

Sexual and vertical transmission of visceral leishmaniasis

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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.

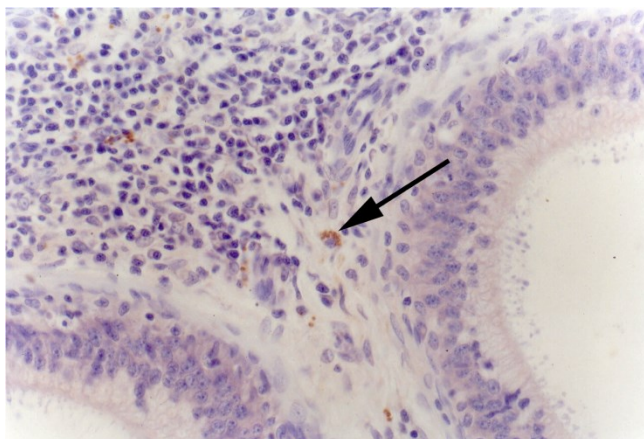
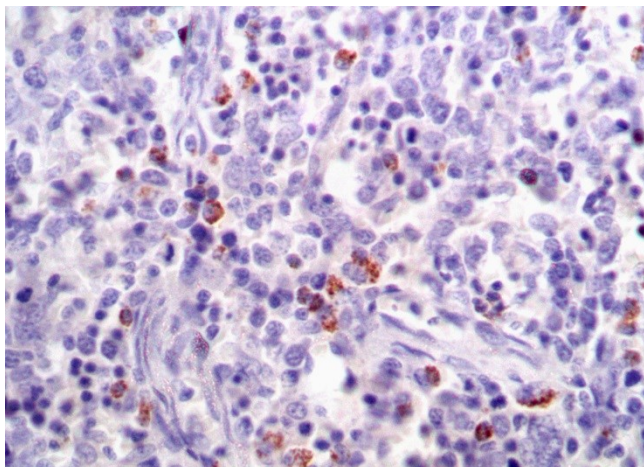


Figure 2. Spleen from a fetal dog with visceral leishmaniasis. Immunolabeling of intracellular *Leishmania* sp. amastigotes in macrophages. Streptavidin-peroxidase complex, 200x.



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⁺ 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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References

- Baneth G, Koutinas AF, Solano-Gallego L, Bourdeau P, Ferrer L (2008) Canine leishmaniosis - new concepts and insights on an expanding zoonosis: part one. Trends Parasitol 24: 324-330.
- Owens SD, Oakley DA, Marryott K, Hatchett W, Walton R, Nolan TJ, Giger U (2001) Transmission of visceral leishmaniosis through blood transfusions from infected English foxhounds to anemic dogs. J Am Vet Med Assoc 219: 1076-1083.
- Morillas-Marquez F, Martin-Sanchez J, Acedo-Sanchez C, Pineda JA, Macias J, Sanjuan-Garcia J (2002) *Leishmania infantum* (Protozoa, kinetoplastida): transmission from infected patients to experimental animal under conditions that simulate needle-sharing. Exp Parasitol 100: 71-74.
- Petersen CA (2009) New means of canine leishmaniasis transmission in North America: the possibility of transmission to humans still unknown. Interdiscip Perspect Infect Dis 2009: 802712.
- Symmers WS (1960) Leishmaniasis acquired by contagion: a case of marital infection in Britain. Lancet 1: 127-132.
- Kapila K, Prakash MB, Mehrota R, Verma K (1994) Testicular leishmaniasis in a boy with acute lymphoblastic leukemia. Acta Cytol 38: 878-879.
- Aste N, Pau M, Biggio P (2002) Leishmaniasis of the prepuce. J Eur Acad Dermatol Venereol 16: 93-94.
- Riera C, Valladares JE (1996) Viable *Leishmania infantum* in urine and semen in experimentally infected dogs. Parasitol Today 12: 412.
- Diniz SA, Melo MS, Borges AM, Bueno R, Reis BP, Tafuri WL, Santos RL (2005) Genital lesions associated with visceral leishmaniasis and shedding of *Leishmania* sp. in the semen of naturally infected dogs. Vet Pathol 42: 650-658.
- Benites AP, Fernandes CE, Brum KB, Abdo MAGS (2011) Presença de formas amastigotas de *Leishmania chagasi* e perfil leucocitário no aparelho reprodutivo de cães. Pesq Vet Bras 31: 72-77.
- Silva FL, Rodrigues AA, Rego IO, Santos RL, Oliveira RG, Silva TM, Xavier MN, Nascimento EF, Santos RL (2008) Genital lesions and distribution of amastigotes in bitches naturally infected with *Leishmania chagasi*. Vet Parasitol 151: 86-90.
- Manna L, Paciello O, Morte RD, Gravino AE (2012) Detection of *Leishmania* parasites in the testis of a dog affected by orchitis: case report. Parasit Vectors 5: 216.
- Mir F, Fontaine E, Reyes-Gomez E, Carlus M, Fontbonne A (2012) Subclinical leishmaniasis associated with infertility and chronic prostatitis in a dog. J Small Anim Pract 53: 419-422.

