

## **Serological study of American *Tegumentary Leishmaniasis* in working horses and contact dogs in the city of Bandeirantes, Parana**

*Estudo sorológico da Leishmaniose Tegumentar Americana em equídeos de trabalho e de cães contactantes na cidade de Bandeirantes, Paraná*

*Estudio serológico de la Leishmaniasis Tegumentaria Americana en équidos de trabajo y perros de contacto en la ciudad de Bandeirantes, Paraná*

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### **Abstract**

The American Tegumentary Leishmaniasis (ATL) occurs in several parts of the world, in Brazil and also in the State of Parana, it is a disease of great importance in public health, since it affects animals and humans, with a zoonotic character. Leishmaniasis is found in two forms, tegumentary and visceral, and has sandflies as vectors and wild animals and domestic dogs as reservoirs. ATL is considered an endemic disease in the city of Bandeirantes, with

human cases occurring in several urban and rural locations, mainly in areas close to forest remnants, peri-urban and peripheral areas, where vulnerable populations live in general, and with precarious basic sanitation conditions. In these regions also live the participants of the wagon driver project, who own several working horses. The project provides guidance on management, good practices, disease prevention, and animal welfare. Since the role of horses as reservoirs and in the chain of transmission of ATL is not well known, the aim of this study was to carry out a serological study of ATL in horses and in the contact dogs of the participants of the "wagon driver" project, at UENP-CLM, to evaluate seropositivity in these species. Blood samples were collected from 20 horses and 12 contact dogs. A serological study, using the ELISAI test for ATL diagnosis, was carried out on the horses and dogs in relation to this study population, to assess seropositivity. In the serological survey, eight horses out of 20 animals (40%) and four dogs out of 12 animals (33.33%) were seropositive, a result considered significant, indicating the presence of the disease and the risk of transmission where they live. The occurrence of human cases and the presence of ATL vectors have already been identified in several locations in the city. Due to the lack of knowledge about the disease and the difficulty in controlling the vectors, health education is necessary in order to provide the population with orientation on how to prevent the disease, as well as entomological surveillance and new serological studies in areas at risk for ATL transmission. The results indicate that there may be importance in the role of horses as reservoirs and in the transmission of Tegumentary Leishmaniasis, which still needs to be clarified.

**Keywords:** Phlebotominae; Public Health; Zoonosis.

### Resumo

A Leishmaniose Tegumentar Americana (LTA) ocorre em várias partes do mundo, do Brasil e também no Estado do Paraná, é uma doença de grande importância em saúde pública, pois afeta os animais e o ser humano, apresentando caráter zoonótico. A leishmaniose é encontrada em duas formas, tegumentar e visceral, e tem como vetores os flebotomíneos e como reservatórios os animais silvestres e os cães domésticos. A LTA é uma doença considerada endêmica na cidade de Bandeirantes, com ocorrência de casos humanos em várias localidades urbanas e rurais, principalmente em áreas próximas a remanescentes florestais, periurbanas e periféricas, onde em geral habitam populações vulneráveis, e com precárias condições de saneamento básico. Nestas regiões também moram os participantes do projeto carroceiro, que possuem vários equinos de trabalho, o projeto fornece orientações sobre manejo, boas práticas, prevenção de doenças e bem-estar animal. Como não se conhece bem o papel dos equinos como reservatórios e na cadeia de transmissão da LTA, com este trabalho objetivou-se realizar um estudo sorológico da LTA nos equídeos e nos cães contactantes dos participantes do projeto carroceiro, da UENP-CLM, para avaliar a soropositividade nestas espécies. Foi realizada a coleta de sangue nos animais, correspondendo a 20 equinos e 12 cães contactantes. Foi realizado um estudo sorológico, utilizando-se o teste de ELISAI para diagnóstico da LTA, nos equinos e cães em relação a esta população de estudo, para avaliação da soropositividade. No levantamento sorológico, oito equídeos em 20 animais (40%) e quatro cães em 12 animais (33,33%) foram soropositivos, resultado considerado significativo, indicando presença da doença e risco de transmissão nos locais onde convivem. Já foi identificada a ocorrência de casos humanos e presença dos vetores da LTA em várias localidades da cidade. Devido à falta de conhecimento sobre a doença e dificuldade em realizar o controle dos vetores, torna-se necessário um trabalho de educação em saúde para realizar a orientação para a prevenção da doença na população assim como estabelecer vigilância entomológica e novos estudos sorológicos em áreas de risco para a transmissão da LTA. Os resultados indicam que pode haver importância no papel dos equinos como reservatórios e na transmissão da Leishmaniose Tegumentar, que ainda precisa ser esclarecido.

