

## Seroepidemiology of *Leishmania* spp. in equids from Uberlândia, Minas Gerais, Brazil

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**ABSTRACT:** *This study evaluated the occurrence of Leishmania spp. in equids from Uberlândia, Minas Gerais, Southeastern Brazil. Serum samples from 257 healthy equids of Uberlândia were assessed for the presence of antibodies against Leishmania spp. by using the indirect fluorescent-antibody test. Additionally, an epidemiological survey was done to evaluate the possible risk factors (species of equids, age, gender, economic activity, and contact with domestic and wild animals) associated with the presence of antibodies against Leishmania spp. The total seroprevalence against Leishmania spp. was 24.1% (62/257); representing seroprevalence levels of 22.7% (55/242) in horses, 50% (4/8) in mules, and 42.9% (3/7) in ponies from all regions evaluated. The species of equid was the only factor that contributed to an elevated seroprevalence of Leishmania spp.*

**Key words:** *Leishmania spp., equids, serology, epidemiology.*

## Soroepidemiologia de *Leishmania* spp. em equídeos de Uberlândia, Minas Gerais, Brasil

**RESUMO:** *O estudo avaliou a ocorrência de Leishmania spp. em equídeos do município de Uberlândia, Minas Gerais, região Sudeste do Brasil. Amostras de soro foram coletadas de 257 equídeos clinicamente saudáveis em Uberlândia, para avaliar a presença de anticorpos contra Leishmania spp. pela técnica de reação de imunofluorescência indireta – RIFI. Afora isto, um inquérito epidemiológico foi realizado para avaliar possíveis fatores de risco (espécie, idade, sexo, atividade econômica e contato com animais domésticos e selvagens) associado com a presença de anticorpos contra Leishmania spp. A soroprevalência total contra Leishmania spp. foi 24,1% (62/257), representando 22,7% (55/242) de equinos, 50% (4/8) de mulas e 42,9%(3/7) de pôneis. A variável espécie foi o único fator de risco significativo na soroprevalência da Leishmania spp.*

**Palavras-chave:** *Leishmania spp., equídeos, sorologia, epidemiologia.*

Leishmaniosis is important zoonotic disease, caused by protozoa of the genus *Leishmania*, with canids, rodents, and humans being the main species affected in the urban cycle. However, a large number of mammalian species have been reported as hosts of *Leishmania* (BRANDAO-FILHO et al., 2003) with reports occurring in domestic animals, increasing worldwide (SOARES et al., 2013) including in horses (BARBOSA-SANTOS et al., 1994). Clinical cases of cutaneous leishmaniosis in domestic animals such as dogs and horses are becoming relatively common in South (BARBOSA-SANTOS et al., 1994) and North America (RAMOS-VARA et al., 1996). However, despite the occurrence of these cases, the role of equids in the transmission of leishmaniosis

has not been fully elucidated, even though these animals are considered as putative host of *L. braziliensis* in the Americas (TOLEZANO, 1994).

This study evaluated the serological evidence of *Leishmania* species in equids from Southeastern Brazil and the possible effects of species, gender, age, utility, and contact with carnivores and wild animals relative to the risk to be infected by this disease.

This study was done within the limits of the city of Uberlândia, in a geographical region of 4,116.09km<sup>2</sup>, which is limited by 18°30' and 19°30' latitude in the south, and 47°50' and 48°50' longitude in the west. This region has a tropical climate, with two well-defined seasons, a rainy and

a dry winter, with an annual pluviometric index of 1500mm, and an average temperature of 22°C (DUARTE et al., 2005).

Blood samples (8mL) were collected by jugular venipuncture from healthy horses (*Equus caballus caballus*; n=242), mules (*E. asinus caballus*; n=8), and ponies (*E. ferus caballus*; n=7), of various breeds, both sexes and different ages from the urban (n=112) and rural (n=145) regions of Uberlândia, Minas Gerais, Southeast Brazil.

Biological data (species, gender, and age) as well as information relative to the functional activity of all animals were documented and tabulated. Risk factors, including equid species (horse, mule, pony), gender, age (<5years; 5-14 years; >14 years), activity (reproduction; work; sport) were investigated to determine a possible relationship with the presence of anti-*Leishmania* spp. antibodies in equids from both regions. The contact with cats, dogs and wild animals were evaluated only in the rural area, and it was assumed that all equids had contact with domestic animals in the urban region.

Sera were obtained by centrifugation of clotted blood and stored at -20°C until used. The presence of antibodies against *Leishmania* spp. was determined by using the indirect fluorescent-antibody test (IFAT) as described by (OLIVEIRA et al., 2008), with glass slides containing promastigotes of *L. amazonensis* (PH 8 strain). The anti-IgG equine conjugate (Sigma-Aldrich Ltd, Dorset, UK) at a 1:2000 dilution was used. The end-point titer was considered as the final dilution that resulted in a complete, bright green membrane and flagellum fluorescence. Positive and negative serum controls against *Leishmania* spp., kindly provided by Dr Thomaz-Socol, V. (Universidade Federal do Paraná) were used in each IFAT assay. The cut-off level to determine a positive reaction was established at  $\geq 1:40$ , starting with 1:20 dilution (OLIVEIRA et al., 2008).

