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Visceral Leishmaniasis: A Brazilian Perspective

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Abstract

Objective: The scope of this paper is to present the main aspects related to the causative agent of visceral leishmaniasis and implications of this interaction with the human host, allowing the improvement of health care actions.

Methods: The paper was put together based on a literature review with a defined search strategy. To obtain the articles we consulted the Scientific Electronic Library Online (SciELO) and the U. S. National Library of Medicine (PubMed).

Results: The leishmaniases are a group of diseases that pose a serious public health problem in at least 88 countries, and are included among the six priority endemic diseases in the world, according to the World Health Organization (WHO). In this scenario the visceral form stands out kala-azar (visceral leishmaniasis) which, despite its endemic nature, has been reported in different areas of Latin America associated with the process of expansion and urbanization, as well as the unchecked deforestation.

Conclusion: The importance of this discussion of the clinical and diagnostic aspects of visceral leishmaniasis relates particularly to delayed diagnosis of cases by professionals unfamiliar with the disease – which is becoming quite common due to the intense flow of people in the world resulting in the delayed initiation of therapy.

Keywords: Visceral leishmaniasis; Epidemiology; Diagnosis; Treatment; Control

Introduction

Visceral leishmaniasis (VL) or kala-azar, also known as dumdum fever, is an overlooked tropical, systemic infectious, non-contagious, potentially serious disease. It is vector transmitted and evolves chronically with pathological alterations in the mononuclear phagocyte system, and can occur systemically or endemically [1,2]. It includes a broad spectrum of clinical manifestations whose course may vary from acute to sub-acute, to chronic, with an incubation period that varies from weeks to years. The disease is transmitted by female sandflies members of the *Psychodidae* family, subfamily *Phlebotominae*, gena *Phlebotomus* (Old World) and *Lutzomyia* (America) [1].

The main parasitic reservoirs are foxes, dogs, and opossums [1]. It is considered an emerging or re-emerging illness with worsening incidence in various parts of the world, especially in tropical areas [3]. In Brazil, despite underreporting, there has been a significant increase in the number of cases of visceral leishmaniasis [4-6] owing to several reasons including precarious economic and social conditions [7] including the nutritional status of the exposed population [8], migratory movement, urbanization, and predatory occupation of previously preserved areas associated with poor health care, which may have favored the peridomiciliary movement of the vector *Lutzomyia longipalpis* [9,10] and the expansion to infected dogs [11,12] transforming VL into a previously unheard of urban disease [13].

Additionally, failures in disease control strategies based on:

- Early detection and treatment in human cases;
- Control of domestic reservoirs; and
- Control of vectors [14] contribute to the spread of the disease [15,16].

Another factor that has been gaining importance is the increased incidence of co-infection with human immunodeficiency virus (HIV) [17,18].

The disease presents significant clinical and epidemiological diversity arising from the multiplicity of possible ecological relations, with the involvement of different species of *Leishmania,* sandflies, and hosts, with zoonotic and anthroponotic cycles [4]. Most transmission processes are classified as zoonotic, with the exception of the anthroponotic cycles of *Leishmania donovani* in Southeast Asia and West Africa and *Leishmania tropica*. Some cycles, previously thought to be exclusively zoonotic, may in fact also are anthroponotic, and vice-versa. This phenomenon has its origins in environmental [19] and epidemiological [5] alterations.

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VL is a chronic and systemic disease whose characteristics include: irregular long term fever, weight loss, asthenia, adynamia, anemia with visible cutaneous and mucosal pallor, splenomegaly, hepatomegaly, leucopenia, thrombocytopenia and complications of bacterial infections. Malnutrition is also common, noted by cachexy upon physical examination as well as laboratory evidence of hypoalbuminemia associated with hypergammaglobulinemia [20]. As a result of various anthropic, environmental, political, economic, and social factors, VL is no longer an illness typical of isolated rural areas, and is now present in urban areas in a pattern of frank geographic expansion [13].

The importance of publicizing this infectious disease lies in the difficulty of unfamiliarized physicians to recognize this illness, especially given its oligosymptomatic characteristics and also because of the existence of effective and easily accessed treatment, allowing for a considerable reduction in fatalities. Furthermore, the disease is difficult to control and causes epidemic outbreaks, which makes it an important public health issue [20].

The objective of this paper is to present the principal aspects of *Leishmania* species that cause VL and the clinical and therapeutic implications of its interaction with the human host, emphasizing the aspects of the VL in Brazil.

Methods

The paper was written based on a literature review with a defined search strategy. To obtain reference material we consulted the Scientific Electronic Library Online (SciELO) and the U. S. National Library of Medicine (PubMed), up to the date of 20/01/2016. The search terms used were:

Strategy 1 – visceral leishmaniasis + epidemiology

Strategy 2 – visceral leishmaniasis + diagnosis

Strategy 3 – visceral leishmaniasis + treatment

Strategy 4 – visceral leishmaniasis + control

In addition to the papers, we also consulted Internal Medicine and Infectious Diseases textbooks as an integral part of the bibliographic search.

The search yielded citations distributed as shown in (Table 1). Of the total manuscripts consulted, after reading the abstracts, we selected 102 texts opting for those focused primarily on the clinical approach to the patient, which subsidized the present work.

SEARCH STRATEGY	DATABASE CONSULTED	
	PUBMED*	SCIELO
Strategy 1 visceral leishmaniasis + epidemiology	1949	30
Strategy 2 visceral leishmaniasis + diagnosis	3896	26
Strategy 3 visceral leishmaniasis + treatment	3396	23
Strategy 4 visceral leishmaniasis + control	1525	56

*For the PubMed search using search terms in English we used the following filters: Humans, adult: 19+ years, publication dates between 01/01/2000 e 20/01/2016.

Table 1: Number of papers obtained in the bibliography search.