**Palavras-chave:** Flebotomíneos; Saúde Pública; Zoonose.

### Resumen

La Leishmaniasis Tegumentaria Americana (LTA) ocurre en varias partes del mundo, en Brasil y también en el Estado de Paraná, y es una enfermedad de gran importancia en la salud pública, ya que afecta a los animales y al hombre, presentando un carácter zoonótico. La leishmaniosis se presenta en dos formas, tegumentaria y visceral, y tiene como vectores a los flebótomos y como reservorios a los animales salvajes y a los perros domésticos. La LTA es considerada una enfermedad endémica en la ciudad de Bandeirantes, con ocurrencia de casos humanos en varias localidades urbanas y rurales, especialmente en áreas cercanas a remanentes forestales, zonas peri urbanas y periféricas, donde suelen vivir poblaciones vulnerables y con precarias condiciones de saneamiento básico. En estas regiones también viven los participantes del proyecto cochero, que son propietarios de varios caballos de trabajo; el proyecto ofrece orientación sobre gestión, buenas prácticas, prevención de enfermedades y bienestar animal. Como el papel de los caballos como reservorios y en la cadena de transmisión de ATL no es bien conocido, este estudio tuvo como objetivo realizar un estudio serológico de ATL en caballos y en los perros de contacto de los participantes del proyecto "cochero", de la UENP-CLM, para evaluar la seropositividad en estas especies. Se recogieron muestras de sangre de 20 caballos y 12 perros de contacto. Se realizó un estudio serológico, mediante la prueba ELISAI para el diagnóstico de ATL, en caballos y perros en relación con esta población de estudio, para evaluar la seropositividad. En la encuesta serológica, ocho caballos de 20 animales (40%) y cuatro perros de 12 animales (33,33%) fueron seropositivos, resultado considerado significativo, ya que indica la presencia de la enfermedad y el riesgo de

transmisión en los lugares donde viven. Ya se ha identificado la aparición de casos humanos y la presencia de vectores de ATL en varias localidades de la ciudad. Debido al desconocimiento de la enfermedad y a la dificultad de control de los vectores, es necesaria una labor de educación sanitaria para orientar la prevención de la enfermedad en la población, así como establecer una vigilancia entomológica y nuevos estudios serológicos en las zonas de riesgo de transmisión de la LTA. Los resultados indican que puede haber importancia en el papel de los caballos como reservorios y en la transmisión de la leishmaniosis tegumentaria, lo que aún debe aclararse.

**Palabras clave:** Flebótomos; Salud Pública; Zoonosis.

## 1. Introduction

The leishmaniasis are considered anthroponoses and constitute a serious public health problem and present tegumentary (LT) and visceral (VL) forms of occurrence, with variations in the clinical presentation and ecological diversity. According to estimates by the World Health Organization (WHO), more than a billion people live in areas at risk of infection. It has a worldwide distribution, occurring in 92 countries on four continents (Americas, Africa, Asia, and Europe) with an annual record of 700 thousand to 1.3 million new cases. It is considered to be one of the six most important infectious diseases in the world, due to the large coefficient of occurrence and the capacity to produce deformities (WHO 2020; Brasil 2017; Brasil 2021).

In the Americas, leishmaniasis occur in 18 countries and the most common clinical form is cutaneous leishmaniasis (CL), while visceral leishmaniasis (VL) is the most severe form and almost always fatal if not treated. Mucosal/mucocutaneous leishmaniasis (MCL) has a chronic course and can cause deformities and sequels (OPAS 2019; OPAS 2020).

The occurrence of ATL (American Tegumentary Leishmaniasis) can be observed from the extreme south of the United States to the north of Argentina; it does not occur in Chile and Uruguay (Brasil 2017; OPAS 2019; OMS 2019). In Brazil, LT is a disease of public health importance, due to its magnitude of occurrence and the deformities it produces in human beings, besides the psychological aspect, affecting social and economic life, since it can be treated as an occupational disease. Cases have been recorded in all Brazilian regions (Brasil 2017).

