Visceral Leishmaniasis. A Neglected Parasitic Infection Worldwide

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Visceral leishmaniasis (VL), or kala-azar, is a vector-borne infectious disease caused by the protozoan parasites *Leishmania donovani* and *L. infantum* (*Leishmania donovani* complex), (Kinetoplastida, Trypanosomatidae). The transmission of *Leishmania* to mammals occurs through the bite of hematophagus vectors of the genus *Phlebotomus* (Old World) and *Lutzomyia* genus (New World) infected by biting and sucking blood an infectious mammals being [1]. *L. donovani* complex species are intracellular parasites of macrophages of lymphoid organs such as the spleen, lymph nodes, bone marrow and liver. Several species of vertebrate mammals may be infected naturally with *Leishmania*. The primary reservoir hosts of *Leishmania* species are sylvatic mammals such as forest rodents, hyraxes and wild canids [2]. The dogs are the main domestics reservoirs of the parasite for the viscerotropic species in the Mediterranean, Asia, North Africa and South America and constituting a important link of the epidemiological cycle of human transmission. Considering the presence or absence of animal reservoirs for *Leishmania*, there are two basic types of epidemiological cycles: zoonotic (ZVL), from canine reservoir hosts to humans or anthroponotic (AVL), from human to human [3]. These parasites have also been transmitted via blood transfusions in people and dogs [4], and by transplacental transmission in dogs, mice and humans [5].

Leishmania have a dual-form life cycle (digenetic), because is found in vertebrates and invertebrates hosts [6,7]. These parasites have two basic life cycle stages: promastigote is an extracellular stage within the invertebrate host (phlebotomine sand fly), and amastigote is an intracellular stage within a vertebrate host. During the life-cycle of *Leishmania* the promastigotes in the insect vector are inoculated into the dermis of the mammalian host during a blood meal. The promastigotes are then phagocytised by the cells of the mononuclear phagocyte system, most by macrophages, that are in the vicinity of the site of the bite. Inside the phagosome, the promastigotes evolves into amastigotes, spherical intracellular forms without flagellum. The multiplication of the parasites by binary fission occurs inside the macrophages. The amastigotes break up the macrophages and are released within the extracellular medium, the cycle continue when other hosts' phagocytes are being infected [8,9]. During the development of the VL, all organs containing macrophages and phagocytes can be infected, especially the lymph nodes, spleen, liver, and bone marrow [10,11].

The incubation period after de initial infection is usually from 2 weeks to 18 months. After of 2 - 8 months often they appear inflammatory reactions within the visceras, nevertheless, VL symptoms can take years to appear. This infection is characterized by prolonged fever, fatigue, splenomegaly, hepatomegaly, substantial weight loss, progressive anemia, pancytopenia, and hypergammaglobulinemia and is complicated by serious infections. The disease is fatal if untreated, with a mortality rate of 75 - 95% [12]. Parasites proliferate wherever there are cells of the mononuclear phagocyte system, most often in macrophages. These are most abundant in the spleen and liver and, consequently, infection leads to an enlargement of both of these organs. Bone marrow cells become infected, and patients develop pancytopenia (namely, depressed production of red blood cells, white cells, and platelets) and immunosuppression, making them susceptible to superinfections [13,14].

VL is a serious public health problem worldwide, occurs five continents with endemic transmission reported in 88 countries, with an estimated annual incidence of 500 thousand new cases. This infection causes an estimated 50,000 deaths annually, a rate surpassed

among parasitic diseases only by malaria, and 2,357,000 disability-adjusted life years lost, placing leishmaniasis ninth in a global analysis of infectious diseases [15]. It is estimated that 350 million people are at risk of infection in the six most heavily affected countries. Most of the VL burden is in India, where 40 percent of all new cases are found [16]. More of 90% of cases of visceral leishmaniasis are concentrated occur in five countries across three continents: north eastern India, Bangladesh, and Nepal in the Indian subcontinent, Sudan in Africa, and north eastern Brazil in South America [17]. The epidemiological data are incomplete, and official figures are likely to underestimate grossly the real prevalence of the disease [18]. Both the number of recorded cases and the geographical affected have grown in the past two decades [19]. However, inadequate surveillance and misdiagnosis mean that the true incidence, mortality, and morbidity of VL are unknown.