Results and Discussion

The genus *Leishmania*

The species belonging to *Leishmania* are included in the kingdom *Protozoa*, phylum *Mastigophora*; order *Kinetoplastida*; family *Trypanosomatidae* (as *Trypanosoma cruzi*) [21]. The genus *Leishmania* is subdivided into three sub-genera (*Leishmania, Viannia e Sauroleishmania*), the first two according of the location of the promastigote forms in the vector intestine. The addition of *Sauroleishmania*, a parasite exclusive to lizards, is more recent. The species belonging to the subgenus *Leishmania* are limited to the midgut and anterior intestine of the sandfly, while the *Viannia* develop in the large intestine [22,23].

The genus unites species of unicellular protozoa, obligate intracellular parasites with a heteroxenous life cycle, with one vertebrate and one invertebrate host, in which reproduction occurs by simple binary division [22]. In mammalian hosts, represented in nature by numerous orders and species, including *Homo sapiens sapiens*, we find the amastigote form – round, immobile, and reproducing obligatorily within the cells of the monocyte phagocytic system. The invertebrate hosts are represented exclusively by small insects belonging to the order Diptera, family *Psychodidae* and genus *Lutzomyia*, in whose digestive tract are found the mobile promastigote: the extracellular, flagellated and infectious form [24].

Among the species of *Leishmania* are *Leishmania donovani*, linked to VL in India and other eastern regions, *Leishmania infantum* (cause of VL in Mediterranean areas), and *Leishmania chagasi* (cause of american VL) [23].

The life cycle of the protozoa has two stages: one in the vertebrate host and the other in the vector. During a blood meal, the infected vector regurgitates part of the content of its intestinal tract and inoculates the infective metacyclic promastigote forms of the parasite together with its saliva – the latter with the capacity to alter the host immune response allowing for persistence of the infection [23]. The vector's salivary proteins possess immunogenic properties, characterized by the presence of IFN-γ, and can determine outcome of the disease [25].

The promastigote (infective) forms of the protozoa bind to macrophage receptors and are quickly phagocytized. This result in reduction of the pathogen, loss of the flagella, and change to the amastigote form, which multiplies and can infect other macrophages, that when ingested together with the host's blood by the sandfly can continue the cycle. Subsequently, there is hematogenous dissemination of the protozoa with tropism for viscera, especially the spleen and the liver. Upon biting the infected host, the vector ingests a small quantity of blood with macrophages containing amastigotes. In the digestive tract of the vector, the amastigotes transform into promastigotes, and after adhering to the intestinal epithelium of the host, differentiate into metacyclic promastigotes, completing the cycle [24,25].

Epidemiological aspects

The leishmaniases – including VL – constitute a group of diseases that represent a serious public health problem in at least 88 countries, considered one of the six priority endemic diseases in the world [20]. VL is a zoonosis typical of tropical areas, occurring in various regions of the world, however more than 90% the cases that occur in the world are registered in six countries: India, Bangladesh, Sudan, Southern Sudan, Ethiopia, and Brazil [26,27]. However, in other regions including Kenya, the Middle East, and some countries of Latin Ameria (Colombia, Venezuela, Bolivia, El Salvador, and Honduras) the disease is also present [3]. An estimated 0.2 to 0.4 million cases occur annually worldwide [26], with roughly 12 million people currently infected

[27]. Specifically in the Americas, the disease has been registered in 12 countries, most recently in Paraguay and Argentina, though roughly 96% of cases are described in Brazil [26]. Although it is an epidemic disease, there have been frequent outbreaks in the five regions of Brazil, with the states of Bahia, Ceará, Rio Grande do Norte and São Luís do Maranhão having the highest recorded number of cases, especially in rural areas [1]. Children under the age of 10 are the most commonly affected (41.9%), and the male gender is proportionally more affected, with 62.8% of cases [20]. The annual mean number of new cases of VL in Brazil between 2003 and 2012 was 3,565 cases and the incidence was of 1.9 cases per 100,000 inhabitants, with a mean lethality of 6.4% [20]. Between 2004 and 2014 Brazil experienced a process of expansion and urbanization of VL, resulting from increased proximity between dwellings, high population density, and the considerable susceptibility of the population to parasitosis [20]. In this period the main outbreaks in the country occurred in the cities of Rio de Janeiro (RJ), Belo Horizonte (MG), Araçatuba (SP), Santarém (PA), Corumbá (MS), Teresina (PI), Natal (RN), São Luís (MA), Fortaleza (CE), Camaçari (BA), and the epidemics in the municipalities of Três Lagoas (MS), Campo Grande (MS), and Palmas (TO) [20].

VL is included in the group of neglected diseases – also known as neglected tropical diseases – that share the hallmark of affecting primarily the more vulnerable populations and that contribute to the perpetuation of poverty, mainly due to their impact on infant mortality and morbidity, reduced productivity of economically active individuals, and the social stigma [8].

The sandfly vectors – in Brazil *Lutzomyia longipalpis* and *Lutzomyia cruzi* – are crepuscular and nocturnal, with *L. longipalpis* found intra- and peridomicile [14,20]. The lifestyle of *L. longipalpis*, the main vector of VL, is in part responsible for the expansion process of the disease, given that it adapts to the domestic environment, where there is availability of organic residues and areas with humidity and shade, in addition to the intermediate host – the dog [20,28]. These insects are small, measuring between 1 and 3 centimeters in length, covered by light colored hairs, and are easily recognizable by their peculiar habit of flying short distances and landing with their wings open [10]. There is the possibility of a third transmitting species, *Lutzoyia migonei*, indicated by high density in areas absent of *L. longipalpis* and/or *L. cruzi* with consequent registration of indigenous VL cases, a fact that remains to be confirmed [20].

In urban areas, the main reservoir is the dog – *Canis familiaris* – among which a high prevalence has been observed in epidemiological investigations [29,30]. In the wild, the main vertebrate hosts with an important epidemiological role are foxes – *Dusicyon vetulus* and *Cerdocyon thous* – and marsupials – *Didelphis albiventris* [11].

Clinical aspects

The incubation period – from 10 days to 24 months, with an average of two to six months [20] and the clinical manifestations of VL vary widely depending on the host immune response. There are often periods of fever remission and illusory improvement of the other symptoms of the morbid process, a fact that not infrequently leads to a patient seeking medical treatment only after months of disease evolution [2]. The disease can be classified into the following forms:

Asymptomatic form: Although infected, subjects do not present signs and symptoms, however it should be noted that the intradermal test is reactive and serum or polymerase chain reaction testing is positive [31].

