Transmission routes of visceral leishmaniasis in mammals

Vias de transmissão da leishmaniose visceral em mamíferos

Vinícius Vasconcelos Gomes de Oliveira[I*]   Leucio Câmara Alves[II]
Valdemiro Amaro da Silva Junior[I]

– REVIEW –

ABSTRACT

Visceral leishmaniasis (VL) is a chronic disease caused by **Leishmania infantum**. The major sites of parasite localization in infected animals are the secondary lymphoid organs, bone marrow and cutaneous tissue. However, reports exist on the detection of the parasite in the organs of the male and female genital system. The main route of transmission is related to the hematophagous sandfly vectors of the genus **Lutzomyia** (New World) and **Phlebotomus** (Old World). However, other routes of transmission may be mentioned, such as sexual, vertical, hematogenic without vector and others involved in VL epidemiology. Thus, the current article reviews the main forms of transmission of visceral leishmaniasis in mammals.

Key words: *Leishmania sp, dogs, sexual transmission, vertical transmission.*

RESUMO

A leishmaniose visceral (LV) é uma doença crônica causada pelo protozoário **Leishmania infantum**. Os principais sítios de localização do parasito nos animais acometidos pela LV foram órgãos linfoides primários, secundários e tegumento. Contudo, existem relatos da detecção do parasito em órgãos do sistema genital masculino e feminino. A principal via de transmissão decorre da ação hematófaga de vetores flebotomíneos pertencentes aos gêneros **Lutzomyia** (Novo Mundo) e **Phlebotomus** (Velho Mundo). Todavia, outras formas de transmissão podem ser relacionadas, como as vias sexual, vertical, hematógena não vetorial e ainda existe a possibilidade de outros vetores estarem envolvidos na epidemiologia da doença. Dessa forma, a proposta deste artigo foi revisar as principais formas de transmissão da leishmaniose visceral em mamíferos.

Palavras-chave: *Leishmania sp., cães, transmissão sexual, transmissão vertical.*

INTRODUCTION

VL is an important parasitic zoonosis and domestic dogs are the main reservoir of the disease, particularly in urban areas (DINIZ et al., 2008). It is caused by **Leishmania infantum** (synonym to **L. chagasi**) with tropism for the mononuclear phagocyte system (NEVES, 2005; CHAPPUIS et al., 2007).

The disease is usually transmitted by the hematophagous activities of sandflies belonging to the genera **Lutzomyia** (New World) and **Phlebotomus** (Old World) (MURRAY et al., 2005). However, alternative routes of transmission have been described in dogs and man (TURCHETTI et al., 2014). In dogs, there are reports of sexual (SILVA et al., 2009a) and vertical transmission (NAUCKE & LORENTZ, 2012), by blood transfusion (DE FREITAS et al., 2006) and by other vectors such as fleas and ticks which may be involved in the epidemiology of the disease (MORAIS et al., 2013).

Alternative routes of infection jeopardize the control strategies and eradication of VL based on vector elimination. In fact, they are inefficient since the parasite **L. infantum** would still maintain its lifecycle transmitted by the breeding of positive stray dogs (SILVA et al., 2009a).

Epidemiologically, infection in dogs is more prevalent than in man, and there is a large number of asymptomatic animals which host parasites in the dermis (BONATES, 2003). The VL in dogs is considered a chronic disease characterized

[I]Departamento de Morfologia e Fisiologia Animal, Universidade Federal Rural de Pernambuco (UFRPE), Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, 52171-900, Recife, PE, Brasil. E-mail: vinicius-vasconcelos@hotmail.com. *Corresponding author.
[II]Departamento de Medicina Veterinária, UFRPE, Recife, PE, Brasil.

by the emergence of several clinical signs such as lymphadenomegaly, dermatopathies (SOLANO-GALLEGO et al., 2009), hepatosplenomegaly, onychogryphosis (ALBUQUERQUE et al., 2007) and ophtalmopathies (BRITO et al., 2006). In addition to these classical lesions, the parasite eventually causes damage to the reproductive system of male (MIR et al., 2012) and female dogs (OLIVEIRA, 2013). It may even be detected in the semen (DINIZ et al., 2005) and the placenta (DUBEY et al., 2005), respectively featuring the occurrence of sexual and vertical transmission.

Since different alternative routes of VL transmission exist in mammals, current analysis will deal with the main forms of transmission of *L. infantum* due to the epidemiological importance for the maintenance of the parasite´s life, especially in vector-free areas.

