Eco-epidemiological Aspects of Visceral Leishmaniasis in the Municipality of Diamantina, Jequitinhonha Valley (Minas Gerais State, Brazil)

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**Background:** The present study was carried out in the rural and urban area of Diamantina/Minas Gerais (MG), an endemic municipality for visceral leishmaniasis (VL) in Brazil. **Methods:** Patient notification records, canine prevalence, and phlebotomine fauna were evaluated. **Results:** In the period from 2016 to 2018, eight human cases were confirmed, with three deaths, predominantly in males. In the same period, a total of 1,388 dogs resided in the rural and urban area of the municipality were submitted to the DPP® and ELISA, with a percentage of confirmed canine cases of 29.9% and 29.4%, respectively. The entomological study conducted in the municipality revealed the presence of 10 species of sand flies, with a predominance of *Lutzomyia longipalpis* (55.75%), mainly in the rural area. **Conclusions:** Unlike what is happening in urban centers, the results of this study suggest that the VL in Diamantina is in the process of urbanization, given the high percentage of confirmed canine cases and the high density of *Lu. longipalpis* in the rural area of the municipality. These risk factors warn about the need for continuous surveillance and the need to control actions of VL in this area.

**INTRODUCTION**

Visceral leishmaniasis (VL) or kala-azar is one of the parasitic diseases with the greatest impact on global public health, with an estimated incidence of 500,000 new cases and 60,000 deaths annually [1,2]. In the Americas, about 90% of human cases of VL have been registered in Brazil, with distribution in the five regions and in 26 of the 27 states [3].

In the life cycle of *Leishmania infantum* *chagasi*, the...
etiological agent of VL in the Americas, the transmission occurs mainly through the bite of female sand flies of the *Lutzomyia longipalpis* species (Lutz & Neiva 1912) [4,5]. The reservoirs include a wide variety of mammalian hosts, being a common occurrence in rodents, marsupials, canids and humans, which are accidentally affected [6,7], and may have irregular fever, progressive weight loss, hepatosplenomegalgy, and anemia.

The main reservoir of *Le. infantum chagasi* in the home environment is the dog (*Canis familiaris*), which contributes to the maintenance of the disease cycle [8,9]. Canine enzooty has preceded the occurrence of human cases and the infection in dogs has been more prevalent than in humans. In addition, the presence of dogs and other domestic animals has been identified as one of the main risk factors for the occurrence of infection in humans [10,11].

The environmental changes caused by humans: deforestation, fire, the disordered city growth, the migration of people to the periphery of urban centers, the constant presence of domestic animals and vectors, allied to the problems of basic sanitation, housing, and malnutrition have been identified as determining factors for the urbanization and geographical expansion of VL in Brazil [12].

The municipality of Diamantina/ Minas Gerais (MG), located in the Jequitinhonha River Valley, is considered an endemic area for visceral leishmaniasis with moderate transmission (average number of reported human cases in the last 3 years between 2.4 and 4.4 cases) [12]. However, there are no data about the epidemiology of VL in the municipality. Thus, the objective of this study was to investigate the registration of human cases, to evaluate the prevalence of visceral leishmaniasis in dogs attended by demand and to characterize the phlebotomine fauna in order to provide the first data on the epidemiological triad of VL in the municipality of Diamantina.

**Study Area**

Diamantina is located in the mesoregion of the Jequitinhonha River Valley, in the State of MG. It has a population of 45,880 inhabitants [13], distributed over an area of approximately 3,900 km², including 10 districts: Conselheiro Mata, Desembargador Otoni, Extração, Guinda, Inhaí, Mendanha, Planalto de Minas, São João da Chapada, Senador Mourão, and Sopa (Figure 1).

The Jequitinhonha River Valley is one of the regions of MG with the highest percentages of poverty, illiteracy, infant mortality, fertility rate, and low incomes. The region has one of the lowest Human Development Indexes (HDI) in the State of MG and occupies one of the worst positions in the country [14]. Diamantina is a small urban city, but is characterized by being the hub of the region, with HDI=0.716 [13]. The city offers health and educa-
tion services to the local and surrounding population. However, the neighborhoods that make up the municipality, characterized as rural (defined by Master Plan of Municipality), have a high concentration of poverty and difficulty in accessing health services [15,16]. Commonly, the population practices subsistence agriculture and domestic animal breeding, and its residents live in modest homes and in inadequate sanitary conditions.

