

Leishmania infantum surveillance on dogs from Primavera do Leste, Mato Grosso state, Brazil

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Sandflies,
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Summary

The objective of this research was to determine the seroprevalence of *Leishmania infantum* in dogs in the city of Primavera do Leste, Mato Grosso, Brazil. A total of 109 serological samples from dogs were analyzed, collected between August 2021 to March 2022, using rapid immunochromatographic test, TR DPP® (LVC BioManguinhos), and Immunoenzymatic Assay (ELISA), EIE-Canine Visceral Leishmaniasis (Bio-Manguinhos®, Fiocruz). IgG anti-*Leishmania infantum* antibodies were detected 4.6% (5/109) by DPP test. Regardless of results, all samples were submitted to ELISA test, of those 0.9% (1/109) was positive, the same having also been positive for the DPP, from asymptomatic, adult dog domiciled in the urban area. This was the first autochthonous report of CVL in the municipality. Since Visceral leishmaniasis caused by *L. infantum* is a disease of *One Health* concern the results obtained are essential to fill the epidemiological gap of in the region. Furthermore, the health authorities should implement surveillance actions in order to monitoring of dogs, as well as survey of sand flies, and health education programs to stimulate the prevention of the disease.

The canine visceral leishmaniasis (CVL) is an anthroponotic disease caused by protozoa, mandatory intracellular parasites, belonging to the Kinetoplastid, Trypanosomatidae Family, from the *Leishmania* Genus (Brasil 2014), by having the *Leishmania infantum* as the main etymological agent of the CVL (Dantas-Torres 2009).

In South America, the parasite is transmitted through the blood meal made by vectors called sandflies, belonging to the *Lutzomyia* genus, having as the main involved species *Lutzomyia longipalpis* (Dantas-Torres 2009), popularly known in Brazil as straw mosquito, *birigui* or *tatuquira* (Brasil 2014).

At Brazil, the CVL possess bigger prevalence in

the Northeast Brazilian region (Bavia *et al.* 2005), although the occurrence of the disease has been reported in other regions of the country (Aguiar *et al.* 2010; Almeida *et al.* 2012; Bianchi *et al.* 2016).

The sand fly *L. longipalpis* was originally found in forest areas, participating in the primary cycle of parasite transmission. However, through environmental changes and human actions, this sand fly has progressively adapted to rural and urban environments, the latter with remarkable adaptation by the vector, and can be found in different areas, such as the outskirts of large centers, in the peridomicile, in chicken coops, pigsty, kennel barn, among other environments and also in the

home (Brasil 2014). This species has already been detected in the state of Mato Grosso (Missawa and Lima 2006), in addition to *L. cruzi* (Missawa *et al.* 2011).

The state of Mato Grosso stands out for its high agricultural production. However, the degradation of ecosystems for greater implementation of arable areas should be a point of alert. According to INPE (INPE 2022), Mato Grosso is one of the frontiers of the Amazon with the greatest deforestation activity in recent years.

In the same sense, Primavera do Leste, a municipality that is only 36 years old, is one of the largest agricultural producers in Brazil. In 2020, corn and soybean production reached 660,000 t and 894,360 t, respectively (IBGE 2020). In this way, stimulated by a strong economy and high job offer, the movement of people and their animals is constant, which, many times, end up occupying peri-urban areas of the city, places that are closer to forest areas.

Cases of human leishmaniasis occur in Primavera do Leste, and in neighboring municipalities such as Poxoréu (Mestre and Fontes 2007). In addition, deaths from leishmaniasis in the region were recently reported, in the municipality of Rondonópolis, located 130km from Primavera do Leste (G1 2023).

Given the characteristics of the municipality, the dynamics of the disease and the risk factors, the present study aimed to determine the seroprevalence of *Leishmania infantum* infection in dogs in the city of Primavera do Leste, Mato Grosso, Brazil.