Oligosymptomatic or subclinical form: This is the most frequent expression of the disease in endemic areas, characterized by nonspecific manifestations such as low fever, unproductive cough, diarrhea, adynamia, in some cases with the presence of discrete hepatomegaly, and palpable spleen in a minority of cases. Generally, most patients (85%) presenting both the asymptomatic and oligosymptomatic forms present spontaneous cure even without treatment after three to six months. A smaller proportion of patients (15%), especially malnourished children, evolve to the classic form [32]. Most oligosymptomatic patients harbor viable parasites throughout their lifetimes, and can develop the disease in the event of immunosuppression reactivation [33].

Acute form: Characterized by high fever, cough, and severe diarrhea without hepatosplenomegaly or important hematological alterations, with a mean duration of two months and significantly elevated anti-*Lieshmania* IgM and IgG (titers ≥ 1:80 considered positive) [32]. The principal differential diagnoses of this form are typhoid fever, malaria, schistosomiasis, and other acute fevers accompanied by hepatosplenomegaly [1].

Classic form: Prolonged disease progression presenting as principal characteristics protein-energy malnutrition, edema, brittle hair, and elongated eyelashes. Alopecia also occurs. The initial signs include persistent or intermittent fever, diarrhea, asthenia, adynamia, nonproductive cough, somnolence, and progressive weight loss. The disease evolves with anemia, dry and brittle hair with more than one color (flag signal), elongated eyelashes, and alopecia may be present. Edema and eventually anasarca may occur. Volumous splenomegaly, variable hepatomegaly, abdominal distention due to the hepatosplenomegaly and some degree of ascites are frequent alterations [32]. Hemorrhagic alterations (epistaxis, ecchymosis, petechia, haemorrhagia) occur in roughly 32.7% of cases [32]. Delayed puberty and growth are observed among children and adolescents, as are hypergammaglobulinemia and hypoalbuminemia. There are rare reports of neurological involvement in VL, clinically expressed primarily by extremity tremors. The reduction of cerebral volume is also described especially in the frontal lobe – evidenced by cranial computerized tomography, in addition to atrophic alterations in the white matter and increased third ventricle on magnetic resonance imaging [34].

In the final stages of this condition, patients may present dyspnea upon minimal effort and accentuated pancytopenia. The association of comorbidities such as bacterial infections – mainly by *Staphylococcus aureus* and *Pseudomonas aeruginosa* –, malnutrition, bleeding, and gangrenous stomatitis may contribute to increased lethality [35]. Although the risk factors for secondary bacterial infections are well-known, a recent study in Brazil carried out in urban areas highlighted AIDS as the most commonly reported comorbidity among patients with VL, followed by tuberculosis, systemic lupus erythematosus, and chronic myeloid leukemia [36]. The susceptibility of HIV-positive patients to VL is attributed to reduced humoral and cellular response to *Leishmania*, and coinfection may accelerate disease progression and increase the pathogenic effects due to interference with macrophage function [37].

Cutaneous post-calazar form: This is a complication of visceral leishmaniasis occurring in patients who have recovered from the disease. These patients present with macular, maculopapular, cutaneous, and nodular eruptions. The eruption initially affects the area around the mouth, from where it can spread to other body regions depending on severity. This form of VL manifestation occurs primarily in the Sudan and in India [38]. Although considered rare in South America, there are reports in

Brazil. In one report, the patient from an endemic area exhibited clinical manifestations of VL three years after being diagnosed with AIDS [39]. The cutaneous lesions manifested mainly on the face, abdomen, and lower extremities, as densely distributed papulonodular lesions [40].

Infection in immunocompromised patients (HIV): coinfection presents peculiar characteristics in clinical manifestations, diagnostic laboratory findings, and treatment response [36,41]. The clinical findings are generally not characteristic; however there may be a rapid, sometimes fatal progression, with T CD_4+ cells as important prognostic factor [42,43]. Usually, the classic triad of febrile hepatosplenomegaly, pancytopenia, and hypergammaglobulinemia are the most frequently observed alterations, occurring in approximately 75% of patients [37,44]. In some cases in which the T CD_4+ count is below 200 cells/mm^3, digestive, pulmonary, and cutaneous manifestations may be observed [44,45]. In cases of coinfection with HIV, there is a persistent doubt of whether it is a primary infection or reactivation, considering the immunosuppression triggered by the HIV [40,46]. The risk of acquiring VL in endemic areas is roughly one hundred times higher in a patient infected with HIV, a context in which the virus acts as an opportunist infective agent. Coinfection with HIV also compromises the therapeutic response, and increases the probability of relapse given that both conditions compete for immune system depletion [47]. This group of patients also presents the possibility of visceralization of cutaneous and mucocutaneous leishmaniasis, and thus it is important to focus on establishment of the differential diagnosis [42]. Other opportunistic infections may often be associated, including esophageal candidiasis, *Pneumocystis jiroveci* pneumonia, tuberculosis, and criptococosis of the central nervous system [44]. Therefore, when considering the high rates of mortality, all patients VL patients should be checked for HIV and the pathogens involved in possible bacterial infections [36]. Table 2 summarizes the clinical manifestations of VL in potentially immunodeficient patients, for whom HIV serum testing should be requested.

Diagnosis

Diagnosis of human VL is still challenging for physicians given the wide variety of clinical signs common to other diseases [48]. Furthermore, the methods used in VL diagnosis are not 100% sensitive and specific. Routine diagnosis is usually based on clinical, epidemiological, and serological parameters; however, evidence of the protozoa via parasitological methods or DNA testing – by molecular methods – is required for definitive diagnosis [48,49].

For the purpose of reducing mortality rates, Table 3 lists the criteria which may be used for the definition of suspected and confirmed cases, as well as warning signs or disease severity, according to the Ministério da Saúde (Ministry of Health) of Brazil.

