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Short Communication

Molecular detection of trypanosomatids in neotropical primates in the state of Mato Grosso, Midwest, Brazil

Detecção molecular de tripanossomatídeos em primatas neotropicais no estado de Mato Grosso, Centro-Oeste, Brasil

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Abstract

Trypanosomatids are uniflagellate protozoa belonging to the Trypanosomatidae family. The genera *Trypanosoma* and *Leishmania* are of paramount importance as they contain species that cause serious diseases, such as Chagas disease and Leishmaniasis, respectively. The objective of the present study was to identify trypanosomatids present in the whole blood of free-living and captive neotropical primates in Mato Grosso State, Midwest Brazil. Between 2017 and 2019, 38 blood samples were collected from seven different neotropical primate species in seven cities in the state. Through molecular techniques, including polymerase chain reaction (PCR) to amplify a fragment of the kinetoplast DNA (kDNA) and 18S ribosomal RNA (18S rRNA) gene, sequencing, and phylogenetic analysis, nine *Leishmania* spp. [seven *L. infantum* and two *L. (Leishmania) amazonensis*] and two *Trypanosoma* spp. (*T. minasense* and *T. rangeli*) were identified. This study contributes to understanding the occurrence and epidemiology of trypanosomatids in Mato Grosso State and the importance of neotropical primates as trypanosome hosts and possible infection sources for other animals and humans. Future identification of other blood pathogens in neotropical primates will assist in disease control and prevention strategies.

Keywords: PCR, protozoan, phylogenetic analysis, zoonosis, Leishmaniasis.

Resumo

Tripanossomatídeos são protozoários uniflagelados pertencentes à família Trypanosomatidae. Os gêneros *Trypanosoma* e *Leishmania* são de suma importância por conterem espécies causadoras de doenças graves, como doença de Chagas e Leishmaniose, respectivamente. O objetivo do presente estudo foi identificar tripanossomatídeos presentes no sangue total de primatas neotropicais de vida livre e cativos no Estado de Mato Grosso, Centro-Oeste do Brasil. Entre 2017 e 2019, foram coletadas 38 amostras de sangue de sete diferentes espécies de primatas neotropicais em sete cidades do Estado. Foram identificados por meio de técnicas moleculares, incluindo reação em cadeia da polimerase (PCR), para amplificar um fragmento do DNA do cinetoplasto (kDNA) e do gene do RNA ribossômico 18S (rRNA 18S), sequenciamento e análise filogenética, nove

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Leishmania spp. [sete *L. infantum* e dois *L. (Leishmania) amazonensis*] e dois *Trypanosoma* spp. (*T. minasense* e *T. rangeli*). Este estudo contribui para o entendimento da ocorrência e epidemiologia dos tripanossomatídeos no Estado de Mato Grosso e a importância dos primatas neotropicais como hospedeiros tripanossômicos e possíveis fontes de infecção para outros animais e humanos. A identificação futura de outros patógenos do sangue em primatas neotropicais ajudará no controle de doenças e em estratégias de prevenção.

Palavras-chave: PCR, protozoário, análise filogenética, zoonoses, Leishmanioses.

Trypanosoma and *Leishmania* are uniflagellate protozoa belonging to the Trypanosomatidae family (Ortiz & Solari, 2019). Some species are pathogenic to animals and humans, such as *Trypanosoma cruzi* and *Leishmania infantum,* which are responsible for Chagas disease and Visceral Leishmaniasis, respectively (Kaufer et al., 2017).

Wild animals are susceptible to infection, and as a group, neotropical primates are of great significance as they can act as reservoirs for these pathogens (Solórzano-García & Pérez-Ponce de León, 2018). The growth of cities, deforestation, expansion of agriculture, and maintenance of wild fauna in captivity have promoted interaction between neotropical primates and humans, increasing the risk of spreading anthropozoonoses. Therefore, studying and understanding this relationship provides pertinent information within an epidemiological scope, allowing knowledge acquisition of transmission dynamics, the sentinel role of the host, and the risks of disease emergence (Aysanoa et al., 2017).

In Brazil, information regarding the potential role of primates as a reservoir of trypanosomatid infections is scarce, and diagnosis may represent a prophylactic measure for zoonoses control, especially for non-human primates because of their physiological, genetic, and geographic proximity to humans (Wolfe et al., 1998; Coimbra et al., 2020).

Therefore, the present study aimed to detect trypanosomatid infection from the whole blood of free-living and captive neotropical primates in Mato Grosso State, Midwest Brazil, using molecular techniques, including polymerase chain reaction (PCR), sequencing, and phylogenetic analysis.

