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PARASITOLOGY

Trypanosoma evansi in dogs from Barão de Melgaço, Mato Grosso: Molecular prevalence

Ana Lúcia Vasconcelos[1] Melissa Harumi Sumiyoshi[1] Arlyson Sousa Ferreira[1] Valéria Régia Franco Sousa[1] Valéria Dutra[2] Luciano Nakazato[2] Eveline da Cruz Boa Sorte Ayres[1] Arleana do Bom Parto Ferreira de Almeida[1*]

[1]Laboratório de Leishmanioses, Universidade Federal de Mato Grosso (UFMT), 78060-900, Cuiabá, MT, Brasil. E-mail: arleferreira@gmail.com. [*]Corresponding author.
[2]Laboratório de Biologia Molecular, Universidade Federal de Mato Grosso (UFMT), Cuiabá, MT, Brasil.

ABSTRACT: *Trypanosoma evansi*, the hemoflagellate pathogen that causes "surra," is a protozoan that infects the widest variety of mammals worldwide, primarily horses, camels, dogs, and wild animals. Cases of canine trypanosomiasis caused by *T.evansi* have been reported in the Brazilian pantonal region. This study determined the prevalence of *T.evansi* and investigate risk factors. The study used blood samples from dogs in the municipality of Barão de Melgaço in the pantonal of the state of Mato Grosso, Brazil. The owner was given a structured epidemiological questionnaire containing information about the risk factors. The quantitative polymerase chain reaction technique was used to detect *T.evansi* DNA using species-specific oligonucleotides TevF and TevR of the gene encoding a 227-bp portion of the glycoprotein of the variant surface (VSG) *T.evansi* Rode Trypanozoon (RoTat) 1.2. Two (prevalence of 0.5%) genetic materials of *T.evansi* were reported in the403 samples. Although, no risk factor was associated with infection (P > 0.05), proximity to vegetation and streams, as well as the presence of tabanids, rodents, and marsupials, which are factors in the occurrence of infection, were observed in positive dogs, implying that the municipality of Barão de Melgaço has epidemiological characteristics that allow canine infection by *T.evansi*.
Key words: canine trypanosomiasis, Pantanal, protozoa, qPCR.

Trypanosoma evansi em cães de Barão de Melgaço, Mato Grosso: prevalência molecular

RESUMO: *Trypanosoma evansi*, o patógeno hemoflagelado que causa a "surra", é um protozoário que infecta a mais ampla variedade de mamíferos em todo o mundo, principalmente cavalos, camelos, cães e animais selvagens. Casos de tripanossomíase canina causada por *T. evansi* têm sido relatados na região pantaneira brasileira. Este estudo tem como objetivo determinar a prevalência de *T. evansi* e investigar fatores de risco. O estudo utilizou amostras de sangue de cães do município de Barão de Melgaço, no pantanal do estado de Mato Grosso, Brasil. O proprietário recebeu um questionário epidemiológico estruturado contendo informações sobre os fatores de risco. A técnica quantitativa de reação em cadeia da polimerase foi utilizada para detectar DNA de *T. evansi* utilizando oligonucleotídeos específicos da espécie TevF e TevR do gene que codifica uma porção de 227 pb da glicoproteína da superfície variante (VSG) *T. evansi* Rode Trypanozoon (RoTat) 1.2. Dois (prevalência de 0,5%) materiais genéticos de *T. evansi* foram encontrados nas 403 amostras. Embora nenhum fator de risco tenha sido associado à infecção (P > 0,05), a proximidade de vegetação e riachos, bem como a presença de tabanídeos, roedores e marsupiais, fatores na ocorrência de infecção, foram observadas em cães positivos, implicando que o município de Barão de Melgaço apresenta características epidemiológicas que permitem a infecção canina por *T. evansi*.
Palavras-chave: tripanossomíase canina, Pantanal, protozoário, qPCR.