The study was carried out in the state of Mato Grosso, municipality of Primavera do Leste, located 235 km from the capital Cuiabá, at latitude 15°33'32" south and longitude 54°17'46" west, at an altitude of 636 meters, composed of the Cerrado biome in 100% of its territory. Primavera do Leste has Paranatinga, Santo Antônio do Leste, Poxoréu, Dom Aquino, Campo Verde, Planalto da Serra and Nova Brasilândia as bordering municipalities (Figure 1)

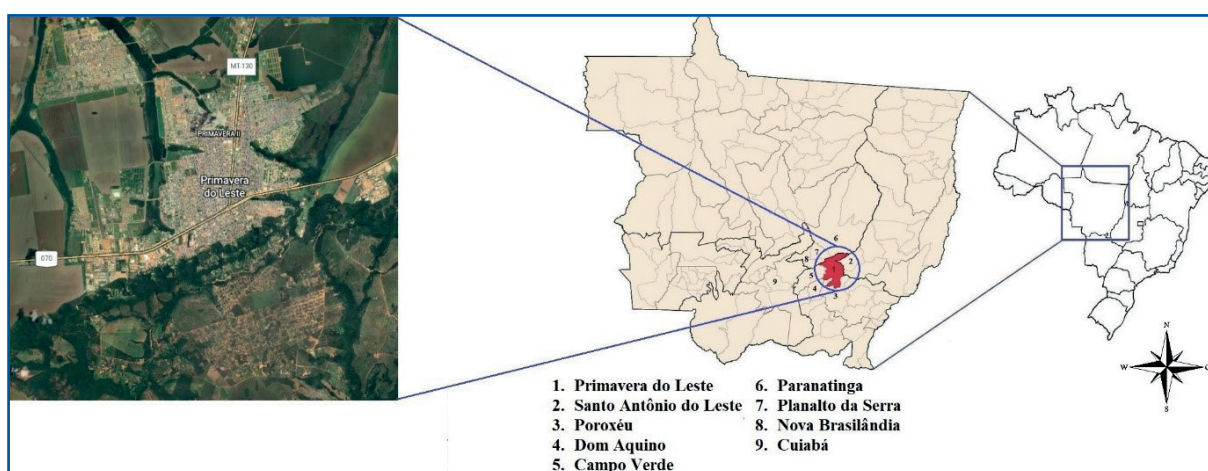


Figure 1. Geographic position of Primavera do Leste, Mato Grosso, Brazil.

Sampling was based on rabies vaccination campaigns carried out in the municipality, considering an approximate population of 8,000 animals (Primavera do Leste, 2018). The sample size calculation was performed considering a prevalence of 7.8% for CVL (Azevedo *et al.* 2008), 5% statistical error and a confidence level of 95%, resulting in a minimum sampling of 109 samples.

From August 2021 to March 2022, blood samples were collected from animals, domiciled, sent by their tutors or guardians for consultations or vaccination at veterinary clinics in the city (n=46). In addition, blood samples were also collected from animals coming from shelters in the city (n=63), with the cutoff for the election of those shelters, those that had at least 10 animals. Animals with a

history of vaccination for leishmaniasis and under 3 months of age, as well as animals residing outside the municipality or accompanied by a person under 18 years of age, were excluded.

A volume of 5mL was collected by venipuncture, using appropriate sterile and disposable materials. The tubes with biological material were centrifuged at 3500x g for 10 minutes.

The DualPathPlatform-TRDPP-LVC, BioManguinhos® (Fiocruz) and ELISA - EIE-LVC kit, BioManguinhos® (Fiocruz) were used as diagnostic method in the present study.

The DPP® is a dual-platform immunochromatographic test, with a combination of protein A conjugated with colloidal gold particles and specific *Leishmania*

antigens, capable of recognizing anti-*Leishmania* antibodies in the analyzed sample (Grimaldi Jr et al. 2012). The methodology used was based on the manufacturer's recommendations.

The ELISA test consists of the reaction of antibodies present in sera with soluble and purified *Leishmania* antigens obtained from *in vitro* culture. This antigen is adsorbed onto microplates and diluted sera are added later. The presence of specific antibodies in the serum will result in binding to the antigens (Brasil 2006). The methodology used was based on the manufacturer's recommendations. In the present study, the positivity criterion was to be reactive in both techniques.

Data were tabulated in Microsoft Excel® spreadsheets for verification and organization and analyzed using IBM SPSS Statistics 28.0.1® and NCSS® (NCSS statistical software, Kaysville, UT, USA) software.

