Leishmaniasis in the United States

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Leishmaniasis, a chronic and persistent intracellular protozoal infection caused by many different species within the genus Leishmania, is an unfamiliar disease to most North American providers.

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1. Introduction

Leishmaniasis is more common in the United States (U.S.) than most Americans realize, involving both autocthonous and imported infections. The recent deployment of millions of Americans to Iraq and Afghanistan has been associated with thousands of cases of cutaneous leishmaniasis (CL) [1], but even more concerning is the likelihood of large numbers of persons with unrecognized asymptomatic visceral leishmaniasis (VL) [2].

2. Asymptomatic Visceral Leishmaniasis: Emerging Issues in the United States

2.1. Introduction

Visceral leishmaniasis is a spectrum of chronic infection from asymptomatic (latent) to oligosymptomatic (e.g., viscerotropic leishmaniasis) to symptomatic disease, likely resulting from an interplay between the host's immune response to contain the parasite and the amount of *Leishmania* protozoa in the blood/tissues. In this respect, an analogous comparison can be drawn to tuberculosis, another disease wherein many persons may be infected, however most will only manifest with positive skin tests and while far fewer will have active disease [3][4][5][6]. *Leishmania* infection is chronic, likely persisting for the life of the host. Most VL infections remain subclinical, with overt symptomatic disease seen mainly in infants, young children, and immunocompromised hosts. Symptomatic leishmaniasis is generally associated with levels of up to 200,000 parasites/mL of blood, while asymptomatic leishmaniasis may only have 0–50 parasites/mL [7][8]. Symptomatic VL is characterized by a wasting illness with chronic fever, hepatosplenomegaly, and pancytopenia, and can result in death if not appropriately treated. Like malaria and American trypanosomiasis, symptomatic VL is a major global parasitic cause of morbidity and mortality [9]. Despite this, VL is an infection not commonly managed by American practitioners, a fact that could result in delayed recognition of or inappropriate therapy for undiagnosed reactivated VL, potentially leading to poor clinical outcomes.

Persons with asymptomatic VL are thought to greatly outnumber those with symptomatic VL by a factor of 4:1 in some locations (East Africa), and as high as 50:1 in others (Spain) [10][11]. We have established that potentially large numbers of previously deployed U.S. servicemembers [2] and likely immigrants from endemic global regions (see Table 1) with asymptomatic VL now reside in the U.S. Asymptomatic VL has medical relevance because of the potential for secondary transmission via blood transfusion and organ donation, the possible risk of domestic U.S. vector acquisition and subsequent transmission, and the reactivation risk associated with increasing use of immune modulating treatments and immunocompromising conditions. A review of *L. infantum* (syn. *chagasi*) worldwide presents varying rates of asymptomatic infection, with specific results depending on the geographic region and identifying assay used, but range from 5–54% [12][13][14]. Asymptomatic VL rates among blood donors in endemic areas of the Mediterranean are reported as between 1–22% [12].

Table 1. *Leishmania* Species Causing VL and Their Geographic Distribution * ± [15][16][17].

ishmania donovani • Includes species formerly known as *L. archibaldi.*	**East Africa and Southern Arabia** • **Sudan, Ethiopia, Eritrea, Kenya,** Uganda, **Somalia, South Sudan**
	Northwestern China • Xinjiang Autonomous Region
	South Asia • **India**, **Bangladesh**, Nepal, Sri Lanka, Pakistan
***Leishmania infantum* (synonym: *L. chagasi*)**	**Central and South America** • Primarily **Brazil**; also Argentina, Paraguay, Colombia, Venezuela, Honduras, Guatemala, Bolivia, Mexico, Uruguay
	Arabian Peninsula • Yemen and Saudi Arabia
	Mediterranean, North Africa, and Middle East • Spain, France, Greece, Italy, Portugal • Tunisia, Balkans, Algeria, Libya • Israel, Turkey, Iran, Iraq, Kuwait, Syria
	Western Asia and China • Afghanistan • Provinces of Gansu, Shaanxi, Shanxi and Sichuan; Xinjiang Region
***L. (Mundinia)* species** • Primarily *L. martiniquensis*; additional genus members include *L. enriettii* complex, *L. orientalis*	**Thailand, Myanmar, Grenada, Martinique**

* List is not all encompassing; per WHO, VL is endemic in ~79 countries worldwide [18]. ± Bolded nations estimated to harbor >90% of the global burden of VL.

The geographical distribution of VL in both the Old and New Worlds mirrors the endemicity of the implicated pathogens, specifically *L. donovani*, *L. infantum*, and *L. Mundinia* species. The greatest numbers of cases are reported from Brazil, India, Sudan, Ethiopia, and Kenya (Table 1) [15]. Globally, cases of VL have declined over the last decade; the World Health Organization's (WHO) estimates between 50,000–90,000 VL cases annually, with a death rate of 95% in untreated cases [15]. The overall decline has been attributed primarily to VL control efforts made on the Indian subcontinent [19].

