ORIGINAL ARTICLE

High seroprevalence and peripheral spatial distribution of visceral leishmaniasis among domestic dogs in an emerging urban focus in Central Brazil: a cross-sectional study

Amanda Gabriela de Carvalho^{a,b}, João Gabriel Guimarães Luz^{a,b}, Luane Dantas Rodrigues^a, João Victor Leite Dias^c and Cor Jesus Fernandes Fontes^{b,d}

^aSchool of Medicine, Institute of Exact and Natural Sciences, Federal University of Mato Grosso, Rondonópolis, Brazil; ^bSchool of Medicine, Post-Graduation Program in Health Sciences, Federal University of Mato Grosso, Cuiabá, Brazil; ^cSchool of Medicine, Federal University of Jequitinhonha and Mucuri Valleys, Teófilo Otoni, Brazil; ^dJúlio Müller University Hospital, University of Mato Grosso, Cuiabá, Brazil

ABSTRACT

The Brazilian municipality of Rondonópolis is an emerging urban focus of intense transmission of visceral leishmaniasis (VL), where few investigations have addressed canine reservoirs. This study assessed the seroprevalence and spatial distribution of canine visceral leishmaniasis (CVL) in the urban area of Rondonópolis. A CVL serosurvey was conducted between October 2016 and February 2017 using an immunochromatographic rapid test and enzyme-linked immunosorbent assay. Domestic dogs were sampled from 25 heterogeneous regions previously defined by the Spatial 'K'luster Analysis by Tree Edge Removal algorithm, which considered the socioeconomic and environmental features from the last demographic census. The CVL spatial distribution was analyzed by kernel density estimation (KDE) and spatial scan statistic. All the autochthonous human VL cases reported between 2014 and 2016 were georeferenced. Of the 600 dogs tested, 115 were seropositive in both tests. The overall CVL prevalence was 19.2% (95%CI: 16.1–22.3%), which varied widely among the evaluated regions (0.0–35.1%). Almost 25% of the sampled households ($n = 405$) had at least one infected dog. KDE demonstrated that positive CVL households were concentrated in the peripheral areas of the city. Spatial scan statistics detected a spatial cluster with significantly low CVL prevalence in the central region (relative risk = 0.37; $p = 0.04$), where only one human VL case was reported. Thus, we demonstrated a high prevalence of CVL in domestic dogs from diverse socioeconomic and environmental urban contexts in Rondonópolis. The CVL cases were peripherally distributed and occurred more frequently in areas that had reported human VL.

KEYWORDS

Visceral leishmaniasis; *Leishmania infantum*; dogs; seroprevalence; spatial analysis; epidemiology; disease control; Brazil

Introduction

In Brazil, visceral leishmaniasis (VL), or kala-azar, is a zoonosis caused by the protozoan *Leishmania* (*Leishmania*) *infantum*, which is transmitted by the bite of female phlebotomine sandflies specifically those of the species *Lutzomyia longipalpis* and *Lutzomyia cruzi*. The disease has great relevance in Brazilian public health due to its recent expansion to urban areas and the consequent increase in the number of confirmed cases [1]. This process of urbanization is thought to have started in the 1980s in the Northeast region of the country because of the changes in the eco-epidemiological features of the disease [2]. Subsequently, VL has spread throughout Brazil, occurring as epidemic outbreaks and/or persistent endemics [3]. Currently, autochthonous VL cases have already been reported in 25% of Brazilian municipalities in 22 states, with approximately 3500 new reports annually and an incidence of 2.0 cases/100,000 inhabitants [4,5].

In the State of Mato Grosso, located in the Brazilian Midwestern region, urban transmission of VL became frequent and expansive in 1998 [6]. In this State, the municipality of Rondonópolis deserves attention as the most important VL endemic area because it concentrates almost half of human cases there reported [7]. In addition, Rondonópolis is the unique municipality classified as intense transmission for VL according to the epidemiological stratification criteria of the Brazilian Ministry of Health [8]. In terms of local morbidity and mortality, the incidence and lethality rates recently reached peaks of 12.1 cases/100,000 inhabitants and 20.0%, respectively [9]. Moreover, Rondonópolis was among the ten cities that reported 15.0% of all VL cases in Brazil between 2001 and 2012 [4]. However, even though the disease is relevant in this urban locality, few studies have addressed its occurrence, especially regarding reservoirs.