The disease is caused by several species of the genus *Leishmania*, mainly by the following species: *Leishmania (Viannia) braziliensis*, *Leishmania (V.) guyanensis*, *Leishmania (V.) naiffi*, *Leishmania (V.) shawi*, *Leishmania (V.) lainsoni*, *Leishmania (Leishmania) amazonenses*. The vectors, called sandflies (Diptera, Phlebotomidae), are responsible for the transmission of the protozoa between one mammal and another (Brasil 2017).

ATL is associated with environmental factors and knowledge of its reservoirs is of great importance to establish risk factors for transmission and infection of the disease. In areas without deforestation peridomestic animals such as dogs, horses and rodents may act as reservoirs, therefore, it is important to conduct studies to investigate the role of these animals in the prevalence of leishmaniasis (Truppel et al. 2014).

In endemic areas for leishmaniasis it is frequent the presence of infected dogs and phlebotomine sandflies in the peridomicile, and considering that horses are long-lived animals and are in endemic areas, they have great chances of becoming infected by several organisms, including the *Leishmania spp.*, and for being one of the food sources of the sandflies, they can contribute to the maintenance of the peridomiciliary cycle of ATL (Silveira et al. 1996; Evers et al. 2017).

ATL is considered endemic in the northern region of Paraná, as well as in the city of Bandeirantes, where phlebotomine sand fly vectors have already been identified in households in several locations in the city, including peri-urban and rural areas. The proximity of domestic animal shelters, the accumulation of organic matter, and soil moisture due to domestic water disposal are risk factors that may favor the presence of vectors and the transmission of the disease (Cruz et al. 2013; Massafera et al. 2005).

Tegumentary Leishmaniasis can affect animals and humans, and dogs and horses can serve as reservoirs. Equine leishmaniasis is still a neglected tropical disease and its importance in the transmission cycle of human leishmaniasis is still unclear. Leishmaniasis should be included in the differential diagnosis of equine skin diseases that do not respond to antibiotic and antifungal treatments (Vieira et al. 2020).

This study aimed to perform a serological study to evaluate the seropositivity for ATL in horses and contact dogs of owners participating in the wagon driver project at the Northern Parana State University (UENP) Luiz Meneghel campus (CLM), in the city of Bandeirantes, Parana State.

## 2. Methodology

The study was conducted in the municipality of Bandeirantes, located in Northern Parana and southern region of Brazil, according to IBGE (2017), it has a population of 31,211 inhabitants and a demographic density of 72.29 inhabitants/kilometer and according to the Koppen-Geiger climate classification (Koppen classification: Cfa), atlantic forest biome and subtropical climate. The city of Bandeirantes is located in an endemic region for the occurrence of Tegumentary Leishmaniasis. The study was approved by the Ethics Committee for Animal Use of UENP, under number CEUA/UENP-0011-2018.

The horses that participated in the study belonged to six different owners participating in the wagon driver project (UENP-PR), which provides equine evaluation and clinical care, as well as guidance on management and prevention of endemic diseases. They came from the neighborhoods of Habitar Brasil (periruban), Vila São Pedro (periruban) and Bairro rural Yara, places with remaining forest areas, animal breeding and the presence of organic matter near the houses.

Blood samples were collected from 20 horses used for work or leisure. The animals belonged to owners living in the city of Bandeirantes, who participated in the wagon driver project of the Universidade Estadual do Norte do Paraná. The owners and animals lived in periurban and rural areas near the city, where there were forest remnants, with poor sanitation, constituting a vulnerable population, with low socioeconomic and cultural power.

The collections occurred during the period from January to July 2019 and a 30x8 needle and 5 milliliter (mL) syringe were used as materials, and the access was made in the jugular vein for blood collection, which after removal was immediately deposited in a dry tube and stored in a thermal box with ice packs until the moment of centrifugation, which was performed 2 hours after collection in the parasitology laboratory of the UENP. After centrifugation, the serum was deposited in eppendorfs and frozen in a freezer at -20 degrees Celsius (C°) and sent to the State University of Londrina (UEL) in an isothermal box with ice packs to perform the serology for ATL.