2.2. Asymptomatic VL: Immunity and Indicators of Progression

Asymptomatic visceral leishmaniasis (AVL) is variably defined, but it is considered present when a person demonstrates a positive *Leishmania* serological, culture, or nucleic acid-based test implying the presence of parasitic organisms in the absence of clinical signs or symptoms of active disease. Immune control rather than total eradication of the parasite is considered to be the most likely outcome following inoculation, so the presence of anti-leishmanial antibodies (including a positive rK39 assay or direct agglutination test [DAT]), positive interferon-gamma release assay (IGRA), leishmanin skin test, or polymerase chain reaction (PCR) are considered indicators of infection. Diagnosis of asymptomatic or subclinical infections can be obtained by histopathological methods and culture, though these more invasive tests are not recommended in the absence of clinically evident illness [11].

Accepting that asymptomatic persons with positive leishmaniasis tests have chronic infection and not just evidence of an immune response to prior infection implies that the host immune response required to achieve complete eradication is ineffective. *Leishmania* species achieve immune evasion and modulation by modifying cell signaling, surviving inside the phagosome, attenuating antigen presentation, and overall dampening of the normal immune response [20]. Indeed, immunomodulation is present from the first stages of infection, as demonstrated by studies showing the immunosuppressive effects of sand fly saliva and how its co-inoculation enhances the parasites' ability to establish early infection [21]. The *Leishmania* parasite has been shown to persist in the spleen and bone marrow of mouse and non-human primate models [22][23][24]. A study of *L. infantum* infection in rhesus macaques showed that despite immunological control of early parasitemia, complete eradication from the reticuloendothelial system was not achieved, ultimately resulting in an inability to produce and sustain an effective, highly specific IgG antibody response, leading to parasite spread and disease progression [24]. Thus, in the presence of a reasonable exposure history and a positive leishmanial test result, it is prudent to assume that asymptomatic VL is present.

The natural history of VL may involve chronic asymptomatic infection (disease control), or progression to symptomatic disease. Studies in animal models have found that the interplay of IL-10, IL-12, INF-γ, and TNF-α was crucial to infection control [25]. Interleukin-10 signaling was needed for parasite persistence and latency, whereas IL-10 knockout mouse models are resistant to infection [24][26]. A Th1-type immune response driven by IL-12, INF-γ, and TNF-α is required for disease control; fatal leishmaniasis infection occurred in TNF-α knockout models [24][25].

Several studies assessed laboratory markers that correlated with progression from asymptomatic to symptomatic VL (Table 2). Among 1600 persons in endemic regions of India followed for three years by Chakravaty et al., 17 (1%) new cases of VL were identified. DAT, rK39, *Leishmania* IGRA, quantitative PCR, and genotyping were analyzed as possible biomarkers for progression to symptomatic VL. Those with a positive blood qPCR or strong positive DAT and/or rK39 assay results showed a statistically significant increased odds of progression to symptomatic disease (odds ratios of 20.9, 19.1, and 30.3, respectively); symptomatic VL tended to occur quickly after seroconversion (median 5 months) [27]. In another study, Das et al. determined immunological risk factors for AVL progression utilizing different parameters [28]. Screening 5794 persons from endemic villages in India with rK39, DAT, and qPCR blood testing, they determined the risk of progression to symptomatic disease based on how many of these individuals were positive for one, two, or all three of these markers. This study identified 42 persons with positive results on all three of these assays, and 23.8% of these individuals progressed to active VL over the course of 6 months (Table 2) [28].

Table 2. AVL and Biomarkers for Progression to Symptomatic VL.

Study Name (Year) [Reference]	Location	Species	Study Size	Tests Used	Follow-Up Duration	Risk of Progression	Factors Associated with Risk of Progression
Evans et al. (1995) [29]	Brazil	*L. infantum*	653 (children)	Anti-leishmanial antibodies	5 years	6.1%	Seroconversion; living in household with prior VL case
Hasker et al. (2014) [30]	India, Nepal	*L. donovani*	32,529	rK39, DAT	1 year	6.4% (India; high baseline DAT) 9.8% (Nepal; high baseline DAT) 7.3% (India; high baseline rK39) 7.7% (Nepal; high baseline rK39) 12.5% (India; new seroconversion) 9.1% (Nepal; new seroconversion)	High titers of rK39 and/or DAT; new seroconverters
Chapman et al. (2015) [31]	Bangladesh	*L. donovani*	2410	rK39	3 years	14.7%	High titer rK39, especially in new seroconverters

Study Name (Year) [Reference]	Location	Species	Study Size	Tests Used	Follow-Up Duration	Risk of Progression	Factors Associated with Risk of Progression
Chakravaty et al. (2019) [27]	India	*L. donovani*	1606	rK39, DAT, IGRA, qPCR	3 years	1.6% (8/476 known seroconverters)	High titer DAT; high titer rK39; +qPCR
Das et al. (2020) [28]	India	*L. donovani*	5794	rK39, DAT, qPCR	6 months	3.27% (+ rK39 only) 8.33% (+rK39 and DAT) 23.8% (+rK39, DAT, and qPCR)	+ for mid-high titer rK39 and DAT plus + qPCR