14. Catone G, Marino G, Poglayen G, Gramiccia M, Ludovisi A, Zanghi A (2003) Canine transmissible venereal tumour parasitized by *Leishmania infantum*. *Vet Res Commun* 27: 549-553.
15. Marino G, Gaglio G, Zanghi A (2012) Clinicopathological study of canine transmissible venereal tumour in leishmaniotic dogs. *J Small Anim Pract* 53: 323-327.
16. Silva FL, Oliveira RG, Silva TM, Xavier MN, Nascimento EF, Santos RL (2009) Venereal transmission of canine visceral leishmaniasis. *Vet Parasitol* 160: 55-59.
17. Rosypal AC, Lindsay DS (2005) Non-sand fly transmission of a North American isolate of *Leishmania infantum* in experimentally infected BALB/c mice. *J Parasitol* 91: 1113-1115.
18. Mancianti F, Sozzi S (1995) Isolation of *Leishmania* from a newborn puppy. *Trans R Soc Trop Med Hyg* 89: 402.
19. Andrade HM, de Toledo VP, Marques MJ, França Silva JC, Tafuri WL, Mayrink W, Genaro O (2002) *Leishmania (Leishmania) chagasi* is not vertically transmitted in dogs. *Vet Parasitol* 103: 71-81.
20. Masucci M, De Majo M, Contarino RB, Borruto G, Vitale F, Pennisi MG (2003) Canine leishmaniasis in the newborn puppy. *Vet Res Commun* 27 Suppl 1: 771-774.
21. Rosypal AC, Troy GC, Zajac AM, Frank G, Lindsay DS (2005) Transplacental transmission of a North American isolate of *Leishmania infantum* in an experimentally infected beagle. *J Parasitol* 91: 970-972.
22. Dubey JP, Rosypal AC, Pierce V, Scheinberg SN, Lindsay DS (2005) Placentitis associated with leishmaniasis in a dog. *J Am Vet Med Assoc* 227: 1266-1269.
23. Pangrazio KK, Costa EA, Amarilla SP, Cino AG, Silva TM, Paixão TA, Santos RL (2009) Tissue distribution of *Leishmania chagasi* and lesions in transplacentally infected fetuses from symptomatic and asymptomatic naturally infected bitches. *Vet Parasitol* 165: 327-331.
24. Gibson-Corley KN, Hostetter JM, Hostetter SJ, Mullin K, Ramer-Tait AE, Boggiatto PM, Petersen CA (2008) Disseminated *Leishmania infantum* infection in two sibling foxhounds due to possible vertical transmission. *Can Vet J* 49: 1005-1008.
25. Freeman KS, Miller MD, Breitschwerdt EB, Lappin MR (2010) Leishmaniasis in a dog native to Colorado. *J Am Vet Med Assoc* 237: 1288-1291.
26. Boggiatto PM, Gibson-Corley KN, Metz K, Gallup JM, Hostetter JM, Mullin K, Petersen CA (2011) Transplacental transmission of *Leishmania infantum* as a means for continued disease incidence in North America. *PLoS Negl Trop Dis* 5: e1019.
27. Meinecke CK, Schottelius J, Oskam L, Fleischer B (1999) Congenital transmission of visceral leishmaniasis (Kala Azar) from an asymptomatic mother to her child. *Pediatrics* 104: e65.
