Journal of Veterinary Science and Public Health

J. Vet. Sc. Public Health, v. 8, n. 2, p. 096-103, 2021

SPATIAL ANALYSIS OF THE OCCURRENCE OF CANINE VISCERAL LEISHMANIASIS CASES USING DIFFERENT PARAMETERS FOR INFECTION CASE DEFINITION

Ana Izabel Passarella Teixeira[1], Debora Marcolino Silva[1], Gustavo Adolfo Sierra Romero[1]

[1] Núcleo de Medicina Tropical, Universidade de Brasília, Brasília, Brasil
Autor para correspondência: ana.teixeira@unb.br
DOI: 10.4025/revcivet.v8i2.54921

ABSTRACT

The application of spatial analysis tools for the study of canine visceral leishmaniasis can be influenced by the method used to detect dogs with leishmaniasis due to a large variety of available tests with distinct accuracy characteristics. Thus, a spatial comparison using two criteria of CVL infection/disease will be presented. The first has a combination of parasitological, molecular and serological techniques. The second uses serological tests recommended by the Ministry of Health in Brazil. It was observed that, indeed, the use of different diagnostic tests can have an important impact on spatial analyses.

Keywords: Visceral leishmaniasis. Dogs. Spatial analysis.

INTRODUCTION

Spatial analysis has been used in the study of infectious diseases with the aim to understand their geographic patterns of morbimortality, as well as to identify risk areas and consequently potentialize actions of epidemiological surveillance (WERNECK et al. 2012; SANTOS and SOUSA 2007).

The concepts of spatial analysis applied to the studies of both canine and human visceral leishmaniasis (HVL) allowed to identify the spatial dependency relationship between human and canine cases, to compare the vector geographic distribution over

areas with higher occurrences of cases, as well as areas of higher morbidity (OLIVEIRA et al. 2001; WERNECK et al. 2012; COSTA et al. 2018).

However, in studies about spatial distribution of canine visceral leishmaniasis (CVL) cases, special attention must be paid regarding the criteria used to define each case. That is because results can be directly influenced by the method used to diagnose dogs. There is not yet a consensus on which method of canine infection diagnosis would be the best, especially in cases of asymptomatic dogs, as there is a diversity of diagnostic methods with varying accuracy (PEIXOTO et al. 2015; DIAS et al. 2018; TRAVI et al. 2018).

Currently, the Brazilian Ministry of Health recommended CVL diagnosis protocol involves serial use of two serological tests, the TR-DPP and the EIE-CVL. However, these tests have been criticized regarding its accuracy characteristics. For this reason, the development of new tests is still a higher priority (LOPES et al. 2017; TRAVI et al. 2018).

Therefore, it is presented here a spatial comparison integrated into a cross-sectional study developed with the aim to identify factors associated to canine infection, which brought forth differences depending on the criteria used in the infection case definition. The first one with a combination of parasitological, molecular and serological techniques and the second one with serological tests recommended by the Brazilian Ministry of Health (MoH).

METHODOLOGY

A descriptive study was conducted in the administrative region number 31 (Fercal) located in the Federal District of Brazil, admittedly endemic for CVL and HVL (CARRANZA-TAMAYO et al. 2010; BRASIL, 2013).

The study was carried out based on the test results for the detection of CVL infection evaluated in a cross-sectional study. Based on the size of human population, the canine population was estimated at 342 dogs (BOGEL et al. 1990; BRASIL, 2013). Also, based on previous work (SILVA et al. 2017), the infection frequency was estimated at 10%. Other parameters used were margin of error of 5%, confidence interval of 95%, effect of design 1.5 [correction on the basis of the possibility of cluster effect, according to Raggio and Magnanini (2000), estimated loss rate of 10%, resulting in a sample size of 270 dogs. All sampling calculation was made on the software *Stat Calc* from Epi Info, version 7.2.0.1.