INTRODUCTION

Trypanosoma evansi, a hemoflagellate protozoan that causes the disease known as "surra" or "mal daschairs," has been described to affect various species of domestic and wild animals in Africa, Asia, and Latin America, including horses, cattle, canines, camelids, and buffaloes (NGUYEN et al., 2021; SAZMAND et al., 2022). Dogs are considered sentinel animals because of their high susceptibility to infection and rapid death (AREGAWI et al., 2019). In addition to the description of human involvement, *T.evansi* is now considered the species of pathogenic *Trypanosoma* with the largest geographic distribution worldwide (NGUYEN et al., 2021). Mechanical transmission occurs during blood meals of biological vectors of the genera *Stomoxys* spp. and *Tabanus* spp. or by vampire bats *Desmodus rotundus* (NGUYEN et al., 2021). Furthermore, predation on contaminated wild mammal carcasses, such as those of capybaras, which are also considered reservoirs of *T.evansi*, isa risk factor for domestic canine infection in

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pantonal regions of Brazil (FRANKE et al., 1994; FILGUEIRAS et al., 2018).

In Brazil, the prevalence varies depending on the region and research methodology used. Prevalence rates of 29%–42.8% have been reported using the polymerase chain reaction (PCR) technique (PORFIRIO et al., 2018; BILHEIRO et al., 2019; ECHEVERRIA et al., 2019). Rates ranged from 20.5% to 57.1% when using the indirect immunofluorescence technique (SANTOS, 2017).

The diagnosis of *T.evansi* infection is limited by intermittent parasitemia in hosts, which makes direct visualization of the parasite difficult, as well as the possibility of cross-reactions with other hemoparasites, such as *Leishmania* spp. and *Ehrlichia* spp. in serological tests (RECALDE et al., 2021). Therefore, infection prevalence data may be underestimated, mainly when using low-sensitivity techniques, such as visualization in blood smears and microhematocrit tests (NGUYEN et al., 2021). Thus, molecular techniques, such as PCR and its variants, have been used to achieve a more accurate diagnosis, particularly in the early stages of the disease or in cases of subclinical infections (RECALDE et al., 2021).

Because of its extensive forest area and rich diversity of mammals, the Brazilian Pantanal biome is a promising field for detecting *T.evansi*, which allows mechanical vectors of pathogenic agents to be in frequent contact with their possible wild hosts (SANTOS, 2017; FILGUEIRAS et al., 2018). This study determined the prevalence of *T.evansi* infection in dogs in the municipality of Barão de Melgaço, Pantanal region of Mato Grosso, and the potential risk factors for canine infection.

MATERIALS AND METHODS

A cross-sectional study was conducted in Barão de Melgaço, which is located in the pantonal region of the state of Mato Grosso, Brazil, at a latitude of 16°11′40″ south and a longitude of 55°58′03″ west, at an altitude of 156m. Previous studies for canine visceral leishmaniasis obtained blood samples from dogs in all urban areas and 11 rural communities during home visits and with the presence and authorization of the person responsible for the dog (Figure 1; DIAS et al., 2017). A dog-to-human ratio of 7:1,a projected population of 8,164 individuals in 2020 (IBGE, 2023), a prevalence of 50%, an acceptable error of 5%, and a confidence level of 95%was used to calculate the sample size of 403 dogs.

Previously, dogs of both sexes, different breeds, and aged 6months or more were examined by inspection and palpation, and clinical changes were recorded on an individual form. A structured epidemiological questionnaire containing information on race, sex, role in the residence, place of stay in the house (intradomestic or peridomiciliar), access to the street, proximity of the house to forests and rivers, location of the house (urban or rural