The disease should always be investigated in patients presenting a history of prolonged fever associated with hepatosplenomegaly, especially if coming from endemic areas, and also in cases of travelers who have returned from such regions and present prolonged febrile conditions [19].

Parasitological diagnosis: Parasitological diagnosis confirms infection [20]. This may be performed by stained smears of peripheral blood, in which the amastigote forms of *Leishmania* can be seen [50]. However, splenic fine needle aspiration is the most sensitive (90% to 95%) method to detect the parasite. Other sites such as the bone marrow and liver may also be aspirated, taking into account that in HIV positive patients the parasite may be located in unusual locations such as the gastrointestinal tract. Because it is considered a safer procedure, bone marrow aspiration is recommended, and the material obtained should be examined in the following order [51]:

- Any clinical form without recent (within the past year) exposure to an endemic area
- Injection drug use
- Absence of anti-*Leishmania* antibodies in the current disease state
- Presence of amastigote forms in peripheral blood
- Involvement of organs rarely affected by VL*
- Treatment failure** or relapse*** after use of pentavalent antimony
- Development of infections suggestive of immunodeficiency**** during or after treatment
- Isolation of dermotropic *Leishmania* strains or those not described as causing visceral involvement

*Involvement of respiratory tract, stomach, esophagus, duodenum, and skin.**Absence of clinical cure after two attempts at treatment with meglumine antimoniate (20mg SbV/kg/day for 30 days).***Reappearance of symptoms within 12 months of clinical cure.****Herpes zoster, miliary tuberculosis, among others.

Table 2: Conditions in which co-infection with Leishmania-HIV should be considered in the clinical diagnosis. Brasil. Manual de recomendações para diagnóstico, tratamento e acompanhamento da co-infecção Leishmania-HIV. Brasília: Ministério da Saúde, 2015. rasil. Manual de recomendações para diagnóstico, tratamento e acompanhamento da co-infecção Leishmania-HIV. Brasília: Ministério da Saúde, 2015.

Suspected case of visceral leishmaniasis
• Any patient with fever and splenomegaly, from an area with occurrence of VL transmission; • Any patient with fever and splenomegaly, from an area without occurrence of transmission, once the most common differential diagnoses for the region are discarded.
Confirmed case of visceral leishmaniasis
Laboratory clinical criteria: confirmation of clinically suspected cases must meet at least one of the following criteria: • Identification of *Leishmania* in direct parasitological exams or culture • Reactive immunofluorescence with a title of 1:80 or more, once other diagnoses are excluded. **Epidemiological clinical criteria**: suspected patients, without laboratory confirmation, from areas with occurrence of VL transmission, but with a favorable response to therapeutic trial.
Patients with signs of severity and alert
• Age under six months or over 65 years • Severe malnutrition; • Comorbidities or one of the following clinical manifestations: jaundice, hemorrhagic phenomena (excluding epistaxis), generalized edema, signs of toxemia (lethargy, poor perfusion, cyanosis, tachycardia or bradycardia, hypoventilation or hyperventilation and hemodynamic instability).

Table 3: Diagnostic criteria for visceral leishmaniasis. Brasil. Manual de recomendações para diagnóstico, tratamento e acompanhamento da co-infecção Leishmania-HIV. Brasília: Ministério da Saúde, 2015.

- Direct examination: A smear is made of a drop of aspirated material on at least four slides. Using Giemsa, Wright, Leishman or Panoptic stains, it is possible to view the amastigote forms of the parasite. Under light microscope oil immersion magnification, smears stained with panoptic acid show elliptical or rounded amastigotes, measuring 3-4μm in diameter, as shown in Figure 1.
- Isolation in culture medium (*in vitro*): Special culture mediums, especially NNN (Novy-MacNeal-Nicolle), are used to inoculate amastigote forms of the parasite, which transform into promastigote. The cultures are observed weekly over four weeks using light microscopy. When positive, the tubes should be sent to reference laboratories for species identification.

Parasite DNA detection methods: The molecular techniques

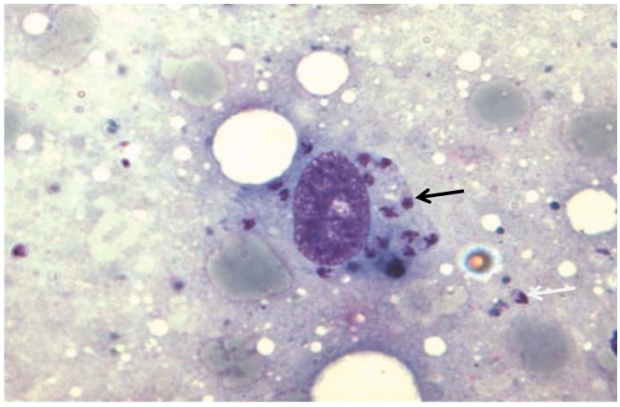

Figure 1: Parasitological analysis of a liver sample from a dog with VL. The black arrow indicates an amastigote in the interior of a macrophage and the white arrow indicates an extracellular amastigote. Blood smear stained using the panoptic method. (Photo by Ronny Francisco de Souza).

used after DNA extraction were developed for more precise detection and identification of the protozoa that cause VL, without the need for isolation of the pathogen in culture [52]. As in the parasitological investigation, various biological materials can be used in these reactions: aspirate from lymph nodes, bone marrow, spleen, total blood, culture, and blood collected on filter paper [53]. These methods are highly sensitive and include polymerase chain reaction (PCR), real-time PCR, reverse transcriptase associated PCR (RT-PCR) for RNA detection, nucleic acid sequence based amplification (NASBA), and loop-mediated isothermal amplification (LAMP) [53] and sequencing [54]. The targets for the PCRs have been primarily repeated sequences of conserved mitochondrial or kinetoplast DNA (conserved region of kDNA DNA) [55]. Other studies identify additional targets for pathogen detection, such as the small-subunit ribosomal RNA (SSU-rRNA), internal transcribed spacer 1 (ITS1), cysteine protease B (cpb), cytochrome b (cyt b), and heat shock protein 70 (HSP70), among others [54].

