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Visceral Leishmaniasis in Ethiopia: A Review

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Abstract: Visceral leishmaniasis (VL) or kala azar is a fatal neglected tropical disease caused by protozoan parasites of the genus Leishmania, belonging to the family Trypanosomatidae.L. donovani complex (L. donovani and L.infantum) are the causative agents of VL in Ethiopia.L. donovani is regarded as the major cause of VL in Ethiopia, although L. infantum was identified as a main causative agent of the recent VL outbreak inLiboKemkem, in the Amhara regional state.In Ethiopia, the burden of leishmaniasis especially that of visceral leishmaniasis (VL) is a major problem to public health development. Every year, health facilities report thousands of cases and hundreds of deaths. In Ethiopia leishmaniasis especially VL is highly association with dog ownership, seasonal migration of labours to and from endemic areas and poor nutritional status. Transmission of *Leishmania*parasites can be zoonotic or anthroponotic; however, the principal way of transmission in Ethiopia remains unclear. Furthermore, the reservoir hosts for this disease are not well known and its transmission ways are not clearly set. However, dogs believed to play a significant role in the spread and maintenance of leishmaniasisin endemic areas.In the Ethiopia´s resource-limited context, there is a need for effective and focused control strategies for leishmaniasis. This requires knowledge of current and potential distribution range of its parasite and vectors as well as its transmission dynamics.So, in-depth studies of its transmission dynamics and mapping areas endemic to this disease and those likely to be affected are very crucial to provide evidences needed for devising effective control methods.

Key words: *Anthroponotic · Leishmania · Transmission dynamics · Zoonotic*

INTRODUCTION

Leishmaniases are a group of diseases caused by more than 20 species of the protozoan genus *Leishmania* that are transmitted between humans and other mammalian hosts by phlebotomine sand flies [1]. The disease endangers some 350 million people in 88 countries, most of them in the poorer regions of the globe. It is widely distributed around the world. The distribution ranges covers inter tropical zones of America, Africa and extend in to temperate regions of South America, southern Europe and Asia. Geographical distribution of the diseases depends on sand fly species acting as vectors, their ecology and the conditions of internal development of the parasite [2].

There are two major clinical forms of leishmaniasis, cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) [3]. VL, also called Kala-Azar, is the most severe form of leishmaniasis, almost always fatal if untreated. VL is the most prevalent form in eastern Africa in general and in Ethiopia in particular. In Ethiopia, VL is reported to be widespread over the arid and semi-arid parts of the country. Cases of VL have been reported from six regions (Tigray, Amhara, Oromia, Southern Nations and Nationalities People's Region (SNNPR), Somali and Afar regional states) and serologically positive cases were reported from Gambela and BenshangulGumuz regional states [4].

Leishmaniadonovani complex (L. donovani and L.infantum) are the causative agents of VL in Ethiopia[5]. Man and dogs are the most commonly affected hosts and they can also be a potential reservoir [6]. Dogs are the most important species among domesticated animals in the epidemiology of this disease; hence, dogs are reservoir hosts for L. infantum, which is one of the two most important organisms in human VL [6, 7]. There are about 30 species of phlebotominesand flies known to transmit leishmaniasisPhlebotomusorientalis, Phlebotomus martini and Phlebotomusceliae have been confirmed as vectors of VL in Ethiopia [8].

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In Ethiopia, although some studies propose that there is evidence of zoonotic transmission due to isolation of *L. donovanicomplex* in dogs[9, 10]. No firm evidence has been reported so far about the principal transmission ways. On the other hand, insights derived from recent research finding indicate that humans are probably the most important domestic reservoirs of Leishmania. In Ethiopia, there is no conclusive evidence whether the principal way of transmission of *Leishmania*parasites can be zoonotic or anthroponotic [10].