The collection was carried out systematically, in a way that the entire Fercal territory was covered. Material was collected from dogs from one house per block as previously described (TEIXEIRA et al. 2018). Blood was collected via peripheral venipuncture and bone marrow was collected in the sternum bone while sedated with ketamine (8 mg/kg) associated with acepromazine (0.1 mg/kg). The owners signed a term of free and informed consent and allowed their animals' blood and bone marrow collection. After they accepted to participate in the project, the location of the residence was identified through coordinates with the application *Locus Map* version 2.1.3 (available for free in the *Google Play Store*). All these protocols were approved by the Animal Use Ethics Committee at the University of Brasília, and numbered UnBDoc 11253/2015.

The samples were submitted to an ensemble of parasitological tests (parasitological staining with Giemsa and culture), serological tests (TR-DPP, EIE-CVL and ELISA rK39) and molecular tests (screening with PCR with kDNA target and confirmatory testing with PCR ITS1 target), according to the protocols described by Teixeira et al. (2018).

From the laboratory results were established two case definition criteria. The first case definition criterion (Criterion 1) considered infected the dog that showed at least one positive result in the performed diagnostic tests, that were: parasitological staining, culture, sequential TR-DPP and EIE-CVL, sequential TR-DPP and ELISA rK39 and molecular tests (screening with PCR kDNA and confirmatory testing with PCR ITS1), and not infected all dogs that did not show any positive results in the tests described above. The second case definition criterion (Criterion 2) considered infected the dog that showed a positive result according to the sequential protocol TR-DPP and EIE-CVL recommended by the MoH, and not infected the dogs that did not show any positive results in the serological sequential protocol described above.

With the cartographic base, the available images and the data collected in field were generated maps with the aid of the software ArcGIS 10.3.1. The cartographic base used and produced in the research was processed in the Universal Transverse Mercator (UTM) projection system, geodetic datum SIRGAS 2000, meridian of reference 51°W (Zone 23 S).

For spatial distribution analysis of dogs, according to the infection criteria previously established, the coordinates marked in the collection point with the GPS (Global Positioning System) from *Locus Map* Version 2.1.3 (available for free in the

Google Play Store) were organized in a table in the Excel software in which the data of each criteria have been attributed. They were then imported into the software ArcGIS 10.3.1. and with the aid of the *Kernel Density* tool a density map was generated for each of the criteria. They were carried out directed to the Fercal urban area, since the larger is occupied by an environmental protection area (APA Cafuringa), for each of the groups.

RESULTS AND DISCUSSION

The studied region Fercal and the limits of the environmental protection area are presented in the Figure 1.