Leishmaniasis is a neglected vector-borne tropical infection that is highly correlated with war, poverty and failed health systems [20,21]. The parasite is of great medical and veterinary public health significance, for it infects numerous mammal species, including humans. The World Health Organization (WHO) classified Leishmaniasis as one of most neglected tropical diseases and VL is classified as the second-largest parasitic killer in the world after Malaria according to published disease burden. Moreover Leishmaniasis disease is considered to be a disease of the poor [22]. In India, VL is concentrated in Bihar, one of the poorest states of the country, while in East Africa, the incidence of VL is very high among migrant and displaced populations in places like Ethiopia and South Sudan. MSF programmes are usually housed in isolated primary health care facilities. If not for MSF's logistical support, these facilities would likely lack electricity, making it difficult to establish appropriate laboratory services or to store medicines at the low temperatures they require.

The diagnosis of VL infections can be approached by two ways: parasitological and serological. The demonstration of parasite in the tissue of relevance is done by

a) The visualization of the amastigotes forms of the parasite by light microscopy examination of the Giemsa-stained smears from tissue aspirate or biopsies (spleen, lymph node and bone marrow aspirate) although is a method very specificity, this procedure demand long time, is risky, invasive and very difficult to perform in field conditions, it suffers from variability of detection sensitivity and the need for an expert microscopist [23,24].

b) Animal inoculation and Culture: *Leishmania* spp. can also be cultured. However, each species will grow only in certain media, and some species can be difficult to isolate. Novy-MacNeil-Nicole medium, Grace's medium and Schneider's Drosophila medium might be used initially. Culture from splenic or bone marrow aspirates has high specificity, but is tedious, time-consuming, requires expertise, expensive equipment and its use is restricted to referral research centers and hospitals thus seldom used for clinical diagnosis [25].

c) Enzyme linked immunosorbent assay (ELISA) has been used as a potential serodiagnostic tool in almost all infectious diseases including leishmaniasis. The sensitivity and specificity of ELISA depends on the antigen employed. ELISA with crude or soluble antigens of promastigotes or amastigotes was applied, however cross reactivity was prevalent resulting in providing it the lowest consideration in diagnosis. One of the most commonly used antigens is a crude soluble antigen (CSA). The sensitivity and specificity of the method using CSA range from 80 to 100% and from 84 to 95%, respectively, while cross-reactivity among patients with tuberculosis, trypanosomiasis and toxoplasmosis has been reported. The use of extracts and semipurified fractions of *Leishmania,* has caused problems such as the presence of cross-reactions (falses positives), i.e. low specificity [26], the presence of falses negatives, i.e. low sensitivity [27], besides the risk of obtain this type of antigens from culture and the high cost and time required. In the last 15 years, the developments in molecular biology in diagnosis, have triggered advances and a great deal of knowledge, with more effective immunochromatographic tools, so that the use of these techniques has strengthened VL control, especially in massive screening studies, inducing very significant progress in surveillance, diagnosis, and epidemiology in human VL all over the world. Advances in recombinant DNA technology have allowed the expression and purification of proteins in large quantities, quickly and efficiently, in a few steps, at a low cost, through the use of the metabolic machine of other microorganisms.In the last two decades, several *Leishmania* antigens have been genetically and antigenically characterized, but the most promising one has been the recombinant antigen rK39. The rK39 is a 39-amino-acid repetitive immunodominant B-cell epitope of the 230 kDa is a