In the DPP test, 5/109 (4.6%) samples were positive for anti-*L. infantum* antibodies. All samples were submitted to the ELISA test, in which 1/109 (0.9%) positive sample was obtained, resulting in a prevalence of 0.92% (Table I).

The positive sample came from an adult, asymptomatic dog, domiciled in the urban area, autochthonous, male, of the Siberian Husky breed.

Table I. Results of the DPP® and ELISA serological methods, performed on 109 dogs from the city of Primavera do Leste, Mato Grosso, Brazil, in 2022. A.F. – Absolute Frequency; R.F. – Relative Frequency.

DPP	ELISA					
	Negative		Positive		Total	
	A.F.	R.F.	A.F.	R.F.	A.F.	R.F.
Negative	104	95,41%	0	0%	104	95,41%
Positive	4	3,67%	1	0,92%	5	4,59%
Total	108	99,08%	1	0,92%	109	100%

The results obtained in the present study are the first record of *Leishmania infantum* infection in dogs in the municipality of Primavera do Leste, MT, Brazil, one of the main agricultural regions of the country. The prevalence obtained can be explained by the absence of symptomatic animals. The compartmentalization of territory between the municipalities of Primavera do Leste and Poxoréu may contribute to the spread of the parasite in the region, since the neighboring municipality is endemic for leishmaniasis with a prevalence of 7.8%

(Azevedo et al. 2008), which poses a risk to the local population. One of these shared areas is the Vale Verde region, which is characterized by having a vast area of forest and belonging to the municipality of Poxoréu, making it difficult to detect the disease and its proper mapping, mainly due to stray dogs, commonly abandoned in this region. Tracking, monitoring and diagnosing infected dogs belonging to neighboring municipalities is essential to avoid free movement of animals that could contribute to the spread of the parasite (Julião et al. 2007).

The results obtained in this study are inferior from other serological surveys carried out in municipalities in the state of Mato Grosso, such as Rondonópolis, with a prevalence of 48.4% (Duarte 2010), Cuiabá 22.1% (Almeida et al. 2012) and Nossa Senhora do Livramento 14 % (Carvalho et al. 2020), since in these regions the presence of vectors (Duarte 2010) and symptomatic animals (Almeida et al. 2012; Carvalho et al. 2020) has already been reported. Although the present study did not carry out an entomological survey, the presence of *L. cruzi* has already been reported in the municipality of Primavera do Leste and region, such as Poxoréu and Dom Aquino, and *L. longipalpis* in Paranatinga (Mestre and Fontes 2007).

The characteristics of the region and the population must also be considered. The municipality of Primavera do Leste has only 36 years of foundation, an economy based on agriculture, poultry, livestock and commerce. As one of the largest soybean producers in Brazil, the city is one of the most important agribusiness routes in the country and, throughout the year, several agricultural events are held. Due to the short time, it was founded and driven by agribusiness, the city is an attraction for many people looking for employment opportunities, coming from different regions of the country, encouraging the real estate sector to expand housing, many of them in more distant points from the center of the city. These characteristics make the city a great gateway for people and animals, with significant proximity to the native environment, which can contribute to the emergence of cases of CVL, exemplifying the importance of One Health.

In this sense, in a study that evaluated the expansion of the visceral leishmaniasis epidemic in the State of Mato Grosso, 1998-2005, it was possible to verify the progression of the disease from the municipality of Várzea Grande to other municipalities in the Center-South of the state, a region characterized for presenting great population mobility, facilitated by the large network of side roads present in the Center-South and Southeast regions of the state. During this period, human infection in the municipality of Poxoréu had high numbers of cases (n=16 cases) (Mestre and Fontes 2007). According to data obtained from the Notifiable Diseases Information

System (SINAN), between 2001 and 2020, 875 cases of human leishmaniasis were reported in Primavera do Leste. In addition, it should be borne in mind that canine infection precedes the occurrence of cases in humans (Góes *et al.* 2012). However, the underreporting of cases of leishmaniasis, human and animal, is still an epidemiological problem, which prevents a better allocation of resources, collaborators and strategies in areas with active transmission of the disease or at risk. In this sphere, a problem observed in the municipality is the non-accounting of CVL cases diagnosed in clinics to the public health service, which contributes to the underreporting of cases and, in addition, culminates in the computation only of cases diagnosed by the public power, which is responsible and burdened by several other demands, such as dengue and COVID-19. Furthermore, the proximity of Primavera do Leste to endemic municipalities may be related to non-autochthonous cases. The geographic characteristics of the region may also suggest human infection in areas close to forests, without the presence of dogs, which could be related to other mammals. However, with the confirmation of CLV in the municipality, new strategies can be adopted together with the public authorities, through the measurement of the dog population, identification of risk areas and vulnerable people, in order to carry out entomological research of potential vectors and serological monitoring of dogs and wild species.