Sample collection procedures were approved by the Ethics Committee on Animal Research of the Federal University of Mato Grosso (UFMT) under protocol no. 23108.015878/2019-65 and Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), permit no. 40617-1 and 42303.

Animals captured by the Environmental Agency of the State of Mato Grosso (SEMA) and Zoonosis Disease Control (CCZ) of Cuiabá municipality were admitted at the UFMT veterinary hospital between 2017 and 2019. The primates were immobilized using ketamine and midazolam according to the body weight of each animal (Cubas et al., 2014). Whole blood samples were collected from free-ranging and captive neotropical primates, stored in tubes containing ethylenediaminetetraacetic acid (EDTA), regardless of clinical signs, and sent to the Veterinary Microbiology and Molecular Biology Laboratory at the UFMT, Cuiabá, Brazil.

The samples were stored at –80°C before molecular tests, and DNA extraction was then conducted using the phenol/chloroform/proteinase K method, as previously described (Sambrook & Russell, 2001). To verify the presence of PCR inhibitors in the DNA samples and extraction success, we tested for the presence of the mammalian glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, with the oligonucleotides GAPDHF and GAPDHR, following the method by Birkenheuer et al. (2003).

All DNA-extracted samples were screened for *Leishmania* by conventional PCR performed with LeishF and LeishR primers, as described by Degrave et al. (1994). Two additional PCR protocols were applied for further testing of positive samples: one using the primers RV1 and RV2, which amplify a fragment of the kinetoplast DNA (kDNA) of *L. infantum* (Lachaud et al., 2002), and another using LSPF and LLAR primers to amplify a region from the kDNA minicircle of *Leishmania (Leishmania) amazonensis* (Conter et al., 2018). For molecular identification of *Trypanosoma*, a fifth PCR targeting a fragment of the 18S ribosomal RNA (18S) rRNA gene was performed as previously described (Silva et al., 2004). Amplicons previously sequenced for each *Leishmania* parasite [*L. infantum* and *L*. (*L.) amazonensis*] were used as positive controls for PCR reactions, and *T. vivax* was used for *Trypanosoma* with ultrapure water acting as a negative control.

PCR products were resolved in 1% agarose gels stained with GelRed™ Nucleic Acid Gel Stain (Biotium Inc., Fremont, CA, USA) and visualized using a ChemiDoc XRS system (Bio-Rad Laboratories, Hercules, CA, USA). The expected-size amplicons of *Trypanosoma* were purified using the Illustra GFX PCR DNA and Gel Band Purification Kit (Cytiva, Chicago, IL, USA) and prepared for sequencing using a BigDye™ kit (Applied Biosystems, Foster, CA, USA). An ABI-PRISM 3500 Genetic Analyzer (Applied Biosystems) was used for sequencing using the same primers used for

PCR. The obtained sequences were compared with the DNA database using the BLAST® algorithm (version 2.8.0) from the National Center for Biotechnology Information (NCBI, 2021) to determine the closest identities with congeneric organisms available in GenBank®.

Sequences of the V7-V8 region of the small ribosomal subunit (SSU) rRNA gene generated in this study and homolog sequences retrieved from GenBank® were used for phylogenetic analyses to characterize *Trypanosoma* species. A cladrogram was built based on the neighbor-joining technique with the bootstrap method with 1,000 replicates using MEGA-X (Molecular Evolutionary Genetics Analysis) version 10.1.8 (Tamura et al., 2004). GenBank® DNA sequences of *Trypanosoma* spp. were included for phylogenetic analysis. *Leishmania* species were allocated as the out-group (Figure 1).