Enzyme Linked ImmunonoSorbent Assay (ELISA) was performed as described by Szargiki et al. (2009) and Ferreira et al. (2018) with adaptations, using *L. amazonensis* antigen, serum and peroxidase-conjugated protein A conjugate (Sigma Aldrich, USA). Samples were tested in duplicate and the test cut-off point was obtained using the mean absorbance of the negative serums with the addition of three standard deviations. To estimate the corrected cut-off point, the Roc curve from the MedCalc Statistical Software program was used as described by Schoonjans, Zalata, Depuydt and Comhaire (1995).

A general physical examination of the horses was performed to assess their general condition, the examination included measuring heart rate, respiratory rate, rectal temperature, capillary perfusion time in oral mucosa, evaluation of oral mucosal staining, and size and consistency of palpable lymph nodes. The results were compared with the parameters of normality found in the literature of Muir and Hubbell (2009).

The body condition score of the animals was also identified by visual analysis and palpation of the areas where fat deposition occurs, where number nine is considered an extremely obese animal and number one is a very thin animal, using the methodology described by Bender et al. (2014).

Blood samples were also collected from 12 dogs that had direct contact with these horses and lived in the same place and belonged to the same owners. Blood was collected with a 25x7 needle and a 5 mL syringe, using the jugular or cephalic vein as access. After the collection, the blood was deposited in a dry tube and refrigerated until centrifugation, which was performed in the parasitology laboratory at UENP, using the same methodology described above.

For the serology of the dogs, the Enzyme Linked ImmunonoSorbent Assay (ELISA) immunoassay technique was also used as described by Benitez et al. (2018) with adaptations, using the antigen *L. amazonenses*, tests performed in duplicate with positive and negative control on each blade and using the statistical analysis of the ROC curve with MedCal Statistical Software program, as described by Metz (1978).

### 3. Results

Of the 20 horses used for the study, 55% were male (11/20) and 45% were female (9/20), 15% (3/20) were used for traction and 85% (17/20) were used for leisure.

**Table 1.** Serology results for LTA (ELISAi), Bandeirantes, 2019.

<b>Animal</b>	<b>LTA serology 3rd cut-off point (0.147465)</b>	<b>Neighborhood</b>
Eq 1	0,169* <sup>1</sup>	Habitar Brasil
Eq 2	0,585	Habitar Brasil
Eq 3	0,0405	Habitar Brasil
Eq 4	0,3595* <sup>1</sup>	Vila São Pedro
Eq 5	0,1865* <sup>1</sup>	Habitar Brasil
Eq 6	0,1135	Habitar Brasil
Eq 7	0,2165* <sup>1</sup>	Habitar Brasil
Eq 8	0,095	Habitar Brasil
Eq 9	0,034	Vila São Pedro
Eq 10	0,0595	Bairro RuralYara
Eq 11	0,1505* <sup>1</sup>	Bairro RuralYara
Eq 12	0,3705* <sup>1</sup>	Bairro Rural Yara
Eq 13	0,1415	Bairro Rural Yara
Eq 14	0,129	Bairro RuralYara
Eq 15	0,1625* <sup>1</sup>	Bairro RuralYara
Eq 16	0,0585	Bairro RuralYara
Eq 17	0,059	Habitar Brasil
Eq 18	0,176* <sup>1</sup>	Habitar Brasil
Eq 19	0,0785	Vila São Pedro
Eq 20	0,049	Vila São Pedro

\*<sup>1</sup> Animals positive for ATL serology at the third cut-off point. Source: authors.

#### 4. Discussion

Regarding the physical examination performed, all horses were within normal vital and clinical parameters and only four animals had a low body score, score between 2 and 3, after visualizing low fat in areas such as neck and tail base, according to the description of Muir and Hubberl (2009) and Bender et al. (2014).

The dogs belonged to three owners, and the other participating equine owners reported not owning any dogs. 50% (6/12) of the dogs were female and 50% (6/12) were male. About the serology, there was positivity in 33.3% of animals (4/12), but no animal had skin lesions.

In the ELISAI technique for LTA serology, three cut-off points were used, following the methodology of Schoonjans et al. (1995) for the calculation, where the results were 0.09125 as the first cut-off point, 0.018738 as the second cut-off point, and 0.147465 as the third cut-off point.

The results in horses showed seropositivity of 40% (8/20) at the third cut-off point (Table I), such animals belonged to 4 different owners and no animal had skin lesions.