The region’s climate regime is typically wet temperate (Cwb - Köppen climate classification), characterized by mild and humid summers (October to April) and cooler, drier winters (June to August) [17]. The average annual rainfall varies from 1250 to 1550 mm, with the months of October to April being the rainiest in the last 2 years. The city is situated at an altitude of 1,280 m. The average annual temperature is in the range of 18° to 19°C, being predominantly mild throughout the year. The relative humidity of the air is almost always high, with annual averages of 75.6%.

### Human Cases of VL

Data concerning the number of human cases of VL in Diamantina between 2016 and 2018 were obtained from the Municipal Health Department, observing the following characteristics: age group, sex, area of residence, and lethality.

### Canine VL Survey

In the period between 2016 and 2018, serological surveys were carried out in order to determine the prevalence of VL in dogs domiciled in the urban and rural areas of the municipality of Diamantina. By phone, the owners of dogs that showed any suggestive sign for CVL contacted the endemic agents at the Zoonosis Control Center, who went to the dog’s place of residence to proceed with the clinical investigation and diagnosis. The serological diagnosis of these dogs followed Technical Note N° 01/2011 CGDT-CGLAB/DEVIT/SVS/MS), using the TR-DPP® immunochromatographic test (Bio-Manguinhos/FIOCRUZ/RJ, Brazil) as a screening method and the immunoenzymatic ELISA EIE® assay (Bio-Manguinhos/FIOCRUZ, RJ, Brazil), as a confirmatory method.

### Entomological Studies

Entomological captures were made in six neighborhoods in the municipality of Diamantina, three of which were considered to be rural (Jambreiro, Maria Orminda, and Santo Antônio) and three belonging to the urban area (Consolação, Gruta de Lourdes, and Palha). Twelve HP light traps [18] were exposed in peridomicile, which presented canines cases and favorable ecological conditions for the development of sand flies, such as the presence of trees, domestic animals, and organic matter.

The traps were exposed from 4:00 pm to 8:00 am the next morning, monthly from February to December 2019, for two consecutive nights per month, in urban and rural areas. The captured specimens were placed in hemolysis tubes containing 70% alcohol, prepared, mounted on a slide [19], and identified according to the classification proposed by Young & Duncan (1994) [20]. The captured sandflies were deposited in the Collection of the Laboratory of Parasitology, Department of Biological Sciences, Federal University of Vales do Jequitinhonha and Mucuri.

### Table 1. Distribution of human VL cases according to age group, residence zone, and lethality of the municipality of Diamantina/MG in the period from 2016 to 2018.

| Age group (years) | Urban area | Rural area | Total | % | Lethality |
|-------------------|------------|------------|-------|---|-----------|
| Female Male       | Female Male| Female Male|       |   |           |
| 0 - 9             | 0 0 0 2 2  | 2 2 25 1   |       |   |           |
| 10 - 19           | 0 0 0 1 1 | 1 12.5 0 0 |       |   |           |
| 20 - 29           | 0 0 0 1 1 | 1 12.5 0 0 |       |   |           |
| 30 - 39           | 0 0 0 0 1 | 1 12.5 0 0 |       |   |           |
| 40 - 49           | 1 0 0 0 1 | 1 12.5 0 0 |       |   |           |
| 50 - 59           | 0 0 0 0 1 | 1 12.5 1 1 |       |   |           |
| 60 - 69           | 0 0 0 0 0 | 0 0 0 0 0 |       |   |           |
| 70 - 79           | 0 0 0 0 0 | 0 0 0 0 0 |       |   |           |
| 80 - 89           | 0 0 0 0 0 | 0 0 0 0 0 |       |   |           |
| 90 - 99           | 1 0 0 0 1 | 1 12.5 1 1 |       |   |           |
| Subtotal          | 2 2 1 3   |            | 8 100 3 |   |           |

TOTAL 4 4 8 100 3
RESULTS

The municipality of Diamantina presented eight autochthonous cases of VL in the period from 2016 to 2018, with four cases recorded in the urban area and four cases in the rural area. The disease prevailed in males (62.5%), with the majority of patients aged 0-9 years (25%) and a lethality rate of 37.5% (Table 1).