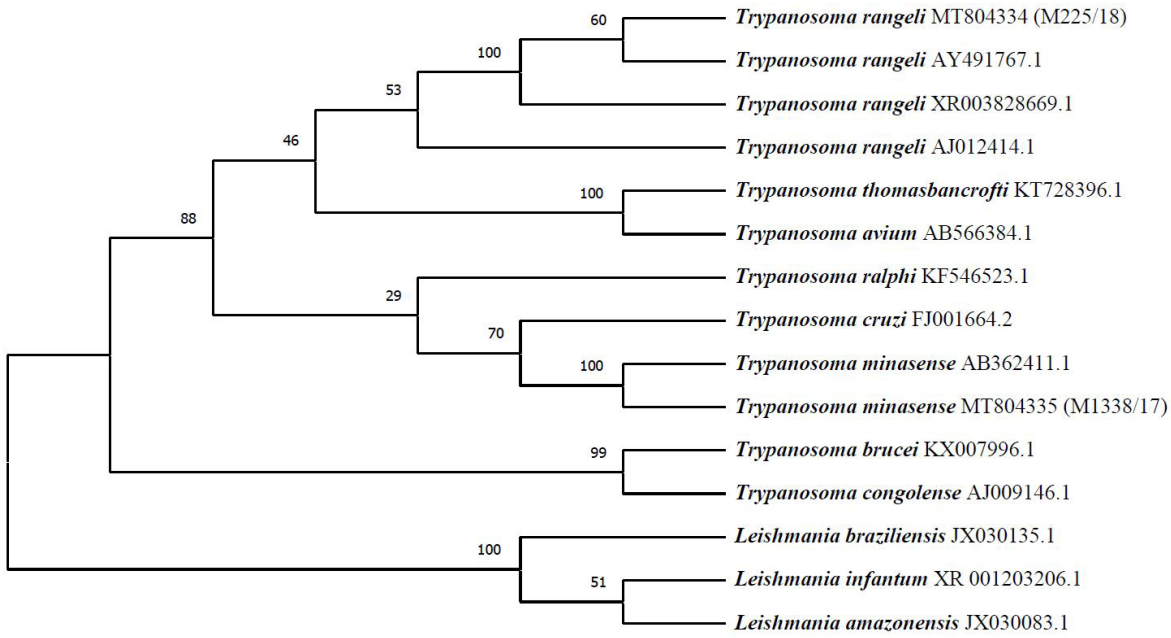

Figure 1. Cladogram of *Trypanosoma* spp. detected in neotropical primates, constructed using MEGA-X version 10.1.8. The cladogram was generated using the neighbor-joining technique performed with 805 base pairs of the V7-V8 region of parasite SSU rRNA genes. The initialization values are shown next to the nodes (1,000 replicates). All sequences obtained in this study are within one group, and *Leishmania* species were used as an out-group.

Thirty-eight neotropical primates representing *Alouatta caraya, Aotus azarae, Aotus infulatus, Ateles marginatus, Mico melanurus,* and *Sapajus apella* species were captured from Cuiabá (15°35'46.0"S 56°05'48.0"W), Chapada dos Guimarães (15°27'38.0"S 55°44'59.0"W), Diamantino (14°24'31.0"S 56°26'46.0"W), Nobres (14°43'13.0"S 56°19'39.0"W), Poconé (16°15'24.0"S 56°37'22.0"W), Tangará da Serra (14°37'40"S 57° 30' 25"W), and Várzea Grande (15°38'48.0"S 56°07'57.0"W) municipalities, Mato Grosso (Figure 2).

PCR results showed that among the 38 samples evaluated, 11 (28.94%) contained trypanosomatid DNA (Table 1); 9 of these were from free-ranging animals (23.38%) that were positive for *Leishmania* spp. Among these, seven individuals (18.42%; including three *M. melanurus*, three *A. azarae*, and one *S. apella*) were infected with *L. infantum*, whereas two animals (5.26%; *S. apella* and *M. melanurus*) tested positive for *L. (L.) amazonensis*.

Furthermore, two (5.26%) of the 18S PCR-positive samples yielded amplicons for *Trypanosoma* species. Partial DNA sequences generated from *S. apella* yielded a haplotype with 99.20% (871/878 bp) similarity to *T. minasense*, designated as *T. minasense* isolate M1338/17 (GenBank number MT804335). Further, a DNA sequence obtained from *M. melanurus* had a 99.36% (938/944 bp) similarity with *T. rangeli*, designated as *T. rangeli* isolate M225/18 (GenBank number MT804334). The similarity of DNA and phylogenetic analysis were conclusive for accurate molecular classification. Coinfection was not observed in this study.

The best method for accurately identifying trypanosomatids is through molecular techniques, such as PCR and sequencing. They have greater specificity and differentiation capacity between species and are performed within a short time frame compared, for example, with blood cultures (Coimbra et al., 2020).