Different risk factors are known to engage in the epidemiology of VL. In endemic areas, more cases occur in younger age groups as they have yet to develop the acquired immunity. Males are more predisposed to develop the disease as they are usually engaged in outdoor activities, which will make them more accessible to the sandflybite.Poor protein, energy, iron, vitamin A and zinc nutritional status increase the risk of VL manifestation [7].A recent study conducted by [9] investigating risk factors associated with the outbreak in LiboKemkem identified dog ownership to be risk factors for infection.

For many years, the public health impact of leishmaniasis has been grossly underestimated, mainly due to lack of awareness of its serious impact. Endemic regions have been spreading further and there has been a sharp increase in the number of cases. As a resultseveral outbreaks occurred in Ethiopia within the last 10 years [9].

The Objective of this Review Is Therefore:

- To review the epidemiology of VL in Ethiopia
- To highlight the role of dog in the epidemiology of VL

Literature Review

Etiology: Leishmaniasis is caused by obligate intracellular protozoa of the genus Leishmania, belonging to the family Trypanosomatidae (order Kinetoplastida). Human infection is caused by about 21 of 30 species that infect mammals. L.donovani complex (L. donovani and L.infantum) are the causative agents of VL in Ethiopia. L. donovani is regarded as the major cause of VL in Ethiopia, although L. infantum was identified as a main causative agent of the recent VL outbreak in LiboKemkem, in the Amhara regional state[7].

Morphology: The parasite exists in two forms: amastigotes and promastigotes. Amastigotes are found in the host and promastigotes in the insect vector. The amastigote are small, round to oval bodies, which measure about 3-5 ìm and found only in the macrophages of infected vertebrate hosts. They are colorless, have a homogenous cytoplasm and are surrounded by a pellicle. The promastigote forms are seen in the gut of the sandfly. They are motile, slender organisms measuring 10-15 ìm in length, with a single anterior flagellum. Amastigotes lack the flagellum, but a short flagellum may be seen arising from the kinetosome [11].

Life Cycle: The life cycle of the Leis mania parasite is completed in two hosts, a vertebrate host and an invertebrate host (phlebotominesandfly) [12].The adult female sandfly is a bloodsucker, usually feeding at night on sleeping prey. When the fly bites an individual infected with Leishmania, the pathogen is ingested along with the prey's blood. The protozoan is in the smaller of its two forms, called an amastigote, which is round, non-motile and only 3–7 micrometers in diameter. Inside the stomach of the sandfly, the amastigotes quickly transform into elongated and motile forms called the promastigotes. Promastigote is spindle-shaped, triple the size of the amastigote and has a single flagellum that allows mobility. The promastigotes live extracellularly in the alimentary canal, reproducing asexually and then migrate to the proximal end of the gut where they become poised for a regurgitation transmission. As the fly bites, the promastigotes are released from the proboscis and introduced locally at the bite site [7].

Once inside the host, promastigotes invade macrophages. Inside the cells they transform back into the smaller amastigote form. The amastigotes replicate in the most hostile part of the macrophage cell, inside the phagolysosome, whose normal defensive response they are able to prevent. After repeated multiplication, they break down their host cell by sheer pressure of mass, but there is some recent speculation that they are able to leave the cell by triggering the exocytosis response of the macrophage [6]. The daughter cells protozoans then migrate to fresh cells or through the bloodstream to find new hosts. In this way the infection is progressive, spreading to the host's mononuclear phagocyte system, particularly the spleen and liver. The free amastigotes in peripheral tissues are then ingested by sandfly to enter another cycle[12].The life cycle of leishmania is presented in Figure 1.