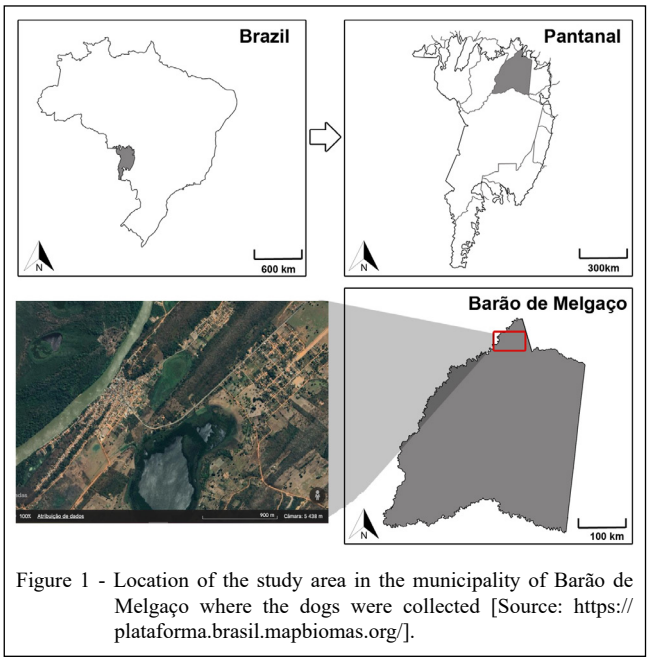

Figure 1 - Location of the study area in the municipality of Barão de Melgaço where the dogs were collected [Source: https://plataforma.brasil.mapbiomas.org/].

area), presence of other animals and/or chicken coops or pigsties, and vegetation around the house was administered to the guardian.

The dogs were venipuncture in the cephalic or jugular veins after the owners were made aware of the procedure and given permission. The blood was placed in a microtube with ethylenediaminetetraacetic anticoagulant, then separated into two 1.5-mL microtubes and stored at −20°C until DNA extraction and PCR were performed (DIAS et al., 2017).

DNA was extracted from blood samples using the phenol–chloroform method and precipitated with isopropanol (SAMBROOK & RUSSEL, 2001). DNA was dissolved in ultrapure water containing RNase at a 20 mg/mL concentration and stored at −20 °C until molecular assays were performed. To ensure the integrity of the DNA and the absence of inhibitors, the samples were subjected to PCR to identify the canine b-globin gene, which amplified a 119-basepair (bp) fragment (QUARESMA et al., 2009).

Real-time PCR was performed using the Step One TM Real-Time PCR Systems Sequence Detection System (Applied Biosystems®) with nonspecific double-stranded DNA intercalators (Sybr Green®). The primers used were species-specific primers (TevF: 5′-TGCAGACGACCTGACGCTACT-3′ and TevR: 5′-CTCCTAGAAGCTTCGGTGTCCT-3′) of the gene that encodes a 227-bp section of the *T.evansi* variant surface glycoprotein (VSG) RoTat 1.2 (AZHAHIANAMBI et al., 2018). The total volume of the protocol was 25 µL, which included 2 µl of Sybr Green®, 12.5µLof ultrapure water, 0.4 µLof primer, 5 µLof DNTP, 2.5µLof buffer, 1.2 µLof MgCl, 0.2 µLof Rox, 0.2 µLof Taq, and 1 µL of biological sample DNA. Ultrapure water was used as the negative control. The amplification regimen was 94 °C for 10 min, followed by 40 cycles of denaturation at 94 °C for 10 s and annealing and extension at 62 °C for 30 s. A *T.evansi* (OL869587.1) sample with a melting temperature of 85.14 °C was used as a positive control.

The data were tabulated and descriptively analyzed. Univariate analysis was performed using the Chi-square or Fisher test to link the detection of *T.evansi* DNA with independent variables, such as sex, race, age, and epidemiological and clinical variables, using statistical software (Epi Information program 3.3.2, CDC, Atlanta, Georgia).