Table 2 shows the percentage of confirmed canine cases of VL and the distribution of human cases by area of residence in the municipality of Diamantina between 2016 and 2018. In this period, 166 out of 565 dogs analyzed presented positive serology for VL in the urban area, with an average percentage of 29.4%. In rural areas, 246 out of 1,388 dogs tested were seropositive for VL, with an average percentage of 29.9%. Of the eight human cases, four were patients living in the urban area and four declared residence in the rural area (Table 2).

The phlebotomine fauna of Diamantina consists of 10 species: Lutzomyia carmelinoi (Ryan, Fraiha, Lainson & Shaw, 1986), Lutzomyia longipalpis (Lutz & Neiva, 1912), Lutzomyia whitmani (Antunes & Coutinho, 1939), Lutzomyia diamantinensis (Barata, Serra-e-Meira & Carvalho, 2012), Lutzomyia evandroi (Costa Lima & Antunes, 1936), Lutzomyia ischyracantha (Falcão & Silva, 1962), Lutzomyia oliveirai (Martins, Silva & Falcão, 1970), Lutzomyia orestes (Fairchild & Trapido, 1950), totaling 4,452 specimens, 2,770 males (62%) and 1,682 females (38%). The rural area had the highest percentage of captured specimens (99.8%), with a predominance of Lutzomyia longipalpis (55.7%) (Table 3).

DISCUSSION

In the last decade, the phenomenon of urbanization has been pointed out as the main responsible for the geographic expansion of VL, mainly in the peripheries of urban areas of Brazilian cities [21-23]. However, the results shown in this work suggest that VL is still in the process of urbanization in the municipality of Diamantina.

Considering the notification of human cases of VL in the rural area, and also the high occurrence of seropositive dogs and Lu. longipalpis in the peridomicile of residences in this area, our data indicate that the profile of transmission of VL in the municipality is mixed of the rural/urban type. In addition, other data that reinforce this hypothesis is the registration of human cases in the urban area, but in peripheral neighborhoods (Consolação, Cidade Nova, and Palha) bordering the rural area.

Analyzing the profile of human cases according to the age group, it is noted that VL was more frequent in children under 10 years old (25%). This finding corroborates that found by other authors [24,25]. The lethality rate was 37.5%, unlike Silva et al. (2001) [10] and Queiroz et al. (2004) [26], who found a lethality of 11.5% and 10.2%, respectively. Further studies need to be undertaken to elucidate this high lethality found in the city.

The percentage of confirmed canine cases during the study period in urban and rural areas was 29.4% and 29.9%, respectively. However, it is important to emphasize that, in the present study, the canine survey was conducted on demand, and not as a census, as in other studies [27-29]. Thus, we believe that the prevalence of VL in this municipality is overestimated, considering that only dogs with any apparent sign/symptom have been subjected to the diagnosis.

In MG, as well as in other Brazilian states, the high density of Lu. longipalpis in urban areas has been evidenced by many authors [28-31]. This sand fly has been identified as the main vector of Le. infantum chagasi in Brazil for complying with the criteria established to be considered a competent vector [32]. Possibly, it is also the...
species responsible for the transmission of *Le. infantum chagasi* in Diamantina.

One of the characteristics of *Lu. longipalpis* is its high adaptive plasticity, being able to adapt easily to different habitats and climatic conditions [33,34]. Although it is considered a highly urbanized species, in this study, this species was found in low numbers in the urban area and high density in the rural environment. In the literature, populations of *Lu. longipalpis* in less anthropized environments have also been reported [35,36].