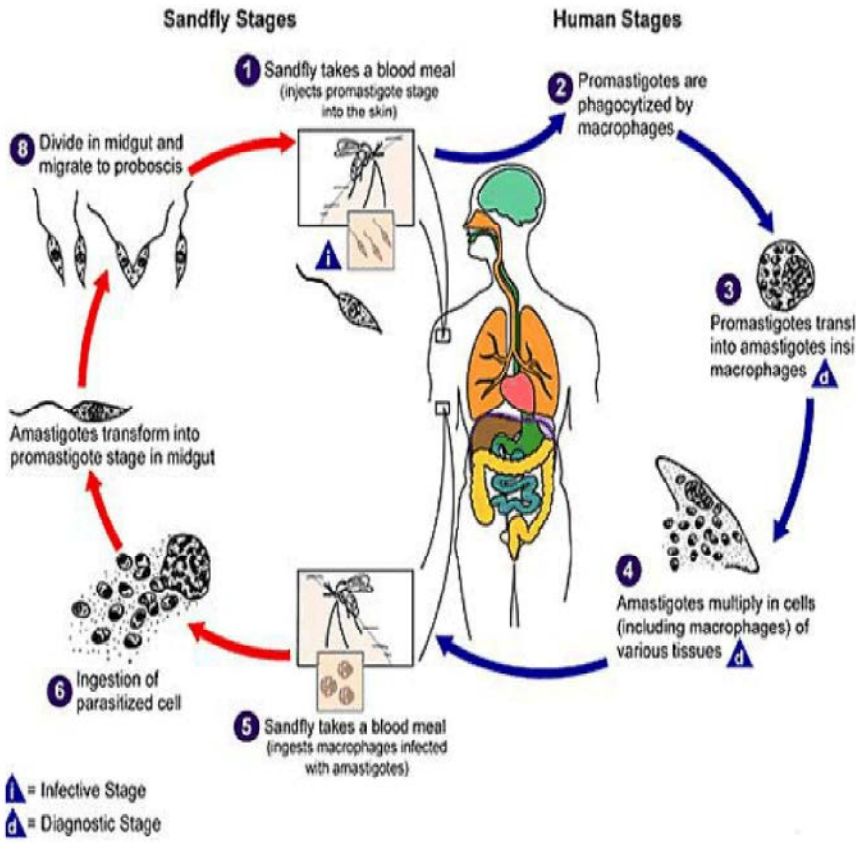

Fig. 1: Life cycle of viscera leshmania. Source:[6].

Epidemiology

Transmission: Dogs are the primary mammalian reservoir for*L. infantum*infection in endemic regions and are the most significant risk factor predisposing humans to infection. VL is classically transmitted to a suitable mammalian host by the bite of an infected sand fly after which the promastigote form of the parasite is phagocytosed by macrophages. Non vector-based mechanisms postulated for transmission of canine visceral leishmaniasis is vertical transmission (transplacental or transmammary) and horizontal transmission by direct contact with infected cells in blood. Transmission has been documented via packed red blood cell transfusion from infected dog and human. It is not known how frequently vertical transmission occurs naturally in endemic areas. There are reports of congenital transmission of VL in humans and during experimental Leishmaniainfection [13].

Host: Man and dogs are the most commonly affected hosts and they can also be a potential reservoir Dogs are the most important species among domesticated animals in the epidemiology of this disease; hence, dogs are reservoir hosts for L. infantum, which is one of the two most important organisms in human VL [6, 7]. Domestic dogs might be the most important reservoir of L. donovani in eastern Africa. In recent time northern Ethiopia and eastern Sudan also reported isolation of L. donovani complex from dogs. Moreover, highly significant attraction of different sandfly species to dog-baited traps was observed. On the other hand, in East Africa, the transmission of L. donovani (the predominant causative agent of VL in the region) is thought to be anthroponotic, indicating that humans are a potential reservoir host for the disease. Hence, the definitive reservoir of VL in East Africa in general and Ethiopia in particular remains to be determined[14].

The ELISA-based blood meal analysis of 273 fresh fed P.orientalis females collected from Metema showed 92% of their blood meal source to be from bovine origin. However, epidemiological significance of bovine in the transmission of leishmaniasis has not yet been investigated [15].

Vectors and Vector Ecology: There are about 30 species of phlebotomine sandflies known to transmit leishmaniases [8]. P.orientalis, P. martini and P.celiae have been confirmed as vectors of VL in Ethiopia. The ecological preference of these flies differs: rainfall, humidity, temperature, soil type and moisture content and land cover type are significantly associated with the distribution pattern of these sandflies, although no universal pattern has been established so far. Changes in temperature, rainfall and humidity can have strong effects on vectors and reservoir hosts by altering their distribution and influencing their survival and population sizes[16].