RESULTS

In this study, 403 blood samples from dogs in Barão de Melgaço were examined for *T.evansi* using real-time PCR, and two (0.5%) samples were positive. Among the dogs examined, 230 (57.1%) were males, 362 (89.8%) lacked a breed definition (SRD), and the age group with the largest prevalence was dogs between 3 and 6years old (113 dogs [28%]; Table 1). The two dogs with *T.evansi* DNA were males; one had no racial classification and was over 6years old, and the other, an American Foxhound breed, was between1and3 years old.

Regarding the epidemiological variables investigated, 247 (61.3%) residences were in urban areas,whereas364 (90.3%) were near vegetation. Tabanids, rodents, and marsupials were reported in 291 (72.2%) houses. The epidemiological variables examined are shown in table 2. The two positive dogs lived in a rural regionnear greenery, where as tabanids, rodents, and marsupials were common. They stayedin the peridomiciliary environment with free access to the street, and one of them had access to the municipality of Barão de Melgaço's rural region.

Regarding clinical symptoms, 157 (39%) of the 403 dogs examined were asymptomatic, and 246 (61%) were symptomatic for trypanosomosis. The main clinical changes associated with *T.evansi* infection observed in the dogs are shown in table 3. The two *T.evansi*-positive dogs were symptomatic, with a regular overall condition and hepatosplenomegaly, and one of them also had pale mucous membranes, apathy, anorexia, weight loss, and edema, primarily of the pelvic limbs.

Table 1 - Univariate analysis of the variables sex, breed, and age of 403 dogs surveyed for *Trypanosoma evansi* infection in the municipality of Barão de Melgaço, Mato Grosso.

Variables	Dogs n (%)	Positives (%)	P-value
----------Sex----------			
Male	230 (57.1)	1 (0.9)	0.33
Female	173 (42.9)	0 (0)	
----------Breed----------			
SRD	362 (89.8)	1 (0.2)	0.19
CRD	41 (10.2)	1 (0.2)	
----------Age groups----------			
Undefined age	64 (15.9)	0 (0)	
< 1 year	31 (7.7)	0 (0)	
1 – 3 years	111 (27.5)	1 (0.2)	0.69
3 – 6 years	113 (28)	0 (0)	
> 6 years	84 (20.8)	1 (0.2)	
Total	403 (100)	2 (0.5)	

SRD – Without defined age; CRD – With defined age.

Table 2 - Univariate analysis of the epidemiological variables researched regarding *Trypanosoma evansi* infection in 403 dogs from the municipality of Barão de Melgaço, Mato Grosso.

Variables	Dogs n (%)	Positives (%)	P-value
----------Location of the house----------			
Urban Area	247 (61.3)	2 (0.5)	0.38
Rural Area	154 (38.2)	0 (0)	
----------Vegetation----------			
Yes	364 (90.3)	2 (0.5)	0.82
No	39 (9.7)	0 (0)	
----------Type of vegetation----------			
No	39 (9.7)	0 (0)	0.82
Forests	137 (34)	1 (0.2)	
Wasteland	38 (9.4)	0 (0)	
Rivers/Streams	92 (22.8)	0 (0)	
Associations	97 (24.1)	1 (0.2)	
----------Presence of tabanids----------			
Yes	291 (72.2)	2 (0.5)	0.52
No	112 (27.8)	0 (0)	
----------Presence of rodents/marsupials----------			
Yes	242 (60)	2 (0.5)	0.36
No	161 (40)	0 (0)	
----------Access to the street----------			
Yes	298 (73.9)	2 (0.5)	0.55
No	105 (26.1)	0 (0)	
----------Dog habitat----------			
Intradomestic	12 (3)	0 (0)	0.86
Peridomiciliar	352 (87.3)	2 (0.5)	
Both	39 (9.7)	0 (0)	
----------Access of rural area----------			
Yes	187 (46.4)	1 (0.2)	0.71
No	216 (53.6)	1 (0.2)	
Total	403 (100)	2 (0.5)	

