

## Sexual and vertical transmission of visceral leishmaniasis

Andréia P. Turchetti<sup>1</sup>, Tayse D. Souza<sup>1</sup>, Tatiane A. Paixão<sup>2</sup>, Renato L. Santos<sup>1</sup>

<sup>1</sup> Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

<sup>2</sup> Departamento de Patologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

### Abstract

Visceral leishmaniasis (VL) is an important zoonosis caused by *Leishmania infantum*, which has in the domestic dog its principal vertebrate host in urban environments. VL is usually transmitted by phlebotomine sand flies, however atypical routes of transmission have been described. In this review we discuss the role of sexual and vertical transmissions, and their implications in the maintenance of VL in canine populations.

**Key words:** *Leishmania infantum*; visceral leishmaniasis; transmission

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### Introduction

Visceral leishmaniasis (VL) is an important re-emerging zoonosis caused by *Leishmania (Leishmania) infantum* (synonym *L. chagasi*) infection. The disease has the domestic dog as its most important vertebrate host and it is usually transmitted by the bite of infected phlebotomine sand flies [1]. However, atypical transmission routes have also been described, including blood transfusion [2] and needle sharing [3]. Two forms of transmission, namely sexual and vertical, are particularly important as they may play a significant epidemiological role in the dissemination and maintenance of the disease, especially in the absence of the invertebrate vector [4]. Therefore, our aim is to review the literature regarding sexual and vertical transmission of VL.

### Sexual Transmission

The first evidence that VL could be sexually transmitted came from a well documented report on a human patient from the United Kingdom, where there was absence of the vector and no report of autochthonous leishmaniasis. A woman, who had never left the country, developed a genital papule with intralesional *Leishmania* sp. Her husband had been diagnosed and treated for VL several years before. At the time of her diagnosis, he presented splenomegaly, hepatomegaly and generalized enlargement of lymph

nodes with myriads of *Leishmania* sp. observed by biopsy [5]. At that time, it was not possible to detect the protozoan in his semen. Although rare, there have been other reports of genital lesions in human patients with VL, including testicular infection in a boy with leukemia [6], and a nodular ulcerative lesion with intralesional *L. infantum* in the prepuce of a man [7].

In dogs, the first evidence of shedding of *Leishmania* sp. in semen was reported by Riera and Valladares [8]. In that study, three male Beagle dogs were experimentally inoculated with *L. infantum*. After confirmation of the infection by serology, direct examination, and culture of lymph node aspirates, semen was collected and cultured in NNN (Novy-MacNeal-Nicolle) medium. *L. infantum* was isolated from the semen of one dog, raising the possibility of venereal transmission. Therefore, we performed a comprehensive study examining genital lesions in naturally *L. infantum*-infected dogs, including three groups: (i) serologically negative; (ii) serologically positive and asymptomatic; and (iii) serologically positive and symptomatic dogs [9]. Naturally infected male dogs have a very high frequency of inflammatory lesions with intralesional amastigotes that were confirmed by immunohistochemistry, particularly in the epididymis (Figure 1), glans penis and prepuce, especially in symptomatic dogs [9]. Benites *et al.* [10] confirmed the presence of *Leishmania* sp. amastigotes

in the canine male reproductive tract by direct observation on tissue imprints. Furthermore, it was shown by polymerase chain reaction (PCR) that infected dogs shed *L. infantum* in the semen [9]. In contrast to what has been observed in males, a study involving naturally infected bitches that had been subjected to a thorough histopathological and immunohistochemical analysis of the internal and external genital organs, demonstrated that vulvar dermatitis was the only important finding. In the female genital system of infected bitches, amastigotes could be observed histologically or by immunohistochemistry, suggesting that *L. infantum* does not have a tropism for the female canine genital system [11].

The clinical importance of *L. infantum* infection affecting the genital tract of the dog has been recently repeatedly reported, including cases of orchitis [12] and chronic prostatitis, both associated with *L. infantum* infection [13]. Additionally, there are reports of *Leishmania* sp. amastigotes in genital organs of dogs with transmissible venereal tumor [14-15]. Interestingly, in these cases the tumor tends to be larger and it often has a more aggressive behavior in dogs with leishmaniasis [15].

Therefore, considering that genital lesions associated with VL have been well documented in dogs [9], it became clear the need to investigate the possibility of sexual transmission of VL. Under experimental conditions, twelve *Leishmania*-free bitches copulated with multiple naturally infected dogs that were shedding *L. infantum* in the semen. All animals were housed in the absence of the insect vector. Six months after the last copulation, three (25%) bitches seroconverted, and six (50%) were PCR positive, clearly demonstrating that *L. infantum* may be sexually transmitted from naturally infected dogs to susceptible bitches in the absence of the biological insect vector [16]. Importantly, considering the tropism of *L. infantum* to the genital system of male dogs [9], and the absence of such tropism in the case of bitches [11], it is reasonable to hypothesize that sexual transmission tends to be unidirectional, with a more efficient transmission from the infected male to a susceptible female, in the case of dogs.