In the endemic areas of Ethiopia, two distinctive ecologic settings have been described. In northwestern foci, the principal vector, P. orientalis, is found in association with black cotton-clay soils and acacia forests. In southern foci, where P. martini and P. celiae are believed to transmit the disease, termite hills are thought to provide resting and possibly breeding sites for sand flies[17-19]. However, the LiboKemkem and Fogera districts, which are affected by the recent epidemic, differ from both of the classic ecologic settings. These areas are located at an altitude of around 1800m, are substantially cooler and have different vegetation from other endemic areas in the lowland areas [9].

Fig. 2: Sand fly. Source: [20]

Risk Factors: Different risk factors are known to engage in the epidemiology of VL. In endemic areas, more cases occur in younger age groups as they have yet to develop the acquired immunity. In East Africa, approximately 65% of VL cases are found in children less than 15 years old [17].However, in outbreaks or areas where the disease has recently been introduced, all age groups are susceptible and most cases occur in groups that have regular contact with sandfly habitats. Males are more predisposed to develop the disease as they are usually engaged in outdoor activities, which will make them more accessible to the sandfly bite [18].

Poor protein, energy, iron, vitamin A and zinc nutritional status increase the risk of VL manifestation [7].Malnourished children, who often suffer simultaneously from associated diseases such as tuberculosis, respiratory and/or intestinal infections are particularly vulnerable. There is significant association between severe malnutrition and intestinal parasitic infection and VL. Malnutrition and intestinal helminth infection down-regulate the Th1 cellular immune response. Down-regulation of Th1 cellular immune response confers susceptibility to VL. Regular deworming reduced malnutrition significantly and supplementation of micronutrients such as zinc and iron, as well as vitamin A, improve malnutrition and enhance Th1 cellular immune response. Thus, periodic deworming and micronutrient and vitamin A supplementation together may reduce the risk of symptomatic VL [19].

Poverty increases the risk for VL. Poor housing and poor sanitary conditions may favor sandfly breeding and resting sites, as well as their access to humans. The flies are mainly attracted to crowded housing, as these provide a good source of blood-meals. Sleeping outside or on the ground may increase the risk of infection. This is especially more evident in nomadic populations and in men who work in agricultural or pastoral settings due to increased time spent outdoors and thus higher exposure to the sandfly. Part of a community that lives and/or has frequent contact with acacia trees and termite hills are at increased risk, since acacia trees and termite hills are common breeding and resting sites for certain species of sandflies [7].

Epidemics of leishmaniasis are often associated with migration and the introduction of non-immune people into areas with existing transmission cycles. In northwestern foci, VL epidemics are associated with migration and the movement of non-immune workers into the VL-endemic extensive farm lands. Workers from the non-endemic highlands are often victims of VL in these foci [20].

The dog ownership is reported to be a risk factor. In Ethiopia, culture and tradition of inhabitants favors the keeping of dogs in urban and rural households often in close association with the family and farm animals. Almost all livestock owners and urban dwellers keep at least one dog to safeguard their properties from wild carnivores and thieves. These and other, socioeconomic realities are considered to be conducive to the maintenance and further propagation of the disease [9].

