

ORIGINAL CONTRIBUTION

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

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Abbreviations: MG, Minas Gerais; VL, visceral leishmaniasis; CVL, canine visceral leishmaniasis; *Lu*, *Lutzomyia*; *Le*, *Leishmania*.

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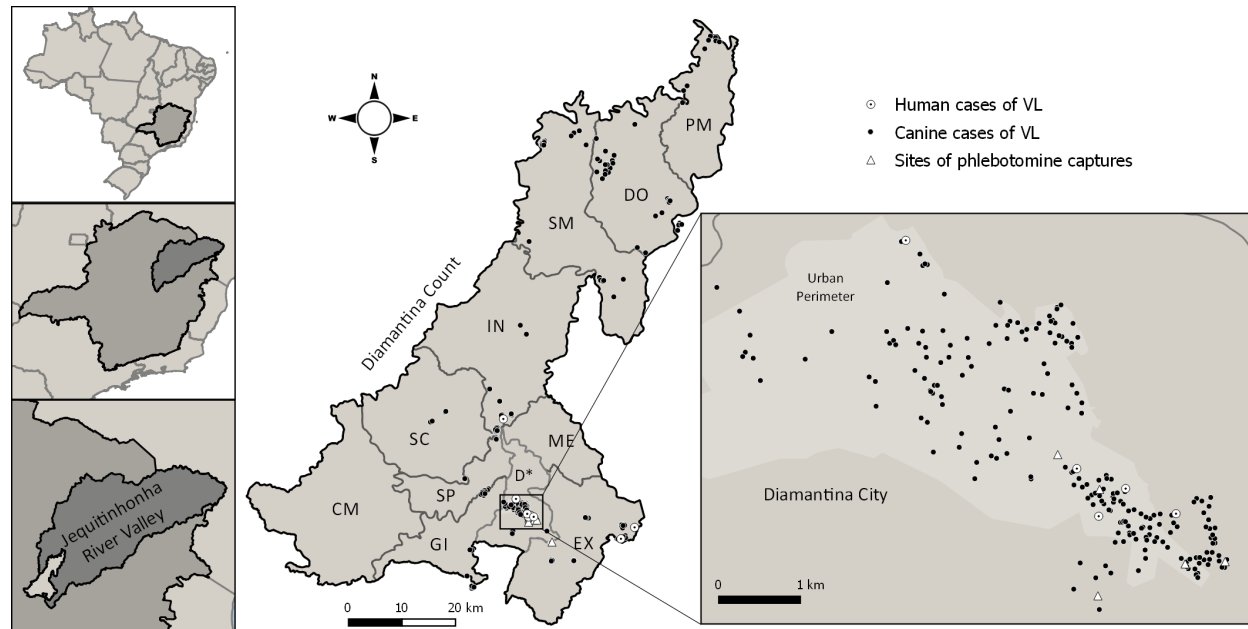


Figure 1. Geographic location of the municipality of Diamantina, Minas Gerais State (MG), Brazil inserted in Jequitinhonha Valley. Inset: Map of the municipality of Diamantina with district subdivision: Diamantina (D*) (study area), Planalto de Minas (PM), Desembargador Otoni (DO), Senador Mourão (SM), Inhaí (IN), São João da Chapada (SC), Conselheiro Mata (CM), Sopa (SP), Guinda (GI), Mendanha (ME), and Extração (EX). In details: Distribution profile of human VL (white dots), canine VL (black dots), and sites of phlebotomine captures (triangles) in the municipality of Diamantina/MG.

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, hepatosplenomegaly, 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-

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			
0 - 9	0	0	0	2	2	25	1
10 - 19	0	1	0	0	1	12.5	0
20 - 29	0	1	0	0	1	12.5	0
30 - 39	0	0	1	0	1	12.5	0
40 - 49	1	0	0	0	1	12.5	0
50 - 59	0	0	0	1	1	12.5	1
60 - 69	0	0	0	0	0	0	0
70 - 79	0	0	0	0	0	0	0
80 - 89	0	0	0	0	0	0	0
90 - 99	1	0	0	0	1	12.5	1
Subtotal	2	2	1	3			
TOTAL		4		4	8	100	3

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 con-

tacted 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 Ormindá, 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 2. Percentage of confirmed canine cases and distribution of human cases of VL by residence area in the municipality of Diamantina/MG from 2016 to 2018.

Area	Localities	Dogs examined (N)	Dogs positive (N)	(%)	VL human cases
Urban	Cidade Nova	11	5	45.5	2
	Consolação	29	12	41.4	1
	Palha	139	38	27.3	1
	Outros	386	111	28.7	-
	Subtotal	565	166	29.4	4
Rural	Jazida	36	12	8.3	2
	Maria Orminda	132	39	29.5	1
	Pinheiro	35	4	11.4	1
	Outros	620	191	30.8	-
	Subtotal	823	246	29.9	4
TOTAL	1,388	412	29.6	8	

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 ischyraantha* (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 geo-

graphic 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

Table 3. Phlebotomine sandflies captured with HP trap in Diamantina/MG according to species, sex, and residence area in period between February to December 2019.

Species	Urban area		Rural area		Total	%
	Female	Male	Female	Male		
<i>Brumptomyia guimaraesi</i>	0	0	0	1	1	0.02
<i>Lutzomyia carmelinoi</i>	0	0	0	6	6	0.13
<i>L. diamantinensis</i>	0	0	1	0	1	0.02
<i>L. evandroi</i>	1	0	58	53	112	2.52
<i>L. ischyracantha</i>	2	0	31	7	40	0.90
<i>L. longipalpis</i>	0	2	312	2,168	2,482	55.75
<i>L. oliveirai</i>	0	0	3	0	3	0.07
<i>L. orestes</i>	0	0	1	0	1	0.02
<i>L. pessoai</i>	0	0	32	32	64	1.44
<i>L. whitmani</i>	0	2	1,238	497	1,737	39.02
<i>Lutzomyia</i> spp.	1	0	2	2	5	0.11
Subtotal	4	4	1,678	2,766		
TOTAL		8		4,444	4,452	100

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 consequent-

ly, 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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