DISCUSSION

The prevalence of dogs positive for *T.evansi* in the municipality of Barão de Melgaço was 0.5%. According to AREGAWI et al. (2019), dogs are considered sentinel animals for *T.evansi* infection because they are highly vulnerable to infection, remain carriers of the protozoan for a short length of time, and have a high mortality rate. ECHEVERRIA et al. (2019) and PORFIRIO et al. (2018) reported that *T.evansi* infection is prevalent in the Brazilian Pantanal, with prevalences of 38% and 29% in dogs from the Mato Grosso do Sul Pantanal region, respectively. These prevalences differ from those reported in this study in Barão's municipality of Melgaço, located in the Pantanal'snorthern region. Lower infection prevalences in dogs have been reported in different regions of Brazil and other countries.

According to VILLENA et al. (2023), *T.evansi* infection in dogs is rare in the Brazilian Amazon, with a prevalence of 1.28% (FILGUEIRAS et al., 2018). In India, NGUYEN et al. (2021) reported a prevalence of 1.8%.In this context, the low prevalence identified in samples from dogs from Barão de Melgaço maybe related to the acute clinical course of the infection, which maymake detecting the protozoan impossible due to the species' high lethality.

Because of the possibility of detection in ill animals and those with subclinical infections, using molecular methods, such as PCR and its variations, to identify *T.evansi* is considered the most accurate method (JAIMES-DUEÑEZ et al., 2017). NGUYEN et al. (2021) reported that the prevalence of *T.evansi* infection in endemic places, such as the Pantanal, is considered to be underestimated due to the low sensitivity and specificity of serological tests and procedures for visualizing the parasite in blood smears. Serological tests can lead to false-positive results due to the possibility of cross-reaction with

Table 3 - Univariate analysis of clinical changes presented by 403 dogs from the municipality of Barão de Melgaço, investigated for *Trypanosoma evansi* infection.

Variables	Dogs n (%)	Positives (%)	P-value
----------Clinical Signs----------			
No clinical signs	157 (39)	0 (0)	0.37
With clinical signs	246 (61)	2 (0.5)	
----------General clinical status----------			
Good	350 (87.8)	0 (0)	
Regular	50 (12.4)	2 (0.5)	0.016*
Bad	1 (0.2)	0 (0)	
----------Mucous membranes----------			
Pale	32 (7.9)	1 (0.2)	
Normocolored	368 (91.3)	1 (0.2)	0.09
Jaundice	3 (0.7)	0 (0)	
----------Apathy----------			
Yes	3 (0.7)	1 (0.2)	0.01*
No	400 (99.3)	1 (0.2)	
----------Anorexia----------			
Yes	2 (0.5)	1 (0.2)	0.01*
No	401 (99.5)	1 (0.2)	
----------Weight loss----------			
Yes	8 (2)	1 (0.2)	0.04*
No	395 (98)	1 (0.2)	
----------Edema----------			
Yes	2 (0.5)	1 (0.2)	0.01*
No	401 (99.5)	1 (0.2)	
----------Splenomegaly----------			
Yes	58 (14.4)	2 (0,5)	0.02*
No	345 (85.6)	0 (0)	
----------Lymphadenomegaly----------			
Yes	88 (21.8)	2 (0.5)	0.05*
No	315 (78.2)	0 (0)	
Total	403 (100)	2 (0.5)	

*Statistically significant difference ($P \leq 0.05$).

other protozoa, whereas failure to detect *T.evansi* in sick animals outside of the parasitemia period can lead to false-negative results (JAIMES-DUEÑEZ et al., 2017; NGUYEN et al., 2021; RECALDE et al., 2021). However, even when using a more sensitive and specific real-time PCR technique than conventional PCR, the prevalence of dog infection in the municipality analyzed was low, reflecting the low prevalence of *T.evansi* infection in dogs.