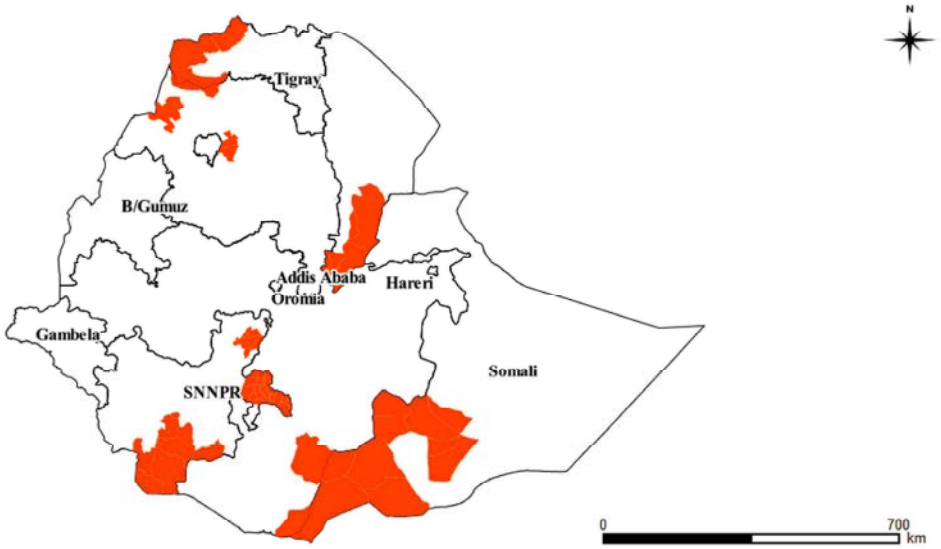

Fig. 3: VL endemic foci in Ethiopia. Source [23].

VL is caused by two leishmanial species, *L. donovani* or *L. infantum*, depending on the geographical area. *L. infantum* infects mostly children and immunosuppressed individuals, whereas *L. donovani* infects all age groups. Human and animal leishmaniases show a wider geographic distribution than previously known [4].

Distribution: Geographical distribution of the diseases depends on sand fly species acting as vectors, their ecology and the conditions of internal development of the parasite [2](Desjeus, 2001). The burden of VL remains unknown worldwide, since several cases are not diagnosed. It has been estimated that there are approximately half a million new cases of VL annually worldwide, with more than 50,000 associated deaths. More than 90% of VL cases occur in just six countries, namely: India, Nepal, Bangladesh, Sudan, Ethiopia and Brazil [21].

VL is reported to be widespread over the arid and semi-arid parts of the country. The most important endemic foci include the Metema and Humera plains in northern Ethiopia, the Omo plains and the Aba Roba focus and Weyto River Valley in SNNPR. The disease was also reported from the Moyale area and Genale river basin in the Oromia regional state, Afder and Liban zones in Ethiopia's Somali region and the Awash Valley in the Afar regional state [22].

The Role of Dog in the Epidemiology of VL: Dogs are the primary mammalian reservoir for *L. infantum* infection in endemic regions and are the most significant risk factor predisposing humans to infection [24]. In both rural and urban areas the presence of seropositive dogs in human dwellings is seen as a possible risk factor for *L. infantum* infection. Indeed, domestic dogs are the main domestic reservoirs of *L. infantum* infection in the transmission cycle of visceral leishmaniasis (VL).Dogs play a significant role in the spread and maintenance of *L. infantum* in endemic areas, although it is not completely clear why dogs are more attractive as a blood source to sand fliesseveral investigators have demonstrated that dogs are an important blood source to the principal vector of visceral leishmaniasis[24].

In many countries, due to the tie of canine infection to human disease, culling of dogs is still used as a means to prevent human disease. Other domestic or synanthropic animals play any role as reservoirs of infection. Recently, a domestic cat (Feliscatus) was found naturally infected with *L. infantum* in an area of transmission of VL. However, the epidemiological role of the cat is still unclear [25].

Pathogenesis: The lifecycle of *L. donovani* has two distinct forms: a promastigoteflagellar form found in the gut of the arthropod vector and an amastigote form, which develops intracellularly in the mammalian host. Only female phlebotomine sandflies transmit the disease, by inoculation of the promastigoteform into the skin. The parasites are internalized by dendritic cells and macrophages in the dermis and transform into amastigotes by losing their flagella. They multiply and survive in

phagolysosomes through a complex parasite–host interaction [26]. The parasites disseminate through the lymphatic and vascular systems and infect other monocytes and macrophages in the reticulo-endothelial system, resulting in infiltration of the bone marrow, hepato-splenomegaly and sometimes enlarged lymph nodes (lymphadenopathy) [26].