Domestic dogs (*Canis familiaris*) are the main reservoir hosts of zoonotic VL responsible for maintaining

the transmission cycle in urban settings. This is because dogs have been proven to be infective to sandflies due to the intense cutaneous parasitism; canine infections are mostly long-lasting and asymptomatic; and dogs are commonly kept as household pets [10,11]. Therefore, assessing the prevalence and spatial distribution of canine visceral leishmaniasis (CVL) is of major importance in order to understand the dynamics of VL transmission [12–14].

The prevalence of CVL has been reported in several Brazilian regions [15–19]. However, since VL occurrence is usually associated with socioeconomic and environmental characteristics [20,21], there is still a need to consider such aspects during the determination of the prevalence of canine disease, mainly because Brazilian cities present remarkable intra-urban differences that may influence its occurrence [22,23].

Epidemiological studies based on geographical information systems and spatial analysis have contributed to the understanding and visualization of epidemiological patterns of VL [24,25]. In the past decade, some researches has focused on CVL spatial distribution in several Brazilian localities [14,26–28]. These contributions are crucial in guiding public health authorities in the resources allocation and control measures

implementation [19]. However, in several endemic areas, CVL pattern distribution still remains unclear.

Thus, the purpose of the present study was to assess the seroprevalence and spatial distribution of CVL in the urban area of Rondonópolis, considering socioeconomic and environmental features as well as human VL cases.

Methods

Study area

The municipality of Rondonópolis (16°28'15" S, 54°38'08" W) is an important agricultural and industrial center in the Southern region of the State of Mato Grosso, West-Central Brazil (Figure 1). In 2017, the estimated human population comprised 222,216 inhabitants. The territory has an area of 4686.622 km², of which 129.2 km² is allocated for the urban area [29]. This urban area is composed of 449 census tracts and 237 neighborhoods that have marked socioeconomic and environmental intra-urban differences [23,30]. Human VL is highly endemic to this locality [7], with 210 autochthonous human cases reported between 2003 and 2016 [9]. Despite this, few investigations have been carried out addressing CVL.

Figure 1. Division of the urban area of the municipality of Rondonópolis, State of Mato Grosso, Brazil, into 25 regions defined by the SKATER (Spatial 'K'luster Analysis by Tree Edge Removal) algorithm. Source: http://servicodados.ibge.gov.br/Download/Download.ashx?u=geoftp.ibge.gov.br/organizacao_do_territorio/malhas_territoriais/malhas_de_setores_censitarios__divisoes_intramunicipais/censo_2010/setores_censitarios_shp/mt/mt_setores_censitarios.zip.

Notes: This regionalization took into account the urban census tract and its socioeconomic and environmental characteristics obtained from the last demographic census [30] (Projection: UTM, Zone 21).

Study design

An observational, cross-sectional study was conducted in the municipality of Rondonópolis to determine the seroprevalence of CVL in domestic dogs sampled from urban areas with different socioeconomic and environmental features. We also analyzed the spatial distribution of the canine infection, as well as its relationship with human VL cases.

Sample size determination

The sample size was calculated at a significance level of 5% and with a 95% confidence interval. The employed expected prevalence of CVL was 23%, which was based on a pilot study conducted in 2016 by our research team in domestic dogs from one restricted area of the municipality during the rabies vaccination campaign. Assuming a ratio of one dog to every seven humans [31], and using the official human urban population from the last demographic census (188,028 inhabitants) [30], the canine population was estimated to be 26,861 individuals. To minimize the design effect, the sample size was inflated by a factor of 1.4, and 10% was added to compensate for any missing data [32]. These parameters determined a sample size of 416 dogs. For sampling purposes, the unit used in this study was the household, and it was considered that each household had at least one dog.

Sampling

Double-stage stratified cluster sampling was performed to assure that the sampled dogs and their owners were representative of the municipality's intra-urban diversity. For this purpose, using Spatial 'K'luster Analysis by Tree Edge Removal (SKATER), the census tracts from the urban area of Rondonópolis were regionalized based on socioeconomic and environmental features. SKATER algorithm is based on recursive partitioning of a minimal spanning tree, creating regions that are internally homogenous, spatially contiguous, and heterogeneous in comparison with each other [33,34]. The SKATER algorithm was implemented in TerraView 4.2.2 (INPE, São José dos Campos, SP, Brazil).