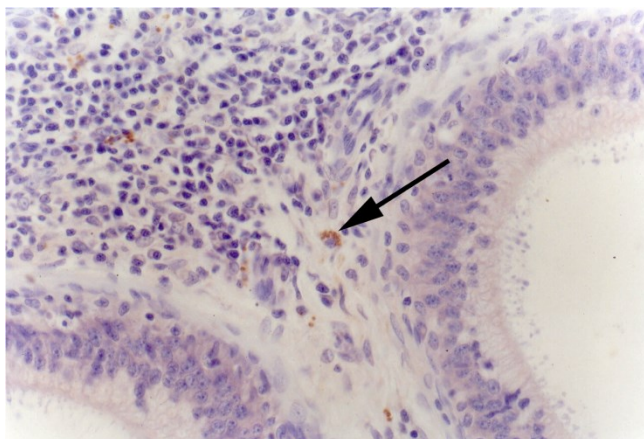
Interestingly, a previous study have demonstrated that one out of eight *Leishmania* free male BALB/C mice that bred with experimentally infected female mice had *Leishmania* DNA detected in the spleen and also developed antibodies against *Leishmania* spp., suggesting that venereal transmission of VL also occur in mice [17], in addition to dogs [16] and humans [5].

## Vertical Transmission

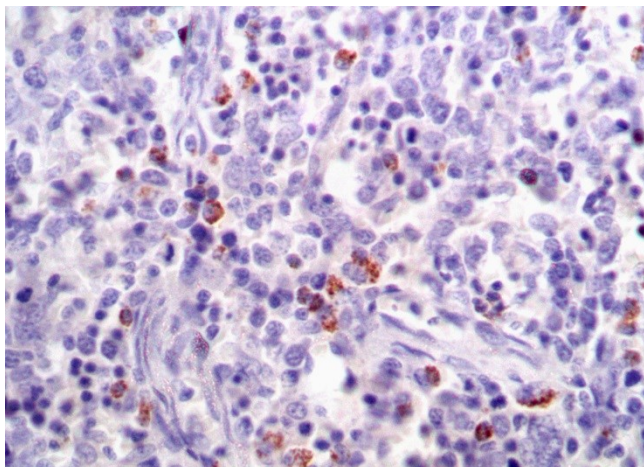
*Leishmania* sp. has been previously isolated from the spleen, liver and lymph node of a newborn from an infected bitch [18]. Although an earlier study had not detected *L. infantum* in tissues of fetuses from naturally infected bitches, and therefore suggested that *L. infantum* was not vertically transmitted [19], more recently several other studies demonstrated that vertical transmission indeed occur [20-26].

*Leishmania* DNA was detected by PCR in the blood of eight out of 31 (26%) newborn puppies from symptomatic or asymptomatic naturally infected bitches [20]. Rosypal *et al.* detected *L. infantum* DNA by PCR in the liver, bone marrow, heart, spleen, lymph node, kidney, and placenta of newborn puppies

**Figure 1.** Epididymitis of a dog with visceral leishmaniasis. Mild diffuse lympho-histio-plasmacytic infiltrate with intracytoplasmic positive immunostained amastigotes (arrow). Streptavidin-peroxidase complex, 200x.



**Figure 2.** Spleen from a fetal dog with visceral leishmaniasis. Immunolabeling of intracellular *Leishmania* sp. amastigotes in macrophages. Streptavidin-peroxidase complex, 200x.



delivered by cesarean section from an experimentally infected Beagle [21]. Interestingly, Dubey *et al.* reported a case of abortion associated with necrotizing placentitis with numerous intralesional *Leishmania* sp., which were observed even within trophoblasts, as demonstrated by transmission electron microscopy [22]. A more recent study including eight pregnant bitches did not detect any gross or microscopic changes in the placenta, but amastigotes were detected by immunohistochemistry (IHC) and PCR in the placenta and lymphoreticular tissues from fetuses (Figure 2) [23]. Additionally, there have been other reports demonstrating infection with *L. infantum* in stillborns or in newborns from naturally infected bitches [24-26]. An interesting report described a case of an infected bitch that gave birth to ten puppies, including four stillborns, two that had neonatal death, and two littermates that were euthanized at one year of age due to poor prognosis of VL [25]. In addition, pups from naturally infected bitches had *L. infantum* specific CD4<sup>+</sup> T cell proliferative responses, suggesting an ongoing immune response specific to the parasite [26]. Together these studies strongly support the notion that canine VL is vertically transmitted. Importantly, there have been a number of case reports of congenital transmission of VL in humans even from asymptomatic mothers [27-30]. Furthermore, vertical transmission has been experimentally demonstrated in mice [17].

It is known that resistance to leishmaniasis is associated with a strong Th1 type response. Immunosuppression is required to prevent immune reactions against fetal antigens during pregnancy. Therefore, the “physiological” shift from Th1 to Th2 type of immune response that takes place during pregnancy may possibly increase the severity of disease as well as the risk of congenital transmission of VL [21].