According to the Ministry of Health, in areas of moderate and intense transmission of VL, the control of the stray canine population must be carried out and, in case of canine prevalence greater than or equal to 2%, an annual canine census survey must be used; in cases where the prevalence is less than 2%, surveillance and monitoring should be maintained. In areas with sporadic transmission, with regard to actions related to the canine reservoir, an annual canine census survey should be used at the place of transmission (Brasil 2006), which justifies the importance and need for the use of more effective policies and actions. in the monitoring and control of diseases of interest to public health.

One of the difficulties observed in carrying out this study was obtaining data from animals allocated to shelters. Many animals had no clinical history or origin, which made it impossible to analyze the data more accurately with the laboratory results. Even so, none of the animals allocated to the shelters chosen to participate in this study tested positive. Although the shelters chosen for the collection of biological samples are located close to forested areas, both submit the dogs collected to a veterinary evaluation upon admission. According to Massis and colleagues (Massis *et al.* 2020), a dog hosted in a kennel does not appear to be a factor that increases the likelihood that dogs will show positivity, even when domiciled

for long periods.

Regarding the collection of dogs from veterinary clinics, this information was obtained by filling in a form by the tutors. Thus, it was possible to describe the positive sample as an asymptomatic male Siberian Husky dog, domiciled in the urban region. However, due to the low prevalence, it was not possible to associate the variables with sex and race.

Asymptomatic animals constitute a problem in the early detection of the disease, since the fact that they do not show clinical signs does not encourage the tutor to seek veterinary assistance. A fundamental point is that most dogs infected by *L. infantum* are apparently healthy, showing no visible clinical signs of visceral leishmaniasis (Dantas-Torres 2009). However, asymptomatic dogs present cutaneous parasitism, being able to live with the parasite for a long time and, consequently, present a greater possibility of transmission to the vector (Magalhães-Junior *et al.* 2006), demonstrating the importance of carrying out epidemiological studies in regions close to endemic areas, such as Primavera do Leste. Thus, the diagnosis and identification of asymptomatic animals are undoubtedly one of the key points in the control of CVL.

The occurrence of more positive animals in the immunochromatographic method in the present study, versus the ELISA method, demonstrates the importance of using diagnostic methods with high sensitivity and specificity, in order to guarantee a more accurate diagnosis and, consequently, avoiding unnecessary euthanasia of animals, making it necessary to search for more accurate techniques.

According to Grimaldi Jr and colleagues (Grimaldi Jr *et al.* 2012), the DPP has high sensitivity in symptomatic animals (98%), however when performed in asymptomatic animals the sensitivity reduces (47%). Better results were found by Ribeiro and colleagues (Ribeiro *et al.* 2019), with DPP® Bio-Manguinhos showing a sensitivity of 97.9% in asymptomatic animals. These data reinforce the idea that this method of serological diagnosis may have different sensitivity and specificities, depending on the clinical stage, type of antigen used and the host's immune response (Grimaldi *et al.* 2012).

Health education and population awareness of the importance of the One Health are the main prevention tools. Although control policies are directed to public areas, in the population of stray dogs and in sand fly breeding sites, it is the citizen's role to carry out their inspections and use appropriate prevention methods in the home environment, a place that is totally adaptable on the part of the vectors of *L. infantum*. Therefore, the availability of more accessible and effective diagnostic and prophylactic methods should not suppress the need

for the population to employ active surveillance and monitoring within their communities.

At the same time, it is the role of the government to make the population aware of its role within the concept of One Health.

Thus, the unprecedented detection of *Leishmania infantum* infection in the present study should encourage health services to carry out greater

surveillance and monitoring of the parasite and entomological research to identify sand flies in the municipality.

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