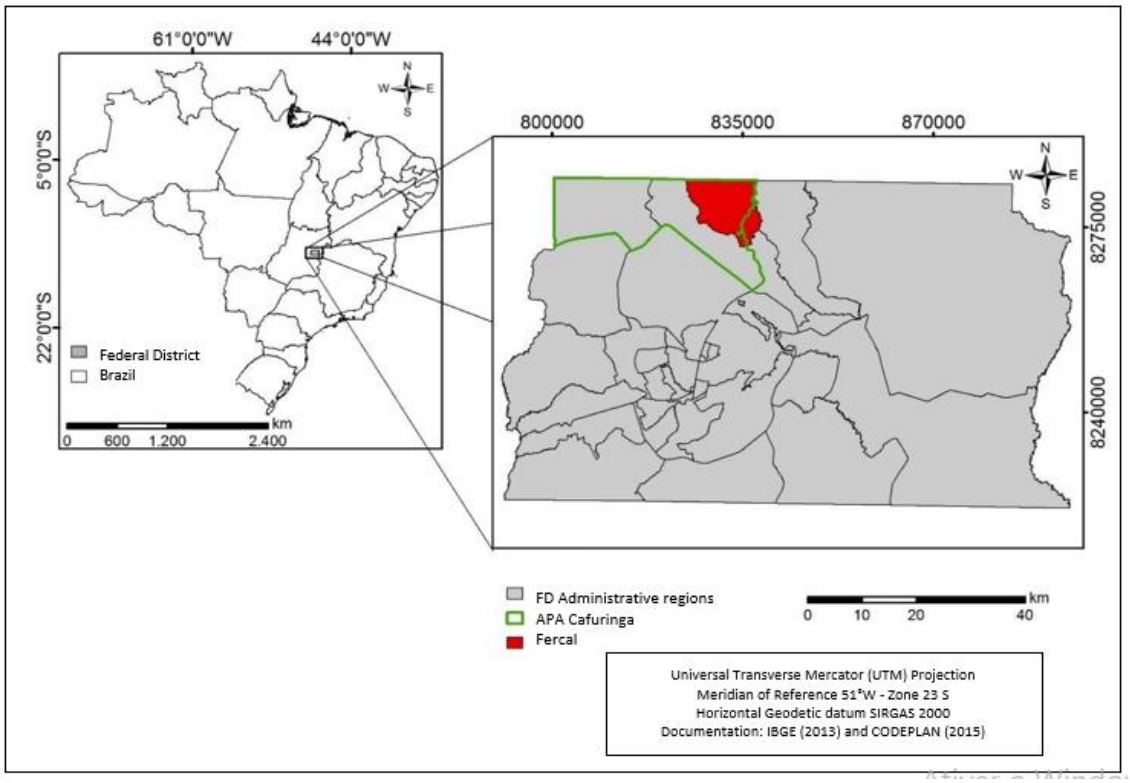

Figure I – Location of Fercal Administrative Region, Federal District, Brazil

The estimated prevalence of infected dogs was 26.25% (95%CI: 20.05 to 33.57) and 9.38% (95%CI: 5.76 to 14.89) using criterion 1 and criterion 2, respectively.

Density maps with both of the established criteria are shown in Figure 2.