### **Importance of sexual and vertical transmission of VL**

Sexual and vertical transmission of VL may play an especially important role in areas where the biological invertebrate vector is absent. Díaz-Espiñeira and Slappendel reported a case of autochthonous VL in the Netherlands, where any known natural vector was absent [31]. The 12-month-old dog developed VL even without ever been to an endemic area. However, his mother was imported from Spain, an endemic area, and developed clinical VL soon after giving birth. Although it has not been proven that transmission actually occurred *in utero*, this report demonstrated

that bitches with VL can be a source of infection for their pups under natural conditions. Naucke and Lorentz reported two cases of autochthonous VL in Germany [32]. A bitch that has never left a region in which there are no phlebotomine sand flies developed clinical VL. She had mated with a dog that had lived in endemic areas. One puppy from one of her three litters tested positive for *Leishmania* sp. infection by indirect immunofluorescence assay (IFA). The authors raise the possibility of both sexual and vertical transmission in this case.

These above mentioned reports exemplify the importance of alternative transmission routes of VL in individual cases. However, the prevalence of VL among Foxhounds in the United States is perhaps the most striking situation in which sexual and vertical transmission possibly play a major role in the maintenance of VL in a non endemic area [33]. VL is an emerging problem among some dog breeds in the United States, particularly in the case of the Foxhound population, which has an estimated prevalence, based in quantitative PCR, greater than 20% [26]. Although phlebotomine sand flies are present in southern United States, it has not been determined if those species are competent biological vectors for *L. infantum*, and therefore, they cannot be held responsible for maintaining the disease in this particular breed. The route of this ongoing *L. infantum* transmission among dogs in the United States is currently unknown, but it is likely due to vertical and sexual transmission [34].

In 1980, VL was diagnosed in an American Foxhound from a kennel in Oklahoma [35]. The remaining Foxhounds were serologically evaluated for anti-*Leishmania* antibodies by IFA, and four of the 16 dogs had high antibody titers [36]. Eight years later, an English Foxhound born and housed in a research colony located in the American state of Ohio was also diagnosed with VL [37]. Sera from eight out of 25 (32%) dogs from that research colony yielded positive results for antibodies against *Leishmania* spp. by IFA [36]. In these reports, clinically affected dogs had not traveled outside of the United States, and an insect vector was not identified. In addition to these reports of autochthonous VL, two other multiple-case outbreaks of leishmaniasis have been identified among Foxhound populations in Michigan and Alabama, in 1989 and 1991, respectively [36].

In 2000, four Foxhounds died due to VL in a kennel in New York, and therefore 112 animals from that kennel were tested by IFA, PCR, protozoal culture, cytologic and/or histologic exams. The diagnosis of VL was made in 41% of those Foxhounds



(46/112). Interestingly, 30 Beagles and Basset Hounds periodically housed in the same kennel tested all negative to *L. infantum* [38].

Since the identification of leishmaniasis in New York, a widespread serosurvey was performed between 2000 and 2003, including over 12,000 Foxhounds that were examined by IFA. This survey revealed the presence of seropositive dogs in 58 kennels from eighteen American states (Alabama, Connecticut, Georgia, Iowa, Illinois, Indiana, Kentucky, Maryland, Michigan, Missouri, North Carolina, New Jersey, New York, Ohio, Pennsylvania, South Carolina, Tennessee, and Virginia) and two Canadian provinces (Nova Scotia and Ontario). Importantly, the study did not detect anti-*Leishmania* antibodies in dogs from other breeds, in wild canids from the same regions or in humans in close contact to positive Foxhounds [39]. Furthermore, so far no cases of autochthonous human visceral leishmaniasis in the U.S. have been reported [40].

In order to verify if VL was restricted to the Foxhound population in the U.S., Grosjean *et al.* performed a serosurvey with more than 900 dogs other than Foxhounds [41]. Only two out of 957 were possibly infected with *L. infantum*. However, infection could not be confirmed due to high titer positivity to *Trypanosoma cruzi*, antibodies that cross-react to the *Leishmania* spp. Importantly, differential diagnosis between *T. cruzi* and *L. infantum* may be quite challenging [42].

No studies have been performed to explore whether foxhounds are genetically more susceptible than other breeds to *Leishmania* spp. infection, although this has been hypothesized [38]. However, sexual and vertical transmission can very well explain the maintenance of the disease restricted to foxhounds, since these routes of transmission would be breed restricted unless random breeding take place. Indeed, the lack of spread of VL from Foxhounds to the wider dog population, local wild canid population or humans [39] strongly suggests that sand fly transmission is not involved, and, thus, VL infection can be maintained by nontraditional transmission routes such as sexual and vertical [43].

### Concluding remarks

Several studies and case reports have clearly demonstrated that canine VL can be sexually and vertically transmitted. The high prevalence of VL among the Foxhound population in the United States brings attention to the potential impact of nontraditional transmission routes, particularly sexual

and vertical transmission, which may play a role in the dissemination and maintenance of VL, especially in the absence of the vector sand fly.

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### Corresponding author

Renato L. Santos  
 Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária  
 Universidade Federal de Minas Gerais  
 Belo Horizonte, MG, Brazil.  
 E-mail: rsantos@vet.ufmg.br

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