Other data that deserves to be highlighted is the remarkable presence of *Lutzomyia whitmani*, one of the vectors of cutaneous leishmaniasis in Brazil, and mainly in the State of MG [37,38]. In this work, this species was found in urban and rural areas, showing its ecological plasticity, as observed by Peterson & Shaw (2003) and Costa et al. (2007) [39,40].

*Lutzomyia pessoai* captured in this study in the rural area, is considered quite anthropophilic, being found frequently inside homes in endemic areas and has been identified as a species suspected of transmitting American tegumentary leishmaniasis (ATL) in other locations [41]. As the municipality of Diamantina is endemic to ATL, the finding of *Lu. pessoai* and *Lu. whitmani* suggests that these are the species responsible for the transmission of dermatotropic *Leishmania* species in the region.

Another observation that accelerates the transmission of *Leishmania* sp. in the municipality is the constant presence of domestic animals, such as dogs, cats, rats, oxen, pigs, and chickens close to homes (data not shown), which facilitates the attraction of sandflies and increases the possibility of vector/human contact, and consequently, of leishmaniasis transmission.

Finally, the results of this work provided the first data on the epidemiological triad in the municipality of Diamantina. Our data suggest that VL in this area is in the process of urbanization, different from what has been happening in other urban centers. The occurrence of human cases, the high percentage of confirmed canine cases, and the high density of *Lu. longipalpis* in the rural area reinforce the need for continuous epidemiological surveillance of VL in Diamantina.