To the regionalization procedure, the following socioeconomic and environmental variables of each census tract were obtained from the last demographic census [30] and used as clustering criteria: mean monthly nominal income of people aged ≥ 10 years, standard deviation of the mean monthly nominal income of people aged ≥ 10 years, mean number of residents in permanent private households (PPHs), standard deviation of the mean number of residents in PPH, percentage of literate people aged ≥ 5 years per PPH, percentage of PPHs with public lighting, percentage of PPHs with pavement, percentage of PPHs connected to the public water supply, percentage of PPHs with open sewer, percentage

of PPHs with accumulated garbage, and percentage of PPHs with urban afforestation.

Hence, by means of the regionalization, 25 regions were created, which were socioeconomically and environmentally heterogeneous (Figure 1). Adopting the estimated ratio of human to canine populations (7:1) present in each region, 416 households were proportionally sampled by spatial randomization using the *Random Points* tool in QGIS 2.10.1 [35]. If a particular sampling point was randomly located in a depopulated region, the nearest residence was chosen for visitation. Additionally, to localize the residences for further data collection, the geographical coordinates of each sampling point were obtained from Google Earth 7.3.0 (Google Inc., Mountain View, CA, U.S.A.)

CVL: data collection and laboratory procedures

To collect data, the sampled homes were visited between October 2016 and February 2017. All households with at least one dog whose owner agreed to join the study were included. Households that did not have a dog, those whose homes were closed, and those with dogs whose owners did not give consent were excluded. In such cases, the next household located to the right in the same block was visited until the inclusion criteria were fulfilled.

After obtaining written consent, we collected blood samples in coded tubes, containing ethylenediamine tetraacetic acid, from all resident dogs aged 6 months or older. The samples were stored in a thermal box to await processing. In the Basic Sciences Laboratory of the Federal University of Mato Grosso, Campus Rondonópolis, the samples were centrifuged at $2325 \times g$ for 10 min for plasma separation, and stored at -20°C until further analysis.

The diagnosis of CVL was achieved according to the guidelines of the Brazilian Ministry of Health, which recommends (1) screening by rapid chromatographic immunoassay based on the dual-path platform (TR DPP[®]) (Bio-Manguinhos/FIOCRUZ, Rio de Janeiro, RJ, Brazil), and (2) confirmation of positivity by enzyme-linked immunosorbent assay (ELISA) (Bio-Manguinhos/FIOCRUZ, Rio de Janeiro, RJ, Brazil) [36]. TR DPP[®] and ELISA assays were performed according to the manufacturer's instructions at the Analysis Laboratory of the Zoonosis Control Center of Rondonópolis and the Central Laboratory of Mato Grosso State (LACEN-MT), respectively.

All surveyed households were georeferenced on location using a handheld GPS device Garmin GPSMAP 76S (Garmin Inc., Lenexa, KS, U.S.A.); WGS84 was used as the reference *datum*. One pair of geographic coordinates was recorded for each household, regardless of the number of dogs undergoing blood collection. A household was considered positive for CVL if at least one seropositive dog was present.

Human VL: data collection

The localizations of all autochthonous human VL cases reported in the municipality between 2014 and 2016 were obtained from the Brazilian Notifiable Diseases Information System, which is coordinated by the Epidemiological Surveillance Sector of the Municipal Health Department of Rondonópolis. Relapses or cases reported in duplicate were excluded. By means of domiciliary visits, the patient's households were also geo-referenced as described above. This 3-year period was chosen because it is the time currently used by the VL Surveillance and Control Program to epidemiologically stratify the transmission areas.

Spatial analysis

Kernel density estimator

The distribution of households with CVL positive and negative animals was analyzed using the ratio of quartic function of kernel density estimation (KDE) [37], using a bandwidth of 400 m, and generating a matrix with a grid cell size of 30 m. KDE is a non-parametric interpolation based on the density of events within a given spatial vicinity. The technique estimates the intensity of occurrence of a particular event by describing the existence and location of agglomerates [38]. The density ratio between positive and negative households provided evidence of CVL hotspots.

Scan statistics

The Kulldorff's spatial scan statistic was used to identify significant local CVL spatial clusters in Rondonópolis [39]. For this purpose, SaTScan™ v. 9.3 software (National Cancer Institute, Bethesda, Maryland, U.S.A.) was used [40]. We adopted a purely spatial analysis that employed a Bernoulli probability model with rates between positive (cases) and negative households (controls). Clusters with high or low rates were considered. Significance level of clustering was 0.05, and it was calculated by means of 999 Monte Carlo replications.

Ethical aspects

This study was approved by the Ethics Committee on Animal Use of Federal University of Mato Grosso (process number: 23108.318103/2016-04) and Ethical Committee for Human Research of Júlio Müller University Hospital (CAAE number: 52023215.5.0000.5541).