Although PCR is a sensitive method for *Leishmania* detection in a variety of human materials [56-59], it is more widely used in epidemiologic studies than in routine diagnosis [60], because serologic tests are still considered more advantageous in terms of cost-benefit [61]. PCR can also be used as a complementary method in the serological approach [62]. Real-time PCR (qPCR) can also be used for diagnosis [63,64]. The advantages in relation to conventional PCR include sensitivity, speed, reproducibility, quantitative ability, and better cost-benefit [65].

Serologic tests: Serologic diagnosis is performed by detection of *Leishmania* antigens or antibodies in serum [66]. Among the various noninvasive tests developed for VL diagnosis, those that employ antibody detection are the most widely used. In this context, it is important to note that VL is characterized by significant polyclonal B lymphocyte stimulation, which results in hypergammaglobulinemia [67]. Indeed, in serological tests the greatest problem that has been faced is the occurrence of false positive results. Several infectious diseases – such as babesiosis, borreliosis, heartworm, ehrlichiosis, and trypanosomiasis – may interfere with the analysis and yield false positive results [68,69].

Among the most commonly used tests are the indirect immunofluorescence test (IF), enzyme-linked immunosorbent assay (ELISA), direct agglutination test (DAT), and the rapid diagnostic test, based on recombinant antigen k39 protein [66]. ELISA has been used as an important tool in serum diagnosis for nearly all infections, including VL. In several studies using DAT in endemic VL areas, the specificity and sensitivity varied from 70.5-100% and 53-100%, respectively [66]. However, one of the difficulties of this method is the time required to complete the test, requiring approximately 18 hours of incubation [70]. IF is considered the gold standard of serum diagnostic tests and the sensitivity and specificity of this method vary between 85.5% and 94,7%, respectively [14].

These techniques employ soluble total antigens, but cross reactions result in numerous false positive results [68,69,71]. One solution to this problem – that has been invested in since the 90's – are recombinant or purified antigens [72-76], especially when using recombinant or purified *Leishmania* molecules [72-76]. One of these is recombinant antigen k39, a member of the kinase family. This recombinant antigen is specific for the species of the *L. donovani* complex [75]. The added use of recombinant antigens has improved diagnostic sensitivity [73].

Nonspecific tests: Other indirect methods that may aid diagnosis include:

- Hematological evaluation: the complete blood count may reveal intense normochromic anemia (less than 3 million red blood cells/mm^3 and hematocrit between 25 and 30%), leukopenia (2000-4000 white blood cells/mm^3) and thrombocytopenia below 100,000/mm^3, Rouleaux formation is common, as is absence of eosinophilia, even when associated with other parasitoses such as schistosomiasis and strongyloidiasis [77].
- Biochemical blood evaluation: the protein assay reveals hypoalbuminemia with increased globulins; cases with inversion of albumin/globulin ratios are not uncommon, and can be as accentuated as those found in multiple myeloma [20]. The aminotransferases are normal or slightly elevated, reaching up to three times the upper reference range. Hyponatremia associated with altered antidiuretic hormone (ADH) secretion [78,79].
- Urinalysis: may reveal proteinuria, hematuria, pyuria, and cylindruria, indicating subclinical glomerulonephritis caused by immune complex deposition [80].

Evaluation by complementary methods: The possible complications of the disease can be identified using some complementary methods, including renal function tests and imaging tests, electrocardiogram and stool parasitology testing. Chest x-rays may reveal interstitial condensation or infiltrate, resulting from concurrent bacterial or viral infection, or caused by the VL itself in cases of leishmaniotic pneumonitis [32,81]. Plain abdominal x-ray may reveal hepatosplenomegaly, evidenced by increased volume with hypotransparent aspect in the upper right quadrant and retraction of the hepatic flexure, with possible presence of free fluid in the peritoneal cavity [82]. Computerized tomography of the abdomen reveals a diffuse enlargement of the liver with periportal hypoattenuation following administration of venous contrast – a finding that indicates inflammation or edema, in addition to revealing ascites, thickening of vesicular wall and lymphadenopathies [83]. The spleen is markedly enlarged with hypodense diffuse nodular lesions, associated with vascular alterations such as vena cava and splenic vein dilation [84]. Doppler echocardiography has been employed to assess cardiac function of patients undergoing treatment with N-methylglucamine antimonate, demonstrating that the occurrence of pericardial effusion is associated with elevated parasitemia [84]. Any clinical manifestation

that signals progression to more severe states should be observed (Table 3). These signs indicate likelihood of development of the severe, life-threatening forms of VL.

Treatment

Specific treatment: Hospitalization of the patient diagnosed with VL is required in order to provide specific parenteral therapy [47]. Drugs currently recommended for treatment of the disease are pentavalent antimonial and liposomal amphotericin B [44,85], the former being the first choice due to its proven therapeutic efficacy [86]. However, pentavalent antimonial is associated with frequent toxicity [87].

It is known that recently, ever increasing doses of antimony have been recommended by the World Health Organization and the Centers for Disease Control and Prevention (USA). This dosage increase has been attributed to the resistance demonstrated by the parasites to these drugs, especially in patients in the Sudan, Kenya, and India [44]. On the other hand, in Brazil, absences of results that confirm this trend justify use of a dose of 20mg of Sb^{+5} kg/day. In this case, the drug is administered intravenously or intramuscularly during 20 to 40 days and cure rates are considered satisfactory [44].

In cases of treatment failure, gestation, contra-indication, need for suspension of pentavalent antimony, or acquired infection in areas of resistance, amphotericin B [47] or miltefosine [88] should be used (however, the cure rate of infection by *L. chagasi* in the treatment with mitefosine is approximately 50%). Treatment using liposomal amphotericin B (LAMB) is very effective and is the most widely used [89]. Table 4 summarizes treatment options for VL.

Treatment of VL can be complicated in cases where the patient develops tumor lysis-like syndrome (TLLS), a metabolic disorder generally manifested after the start of chemotherapy [90]. This syndrome is triggered by "massive death of parasites and amastigote-containing mononuclear phagocytes after the initiation of treatment for visceral leishmaniasis" [91]. The complications are more pronounced among patients with high parasite loads, who may present residual kidney damage within 30 days of starting treatment [90,91]. On the fourth post-treatment day, there are increases in serum levels of uric acid, phosphate, creatinine, and urea. In these cases, metabolic recovery is obtained by hydration, urine alkalinization, and use of allopurinol, and in some cases tissue damage was observed. Awareness of this condition is important, to adopt prophylactic measures and also for early recognition and adequate medication [91].

