, more dose-intensive treatment regimen [85]. A total of 163 patients was accrued for the clinical trial, 84 of whom received the standard treatment of 4 mg/kg PI on days 1, 4, and 7, while 79 patients received the experimental treatment of 7 mg/kg of the drug on days 1 and 3 [85].

The results showed that the 3-day regimen was not non-inferior to the 7-day regimen, producing comparable proportions of clinical cures and therapy failures as the 7-day regimen (around 50 and 20%, respectively; [85]). Adverse events in both arms were also comparable, although the shorter, more dose-intensive regimen produced more toxicity, in particular to liver and kidneys. On the other hand, the 3-day regimen was at least 20% more cost-effective than the 7-day arm. Nevertheless, it was recommended to maintain the 7-day treatment regimen in Suriname because of its lesser toxicity [85].

Closing remarks

After many years of standstill, the last decade has brought considerable advances in our understanding of CL in Suriname. New *Leishmania* species and new sand fly vectors have been identified, and the dog has been implicated as a possible reservoir of the infection. Furthermore, the prevalence of CL over other clinical forms of Leishmaniasis has been corroborated and *L. (V.) guyanensis* has been confirmed as the main causative agent, validating the use of PI against the disease in Suriname.

However, the poor responsiveness to PI of certain patients infected with *L. (V.) guyanensis*, and the identification of *Leishmania* species and sand fly factors implicated in the development of MCL, make it essential to identify more efficacious forms of treatment of both forms of Leishmaniasis. Topical paromomycin with or without gentamicin and high-dose oral fluconazole were efficacious against CL caused by *L. (L.) major* [90] and *L. (V.) braziliensis* [91], respectively, while causing acceptable toxicity. Therefore, evaluation of these agents for their efficacy in CL patients who respond unsatisfactorily to PI may be worthwhile.

Many patients with CL in Suriname's interior cannot afford the costs of treatment and transportation to health centers and instead use traditional herbal substances. Recently, twenty-five such preparations were assessed for their potential anti-leishmanial efficacy using cell culture models [92]. These studies suggested that a preparation from the wolf apple *Solanum lycocarpum* A.St.-Hil. (Solanaceae) was not active against amastigotes from *L. (L.) donovani* but exhibited meaningful cytotoxicity against promastigotes from *L. (V.) guyanensis*, *L. (L.) major*, and *L. (L.) donovani* [92]. Importantly, this preparation did affect the viability of the macrophage-like cells infected with the amastigotes [92] suggesting that it had a good safety index. These encouraging findings raise the possibility of identifying efficacious plant-based anti-leishmanials that are readily available and affordable.

In addition to novel forms of treatment, novel methods for diagnosis of CL are required in Suriname. These methods should not only be sensitive and reliable, but also affordable and easy to perform. For these reasons, the CL Detect™ Rapid Test (http://clinicaltrials.gov/show/NCT01865032) is being assessed for its feasibility to detect *Leishmania* species in skin lesions of suspected CL patients in Suriname. The test performed satisfactory in various countries in both the Old and the New World [93].

Hopefully, these potential advances, together with the above-mentioned new insights into the biology and treatment of CL in

Suriname, will result in more efficacious therapeutic modalities and more effective diagnostic methods in the country.

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