Results

A total of 405 households in the urban area of the municipality of Rondonópolis were visited for data collection, and 600 animals were tested for CVL, at a ratio of 1.5 dogs per household. Of these dogs, 115 were found to be positive using both TR DPP® and ELISA, indicating an overall

CVL seroprevalence of 19.2% (95% CI: 16.1–22.3%). The percentage of seropositive animals varied widely among the SKATER-defined sampling regions, ranging from 0.0 to 35.1% (Table 1).

Of the visited households, 24.9% (101/405) (95% CI: 20.7–29.2%) had at least one seropositive dog and were classified as CVL-positive households. Exploratory analysis by KDE revealed that hotspots for CVL were concentrated in the peripheral regions of Rondonópolis city, where there were more CVL-positive households than CVL-negative households (Figure 2). Scan statistics corroborated these results by detecting a spatial cluster with significantly low CVL prevalence in the central region of the city (relative risk = 0.37; $p = 0.04$) (Figure 3). In addition, the location of the households of the 30 autochthonous human VL cases reported in Rondonópolis between 2014 and 2016 are represented in Figure 3. Notably, they were distributed almost exclusively outside the region of low CVL occurrence. Only one case was reported in the marginal region of the cluster.

Discussion

Domestic dogs constitute the main urban reservoir of *L. infantum* in the zoonotic transmission cycle of VL. Therefore, the evaluation of CVL occurrence is a topic of major interest in endemic areas [14,28,38]. To our knowledge, the present study was the first CVL serosurvey conducted in the municipality of Rondonópolis: the most important VL endemic area in the Brazilian State of Mato Grosso with nationwide relevance [4,7].

Table 1. Distribution of canine visceral leishmaniasis (CVL) by regions from the urban area of the municipality of Rondonópolis, State of Mato Grosso, Brazil (2016–2017).

Region	Sampled dogs (n)	Seropositive dogs (n)	CVL seroprevalence (%)
1	24	7	29.2
2	39	8	20.5
3	14	4	28.6
4	18	2	11.1
5	23	3	13.0
6	16	2	12.5
7	23	6	26.1
8	33	10	30.3
9	40	9	22.5
10	28	4	14.3
11	34	6	17.7
12	20	1	5.0
13	31	5	16.1
14	21	3	14.3
15	17	3	17.7
16	26	7	26.9
17	20	7	35.0
18	37	13	35.1
19	23	3	13.0
20	18	0	0.0
21	13	1	7.7
22	20	3	15.0
23	24	5	20.8
24	18	0	0.0
25	18	3	16.7
Total	600	115	19.2 (16.1–22.3) ^a

^aConfidence interval at 95%.

Figure 2. Kernel ratio density map of canine visceral leishmaniasis in the urban area of the municipality of Rondonópolis, State of Mato Grosso, Brazil (2016–2017). Source: http://servicodados.ibge.gov.br/Download/Download.ashx?u=geoftp.ibge.gov.br/organizacao_do_territorio/malhas_territoriais/malhas_de_setores_censitarios_divisoes_intramunicipais/censo_2010/setores_censitarios_shp/mt/mt_setores_censitarios.zip.

Notes: The red crosses represent households with at least one infected dog (positive households), while the green circles show households without any infected dogs (negative households) (Projection: UTM, Zone 21).

The overall prevalence of CVL (19.2%) in the present survey was higher than that previously detected in other urban areas of Brazil [13,14,41] and abroad [42,43], which indicated a high risk of VL in Rondonópolis. In addition, this may be related to the process of VL urbanization in Brazilian municipalities with more than 100,000 inhabitants that intensified in the 2000s [4]. On the other hand, the prevalence of CVL in Rondonópolis was lower than observed in Jaciara, Mato Grosso State (54.7%) [17], and Governador Valadares, Minas Gerais State (30.2%) [44]. One possible explanation for these differences may be the fact that some authors have sampled dogs from restricted areas, or from where human VL cases have been reported, which could bias the results towards a higher prevalence [16].

To avoid this detection bias and optimize the sampling process, we employed the SKATER algorithm, which determined 25 spatial regions with different socioeconomic and environmental features for random selection of animals [34]. This new way of regionalization in CVL surveys ensured that dogs from various scenarios of the city were sampled. This was particularly desirable in Rondonópolis, which has large intra-urban differences that may affect the occurrence of the infection

[22,23]. Indeed, CVL prevalence varied widely among the evaluated regions (0.0–35.1%). It is well known that, in addition to individual and microenvironmental characteristics, CVL is associated with variables that depend on favorable social, ecological, and demographic contexts [12,45,46]. The areas with lowest prevalence of the canine infection were mainly characterized by high monthly nominal income, high percentage of literate people per PPH, and low percentage of PPHs with open sewer. On the other hand, the areas with highest occurrence of CVL were marked by the predominance of PPHs with high mean number of residents, low public lighting, and poor pavement.