Classical pathway of VL transmission

L. infantum is a biphasic parasite, with heteroxenous life cycle, or rather, completed in two hosts: the sandfly vector (*L. longipalpis* in the New World), which hosts the parasite in a flagellated promastigote extracellular form; and the mammalian host in which the intracellular amastigote form develops (BARATA et al., 2004; TOMÁS & ROMÃO, 2008; SOLANO-GALLEGO et al., 2009).

The primary transmission of VL between mammals occurs indirectly through the vector which is infected during blood meal in an infected human or animal, by ingesting amastigotes of *L. infantum* in the dermis (BARATA et al., 2004). The amastigotes become promastigotes inside the insect gut between 24 to 48 hours after ingestion. In their turn, the promastigotes reproduce and migrate to the esophagus and pharynx. During the next blood meal they are inoculated into the dermis of the vertebrate host (KAMHAWI, 2006). The salivary gland secretions of the *L. longipalpis* have a potent vasodilator peptide, called maxadilan, which is released in the host´s dermis during the blood meal, and thus facilitating the infection (LERNER et al., 1991; LERNER & SHOEMAKER, 1992).

After infection of the vertebrate host by the parasite´s promastigotes, the latter are engulfed by macrophages and become amastigotes. The amastigotes reproduce in the macrophages, break up the macrophages and are released within the extracellular medium, where they are again engulfed by other macrophages (HANDMAN & BULLEN, 2002).

Sexual transmission of VL

The first report on sexual transmission of VL occurred in a human patient in the United Kingdom.

The study reported that in an area without vectors or autochthonous cases of the disease, a woman, who had never been abroad, developed genital lesions containing amastigotes of *L. infantum*. The analysis showed that infection occurred because her husband had been infected by parasite in the Sudan twelve years before (SYMMERS, 1960).

In dogs, RIERA & VALLADARES (1996) suggested that the venereal transmission occurred in this species, since *L. infantum* was isolated in the animals´ semen. In their study, they reported that three Beagle males were experimentally inoculated with *L. infantum* and after infection had been confirmed by serological methods, direct exams and cell culture of lymph nodes obtained by puncture, the semen was collected and cultivated in a NNN medium (Novy-MacNeal-Nicolle).

Besides the isolation of *L. infantum* in canine semen (RIERA & VALLADARES, 1996), several other studies have shown that the parasite features tropism for the male reproductive system of dogs (DINIZ et al., 2005; AMARA et al., 2009; BENITES et. al., 2011) producing a local immune response (BENITES et al., 2011) and favoring the appearance of *L. infantum* in the semen of the animals (DINIZ et al., 2005).

Male dogs naturally infected by *L. infantum* reveal no macroscopic changes in the genital organs, even though histological lesions are extant (AMARA et al., 2009). Lesions are characterized by chronic inflammatory reactions in all organs who belong to this system (OLIVEIRA, 2013), degenerative atrophy of the seminiferous epithelium (PIZARRO et al., 1989; DINIZ et al., 2005; AMARA et al., 2009), interstitial fibrosis with thinning of the epididymal epithelium (AMARA et al., 2009), prostatitis (MIR et al., 2012) and azoospermia (DIAZ et. al., 1982).

Besides lesions in the internal genital organs, amastigotes of *L. infantum* has also been reported in the dog´s glans penis associated to transmissible venereal tumor (CATONE et al., 2003, MARINO et al., 2012) and prepuce (DINIZ et al., 2005), providing an inflammatory reaction with the predominance of macrophages in these regions. These studies become strongest the possibility of the sexual transmission of VL. On the other hand, it is debatable whether disease transmission occurs through the contaminated semen or by the transference of *L. infantum* amastigotes derived from the male´s external genitalia during canine highly traumatic mating (SILVA et al., 2009a).

In female dogs, the vulvar dermatitis was the only important lesion suggesting that *L.*

infantum does not have tropism by female genital system (SILVA et al., 2008). However, other studies showed that all the organs of the genital system from female dogs naturally infected by *L. infantum*, with the exception of the ovaries, have lesions with high parasitic load levels in all the organs (ROSYPAL et al., 2005; OLIVEIRA, 2013). Consequently, the parasite has a tropism for the genital organs of the male (DINIZ et al., 2005) and female dogs (OLIVEIRA, 2013).