Clinical Sign: Following an incubation period that generally lasts between 2 and 6 months, VL patients present symptoms and signs of persistent systemic infection (including fever, fatigue, weakness, loss of appetite and weight loss) and parasitic invasion of the blood and reticulo-endothelial system (that is, the general phagocytic system), such as enlarged lymph nodes, spleen and liver. Fever is usually associated with rigor and chills and can be intermittent. Fatigue and weakness are worsened by anemia, which is caused by the persistent inflammatory state, hypersplenism (the peripheral destruction of erythrocytes in the enlarged spleen) and sometimes by bleeding [27].

The clinical presentation of VL is similar in the various endemic areas but there are some differences. For example, enlarged lymph nodes are rarely found in Indian VL patients but are frequent in Sudanese VL patients. As the disease advances, splenomegaly can increase, causing abdominal distension and pain, which is sometimes increased by concomitant hepatomegaly. Symptoms and signs of bacterial co-infections such as pneumonia, diarrhea or tuberculosis can confuse the clinical picture at the time of initial diagnosis. VL symptoms often persist for several weeks to months before patients either seek medical care or die from bacterial co-infections, massive bleeding or severe anemia [12].

Diagnosis

Conventional Parasite Detection Techniques: The commonly used method for diagnosing VL has been the demonstration of parasites in splenic or bone marrow aspirate. The presence of the parasite in lymph nodes, liver biopsy, or aspirate specimens or the buffy coat of peripheral blood can also be demonstrated. Amastigotes appear as round or oval bodies measuring 2 to 3 ìm in length and are found intracellularly in monocytes and macrophages. In preparations stained with Giemsa or Leishman stain, the cytoplasm appears pale blue, with a relatively large nucleus that stains red. In the same plane as the nucleus, but at a right angle to it, is a deep red or violet rod-like body called a kinetoplast[25].

Serological Methods of Diagnosis: Serological testing is much more frequently used in areas where leishmaniasis is endemic. The rK39 immunochromatographic test gave correct, positive results in 92% of the people with VL and it gave correct, negative results in 92% of the people who did not have the disease [3].

The Indirect fluorescent antibody test is one of the most sensitive tests available. The test is based on detecting antibodies, which are demonstrated in the very early stages of infection and are undetectable six to nine months after cure. If the antibodies persist in low titers, it is good indication of a probable relapse[28].

The direct agglutination test (DAT) is a highly specific and sensitive test[29]. It is inexpensive and simple to perform making it ideal for both field and laboratory use. The method uses whole, stained promastigotes either as a suspension or in a freeze-dried form. The freeze-dried form is heat stable and facilitates the use of DAT in the field. However, the major disadvantage of DAT is the relative long incubation time of 18 hr and the need for serial dilutions of serum[30].

The Enzyme Linked Immunosorbent Assay (ELISA) is a valuable tool in the serodiagnosis of leishmaniasis. The test is useful for laboratory analysis as well as for field applications. However, the sensitivity and specificity of ELISA is greatly influenced by the antigen used [10].

Molecular Methods: Polymerase Chain Reaction (PCR): amongst the molecular methods used for clinical diagnosis, PCR has been proved to be most sensitive and specific technique. The specificity of the PCR can be adapted to specific needs by targeting conserved region of the gene. Gene amplification through the PCR has several advantages compared to traditional techniques, because of its extremely high sensitivity, rapidity and the ability to be performed with a broad range of clinical specimens [31].

Treatment: In human: Patients should be referred to a specialist tropical disease unit for diagnosis and treatment of all forms of leishmaniasis, depending upon the form of the disease. There are several drug treatments available including oral, parenteral and topical medications [32].

Pentavalent antimonials such as stibogluconate and meglumineantimonite, have been the mainstay of treatment since the 1940's, but are complicated by adverse side effects, resistance and cost. Liposomal amphotericin B is more favorable in regions where resistance is common. Research into new antileishmanial drugs such as miltefosine, paromycinand sitamaquine may expand treatment options in the future [33].