The subsequent spatial analysis emphasized these differences in CVL distribution. It was clear that the canine infection was concentrated in the peripheral areas of Rondonópolis, mainly because there was a low prevalence cluster in the central region of the city, as detected by scan statistics. In contrast, Teixeira-Neto et al. [14] reported that CVL was scattered throughout the entire urban network of Divinópolis, while Ursine et al. [38] detected a central distribution in Araçuaí. Both of these studies were in Minas Gerais State. Such differences are to be expected because the distribution of VL

Figure 3. Spatial cluster of low prevalence of canine visceral leishmaniasis (blue circle) detected using Kulldorff's spatial scan statistic, according to Bernoulli model, in the urban area of the municipality of Rondonópolis, State of Mato Grosso, Brazil (2016–2017). Source: http://servicodados.ibge.gov.br/Download/Download.ashx?u=geofp.ibge.gov.br/organizacao_do_territorio/malhas_territoriais/malhas_de_setores_censitarios_divisoes_intramunicipais/censo_2010/setores_censitarios_shp/mt/setores_censitarios.zip.

Notes: The red crosses represent households with at least one infected dog (positive households), while the green circles show households without any infected dogs (negative households). The yellow triangles represent the households where autochthonous cases of human visceral leishmaniasis were reported in Rondonópolis between 2014 and 2016 (Projection: UTM, Zone 21).

is not stable; it depends on the process of occupation and social organization of the geographic space, which tends to differ from one city to another [3,14,47].

In Rondonópolis, the periphery generally corresponds to areas of recent colonization, and it is bordered by pastures or green areas. These scenarios are probably a consequence of the recent accelerated urbanization process, migration waves and population growth, which led to the disorderly occupation of the natural space with formation of areas marked by poor living conditions as well as deficient sanitary and urban infrastructure [23]. Taken together, these factors probably favored the high adaptation of the vector [7], leading to the establishment of VL transmission cycles in such peripheral zones, as similarly reported in Teresina, Piauí State [48]. In addition, Costa [3] suggested that the marginalization of VL in the territory of some Brazilian cities may be related to a possible relationship between the urban transmission cycle and the surrounding sylvatic environment, which in turn requires elucidation in future investigations in Rondonópolis.

Interestingly, the absolute majority of the autochthonous human VL cases reported in the 3 years before the

present canine survey (2014–2016) were also located in the peripheral regions, out of the central low prevalence cluster for CVL. This descriptive overlap strongly suggests an association between human and canine disease in Rondonópolis, as demonstrated in other Brazilian localities [14,38,49]. In fact, it is established that CVL usually precedes the emergence of human VL cases [12], so there are no reports of human disease without the presence of infected dogs [14,22]. Corroborating this, Araújo et al. [21] detected a significant correlation between the relative risk of human VL and the number of infected dogs per inhabitant in an ecological study carried out in Belo Horizonte, Minas Gerais State, Brazil.

The present study has some limitations. Firstly, the characteristics of the neighborhoods that emerged after 2010 were not considered in the regionalization process, since they obviously were not listed in the last demographic census. Secondly, due to the lack of updated data regarding the number of inhabitants per census tracts, the present study employed the human population confirmed in 2010 for sampling procedures. Thirdly, stray dogs were not evaluated; hence, the prevalence of CVL might have been underestimated, because such animals are more exposed

to CVL risk factors [50]. On the other hand, the use of stray dogs could have biased the spatial analysis, because such animals are constantly on the move. Finally, the accuracy of the serological methods herein employed for CVL diagnosis is still controversial [51]. However, the same protocol is currently recommended as an official dog-screening measure in the VL Surveillance and Control Program of the Brazilian Ministry of Health [8,36].

Despite these limitations, the data clearly demonstrated a high CVL burden in the urban area of Rondonópolis. In addition, our sampling model, which was based on regionalization by SKATER, could be easily applied to other urban areas to assess canine infection. Considering that the existence of positive dogs is a great indicator in monitoring VL transmission to humans [52], this study may be useful to authorities in defining priority areas for the implementation and optimization of actions aiming at disease control. Such control measures should be focus on integrated programs for the improvement of urban infrastructure, environmental management, vector and reservoir control, and health education [20,38,53]. Finally, future studies addressing human cases, VL risk factors, sandflies fauna and wild host species should be conducted to clarify the dynamics of VL transmission in Rondonópolis.