In experimental procedures without biologic vectors, female dogs negative to *L. infantum* were mated with male dogs naturally infected resulting in 25% of female dogs serologically positive to *Leishmania* spp. and 50% of them were PCR positive (Silva et al., 2009a). According to SILVA et al. (2009a), the sexual transmission was a one-way event despite the fact that they did not performed the experiment in reverse. However, ROSYPAL & LINDSAY (2005) showed that female BALB/C mice experimentally infected with *L. infantum* and positive to PCR and serological test transmitted the parasite to male mice which shared the same cage.

Vertical transmission of VL

The vertical transmission of VL in humans was described in children born of infected mothers living in endemic areas (LOW & COOKE, 1926; BLANC & ROBERT, 1984). On the other hand, the transmission route is theoretically supported in dogs by the presence of *L. infantum* in pregnant uterus (ROSYPAL et al., 2005), fluid and fetal tissues (CARACAPPA et al., 2000), placental trophoblast (DUBEY et al., 2005) and organs of pups born of infected female dogs (PANGRAZIO et al., 2009).

The first report on the vertical transmission in dogs was given by MANCIANTI & SOZZI (1995) who isolated *L. infantum* in samples of the liver, spleen and lymph node of a neonate born of an infected female dog. This transmission route was investigated by ANDRADE et al. (2002) who used 63 puppies born to 18 infected female dogs to detect the parasite by parasitological, histopathological and PCR tests in samples of the spleen, liver, lymph nodes and bone marrow. According to the authors, no sample from the puppies proved to be positive and thus the vertical transmission of VL could not be confirmed. However, more recent studies demonstrated that this kind of transmission would be possible in dogs (MASUCCI et al., 2003; DUBEY et al., 2005; ROSYPAL et al., 2005; GIBSON-CORLEY et al., 2008; PANGRAZIO et al., 2009; SILVA et al., 2009b; FREEMAN et al., 2010; BOGGIATTO et al., 2011; NAUCKE & LORENTZ, 2012).

L. infantum transmission by infected females to puppies does not seem to be related to clinical status during pregnancy. In this case, infection rarely promotes the emergence of clinical signs during the first month of life in dogs (MASUCCI et al., 2003). The parasite migration from mothers to puppies during the pregnancy may occur through the transplacental route since the DNA of *L. infantum* was detected by PCR in the pregnant uterus (ROSYPAL et al., 2005), Further, amastigotes have already been reported in the placental trophoblast (DUBEY et al., 2005).

Since puppies born of infected mothers hardly have any clinical signs (MASUCCI et al., 2003), there are also no histological lesions related with VL in canine fetal organs from positive female dogs (PANGRAZIO et al., 2009). Although no structural changes in the liver, spleen, lymph nodes, bone marrow, kidney and heart have been registered, amastigotes were reported in all organs, except kidney and heart (PANGRAZIO et al., 2009). Similarly as described by MASUCCI et al. (2003), it was observed that there was no difference in the potential transmission of VL when compared pregnant female dogs symptomatic or not, being also possible to observe a high frequency of placental transmission (PANGRAZIO et al., 2009).

In turn, some reports demonstrated infection by *L. infantum* in stillborns or newborns of naturally infected female dogs (GIBSON-CORLEY et al., 2008; FREEMAN et al., 2010; BOGGIATTO et al., 2011). Further, puppies born of naturally infected female dogs showed specific CD4^{+} T cell proliferative responses against *L. infantum*. This fact suggests a specific immune response against the parasite (BOGGIATTO et al., 2011).

The resistance to leishmaniasis is associated with an intense Th1 type response. On the other hand, immunosuppression is necessary to avoid immune reaction against fetal antigens during pregnancy. Therefore, the natural change that occurs in the immune response pattern during the pregnancy from Th1 to Th2 type may increase the severity of disease in the female dogs and the vertical transmission VL risk (ROSYPAL et al., 2005).

The prevalence of the VL among Foxhound dogs in the United States is remarkable since the sexual and vertical transmission could be responsible for the maintenance of the disease in a non endemic area (PETERSEN, 2009). The VL is an emerging problem among some dog breeds in this country, particularly in the case of this breed dog, with a more than 20% prevalence based on quantitative PCR (BOGGIATTO et al., 2011). This

condition occurs in an area where it was not possible to determine whether the species of sandflies were competent as biological vectors for *L. infantum*. Therefore, they may not have contributed towards the maintenance of the disease in this particular breed. Thus, the manner *L. infantum* has been transmitted in dogs in the United States is currently unknown. However, there are reasons to support sexual and vertical transmissions in the maintenance of the disease (PETERSEN & BARR, 2009).