Use of immunomodulators: The therapeutic approach is being reconsidered using the idea of enhanced immunity, and immunomodulators associated with chemotherapy drugs seems promising as the most effective therapeutic alternative in the future [92,93].

Granulocyte and macrophage colony stimulating factors – G-CSF and GM-CSF – have been used in the treatment of hematological disorders in patients with VL and neutropenia. The exogenous anti-Leishmania effect of GM-CSF is accompanied not only by early macrophage and neutrophil mobilization, but also by the influx of myelocytic cells to the affected organ [93]. Immunotherapy with interferon gamma (IFN-γ) in acute or refractory VL patients under treatment with Sb^{+5} showed encouraging results [93]. The use of this therapy in patients with the severe forms of the disease and important neutropenia (<1,500 neutrophils/mm^3) significantly reduces mortality – usually associated with bacterial or viral infections and septic complications [95].

Support measures: Blood transfusion represents an important therapeutic measure in VL. Referral to a hemotherapy service is indicated for patients with evidence of serious or significant laboratory alterations, including leucopenia <1,000/mL, neutrophils <500/mm^3, thrombocytopenia <50,000/mL, serum hemoglobin <7 g/dL, serum creatinine greater than two times upper reference range, prothrombin activity less than 70%, serum bilirubins above reference range, hepatic enzyme greater than five times reference range, albumin <2.5 mg/mL, and chest X-ray with image suggestive of infection or pulmonary edema [47,85].

Based on the presence of neutropenia – typical of VL in its classic form – the patient is presenting a decreased inflammatory response and is at risk of manifesting bacterial infections, especially on the skin, respiratory tract, and middle ear, with *Pseudomonas aeruginosa* and *Staphylococcus aureus* the most commonly involved agents [95]. Thus, fever has little diagnostic value in secondary infection, and there is no secure method to identify bacterial infection. Since there is no consensus in the literature on the antimicrobial approach in these patients, a prudent approach would be to follow the recommendations outlined for the febrile neutropenic cancer patient [47,95], as summarized in Table 5.

Special circumstances: In the treatment of *Leishmania* and HIV co-infection, the drugs used are the same as those for immunocompetent patients, with the recommended drug of choice being pentavalent antimonial. Alternately, amphotericin B and pentamidine isethionate can be used [47].

During pregnancy, treatment aims to cure the pregnant patient and to prevent transplacental transmission. Because of their teratogenicity, antimonials should not be used, nor should amphotericin B (it is cardiotoxic and nephrotoxic); as such, the drug of choice is aminosidine (12 to 16 mg/kg/day, intramuscularly, for 15 to 20 days) [96].

In patients presenting the cutaneous form post-kala-azar, the drug typically used is sodium stibogluconate (20mg/kg/day for two months) [88].

Cure control: Assessment of cure depends on monitoring of clinical criteria: fever drop (the first symptom to disappear, after the second to fifth day of specific medication), hepatosplenomegaly regression, overall patient improvement and appetite restoration (seen during the first week of treatment, weight gain, urinary volume, lymph node size, increased albumin and decreased serum gamma globulins. Following treatment, the presence of eosinophils in peripheral blood is a marker of a favorable prognosis [47,85].

Nursing care: The change in the epidemiological characteristic of VL points to a need for integration among healthcare workers in treating affected individuals. In this context, physicians, nurses, and other professionals should be aware of the characteristics of the disease, its transmissibility, and be able to recognize new cases. In the case of the supporting healthcare staff, they should follow the steps of the healthcare process proposed by the nurse, the professional who should guide their practice based on care planning.

Among the models proposed for this purpose are those outlined by Wanda de Aguiar Horta [97] in the Theory of Basic Human Needs associated with the North American Nursing Diagnosis Association (NANDA) taxonomy [98] and the use of the Nursing Interventions Classification (NIC) that guides interventions based on the proposed diagnoses [99], as shown in Table 6. The term nursing diagnosis is defined by NANDA as "a clinical judgment about human experience/responses to health conditions/life processes that exist in an individual, family, or community that provide the foundation for definitive therapy