Conclusion

This study demonstrated, for the first time, a high prevalence of CVL in domestic dogs from diverse socioeconomic and environmental contexts in the urban area of the Rondonópolis municipality. Spatial analysis revealed a peripheral distribution of the canine infection, especially in areas with confirmed human VL cases.

Acknowledgments

The authors are grateful to the Municipal Health Department of Rondonópolis, especially the Environmental Surveillance Division (Zoonosis Control Center) and Epidemiological Surveillance Sector, for their cooperation, dedication, and support in data collection, as well as for providing the digital georeferenced database of neighborhoods.

Disclosure of interest

The authors report no conflicts of interest.

ORCID

Cor Jesus Fernandes Fontes <http://orcid.org/0000-0003-2756-0588>

References

- [1] Conti RV, Moura Lane VF, Montebello L, et al. Visceral leishmaniasis epidemiologic evolution in timeframes, based on demographic changes and scientific achievements in Brazil. *J Vector Borne Dis*. 2016;53(2):99–104.
- [2] Harhay MO, Olliaro PL, Costa DL, et al. Urban parasitology: visceral leishmaniasis in Brazil. *Trends Parasitol*. 2011;27(9):403–9.
- [3] Costa CHN. Characterization and speculations on the urbanization of visceral leishmaniasis in Brazil. *Cad Saude Publica*. 2008;24(12):2959–63.
- [4] Werneck GL. Visceral leishmaniasis in Brazil: rationale and concerns related to reservoir control. *Rev Saude Publica*. 2014;48(5):851–5.
- [5] Brasil. Ministério da Saúde. Leishmaniose visceral – Situação epidemiológica. 2016 [cited 2017 Aug 17]. Available from: <http://portalsaude.saude.gov.br/index.php/o-ministerio/principal/leia-mais-o-ministerio/726-secretaria-svs/vigilancia-de-a-a-z/leishmaniose-visceral-lv/11334-situacao-epidemiologica-dados>.
- [6] Mestre GLC, Fontes CJF. A expansão da epidemia da leishmaniose visceral no Estado de Mato Grosso, 1998–2005. *Rev Soc Bras Med Trop*. 2007;40(1):42–8.
- [7] Guimarães AGF, Alves GBM, Pessoa AM, et al. Spatial analysis of visceral leishmaniasis in the municipality of Rondonópolis, in the Brazilian State of Mato Grosso, from 2003 to 2012: human, canine and vector distribution in areas of disease transmission. *Rev Soc Bras Med Trop*. 2015;48(3):291–300.
- [8] Brasil. Ministério da Saúde. Manual de Vigilância e controle de Leishmaniose Visceral. 2006 [cited 2017 Oct 30]. Available from: http://bvms.saude.gov.br/bvs/publicacoes/manual_vigilancia_controle_leishmaniose_viscer.pdf.
- [9] Brasil. Ministério da Saúde. DATASUS. Leishmaniose visceral – Casos notificados no Sistema de Informação de Agravos de Notificação – Mato Grosso. 2016 [cited 2017 Jul 30]. Available from: <http://tabnet.datasus.gov.br/cgi/deftohtm.exe?sinanet/cnv/leishvmt.def>.
- [10] Quinnell RJ, Courtenay O. Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. *Parasitol*. 2009;136:1915–1934.
- [11] Costa AP, Costa FB, Soares HS, et al. Environmental factors and ecosystems associated with canine visceral leishmaniasis in Northeastern Brazil. *Vector Borne Zoonotic Dis*. 2015;15(12):765–74.
- [12] Werneck GL, Costa CHN, Walker AM, et al. Multilevel modelling of the incidence of visceral leishmaniasis in Teresina, Brazil. *Epidemiol Infect*. 2007;135:195–201.
- [13] Oliveira CDL, Assunção RM, Reis IA, et al. Spatial distribution of human and canine visceral leishmaniasis in Belo Horizonte, Minas Gerais State, Brazil, 1994–1997. *Cad Saude Publica*. 2001;17(5):1231–9.
- [14] Teixeira-Neto RG, Silva ES, Nascimento RA, et al. Canine visceral leishmaniasis in an urban setting of Southeastern Brazil: an ecological study involving spatial analysis. *Parasit Vectors*. 2014;7:485.
- [15] Coura-Vital W, Marques MJ, Veloso VM, et al. Prevalence and factors associated with leishmania infantum infection of dogs from an urban area of Brazil as identified by molecular methods. *PLoS Negl Trop Dis*. 2011;5(8):1291.
- [16] Almeida ABPF, Sousa VRF, Cruz FACS, et al. Canine visceral leishmaniasis: seroprevalence and risk factors in Cuiabá, Mato Grosso, Brazil. *Rev Bras Parasitol Vet*. 2012;21(4):359–65.
- [17] Lopes PM, Boa Sorte EC, Gasparetto ND, et al. Seroprevalence and risk factors associated with visceral leishmaniasis in dogs in Jaciara, State of Mato Grosso. *Rev Soc Bras Med Trop*. 2014;47(6):791–5.
- [18] Pimentel DS, Ramos RAN, Santana MA, et al. Prevalence of zoonotic visceral leishmaniasis in dogs in an endemic area of Brazil. *Rev Soc Bras Med Trop*. 2015;48(4):491–3.
- [19] Barbosa DS, Belo VS, Rangel MES, et al. Spatial analysis for identification of priority areas for surveillance and control