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**REFERENCES**

1. Bhattacharya SK, Sur D, Karbwang J. Childhood visceral leishmaniasis. Indian J Med Res. 2006 Mar;123(3):353–6.
2. World Health Organization. Control of the leishmanias: report of WHO Expert Committee. Series WHO. 1992;(793):139–58.
3. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Coordenação-Geral de Desenvolvimento da Epidemiologia em Serviços. Guia de Vigilância em Saúde: volume 3 /1. ed. atual. - Brasília: Ministério da Saúde, 2017.
4. Soares RP, Turco SJ. *Lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotominae): a review. An Acad Bras Cienc. 2003 Sep;75(3):301–30.
5. Lainson R, Rangel EF. *Lutzomyia longipalpis* and the eco-epidemiology of American visceral leishmaniasis, with particular reference to Brazil: a review. Mem Inst Oswaldo Cruz. 2005 Dec;100(8):811–27.
6. Deane LM, Deane MP. Encontro de leishmanias nas vísceras e na pele de uma raposa em zona endêmica de calazar, nos arredores de Sobral. O Hospital. 1954;(45):419-421.
7. Lainson R, Shaw JJ, Ryan L, Ribeiro RS, Silveira FT. Leishmaniasis in Brazil. XXI. Visceral leishmaniasis in the Amazon Region and further observations on the role of *Lutzomyia longipalpis* (Lutz & Neiva, 1912) as the vector. Trans R Soc Trop Med Hyg. 1985;79(2):223–6.
8. Courtenay O, Quinell RJ, Garcez LM, Shaw JJ, Dye C. Infectionousness in a cohort of dog建议 fails to control visceral leishmaniasis in areas of high transmission. J Infect Dis. 2002 Nov;186(9):1314–20.
9. Moreno J, Alvar J. Canine leishmaniasis: epidemiological risk and the experimental model. Trends Parasitol. 2002 Sep;18(9):399–405.
10. Silva ES, Gontijo CM, Pacheco RS, Fiuza VO, Brazil RP. Visceral leishmaniasis in the Metropolitan Region of Belo Horizonte, State of Minas Gerais, Brazil. Mem Inst Oswaldo Cruz. 2001 Apr;96(3):285–91.
11. Borges BK, Silva JA, Haddad JP, Moreira EC, Magalhães DF, Ribeiro LM, et al. Presença de animais associada ao risco de transmissão da leishmaniose visceral em humanos em Belo Horizonte, Minas Gerais. Arq Bras Med Vet Zootec. 2009;61(5):1035–43.
12. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Manual de vigilância e controle da leishmaniose visceral. – Brasília [color – ] [Série A. Normas e Manuais Técnicos]. Editora do Ministério da Saúde. 2006;120:il.
13. IBGE - Instituto Brasileiro de Geografia e Estatística. Anuário Estatístico do Brasil - Instituto Brasileiro de Geografia e Estatística, 2010.
14. Matos R, Garcia RA. A população do Vale do Jequitinhonha. In: Souza JV, Henriques MS, editors. Org. Vale do Jequitinhonha: formação histórica, populações e movimentos. Belo Horizonte: UFMG/PROEX; 2010. pp. 97–127.
15. de Andrade CL, Szwarcwald CL. Desigualdades sócio-espaciais da adequação das informações de nascimentos e óbitos do Ministério da Saúde, Brasil, 2000-2002. Cad Saude Publica. 2007 May;23(5):1207–16.
16. Victora CG, Aquino EM, do Carmo Leal M, Monteiro CA, Barros FC, Szwarcwald CL. Maternal and child health in Brazil: progress and challenges. Lancet. 2011 May;377(9780):1863–76.
17. Vieira EW. Acesso e utilização dos serviços de saúde de atenção primária em população rural do Município de Jequitinhonha, Minas Gerais [Dissertação de Mestrado]. Belo Horizonte: Escola de Enfermagem, Universidade Federal de Minas Gerais; 2010.
18. Pugedo H, Barata RA, França-Silva JC, Silva JC, Dias ES, HP: um modelo aprimorado de armadilha luminosa de sução para a captura de pequenos insetos. Rev Soc Bras Med Trop. 2005 Jan-Feb;38(1):70–2.
19. Langeron M. 1949. Précis de microscopie. Masson et Cie, Libraires de L’Académie de Medicine, Saint-Germain, Paris, 1.
20. Young DG, Duncan MA. Guide to the identification and geographic distribution of *Lutzomyia* sand flies in Mexico, the West Indies, Central and South America (Diptera: psychodidae). Mem Am Entomol Inst. 1994;(54):1–881.
21. Nascimento MD, Costa JM, Fiori BI, Viana GM, Filho MS, Alvim AC, et al. Aspectos epidemiológicos determinantes na manutenção da leishmaniose visceral no Estado do Maranhão, Brasil. Rev Soc Bras Med Trop. 1996;29(2):233–40.
22. Bevilacqua PD, Paixão HH, Modena CM, Castro MC. Urbanização da leishmaniose visceral em Belo Horizonte. Arq Bras Med Vet Zootec. 2001;53(1):1–8.
23. Dantas-Torres F, Brandão-Filho SP. Expansão geográfica da leishmaniose visceral no Estado de Pernambuco. Rev Soc Bras Med Trop. 2006 Jul-Aug;39(4):352–6.
24. Barata RA, Peixoto JC, Tanure A, Gomes ME, Apolinário EC, Bodevan EC, et al. Epidemiology of visceral leishmaniasis in a reemerging focus of intense transmission in Minas Gerais State, Brazil. BioMed Res Int. 2013;2013(3):405083.
25. Coimbra VC, Lima MS, Oliveira FM, Abreu WM, Ferreira JM, Bezerra NP. Leishmaniose visceral: perfil epidemiológico dos casos notificados no município de São Luís-MA, no período de 2014 a 2017. Rev Bras Educ Saude. 2019;(9):87–93.
26. Queiroz MJ, Alves JG, Correia JB. Leishmaniose visceral: características clínico-epidemiológicas em crianças de área endêmica. J Pediatr. 2004(8):141–6.
27. França-Silva JC, da Costa RT, Siqueira AM, Machado-Coelho GL, da Costa CA, Mayrink W, et al. Epidemiology of canine visceral leishmaniasis in the endemic area of Montes Claros Municipality, Minas Gerais State, Brazil. Vet Parasitol. 2003 Feb;111(2-3):161–73.
28. Monteiro EM, da Silva JC, da Costa RT, Costa DC, Barata RA, de Paula EV, et al. Leishmaniose visceral: estudo de flebotomíneos e infecção canina em Montes Claros, Minas Gerais. Rev Soc Bras Med Trop. 2005 Mar-Apr;38(2):147–52.
29. Dias ES, Regina-Silva S, França-Silva JC, Paz GF, Michalsky EM, Araújo SC, et al. Eco-epidemiology of visceral leishmaniasis in the urban area of Paracatu, state of Minas Gerais, Brazil. Vet Parasitol. 2011 Mar;176(2-3):101–11.
30. Barata RA, Silva JC, Costa RT, Fortes-Dias CL, Silva JC, Paula EV, et al. Phlebotomine sand flies in Porteirinha, an area of American visceral leishmaniasis transmission in the State of Minas Gerais, Brazil. Mem Inst Oswaldo Cruz. 2004 Aug;99(5):481–7.
31. Lopes JV, Michalsky EM, Pereira NC, de Paula AJ, Lara-Silva FO, Silva-Lana R, et al. Entomological studies in Itaúna, Brazil, an area with visceral leishmaniasis transmission: fauna survey, natural *Leishmania* infection, and molecular characterization of the species circulating in phlebotomine sand flies (Diptera: Psychodidae). J Med Entomol. 2019 Sep;56(5):1368–76.
32. Killick-Kendrick R, Ward RD. Ecology of *Leishmania*. Parasitology. 1981;(82):143–52.
33. Costa PL, Dantas-Torres F, da Silva FJ, Guimarães VC, Gaudêncio K, Brandão-Filho SP. Ecology of *Lutzomyia longipalpis* in an area of visceral leishmaniasis transmission in north-eastern Brazil. Acta Trop. 2013 May;126(2):99–102.
34. Oliveira AM, Vieira CP, Dibo MR, Guirado MM, Rodas LA, Chiarravalloti-Neto F. Occurrence of *Lutzomyia longipalpis* and human and canine cases of visceral leishmaniasis and evaluation of their expansion in the North-
west region of the State of São Paulo, Brazil. Acta Trop. 2016;(164):233–42.