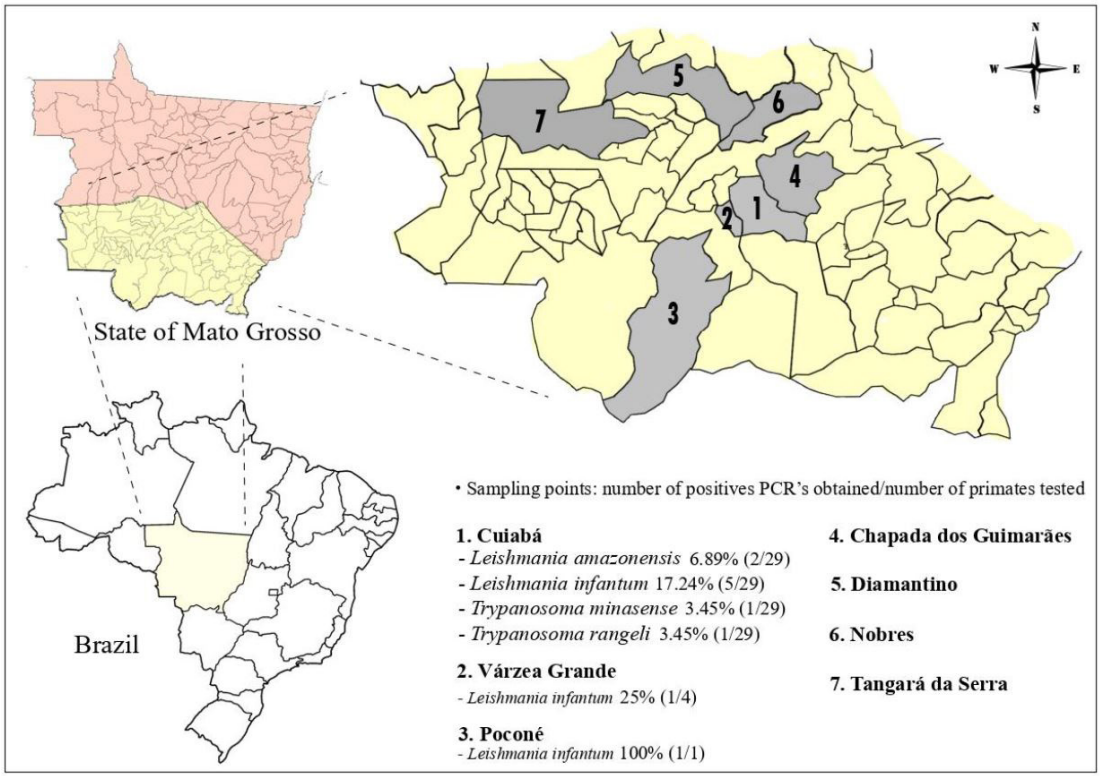

Figure 2. Map of the municipalities within the State of Mato Grosso where samples of the primates were collected for the polymerase chain reaction (PCR) with the respective infection rates (%).

Table 1. Epidemiological data of the animals tested and their respective results (F = Female, M = Male).

Identification	Host	Habitat	Sex	Municipality	*Trypanosoma* spp.	*Leishmania* spp.
M05/17	*Sapajus apella*	Captive	F	Cuiabá	-	-
M08/17	*Sapajus apella*	Captive	F	Cuiabá	-	-
M530/17	*Sapajus apella*	Free-ranging	M	Cuiabá	-	-
M1247/17	*Sapajus apella*	Captive	M	Várzea Grande	-	-
M1249/17	*Sapajus apella*	Captive	F	Várzea Grande	-	-
M1313/17	*Sapajus apella*	Free-ranging	M	Cuiabá	-	-
M1328/17	*Alouatta caraya*	Free-ranging	M	Cuiabá	-	-
M1338/17	*Sapajus apella*	Free-ranging	F	Cuiabá	***T. minasense***	-
M1339/17	*Mico melanurus*	Free-ranging	F	Cuiabá	-	-
M2043/17	*Mico melanurus*	Free-ranging	M	Cuiabá	-	-
M35/18	*Mico melanurus*	Free-ranging	M	Cuiabá	-	-
M64/18	*Aotus infulatus*	Free-ranging	M	Cuiabá	-	-
M81/18	*Aotus azarae*	Free-ranging	M	Cuiabá	-	-
M133/18	*Aotus azarae*	Free-ranging	F	Chapada dos Guimarães	-	-
M180/18	*Mico melanurus*	Free-ranging	M	Cuiabá	-	-
M204/18	*Mico melanurus*	Free-ranging	F	Cuiabá	-	-
M225/18	*Mico melanurus*	Free-ranging	M	Cuiabá	***T. rangeli***	-

Table 1. Continued...