Hematogenous transmission without vector

Blood transfusion is a common practice in veterinary medicine mainly when the animals have clinical signs related to anemia and hemorrhage (HOSGOOD, 1990). In this case there is a great possibility of transmission of the infectious agents, particularly protozoa, due to their long incubation periods, subclinical persistence in infected animals and possibility of remaining viable in blood stocks (OWENS et al., 2001, TABAR et al., 2008).

The VL transmissions through blood transfusion have been documented in different breeds of dogs that received blood from English Foxhounds donors (OWENS et al., 2001). In this study, the authors do not recommend dogs belonging to this breed as blood donors in North America due to the high prevalence of VL in the animals.

In Spain, TABAR et al. (2008) detected DNA of *L. infantum* by quantitative PCR in 19.6% of samples from a canine blood bank in an endemic area and demonstrated that infection by *L. infantum* is commonly found in canine blood donors in the region. Further, hamsters (*Mesocricetus auratus*), inoculated with blood and monocyte suspension from dogs naturally infected by *L. infantum*, developed clinical signs and infection after a 6-month post-inoculation period (DE FREITAS et al., 2006). Results suggest that blood donors should be monitored regularly and rigorously against *L. infantum* so that disease dissemination by blood transfusion would be avoided (DE FREITAS et al., 2006).

Transmission by alternative vectors

The vectorial capacity of the other arthropods may be enhanced by the epidemiological characteristics of VL that allow the close association of ectoparasites with infected dogs (COUTINHO et al., 2005). The above reinforces the theory of SHERLOCK (1964) who reported that transmission could probably be made by other vectors when sandflies are absent. Ticks (*Rhipicephalus sanguineus*) and fleas (*Ctenocephalides felis*

felis) are among these possible alternative vectors (COUTINHO et al., 2005; COUTINHO & LINARDI, 2007; MORAIS et al., 2013).

The hypothesis of transmission of VL in dogs by ticks was studied in France in the early twentieth century by BLANC & CAMINOPTEROS (1930). These authors did an experimental infection in *R. sanguineus,* which were able to maintain viable *L. infantum*. On the other hand, it was demonstrated in the USA, under experimental conditions, that ticks may be infected by *L. infantum* after blood feeding on an infected dog. Amastigotes ingested during the blood meal may develop into promastigotes and survive for several weeks in the gut of the ectoparasite (MCKENZIE, 1984).

In Brazil, the infectivity of *L. infantum* in ticks was confirmed by experimental inoculation of macerated positive ticks in hamsters (*M. auratus*) (COUTINHO et al., 2005).More recently, kDNA was detected in the salivary gland of *R. sanguineus* collected from seropositive dogs to *Leishmania* sp. (DANTAS-TORRES et al., 2010a). Furthermore, the DNA of *L. infantum* has been found in dogs and ticks that were parasitizing these animals (MORAIS et al., 2013). These results, however, did not confirm the vectorial capacity of this species, since the maintenance of the parasite epidemiological cycle and transmissibility for dogs have not been proved. On the other hand, DANTAS-TORRES et al. (2010b) infected teleogines of *R. sanguineus* with promastigotes of *L. infantum* and eggs and larvae of these females were positive by PCR. Results reveal that the parasite may be maintained during the different developing stages.

The *C. felis felis* flea has also been researched due to its probable participation in the epidemiology of VL (COUTINHO & LINARDI, 2007, FERREIRA et al., 2009), but with less scientific basis than those for ticks. In these two studies, fleas collected from dogs naturally infected by *L. infantum* were macerated and inoculated in hamsters (*M. auratus*) orally or intraperitoneally. In the two studies, the hamsters became positive in molecular and serological tests. On the other hand, the possibility of oral transmission of *L. infantum* by fleas cannot be verified due to the possibility of cross-reactivity between *Leishmania* and *Leptomonas* by PCR and indirect immunofluorescence test (COUTINHO & LINARDI, 2007).

CONCLUSION

Although health authorities only consider the transmission of VL by sandflies, other routes may have epidemiological significance, mainly in areas

without biological vectors. Among the transmission routes currently studied, the sexual and vertical routes may be highlighted. The participation of other biological vectors, such as ectoparasites in dogs, in the spread of disease still needs scientific confirmation. In the case of blood transfusion, a strict control and monitoring are necessary for blood donor dogs to prevent infection by *L. infantum*.

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