In the results of the horses, the seropositivity for LT was higher than in the study by Ferreira et al. (2018), who found positivity of 25.4% for indirect ELISAI in the maranhense lowlands and described that the role of horses in the transmission of leishmaniasis is uncertain, but that seroconversion occurs in these animals. The results of seropositivity in this study regarding horses differ from those of Benvenga (2013) in São Paulo, who found much lower positivity of 2.5% of the horses analyzed, although this author used the indirect immunofluorescence technique.

This study also differed from Limeira et al (2019), which with a meta-analysis, showed a prevalence for combined ATL of 25%, but with great heterogeneity among the studies, related to differences in the methods used to diagnose the disease.

The seropositivity of the dogs was four dogs in a total of 12 animals (33.33%), which is higher than the results of Lonardoni et al. (2006) that found 19% of the analyzed animals positive. None of the positive dogs had clinical signs of the disease, and the seropositivity was higher than the results found by Madeira et al. (2000), in the state of Rio de Janeiro, where they found 11.94% of reactors.

**Table 2.** Number of owners, participating animals, and animals positive for ATL according to the neighborhood of habitation in Bandeirantes, 2019.

<b>Neighborhood</b>	<b>n° participants eq.</b>	<b>n° positives eq. *2</b>	<b>n° participating dogs</b>	<b>n° positives dogs *2</b>	<b>n° owners participating</b>
Habitar Brasil	9	4	3	1	1
Bairro Rural Yara	7	3	6	3	2
Vila São Pedro	4	1	3	0	3
<b>Total</b>	<b>20</b>	<b>8</b>	<b>12</b>	<b>4</b>	<b>6</b>

\*2 Number of animals positive for LTA disease by serological test. Source: authors.

All the participating animals lived in peri-domicile areas, in all the neighborhoods analyzed both horses and dogs were positive for ATL, and all the places where there were positive horses had direct contact with positive dogs as well, which may reaffirm the role of horses as a reservoir of the disease and be indicative of the presence of sandflies infecting both dogs and horses.

Cruz et al. (2012) found in peridomiciliary areas of Itambaracá, a town in northern Paraná, near the city of Bandeirantes, five species of phlebotomines considered to be ATL vectors, these being: *Nyssomyia neivai*, *Pintomyia pessoai*, *Migonemya migonei*, *Nyssomyia whitmani* and *Pintomyia fischeri*. Also, the study that showed a high frequency of phlebotomine sandflies in peridomestic areas of the city of Bandeirantes, especially at night, indicating that the phlebotomine sand fly vectors have shown themselves capable of adapting to anthropic environments.

The presence near the peridomicile and work animals such as horses and companion animals such as dogs is attractive to sandflies, which may increase the risk of contracting ATL in transmission areas. Measures are needed to control the ATL vector population, especially in the household environment, in addition to educational measures for the population, observations also described by Cruz et al. (2013).

Studies such as those by Bresalier, Cassidy and Woody (2015) comparing the health of humans and animals on the transmission of zoonosis such as leishmaniasis should be conducted in an integrated and simultaneous manner. Bandeirantes was one of the municipalities that stood out in the study by Melo, Rossoni and Teodoro (2017), due to the high number of cases of ATL in humans, among the 268 municipalities in the state of Paraná with recorded cases.

The present study showed in the serological results found that there are horses and dogs seropositive for tegumentary leishmaniasis in the region studied, indicating the presence of the disease in the areas sampled. Studies already carried out in the region, in which vectors were found and cases of the disease in humans already reported, reaffirm the endemicity of ATL in the city and indicate the need for vigilance and control of vectors and health education activities to make the population aware of the prevention of the disease should be carried out in the region, since there are many rural areas and remaining forest areas, areas with accumulation of organic matter that can be risk factors for the transmission of ATL in the region.

## 5. Conclusions

Both horses and dogs seropositive for American Tegumentary Leishmaniasis (ATL) were found in the region studied, there are other studies that show the presence of the vector and registered cases of the disease in the city, which reaffirms the endemic form of ATL in Bandeirantes and shows the need to prevent the disease and spread forms of prevention to the population.

There is a necessity to realize serological studies for ATL covering a larger number of horses and dogs in the transmission areas where human cases have been reported, for a better evaluation of the risk factors and to better understand the role of horses in the transmission of the disease, considering that there are still few studies on the subject.

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