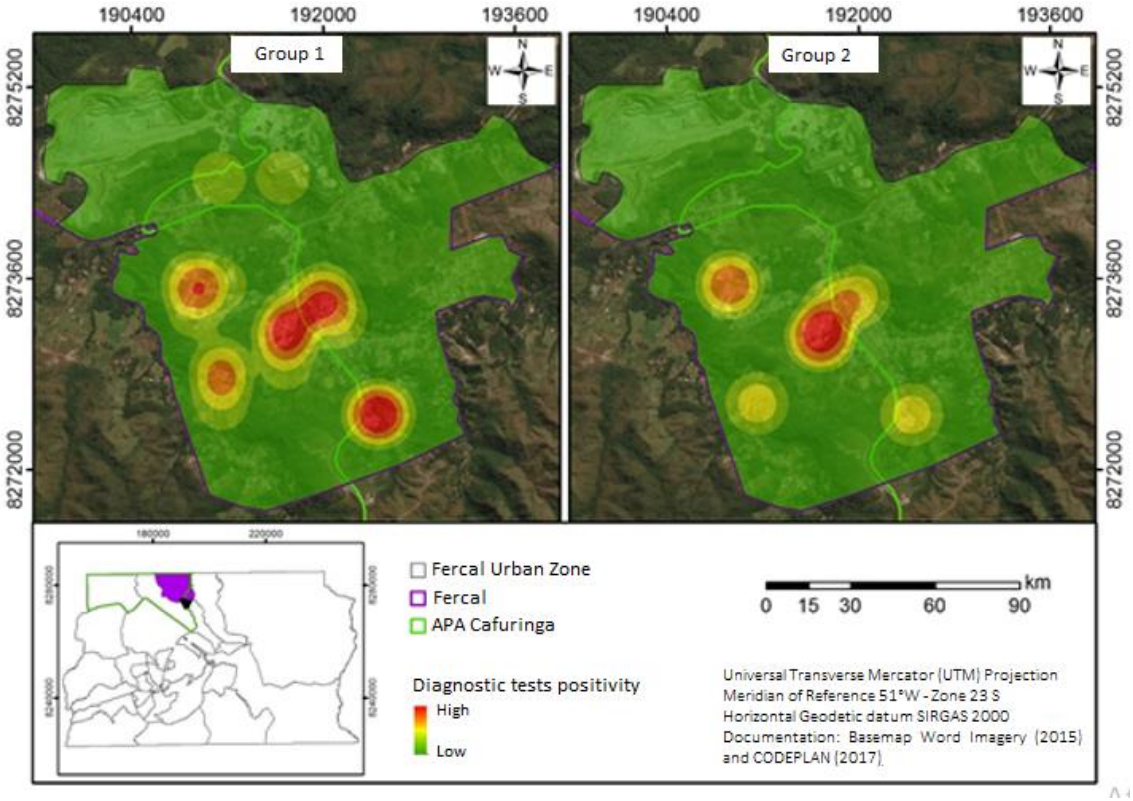

Figure II – Heat Maps with the hot-spots according to both of the criteria to detect dogs with CVL. Federal district, Brazil

The use of maps in epidemiologic investigations can be extremely relevant for the identification of priority intervention areas in order to control a disease (WERNECK et al. 2002; COSTA et al. 2018). This description shows the difference between potential areas of interest for the application of intervention for CVL control based on the results of different diagnostic approaches applied to the canine population. The map built using the case definition criteria recommended by the MoH (Group 2) brings an information that could offer inadequate orientation to the decision makers responsible for the control program if the images were used to decide which locations should be applied the currently available interventions. The map that uses the more complete definition (Group 1) can better detect the endemic extension and intensity, offering better information for the decision making. Thus, this work draws attention to the vulnerability that the analysis may suffer if it incorporates the location variables of the infection cases in the CVL scenario, due to the difficulties that transcend the spatial analysis method and go back to the absence of adequate methods for the accurate detection of canine infection (BARBOSA et al. 2014; PEIXOTO et al. 2015; LOPES et al. 2017; TEIXEIRA et al. 2018). It is concluded that the use of different diagnostic tests can have an important impact on the spatial distribution data of the CVL cases and that the comparative analysis

between several studies that address spatial parameters relating to this problem should take into consideration the diagnostic techniques used. It is also of special relevance to consider also the diagnostic aspects when thematic maps of time series are compared, registering the variation in the diagnostic criteria of canine infection definition over time.

Ethical approval:

All protocols used were approved by the Animal Use Ethics Committee at the University of Brasília, and numbered UnBDoc 11253/2015

Fundings Information:

The study was funded by the Federal District Research Foundation (FAPDF) - process 193.000.867/2015 and the National Council for Scientific and Technological Development (CNPq) process 142247/2015-4

Author's' contribuition:

GASR, and AIPT provided an intellectual framework for the preparation of this study; AIPT and DMS collected the biological samples and conducted the diagnostic tests (PCR and parasitologycal). All authors contributed to data analysis and drafting the manuscript, and all have read and approved the final version

Acknowledgments:

We thank Mr. José Barbosa Bezerra for his assistance during field work as well as the DIVAL laboratory staff responsible for the execution of the serology assays

REFERENCES

BARBOSA, D.S.; BELO, V.S.; RANGEL, M.E.S.; WERNECK, G.L.; Spatial analysis for identification of priority areas for surveillance and control in a visceral leishmaniasis endemic area in Brazil. **Acta Tropical** v.131, p.56-62 2014

BÖGEL, K.; FRUCHT, K.; DRYSDALE, G.; REMFRY, J.; World Health Organization. Veterinary Public Health Unit. et al. (1990). Guidelines for dog population management / preparation; editing co-ordinated by Frucht K, Drysdale G and Remfry J Geneva: **World Health Organization**. Available from:

http://www.who.int/iris/handle/10665/61417 Accessed in: 03 Feb. 2014

BRASIL, DIVAL/GEVAZ/GDF 2013. Informativo Ambiental Das Leishmanioses No DF. Brasília. Available from: http://www.saude.df.gov.br/images/Informativos/DIVAL/Informativo_Ambiental__2013_Leishmanioses_EM_PDF.pdf Accessed in: 8 Mar. 2014.