Drug	Dose	Administration	Side effects	Recommendations
N-methylglucamine antimonate (SbV+)	The prescribed dose references pentavalent antimony (Sb^{+5}) = 20mg/Sb^{+5}/kg/day intravenously or intramuscularly, once a day for 20 to 40 days. Maximum limit of three ampoules per day	Administration by intravenous or intramuscular route over at least five minutes. The dose may be diluted in 5% dextrose to facilitate infusion	Arthralgia, myalgia, loss of appetite, nausea, vomiting, stomach fullness, epigastric pain, pyrosis, abdominal pain, pain at application site, fever, severe cardiac arrhythmias, hepatotoxicity, nephrotoxicity, and pancreatitis	• Monitor hepatic enzymes, renal function, serum amylase and lipase • Electrocardiogram before, during, and at the end of treatment to monitor corrected QT interval, arrhythmias, and T wave flattening • Contraindicated in patients with renal impairment, patients who underwent renal transplantation, and in pregnant women.
Amphotericin B deoxycholate	1mg/kg/day by infusion for14 to 20 days. Maximum daily dose of 50mg.	Reconstitute the powder in 10mL of distilled water. Immediately shake the vial until the solution becomes clear. This initial dilution contains 5mg of amphotericin B per mL and can be stored at temperatures of 2-8°C and protected from light for at most one week, with a minimum loss of potency and limpidity. To prepare the solution for infusion, a new dilution is required. Dilute each 1mg (0.2mL) of amphotericin B from the previous solution in 10mL of 5% dextrose. The final concentration will be 0.1mg/mL of amphotericin B. Infusion time is between two and six hours.	Fever, headache, nausea, vomiting, hyporexia, tremors, chills, phlebitis, cyanosis, hypotension, hypokalemia, hypomagnesemia, impaired renal function, and behavioral disorders.	• Monitor renal function, serum potassium and magnesium • Replace potassium when necessary • Follow orientations for dilution and infusion time • In case of side effects or adverse reactions during infusion, administer an antipyretic one hour prior to administration • In renal dysfunction with creatinine levels above two time reference values, treatment should be suspended for two to five days and restarted on alternating days once creatinine levels drop • Prior to reconstitution, the lyophilized amphotericin B powder should be kept under refrigeration (temperature between 2 to 8°C) and protected from light
Liposomal amphotericin B	3mg/kg/day, for seven days, or 4mg/kg/day, for five days, by venous infusion, in a single daily dose.	Reconstitute the powder in 12mL of sterile water for injection, shaking the vial vigorously for 15 seconds in order to completely dissolve the liposomal amphotericin B. This yields a solution containing 4mg/mL of liposomal amphotericin B. This solution can be stored for up to 24 hours at temperatures between 2 and 8°C. Redilute the calculated dose at the proportion of 1 mL (4mg) of liposomal amphotericin B to one to 19mL of 5% dextrose. The final concentration will be 2 to 0.2mg of liposomal amphotericin B per mL. Infusion shoud begin within six hours of dilution in 5% dextrose. Infusion time is 30 to 60 minutes.	Fever, headache, nausea, vomiting, tremors, chills, and low back pain.	• Monitor renal function and serum potassium • Follow instructions on dilution and infusion times • In case of side effects or adverse reactions during infusion, administer an antipyretic one hour prior to treatment • In renal dysfunction, with creatinine levels two times greater than reference range, treatment should be suspended for two to five days and restarted on alternating days once creatinine levels have dropped • Before reconstitution, the lyophilized liposomal amphotericin B powser should be kept under refrigeration (temperatures between 2 and 8°C) and protected from light

Table 4: Summary of treatments for visceral leishmaniasis.
SOURCE: BRASIL. Ministério da Saúde. Leishmaniose visceral grave: normas e condutas. Brasília: Ministério da Saúde, 2006, p.62.; and Siqueira-Batista R, Gomes AP. Antimicrobianos – guia prático. 2ª ed. Rio de Janeiro: Rubio, 2011.

	Indication	Treatment schedule
Antibiotic prophylaxis	• Patients under two years of age • Patients with neutrophil count below 500neutrophils/mm^3	Ceftriaxone 75-100mg/kg/day intravenously, associated with oxacillin 100-200mg/kg/day intravenously, fractionated in six daily applications
Antibiotic treatment	• Patients with confirmed infection • Patients with signs of systemic inflammatory response syndrome* even when site of infection is undefined	Follow the Comissão de Controle de Infecção Hospitalar guidelines and the local antibiotic treatment protocols, considering site of infection, severity, community or hospital origin of the infection and the culture results

*Poor peripheral perfusion, altered state of consciousness, tachypenia, tachycardia, hypotension, oliguria, and evidence of coagulopathy.
Table 5: Use of antimicrobial drugs in visceral leishmaniasis. SOURCE: BRASIL. Ministério da Saúde. Leishmaniose visceral: recomendações clínicas para redução da letalidade rasília: Ministério da Saúde, 2011.

Psychobiological needs	Nursing Diagnosis (NANDA, 2010)	Nursing Activity/Intervention (NIC, 2010)
HYDRATION	Risk of electrolyte imbalance related to I. Water imbalance II. Diarrhea III. Secondary effects of treatment	- Monitor intake and elimination - Monitor occurrence of manifestations of electrolyte imbalance - Offer liquids as appropriate - Monitor for water loss (diarrhea) - Instruct patient and/or family members on the signs and symptoms of electrolyte imbalance and to seek medical assistance - Monitor occurrence of abnormal serum electrolyte levels
NUTRITION	Imbalanced nutrition: less than body requirements related to: I. Biological factors II. Impaired ability to ingest food	- Offer light, creamy, non-spicy foods - Encourage ingestion of light foods - Monitor caloric and nutritional ingestion - Weigh the patient at specific intervals - Monitor occurrence of dry and thin hair - Monitor occurrence of nausea and vomiting
PHYSICAL ACTIVITY AND EXERCISE	Intolerance to physical activity related to: I. Generalized weakness	- Monitor nutritional ingestion to ensure adequate energy resources - Encourage expression of feelings about limitations - Select interventions to reduce fatigue using combinations of pharmacological and non-pharmacological categories, as appropriate
THERMAL CONTROL	Hyperthermia related to: I. disease (Leishmaniasis) II. dehydration	- Monitor temperature at appropriate intervals - Monitor blood pressure, pulse, and respiration - Monitor ingestion and elimination - Stimulate greater ingestion of oral fluids, as appropriate - Administer antipyretics, per physician's prescription - Administer warm sponge bath, as appropriate - Apply towel-wrapped ice packs to groin and armpits - Administer medication to treat the cause of the fever, per physician's prescription - Closely monitor temperature to prevent treatment-induced hypothermia
VASCULAR REGULATION	Bleeding risk related to: I. impaired hepatic function	- Monitor platelet count, hemoglobin, and hematocrit - Closely monitor the patient for hemorrhage, (verify all secretions for occult or visible blood - When an invasive procedure is necessary, monitor closely for bleeding
IMMUNE REGULATION	Risk of infection related to I. inadequate secondary defenses II. malnutrition	- Monitor systemic and local signs and symptoms of infection - Promote adequate nutritional intake - Monitor absolute granulocyte count, white blood cell count, and differential results
Psychosocial Needs	**Nursing Diagnosis (NANDA, 2010)**	**Nursing Activity/Intervention (NIC, 2010)**
SECURITY LEARNING (health education)	Ineffective health self-control related to: I. learning disabilities Self-neglect related to: I. lifestyle/choices II. maintaining control	- Monitor at-risk populations for adherence to prevention and treatment programs - Monitor environmental factors that influence disease transmission - Instruct on adequate control of vectors and hosts in animal reservoirs - Inform the public about the disease and control-related activities - Implement investigations into environmental health risks - Prepare and make available written educational material at target audience reading level - Involve individuals, families, and groups in the planning and implementation of measures to modify health behaviors and lifestyle

Table 6: Possible basic human needs affected, their respective diagnoses and nursing interventions for the leishmaniasis patient, according to the Theory of Basic Human Needs (Horta, 1979), NANDA (2010), and NIC (2010).

aiming to achieve results in which the nurse is required" [100]. On the other hand, according to NIC, the nursing intervention consists of "any treatment based on the judgment and clinical knowledge by a nurse to improve patient/client outcomes" [100].