- in a visceral leishmaniasis endemic area in Brazil. *Acta Trop.* **2014**;131:56–62.
- [20] World Health Organization (WHO). Control of leishmaniasis. Report of a meeting of the WHO Expert Committee on the Control of Leishmaniasis. Geneva: World Health Organization; **2010**.
- [21] Araújo VEM, Pinheiro LC, Almeida MCM, et al. Relative risk of visceral leishmaniasis in Brazil: a spatial analysis in urban area. *PLoS Negl Trop Dis.* **2013**;7(11):e2540.
- [22] Oliveira CDL, Morais MHF, Machado-Coelho GLL. Visceral leishmaniasis in large Brazilian cities: challenges for control. *Cad Saude Publica.* **2008**;24(12):2953–8.
- [23] Luz JGG, Carvalho AG, Marques AP, et al. Intestinal parasitic infections and associated risk factors in preschoolers from different urban settings in Central-Western Brazil. *Asian Pac J Trop Dis.* **2017**;7(7):405–10.
- [24] Kirby RS, Delmelle E, Eberth JM. Advances in spatial epidemiology and geographic information systems. *Ann Epidemiol.* **2017**;27:1–9.
- [25] Melchior LAK, Brilhante AF, Chiaravalloti-Neto F. Spatial and temporal distribution of American cutaneous leishmaniasis in Acre state. Brazil. *Infect Dis Poverty.* **2017**;6:99.
- [26] Carneiro DDMT, Bavia ME, Rocha WJSF, et al. Application of spatio-temporal scan statistics for the detection of areas with increased risk for American visceral leishmaniasis in the state of Bahia, Brazil. *Geospat Health.* **2007**;2(1):113–126.
- [27] Borges LFNM, Lopes EGP, Freitas ACP, et al. Prevalência e distribuição espacial da leishmaniose visceral em cães do município de Juatuba, Minas Gerais, Brasil. *Cienc Rural.* **2014**;44(2):352–7.
- [28] Dias AFLR, Almeida ABPF, Cruz FACS, et al. Seroprevalence and spatial analysis of canine visceral leishmaniasis in the Pantanal region, Mato Grosso State, Brazil. *J Zoonotic Dis Public Health.* **2017**;1(1):1–7.
- [29] Instituto Brasileiro de Geografia e Estatística (IBGE). Cidades@ – Rondonópolis; **2017** [cited 2017 Sep 29]. Available from: <http://cod.ibge.gov.br/2VD3L>.
- [30] Instituto Brasileiro de Geografia e Estatística (IBGE). Censo Demográfico; **2010** [cited 2016 Jul 27]. Available from: <https://censo2010.ibge.gov.br/>.
- [31] Savani ESM, Schimonsky BV, Camargo MCGO. D'auria SRN. Vigilância de leishmaniose visceral americana em cães de área não endêmica, São Paulo. *Rev Saude Publica.* **2003**;37(2):260–2.
- [32] Luiz RR, Magnanini MMF. A lógica da determinação do tamanho da amostra em investigações epidemiológicas. *Cad Saude Colet.* **2000**;8(2):9–28.
- [33] Lage JP, Assunção RM, Reis EA. A minimal spanning tree algorithm applied to spatial cluster analysis. *Electron Notes Discrete Math.* **2001**;7:162–5.
- [34] Assunção RM, Neves MC, Câmara G, et al. Efficient regionalization techniques for socio-economic geographical units using minimum spanning trees. *Int J Geogr Inf Sci.* **2006**;20(7):797–811.
- [35] QGIS Development Team. Quantum GIS geographic information system. Open source geospatial foundation project, v. 2.10 Pisa; **2012** [cited 01 Aug 2016]. Available from: <http://qgis.org>.
- [36] Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância das Doenças Transmissíveis. Nota Técnica Conjunta nº 1, de Esclarecimentos sobre substituição do protocolo diagnóstico da leishmaniose visceral canina (LVC). Brasília: Coordenação Geral de Doenças Transmissíveis/Coordenação Geral de Laboratórios de Saúde Pública; **2011**.