Identification	Host	Habitat	Sex	Municipality	*Trypanosoma* spp.	*Leishmania* spp.
M656/18	*Sapajus apella*	Free-ranging	F	Cuiabá	-	***L. amazonensis***
M742/18	*Alouatta caraya*	Captive	M	Várzea Grande	-	-
M897/18	*Mico melanurus*	Free-ranging	M	Diamantino	-	-
M970/18	*Mico melanurus*	Free-ranging	M	Cuiabá	-	***L. amazonensis***
M1022/18	*Aotus azarae*	Free-ranging	F	Cuiabá	-	***L. infantum***
M1057/18	*Sapajus apella*	Free-ranging	M	Nobres	-	-
M1098/18	*Sapajus apella*	Captive	F	Tangará da Serra	-	-
M1106/18	*Sapajus apella*	Captive	F	Cuiabá	-	-
M1176/18	*Aotus azarae*	Free-ranging	F	Cuiabá	-	***L. infantum***
M1241/18	*Sapajus apella*	Free-ranging	M	Poconé	-	***L. infantum***
M522/19	*Aotus infulatus*	Free-ranging	F	Cuiabá	-	-
M603/19	*Alouatta caraya*	Captive	F	Cuiabá	-	-
M627/19	*Mico melanurus*	Free-ranging	F	Cuiabá	-	***L. infantum***
M726/19	*Aotus infulatus*	Free-ranging	M	Cuiabá	-	-
M790/19	*Mico melanurus*	Free-ranging	F	Várzea Grande	-	***L. infantum***
M1176/19	*Aotus azarae*	Free-ranging	F	Cuiabá	-	***L. infantum***
M1460/19	*Mico melanurus*	Free-ranging	F	Cuiabá	-	***L. infantum***
M1638/19	*Ateles marginatus*	Captive	F	Cuiabá	-	-
M1639/19	*Ateles marginatus*	Captive	F	Cuiabá	-	-
M1640/19	*Ateles marginatus*	Captive	F	Cuiabá	-	-
M1641/19	*Ateles marginatus*	Captive	F	Cuiabá	-	-

Considering this bias, the results obtained for *Leishmania* spp. indicated a high *L. infantum* occurrence, accounting for 77.77% (7/9) of the species identified. This finding points to a public health concern as non-human primates may be competent in transmitting *L. infantum* to the invertebrate vector *Lutzomyia longipalpis* (Oliveira et al., 2019).

Molecular detection also was been reported for the first time with respect to *L. infantum* in three primates of the species, *A. azarae*, a species reported to be refractory to infection in a study of *in vitro* infection of peritoneal macrophages (Carneiro et al., 2012). This could be attributed to *in vivo* immune responses that have complex characteristics. Several factors, such as the nutritional status of the individual, particular immune response, and influence predisposition to clinical leishmaniasis infection; therefore, the presence of the trypanosome does not necessarily mean the animals had leishmaniasis, and the parasites may be eliminated from the animal (Serafim et al., 2010; Laurenti et al., 2014).

L. (L.) amazonensis is endemic to South America. In the current study, *L. (L.) amazonensis* DNA was detected by PCR in the blood of two primate species (*M. melanurus* and *S. apella*). After experimental infection, *S. apella* exhibited self-healing characteristics against *L. (L.) amazonensis* infection owing to components of their innate and acquired immunity with complete elimination of the disease after 150 days (Laurenti et al., 2014).

T. minasense was identified in free-living *S. apella*. This trypanosome is classified as non-pathogenic, specific to non-human primates, and therefore, there are no reports of human infections (Ziccardi et al., 1996). Moreover, *T. minasense* has been detected in *Alouatta caraya* in captivity in Ilha Solteira, São Paulo, Brazil (Tenório et al., 2014), and three free-living *Callithrix* spp. at a botanical garden in downtown Rio de Janeiro, Brazil (Coimbra et al., 2020). Even with *T. minasense* present in several Brazilian regions, the public health risk is minimal since this protozoan is considered apathogenic; however, the reported finding is of descriptive importance.

In the present study, *T. rangeli* was identified in free-living *M. melanurus*. *T. rangeli* has already been detected in 72 primates of the species *Saguinus bicolor* (Callitrichidae) free-living in the Amazon rainforest (Silva et al., 2008). This protozoan is classified as non-pathogenic to vertebrates. However, unlike *T. minasense*, it has zoonotic potential (Silva et al., 2008). Moreover, detecting this parasite does not represent a public health issue because, in humans and domestic or wild reservoirs, parasitemia has a short duration and is low (Ramirez et al., 1998).

Our findings contribute to understanding the occurrence and epidemiology of diseases caused by *Trypanosoma* and *Leishmania* in Mato Grosso State, Brazil, and the importance of neotropical primates, which may play a role as hosts and possible infection sources of these protozoans for other animals and humans. Taken together, our study encourages further work to identify other pathogens in these animals, which will assist in disease control and prevention strategies.

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