Regarding the sex and breed of dogs, no association was reported between these variables and an increased risk of infection by *T.evansi*, as reported in other studies (CHOWDHURY et al., 2005; PRASAD et al., 2015; ASIF et al., 2020). The two infected dogs were males; one had no breed definition, and the other was an American Foxhound. However, when it comes to the age variable, PRASAD et al. (2015) found a higher prevalence of infection in dogs up to 2 years of age (2.77%) and a lower prevalence in dogs over 8 years old, suggesting that younger dogs with a tendency to decline in immunity may be more susceptible to infection. This analysis was not possible in this study because the positive dogs were of various ages, one over 6 years old and the other between 1and 3 years old. ASIF et al. (2020) believed that reduced infection in adult dogs is attributable to improved immunological response.

There was no statistically significant relationship between the epidemiological variables evaluated and *T.evansi* infection. However, the positive dogs lived in urban areas but near forests and streams, and the presence of tabanids, rodents,

and marsupials was also noted in the areas where the residences were located. These characteristics have been identified as infection risk factors (AQUINO et al., 1999; FILGUEIRAS et al., 2018; NGUYEN et al., 2021). According to KHAN et al. (2022), dogs that move near bodies of water, such as lakes and dams, are at a higher risk of infection due to vector (fly) activity, particularly during the rainy season. However, domestic dogs' predation on infected carcasses of small wild animals is considered a form of transmission (BONO-BATTISTONI et al., 2016; BILHEIRO et al., 2019).

According to RECALDE et al. (2021), the acute nature of the infection in dogs shows that the sources of infection are prevalentin the environment where the dogs live. Although, dogs are highly susceptible to the parasite, the author claims that detecting *T.evansi* is uncommon, highlighting the importance of these dogs' proximity to local reservoirs, which facilitate mechanical transmission by hematophagous insects. Another important aspect is locating marsupials near the houses of positive dogs. HERRERA et al. (2004) reported that the detection of *T.evansi* in small rodents and marsupials emphasizes the importance of other vector species being involved in the transmission because while rodents and marsupials are nocturnal, hematophagous flies from the Tabanidae family are active during the day and at night, increasing the chances of transmission.

Apathy, weight loss, fever, cachexia, hepatomegaly, splenomegaly, lymphadenomegaly, edema, paresis, and, in severe cases, nervous symptoms and death have been reported in sick dogs (BONO-BATTISTONI et al., 2016; FILGUEIRAS et al., 2018; RECALDE et al., 2021; SAZMAND et al., 2022). The dogs in this study who tested positive for *T.evansi* had a normal overall condition, splenomegaly, and lymphadenomegaly, which are common clinical signs of infection (AQUINO et al., 1999; JAIMES-DUEÑEZ et al., 2017). However, one of the dogs showed signs of anorexia, apathy, weight loss, edema, and pale mucous membranes, which are common in *T.evansi* infection (BONO-BATTISTONI et al., 2016; FILGUEIRAS et al., 2018; RECALDE et al., 2021; SAZMAND et al., 2022). Although, many clinical signs were statistically associated with infection ($P \leq 0.05$), these findings should be analyzed cautiously due to the small number of positive dogs.

CONCLUSION

Despite a low prevalence (0.5%), the detection of two dogs positive for *T.evansi* reveals the presence of the protozoan in the canine population of the municipality of Barão de Melgaço. Although, no risk factors were found to be associated with the infection ($P > 0.05$), proximity to vegetation and streams and the presence of tabanids, rodents, and marsupials, which are factors in the occurrence of infection, were observed in positive dogs, implying that the municipality of Barão de Melgaço has epidemiological characteristics that allow canine infection by *T.evansi*.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically reviewed the manuscript and approved its final version.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This research was approved by the Ethics Committee on the Use of Animals of the Federal University of Mato Grosso (CEUA-UFMT), under protocol nº 23108.068363/2021-82, and followed the principles of the Brazilian Government for the use and care of vertebrate animals.

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