35. Chagas AP, Soares DC, Sousa GC, Viana RB, Rebelo JM, Garcez LM. Aspectos ecológicos da fauna de flebotomíneos em focos de leishmaniose na Amazônia Oriental, Estado do Pará, Brasil. Rev Panamazonica Saúde. 2016;7(7):123–32.

36. Cerqueira RF, Simões-Gomes FC, Sincurá YR, Santos T, Barata RA. Phlebotomine fauna (Diptera, Psychodidae) in Rio Preto State Park, Southern Espinhaço Range, Minas Gerais, Brazil. Stud Neotrop Fauna Environ. 2017;(53):1–6.

37. Carneiro AP, Lima ML, Santiago RM, Gama MA, Santos CA, Falcão AL, et al.; Andrade Filho JD. Flebotomíneos de Timóteo, Estado de Minas Gerais, Brasil (Diptera: psychodidae). Cad Saude Publica. 1997 Oct;13(4):767–70.

38. Souza NA, Andrade-Coelho CA, Vilela ML, Peixoto AA; Rangel EF. Seasonality of Lutzomyia intermedia and Lutzomyia whitmani (Diptera: Psychodidae: Phlebotominae), occurring sympatrically in area of cutaneous leishmaniasis in the State of Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz. 2002 Sep;97(6):759–65.

39. Peterson AT, Shaw J. Lutzomyia vectors for cutaneous leishmaniasis in Southern Brazil: ecological niche models, predicted geographic distributions, and climate change effects. Int J Parasitol. 2003 Aug;33(9):919–31.

40. Costa SM, Cechinel M, Bandeira V, Zannuncio JC, Lainson R, Rangel EF. Lutzomyia (Nyssomyia) whitmani s.l. (Antunes & Coutinho, 1939) (Diptera: Psychodidae: Phlebotominae) and the epidemiology of American cutaneous leishmaniasis in Brazil. Mem Inst Oswaldo Cruz. 2007;(102):149–53.

41. Rangel EF, Lainson R. Proven and putative vectors of American cutaneous leishmaniasis in Brazil: aspects of their biology and vectorial competence. Mem Inst Oswaldo Cruz. 2009 Nov;104(7):937–54.