- [37] Gatrell AC, Bailey TC, Diggle PJ, et al. Spatial point pattern analysis and its application in geographical epidemiology. *Trans Inst Br Geogr.* **1996**;21:256–274.
- [38] Ursine RL, Dias JVL, Morais HA, et al. Human and canine visceral leishmaniasis in an emerging focus in Araçuaí, Minas Gerais: spatial distribution and socio-environmental factors. *Mem Inst Oswaldo Cruz.* **2016**;111(8):505–11.
- [39] Kulldorff M, Nagarwalla N. Spatial disease clusters: Detection and inference. *Stat Med.* **1995**;14(8):799–810.
- [40] Kulldorff M. A spatial scan statistic. *Commun Stat Theory Methods.* **1997**;26(6):1481–96.
- [41] Brito FG, Langoni H, Silva RC, et al. Canine visceral leishmaniasis in the Northeast Region of Brazil. *J Venom Anim Toxins Incl Trop Dis.* **2016**;22:15.
- [42] Ferroglio E, Maroli M, Gastaldo S, et al. Canine leishmaniasis. Italy. *Emerg Infect Dis.* **2005**;11(10):1618–20.
- [43] Cortes S, Vaz Y, Neves R, et al. Risk factors for canine leishmaniasis in an endemic Mediterranean region. *Vet Parasitol.* **2012**;189:189–196.
- [44] Barata RA, Peixoto JC, Tanure A, et al. Epidemiology of visceral leishmaniasis in a reemerging focus of intense transmission in Minas Gerais state. Brazil. *Biomed Res Int.* **2013**;2013:405083.
- [45] Silva AVM, Magalhães MAFM, Brazil RP, et al. Ecological study and risk mapping of leishmaniasis in an endemic area of Brazil based on a geographical information systems approach. *Geospat Health.* **2011**;6(1):33–40.
- [46] Reguera RM, Morán M, Pérez-Pertejo Y, et al. Current status on prevention and treatment of canine leishmaniasis. *Vet Parasitol.* **2016**;227:98–114.
- [47] Neto JC, Werneck GL, Costa CHN. Factors associated with the incidence of urban visceral leishmaniasis: an ecological study in Teresina, Piauí State, Brazil. *Cad Saude Publica.* **2009**;25(7):1543–51.
- [48] Werneck GL, Costa CHN, Walker AM, et al. The urban spread of visceral leishmaniasis: clues from spatial analysis. *Epidemiology.* **2002**;13(3):364–7.
- [49] Margonari C, Freitas CR, Ribeiro RC, et al. Epidemiology of visceral leishmaniasis through spatial analysis, in Belo Horizonte municipality, state of Minas Gerais, Brazil. *Mem Inst Oswaldo Cruz.* **2006**;101(1):31–8.
- [50] Fraga DBM, Solcà MS, Silva VMG, et al. Temporal distribution of positive results of tests for detecting *Leishmania* infection in stray dogs of an endemic area of visceral leishmaniasis in the Brazilian tropics: a 13 years survey and association with human disease. *Vet Parasitol.* **2012**;190:591–4.
- [51] Peixoto HM, Oliveira MRF, Romero GAS. Serological diagnosis of canine visceral leishmaniasis in Brazil: systematic review and meta-analysis. *Trop Med Int Health.* **2015**;20(3):334–52.
- [52] Belo VS, Struchiner CJ, Werneck GL, et al. A systematic review and meta-analysis of the factors associated with *Leishmania infantum* infection in dogs in Brazil. *Vet Parasitol.* **2013**;195:1–13.
- [53] Romero GAS, Boelaert M. Control of visceral leishmaniasis in Latin America – a systematic review. *PLoS Negl Trop Dis.* **2010**;4(1):e584.