Prophylaxis and Recommendations for Travelers

There are some behavioral measures that considerably reduce the risk of contracting VL [28] which apply to humans, to the vector, and to other animals (Table 7).

At the moment there are no vaccines or drugs with proven efficacy in immunoprophylaxis or chemoprevention of visceral leishmaniasis in humans.

Based on the insufficient knowledge of the VL transmission chain, strategies for disease control are centered on early detection and treatment of affected patients, reduction of vector population, elimination of sandfly reservoirs, and continuing education of healthcare professionals. A study carried out in the state of Bahia between 1995 and 2000 demonstrated the validity of control actions with the percentage of visited and sprayed buildings [101].

Actions directed at the human population	Actions directed at the vector	Actions directed at the canine population
• Individual protection measures such as fine-mesh mosquito netting, screening of doors and windows, use of repellents, non-exposure at sandfly activity periods (dusk and night)	• Environmental sanitation: urban cleaning, elimination and proper disposal of solid organic wastes, elimination of humidity sources, non-permanence of domestic animals indoors	• Control of errant canine population • Adoption • Application of the canine visceral leishmaniasis vaccine • Use of screens in individual or collective kennels • Use of collars impregnated with 4% deltamethrin

Table 7: Summary of prophylactic measures for visceral leishmaniasis
SOURCE: Brasil. Ministério da Saúde. Secretaria de vigilância em saúde. Departamento de vigilância epidemiológica. Manual de vigilância e controle da Leishmaniose visceral. Brasília: Editora do Ministério da Saúde, 2006.p.120.

	Objectives	Methodology
Entomological surveillance	• Verify the presence of *L. longipalpis* and/or *L. cruzi*, in areas without human VL cases • Verify the presence of *L. longipalpis* and/or *L. cruzi*, in previously uninvestigated areas with sporadic, moderate, or intense transmission • Learn the vector dispersion in the area, in order to identify areas without indigenous VL cases that are receptive to canine sampling surveys; in areas with VL transmission, instruct on vector control measures	• Manual collection using Castro collection tubes • Manual collection using monitored capture • Collection using adhesive trap • Collection using light traps • Traps with animals or pheromones
Surveillance in humans*	• Determine whether the case is autochthonous or imported (if imported, inform the state or municipal epidemiological surveillance of the probable site of infection) • Determine whether the area is endemic or if it is a new transmission site • Learn the epidemiological characteristics of the case • Perform an active search for new cases, characterize them clinically and laboratory	• Identification of the individuals who spontaneously seek health services • Active search for cases in transmission areas • Home visits by the staff of the Estratégia da Saúde da Família (Family Health Strategy) • Referral of suspected cases to reference centers
Surveillance in the dog	• Alert veterinarians about the risk of canine visceral leishmaniasis (CVL) transmission • Alert the population about the occurrence of CVL in the area and warn about the clinical signs and diagnostic services as well as preventive measures for eliminating likely vector breeding sites • For the public sector, initiate and implement urban sanitation actions in public land, squares, gardens, playgrounds, and other areas, adequately disposing of collected organic matter • Upon clinical suspicion of an infected dog, delimit the area to search for the breeding site. Define the search area as a radius of at least 100 dogs to be examined based on the first canine case. Within this area, perform the tasks outlined in the next column	• Active search for symptomatic dogs and parasitological examination and confirmation of *Leishmania* species. If *L. chagasi* is identified, collect serum from all dogs in the area, in order to assess canine prevalence and carry out other measures

*Human visceral leishmaniasis is a mandatorily reportable disease and as such any suspected case in Brazil should be notified using the standardized Sistema Nacional de Agravos de Notificação – SIVAN form and investigated by appropriate health services.

Table 8: Summary of epidemiologic surveillance measures for visceral leishmaniasis

SOURCE: Brasil. Ministério da Saúde. Secretaria de vigilância em saúde. Departamento de vigilância epidemiológica. Manual de vigilância e controle da Leishmaniose visceral. Brasília: Editora do Ministério da Saúde, 2006.

To date there are no available vaccines for the leishmaniasis. However, research has been carried out that may in the near future lead to the development of vaccines for human visceral leishmaniasis. Among the promising results aimed at prevention and control of VL are first, second, and third generation vaccines. The first are based on the attenuated parasite; the second on antigenic or recombinant proteins; and the third are derived from DNA plasmids encoding the antigen. Some of these, such as Leishmune®, Eishtec, and CaniLeish® have already been licensed for the prevention of canine VL [102].

Epidemiologic Surveillance

Epidemiologic surveillance of VL is part of the Brazilian Visceral Leishmaniasis Control Program (Programa de Controle da Leishmaniose Visceral – PCLV), whose priority is to reduce morbidity and mortality rates through early diagnosis. It includes entomological surveillance and monitoring of human and canine cases, as summarized in Table 8.

In this way, epidemiological surveillance may indicate signs for actions and preventions to be adopted in humans. The first goal of the PCLV is to incorporate regions including those that have no recorded cases of this disease.

Final Considerations

Visceral leishmaniasis is a serious public health problem that was previously restricted to rural areas but is currently associated with the process of urbanization. The disease is potentially fatal if not diagnosed in time, but fully curable if detected, in the absence of co-morbidities that may impact overall patient condition. It is therefore important that healthcare professionals possess the capacity to identify a suspected

VL case – especially those among patients in non-endemic areas – in order to reduce the morbidity and mortality associated with the disease through surveillance measures, prevention, and control.

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