CARRANZA-TAMAYO, C.O.; CARVALHO, M.S.; BREDT, A.; BOFIL, M.I.; RODRIGUES, R.M.; Autochthonous visceral leishmaniasis in Brasilia, Federal District, Brazil. **Revista da Sociedade Brasileira de Medicina Tropical**, v.43, n.4, p. 396-9, 2010

COSTA, D.N.C.C.; BLANGIARDO, M.; RODAS, L.A.C.; NUNES, C.M.; HIRAMOTO, R.M.; TOLEZANO, J.E.; Canine visceral leishmaniasis in Araçatuba, state of São Paulo, Brazil, and its relationship with characteristics of dogs and their owners: a cross-sectional and spatial analysis using a geostatistical approach. **BMC Vet Res**. v.14, n.229, p.1-13. 2018

DIAS, R.C.F.; THOMAZ-SOCCOL, V.; PASQUALI, A.K.S.; ALBAN, S.M.; FENDRICH, R.C.; POZZOLO, E.M.; Variables associated with the prevalence of anti-Leishmania spp. antibodies in dogs on the tri-border of Foz do Iguaçu, Paraná, Brazil. **Revista Brasileira de Parasitologia Veterinária**. v.27, n.3, p.338-47; 2018

LOPES, E.G.; SEVÁ, A.P.; FERREIRA, F.; NUNES, C.M.; KEID, L.B.; Serological and molecular diagnostic tests for canine visceral leishmaniasis in Brazilian endemic area: one out of five seronegative dogs are infected. **Epidemiology Infection**. v.145, n.12, p.2436-44, 2017

OLIVEIRA, C.D.; ASSUNÇÃO, R.M.; REIS, I.A.; PROIETTI, F.A.; Spatial distribution of human and canine visceral leishmaniasis in Belo Horizonte, Minas Gerais State, Brasil, 1994-1997. **Caderno de Saude Publica**. v.17, n.5, p.1231-9 2001

PEIXOTO, H.M.; OLIVEIRA, M.R.F.; ROMERO, G.A.S.; Serological diagnosis of canine visceral leishmaniasis in Brazil: systematic review and meta-analysis. **Tropical Medicine Int Health**. v.20, n.3, p.334-52, 2015

RAGGIO, L.R.; MAGNANINI, M.M.F.; A lógica da determinação do tamanho da amostra em investigações epidemiológicas. **Cadernos de Saude Coletiva**. v.8, n.2, p.9-28; 2000

SANTOS, S.M.; SOUZA, W.V.; **Introdução à estatística espacial para**

a saúde pública. Brasília: Ministério da Saúde/Fundação Oswaldo Cruz; 2007

SILVA, G.J.; SILVA, E.T.; COSTA, G.R.T.; SANTOS, I.B.; Vigilância da leishmaniose visceral no Distrito Federal: aspectos organizacionais, situação epidemiológica e medidas intersetoriais. **Comunicação em Ciências Saúde**. v.28, n.2, p.149-155, 2017

TEIXEIRA, A.I.P.; SILVA, D.M.; VITAL, T.; NITZ, N.; CARVALHO, B.C.; HECHT, M.; Improving the reference standard for the diagnosis of canine visceral leishmaniasis: a challenge for current and future tests. **Memórias Instituto Oswaldo Cruz.** v.114, e180452, 2018

TRAVI, B.L.; CORDEIRO-DA-SILVA, A.; DANTAS-TORRES, F.; MIRÓ, G.; Canine visceral leishmaniasis: Diagnosis and management of the reservoir living among us. **PLoS Negleted Tropical Disease**. v.12, n.1, e0006082 2018

WERNECK, G.L.; COSTA, C.H.; WALKER, A.M.; DAVID, J.R.; WAND, M.; MAGUIRE, J.H.; The urban spread of visceral leishmaniasis: clues from spatial analysis. **Epidemiology** v.13, n.3, p.364-367, 2002