member of kinesis family of *L. chagasi* [28, 29]. The rK39 ELISA has proven to be suitable for detecting human and canine visceral leishmaniasis with the ost producing specific antibodies against replicating *Leishmania* [30]. rk39 MUESTRA UNA sensitivity and specificity of 100% and 96% respectively. The antibody titres to this antigen directly correlate with active disease and have potential in monitoring the chemotherapy and in predicting the clinical relapse. In addition, rK39 ELISA has a high diagnostic and prognostic utility in HIV infected patients. Due to the requirement of skilled personnel, sophisticated equipment and electricity, ELISA is not used in the rural endemics areas for the diagnosis of VL [31].

d) Rapid assay: It has developed an immunochromatographic strip test sensitized with rK39 to be employed in field conditions, which eliminates the use of costly infrastructure. The first large-scale evaluation of this rK39 ICT was done in India [32], and 100% sensitivity and 98% specificity were reported. Several other validation studies in different parts of the world, as well as a meta-analysis, confirmed the diagnostic performance of the rK39 ICT combined with a clinical case definition as good to excellent for VL, with a sensitivity in East Africa slightly lower than that in the Indian subcontinent [33]. WHO–Tropical Disease Research (WHO/TDR) directed a multicenter study in 5 countries that confirmed the high diagnostic accuracy of this rK39 rapid diagnostic test (RDT) in India and Nepal [34].

e) Molecular Methods: Detection of parasite DNA in the peripheral blood is emerging as a non-traumatic and sensitive tool for the diagnosis of VL. *Leishmania spp.* is characterized by a prominent kinetoplast structure containing the mitochondrial DNA in the parasites' single mitochondrion. The kinetoplast contains hundreds of DNA maxicircles encoding genes that are destined for RNA editing and thousands of DNA minicircles. The kinetoplast DNA (kDNA) sequences for its their abundance, specificity, and repetitive nature, have frequently been targeted for nucleic acid-based detection [35]. The PCR assay has the capability of detecting low levels of parasitemia from the peripheral blood. This capability can be exploited both for the diagnosis of the disease and for prediction of the disease outcome, since a negative PCR result at the end of treatment is likely to be associated with a favorable outcome [36]. PCR assays with primers which amplify kinetoplast DNA (kDNA) have been evaluated for the diagnosis of VL and have been to shown to have excellent sensitivities and specificities, and they may prove to be useful for correlation of the findings at the time of diagnosis and the final outcome at the end of treatment. The aim of this prospective study was to evaluate the utility of PCR in the diagnosis of VL and to assess its value in identifying the disease outcome after treatment [37]. PCR has proven to be a valuable, rapid and sensitive tool, and can identify the parasite species in different clinical samples from humans, reservoirs and vectors.

Control of leishmaniasis requires a combination of intervention strategies; early diagnosis and treatment is an important aspect. In VL, diagnosis is made by combining clinical signs with parasitological or serological tests (rapid diagnostic tests and others). In addition, an accurate diagnostic test that can identify active VL versus asymptomatic disease remains a key component of measures that aim to control this serious disease [38]. Appropriate diagnostic tests are important in epidemiological studies and control programs, and for that reason the association of techniques may increase the positivity and contribute to the control of this canine disease and human disease [39]. The existence of several methods for the diagnosis of VL enables the distinction between active and asymptomatic infection, the diagnosis of relapse and also the diagnosis of VL-HIV coinfection. As the sensitivity and specificity of a method may vary in different endemic regions, the selection of the diagnostic tests should be based on several parameters, including the sensitivity and specificity as well as the cost, the availability of equipment and qualified personnel, and field applicability [40]. Based on the fact that VL usually is associated with the poverty, illiteracy, displacement, unhygienic and poor housing as well as environmental changes, where all these factor available to sand flies good condition to breed in and easy access to human beings, because the people in the poverty class will not able to afford the simple protection from sandflies like using net or even window screen will not able to afford for poor [41].

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