All statistical analysis was done by using the software SISVAR and Minitab 16. The seroprevalence was calculated with an associated 95% confidence interval. The Chi-square and Fisher's exact were used to evaluate significant risk factors associated with seroprevalence of *Leishmania* spp. All results were considered significant when  $P < 0.05$ .

Antibodies against *Leishmania* spp. were detected in 24.1% (62/257) of serum samples evaluated by IFAT from all equids evaluated during this study, representing seroprevalence of 22.7% (55/242) in horses, 50% (4/8) in mules, and 42.9% (3/7) in ponies. Nevertheless, positive seroreactivity

to *Leishmania* spp. was demonstrated in horses from the urban (20.2%; 22/109) and rural (24.8%; 33/133) regions. However, mules (80%; 4/5) originated only from the rural districts were seropositive. Ponies were derived only from the rural region and 42.9% (3/7) of these were seropositive.

Significant statistical differences were not observed when the genders of seropositive equids from the urban ( $P=0.814$ ) and rural regions ( $P=0.354$ ), the different age groups of equids from the urban ( $P=0.783$ ) and the rural ( $P=0.998$ ) regions, and activity of equids from the urban ( $P=0.954$ ) and rural ( $P=0.222$ ) regions were compared. Significant statistical differences ( $P=0.18$ ) in the seroprevalence of equids to *Leishmania* spp. between the urban (19.6%; 12.7 - 28.2) and rural (27.6%; 20.5-35.6) regions were not observed. Additionally, the relative risk of seropositivity to *Leishmania* spp. between the urban and rural regions was 1.4 (0.89-2.22).

Antibodies against *Leishmania* spp. above the cut-off threshold were detected in equids (19.6%; 22/112) within the urban region: 20 (90.0%) had a titer of 40, while only 2 (9.1%) demonstrated a titer of 80. Alternatively, 27.6% (40/145) of equids from the rural region demonstrated antibodies over the cut-off threshold; 30 (75%) of these had a titer of 40, while 10 (25%) had a titer of 80.

Significant risk ( $P < 0.05$ ) was only associated with the species of animal maintained within the rural region investigated (Table 1). Mules demonstrated the highest level of seroprevalence whereas horses exhibited the lowest. When the risk of mules and ponies to be seropositive was compared relative to horses, the risk of seropositivity to *Leishmania* spp. was 3.22 (1.9-5.47) times higher for mules, but was not significant for ponies. However, the need to utilize a larger number of animals must be emphasized, considering the limited number of mule and ponies utilized during this investigation.

Results from this study demonstrated that equids from all regions evaluated were seropositive for *Leishmania* spp., with seropositivity ranging from 19-45%; these results suggested that equids within this region of Southeastern Brazil were exposed to the pathogen and can probably develop leishmaniosis. Nevertheless, none of the equid species evaluated during this investigation had clinical manifestations of leishmaniosis; similar results were described in a study done within the endemic region of northern Paraná (TRUPPEL et al., 2014).

Results from this study recorded a comparatively lower seroprevalence, in contrast

Table 1 - Relative risk (RR) of equids from the urban and rural regions of Uberlândia being seropositive to *Leishmania* spp.

Regions	Risk Factor - Species	N <sup>a</sup>	Prevalence (%)	95% CI <sup>b</sup>	P value	RR (95%CI) <sup>c</sup>	P value
Urban	Horses	109	20.2% (n=22)	13.1-28.9	1.00	1	1.00
	Mule	3	0% (n=0)	0-70.7	-	-	-
Rural	Horses	133	24.8% (n=33)	17.7-33.0	0.016*	1	-
	Mule	5	80% (n=4)	28.4-99.5	-	3.22 (1.9 – 5.47)	0.018*
	Pony	7	42.9% (n=3)	9.9-81.6	-	1.73 (0.70-4.27)	0.373

Note: <sup>a</sup> Number of samples, <sup>b</sup> Confidence interval, <sup>c</sup> Relative risk (95% confidence interval).

to investigations done in the states of Rio de Janeiro (30.8%, 8/26 by IFAT, OLIVEIRA-NETO et al., 1988) and Paraná (76.3%; 42/55), using the direct agglutination test (VEDOVELLO FILHO et al., 2008). Consequently, these seroepidemiological surveys from different geographical locations have identified *Leishmania* spp. antibodies in horses and might suggest that horses can act as an alternative host for this pathogen.

When the species of equids were analysed, mules residing within the rural regions demonstrated the most elevated risk of being seropositive to *Leishmania* spp.; but this trend was not observed in their counterparts from urban areas. Although, the number of mules available for analyses were relatively low (n=8), four seropositive animals were from three distant rural districts, suggesting that these animals had contact with *Leishmania* spp., and therefore were at risk to develop leishmaniosis. However, all other risk factor evaluated during this study did not significantly increase the chances of equids being seropositive for *Leishmania* spp.: differences in the age, gender, contact with domestic and wild animals, and the economic activity of the equids from this region did not seem to have any effect on seropositivity to leishmaniosis.

The elevated seroprevalence of antibodies against *Leishmania* spp. in equids from the urban and rural regions of the geographical areas studied suggested that these animals were exposed to the pathogen and might be at risk of presenting leishmaniosis.

#### BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The study was approved (protocol # 071/13) by the Bioethics Committee, Universidade Federal de Uberlândia, Brazil.

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