28. Pagliano P, Carannante N, Rossi M, Gramiccia M, Gradoni L, Faella FS, Gaeta GB (2005) Visceral leishmaniasis in pregnancy: a case series and a systematic review of the literature. *J Antimicrob Chemother* 55: 229-233.
29. Boehme CC, Hain U, Novosel A, Eichenlaub S, Fleischmann E, Löscher T (2006) Congenital visceral leishmaniasis. *Emerg Infect Dis*, 12: 359-360.
30. Zinchuk A, Nadraga A (2010) Congenital visceral leishmaniasis in Ukraine: case report. *Ann Trop Paediatr* 30: 161-164.
31. Díaz-Espiñeira MM, Slappendel RJ (1997) A case of autochthonous canine leishmaniasis in The Netherlands. *Vet Q* 19: 69-71.
32. Naucke TJ, Lorentz S (2012) First report of venereal and vertical transmission of canine leishmaniosis from naturally infected dogs in Germany. *Parasit Vectors* 5: 67.
33. Petersen CA (2009a) Leishmaniasis, an emerging disease found in companion animals in the United States. *Top Companion Anim Med* 24: 182-188.
34. Petersen CA, Barr SC (2009) Canine leishmaniasis in North America: emerging or newly recognized? *Vet Clin North Am Small Anim Pract* 39: 1065-1074.
35. Anderson DC, Buckner RG, Glenn BL, MacVean DW (1980) Endemic canine leishmaniasis. *Vet Pathol* 17: 94-96.
36. Schantz PM, Steurer FJ, Duprey ZH, Kurpel KP, Barr SC, Jackson JE, Fox JC (2005) Autochthonous visceral leishmaniasis in dogs in North America. *J Am Vet Med Assoc* 226: 1316-1322.
37. Swenson CL, Silverman J, Stromberg PC, Johnson SE, Wilkie DA, Eaton KA, Kociba GJ (1988) Visceral leishmaniasis in an English foxhound from an Ohio research colony. *J Am Vet Med Assoc* 193: 1089-1092.
38. Gaskin AA, Schantz P, Jackson J, Birkenheuer A, Tomlinson L, Gramiccia M, Breitschwerdt EB (2002) Visceral leishmaniasis in a New York foxhound kennel. *J Vet Intern Med* 16: 34-44.
39. Duprey ZH, Steurer FJ, Rooney JA, Kirchhoff LV, Jackson JE, Rowton ED, Schantz PM (2006) Canine visceral leishmaniasis, United States and Canada, 2000-2003. *Emerg Infect Dis* 12: 440-446.
40. Rosypal AC, Troy GC, Zajac AM, Duncan RB, Waki K, Chang KP, Lindsay DS (2003) Emergence of zoonotic canine leishmaniasis in the United States: isolation and immunohistochemical detection of *Leishmania infantum* from foxhounds from Virginia. *J Eukaryot Microbiol* 50 Suppl: 691-693.
41. Grosjean NL, Vrable RA, Murphy AJ, Mansfield LS (2003) Seroprevalence of antibodies against *Leishmania* spp among dogs in the United States. *J Am Vet Med Assoc* 222: 603-606.
42. Nability MB, Barnhart K, Logan KS, Santos RL, Kessell A, Melmed C, Snowden KF (2006) An atypical case of *Trypanosoma cruzi* infection in a young English Mastiff. *Vet Parasitol* 140: 356-361.
43. Quinnell RJ, Courtenay O (2009) Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. *Parasitology* 136: 1915-1934.

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