In recent decades, the *L. infantum* outbreak in the southwest environs of Madrid, Spain illustrated important risk factors for VL, as well as the prevalence of asymptomatic infection. In the towns of Fuenlabrada, Leganés, Getafe, and Humanes de Madrid, the annual incidence of reported leishmaniasis infections rose >40-fold from 0.5 cases/100,000 persons per year in 2000–2009, to 22.2/100,000 persons per year between mid-2009–2012; the town of Fuenlabrada alone saw 43.5/100,000 cases per year [32][33]. 446 total cases of leishmaniasis were confirmed during the first three years, with VL comprising 35.9% (160 cases); this overall total subsequently increased to 758 by the beginning of 2018 [34]. Interestingly, persons who were identified as being of African origin developed a disproportionately greater amount of VL (89% of leishmaniasis cases among Africans were VL). 31.3% of VL cases were identified as having an underlying immunocompromised state; HIV was present in 10%, and 15.6% were taking some form of immunosuppressive medication [32].

An additional study on asymptomatic VL investigated 804 healthy persons from Fuenlabrada with no history of symptomatic leishmaniasis, using DAT, immunofluorescent antibody (IFAT), PCR, or a whole blood stimulation assay (WBA) with IL-2 quantification for detection of infection. Asymptomatic infection was defined as a positive result on any of these tests in the absence of signs or symptoms of active disease. The WBA-IL2 proved to be the most sensitive test, with 20.7% of the sample testing positive, as compared to 0%, 0.11%, and 1.0% of PCR, IFAT, and DAT tests, respectively [34]. This prevalence (20.7%) is similar to values noted for the leishmanin skin test (LST) when it was used as a screening tool for AVL in Georgia (19.3% LST positive) and Ethiopia (23.1%) [34][35][36].

2.3. Asymptomatic VL: Diagnostic Approach in the U.S.

Testing for asymptomatic visceral leishmaniasis is rarely performed outside of a research setting. Guidelines for the diagnosis of symptomatic leishmaniasis in North America have been published, though the specific assays that are favored may vary depending upon the specific clinical setting, disease endemicity, and available testing capabilities [37].

A practical approach in a low prevalence setting like the U.S. may involve using appropriate serologies for screening symptomatic persons coupled with direct parasite detection via histopathology, parasite culture, and/or PCR for confirmation. Of note, anti-leishmanial antibodies have been associated with cross-reactivity to other protozoan pathogens, including *T. cruzi* in humans as well as *T. gondii* (among others) in canines [38][39]. Serological assays may be less sensitive in those with immunocompromise (especially HIV) [40][41]. However, persons with AIDS often have higher burdens of parasitemia, increasing the sensitivity of parasitological diagnosis via microscopy and PCR testing [40][42][43].

The sole American population surveyed for asymptomatic VL was healthy U.S. servicemembers who had deployed to Iraq; the most frequently positive test (27/39; 69%) was the *L. infantum* interferon gamma release assay (IGRA) [2]. Unfortunately, the rK39 immunochromatographic test (approved by the Food and Drug Administration) has not been found helpful in asymptomatic *L. infantum* in studies in Brazil, Spain, and the U.S. [44][45].

There are currently three reference laboratories for leishmaniasis diagnostic testing in North America: McGill University in Montreal, Canada; the CDC in Atlanta, Georgia; and the Walter Reed Army Institute of Research in Silver Spring, Maryland (military beneficiaries only) [37]. Histopathology review, parasite culture, tissue PCR, rK39 serology, and species identification are offered. However, testing that would assist with diagnosis of asymptomatic VL (IGRA, WBA, blood PCR, Leishmanin skin test) is restricted in the U.S. to *Leishmania* IgG (an assay that has not been tested in any AVL surveillance) and perhaps the Karius test®.

Molecular testing (such as PCR amplification targeting leishmanial gene sequences) is of increasing importance [46]. Quantitative PCR can be performed on multiple tissue types including blood, and shows great promise with very high sensitivity and specificity (both >90%) [44]. This modality also appears to roughly correlate with parasite load, and with further study could potentially help discriminate between latent versus active subclinical disease [47]. Additionally, it may also be useful for monitoring response to therapy and identifying reactivation in immunosuppressed patients, due to the higher infectious burden and greater likelihood of circulating parasites in this population. Unfortunately, this test is not yet available in the aforementioned North American reference laboratories.

2.4. Reactivation, Prophylaxis, and Screening for AVL in Immunosuppressed Populations

There are several clinical settings where leishmaniasis poses significant risk for severe disease or reactivation. Historically, the most experience has been in advanced HIV disease, the risk for which has been greatly ameliorated with effective antiretroviral therapy and associated immune reconstitution. However, with increasing prevalence of organ transplantation and novel immunomodulatory therapies, other sources of immunosuppression are gaining importance. Reactivation is an emerging concern in the U.S. with a new pool of potentially thousands of AVL-infected veterans as well as immigrants from endemic regions (Middle East/North Africa [MENA], Latin America) who have ready access to and may one day require immunosuppressing therapies. Thus, the lifelong risk of reactivation, even remote from initial exposure, plays a critical role in this population.

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