Data on miltefosine use in East Africa are restricted to one study that was conducted in northern Ethiopia, in which it was found to be as safe and effective as sodium stibogluconate in HIV-negative patients and safer, but less effective in HIV co-infected patients. Oral sitamaquine, an 8-aminoquinoline derivative, has been shown to have clinically significant antileishmanial activity. This effective oral anti leishmanialcompound has been tested in Kenya, Brazil and India. Patients should be properly hydrated and given nutritional supplements. Severe anemia should be corrected with blood transfusions and concomitant infections should be treated with appropriate anti-infective agents. Successful therapy improves the general condition, resolves fever, causes regression of splenomegaly and recovery of blood counts towards normal [34].

In Animals: Treatment can produce clinical improvement, although it may not eliminate the parasite. Pentavalent antimonials are often used for treatment, where they are available. Other drugs used, such as, allpurinol, amphotericin B, or second line drugs may also be employed, either alone or in combination. Allopurinol has been used as a maintenance drug to prevent relapses. The prognosis is poorer in dogs that are severely ill and animals with kidney disease [35].

Control and Prevention: Measures to control transmission vary according to local epidemiology. Leishmaniasis control usually relies on case management (case detection and treatment), vector and reservoir control. Early case detection and treatment are essential for both individual patients and for the community. The treatment outcome is better in VL patients who are treated in an early stage. Untreated VL patients act as a source of infection and therefore contribute to disease transmission in anthroponotic VL area[28].

Reservoir Control: dogs are the main reservoir of *L. infantum* in zoonotic VL. Despite evidence from experimental studies showing a decreased incidence of VL in both dogs and children following serological screening of dogs and killing of sero-positive animals, the efficiency and acceptability of this control strategy is increasingly being debated. Treating infected dogs is not an effective control strategy as relapses are frequent and dogs can regain infectivity weeks after treatment, despite being clinically cured. Moreover, the widespread veterinary use of VL drugs might lead to resistance in parasites. Vaccination of dogs would nevertheless be the best strategy if an efficacious vaccine can be developed [28].

Vector Control: Controlling sandflies is an integral component of leishmaniasis control.Sandflies are susceptible to the same insecticides as Anopheles mosquitoes, the malaria vector. Residual insecticide spraying of houses and animal shelters, where the vector (*sandy fly)* is restricted to areas in and around the home. Following the large scale anti-malarial insecticide (dichloro-diphenyl-trichloroethane (DDT)) spraying campaigns that were implemented in the 1950s [36].

Insecticide-Impregnated Materials: the use of insecticide-treated bednets (ITNs) could concomitantly prevent VL and other vector-borne diseases, such as malaria. Depending on the sleeping traditions of the population and the biting habits of the local vector, other insecticide-impregnated materials such as curtains and blankets should be evaluated for use in VL prevention, as some have been shown to provide efficient protection against coetaneousleishmaniasis. Depending on the sleeping traditions of the population and the biting habits of the local vector, other insecticide-impregnated materials such as curtains and blankets should be evaluated for use in VL prevention[36].

CONCLUSION

In Ethiopia, Leishmaniasis is an important vector borne disease whose existence has been documented many decades ago. Given the increasing mobility of people, there is an impending potential for Leshmaniasis to spread to new areas, since the distribution of its vector is known to be more widespread that the disease itself. Moreover, the reservoir hosts for this disease are not well known and its transmission ways are not clearly set. As a result, no sound control measure is implemented so far except the treatment of sick individuals. So, in-depth studies of its transmission and mapping areas endemic to this disease and those likely to be affected are very crucial to provide evidences needed for devising effective control methods.

Based on the above conclusion the following recommendations are rewarded

- Both development and implementation of new control measures are priorities and should be supported by institutions of both public and private sectors.
- The quality of life of the population at risk should be improved to prevent many infectious and parasitic diseases

- Vector control is one of the most important measures in the control of vector-borne diseases
- Veterinarians and medical professionals should work together to minimize the zoonotic transmission.

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