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RESEARCH

Detection of *Trypanosoma evansi* in jaguars (*Panthera onca*): insights from the Brazilian Pantanal wetland

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Abstract

Trypanosoma evansi is a widespread and neglected zoonotic parasite that affects domestic and wild animals, causing a disease commonly known as "surra." The Brazilian Pantanal wetland is recognized as an enzootic area for this protozoan, yet recognizing the importance of reservoir hosts also in order to prevent zoonotic outbreaks. This study aimed to assess the occurrence of *T. evansi* in jaguars (*Panthera onca*) from the Brazilian Pantanal wetland and explore associated clinical and hematological manifestations. A total of 42 animals were screened by PCR and sequenced for species identification when positive. *Trypanosoma evansi* was detected in six free-ranging jaguars (six positive animals of 42 captures and 16 recaptures), representing the first molecular evidence of such infection in this animal species. Our findings suggest that jaguars may act as reservoir hosts of *T. evansi* in the Brazilian Pantanal wetland. The better understanding of the role of wildlife in the epidemiology of *T. evansi* is also of importance to future reintroduction and translocation programs toward wildlife conservation efforts.

Keywords Hemoparasite · Protozoan · Wild felids · Tripanossomiasis · Brazil

Introduction

Trypanosoma evansi, the etiological agent of a neglected disease known as "surra" or in Portuguese "mal das cadeiras," affects a wide range of domestic and wild animals (Franke et al. 1994; Silva et al. 1995; Herrera et al. 2004), with sporadic cases also reported in humans (Joshi et al., 2005; Powar et al. 2006). Previous studies have documented that many animal species, such as cattle (Franke et al. 1994), horses (Rodrigues et al. 2005), dogs (Echeverria et al. 2019; Nguyen et al., 2021), tigers (Upadhye and Dhoot, 2000), camels (Desquesnes et al. 2008), and bats (Herrera et al. 2004), and even humans may be susceptible to *T. evansi* infection. In addition, a zoo outbreak was reported in India, which many felid species, including jaguars (*Panthera onca*), died due to *T. evansi* (Sinha et al., 1971).

The Brazilian Pantanal biome has been identified as an enzootic area for *T. evansi* (Nunes et al. 1993). Therefore, understanding the ecology of this protozoan in the region is advocated, especially considering the potential wild reservoir hosts (Herrera et al. 2004).

The ecosystem complexity and its biodiversity require a comprehensive approach to disease ecology, including studies about potential disease reservoirs among both common and endangered wildlife species. The jaguar, as one of the apex predators in South America, merits particular attention, and although its susceptibility to the infection has been demonstrated in captive animals (Sinha et al., 1971; Khan et al., 2023), the role of free ranging jaguars in the transmission dynamics of *T. evansi* remain understudied. Under the above

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circumstances, this study aimed to assess the occurrence of *T. evansi* infection in free-ranging jaguars within the Brazilian Pantanal wetland.

Material and methods

The study was conducted in the municipality of Miranda, Mato Grosso do Sul state, Brazil, within the Pantanal biome, between 2013 and 2023 (Fig. 1). Jaguars were captured using foot snares (May-Junior et al. 2021) to collect biological samples and set GPS/VHF radio collars. The radio collars had drop-off system after 12 months, so recaptures were not necessary to remove it. In all animals, we assessed the health parameters, including mucosal color, capillary refill, hematocrit, blood smear, rectal temperature, and weight (kg); also, radio or GPS collars were set when possible, according to both collar availability and jaguar size. Only a young female jaguar was not set a radio collar, as she was young and too small (50 kg). Ethical procedures were approved by the Ministry of Environment under license number #42093-1.

Blood samples were collected and processed using the PureLink® Genomic DNA Mini Kit (Invitrogen™, Carlsbad, CA, USA) to extract genomic DNA (200 µL volume) following the manufacturer's instructions. Molecular detection of *T. evansi* involved the use of two specific primer protocols. Firstly, an assay was caried out to amplify a 315-bp fragment following Ventura et al. (2002). Subsequently, an assay was conducted to amplify a ~ 540-bp ITS-1 fragment, as described by Desquesnes et al. (2001). Blood samples from experimentally infected mice and naturally infected dogs were used as positive controls. In order to evaluate the quantity and quality of the extracted DNA, a NanoDrop™ spectrophotometer at an absorbance of 260/280 nm was used.

One random sample was selected to retrieve sequence. Amplicons of the expected size were purified and sequenced in both directions using the Big Dye Terminator v.3.1 chemistry in a 3130 Genetic Analyzer (Applied Biosystems, California, USA) equipped with an automated sequencer (ABI-PRISM 377). The resulting sequence was aligned using the fast Fourier transform algorithm in MAFFT (Katoh et al. 2019) and subsequently compared with reference sequences available in the GenBank database using the Basic Local Alignment Search Tool (BLAST). The sequence was submitted to the GenBank database under the accession number: OR797837.

Results

Six (four females and two males) out of 58 captures (42 animals and 16 recaptures) scored positive at PCR (Table 1) (Supplementary Table 1), representing an occurrence of 14.28% (6/42). At the BLAST analysis, the

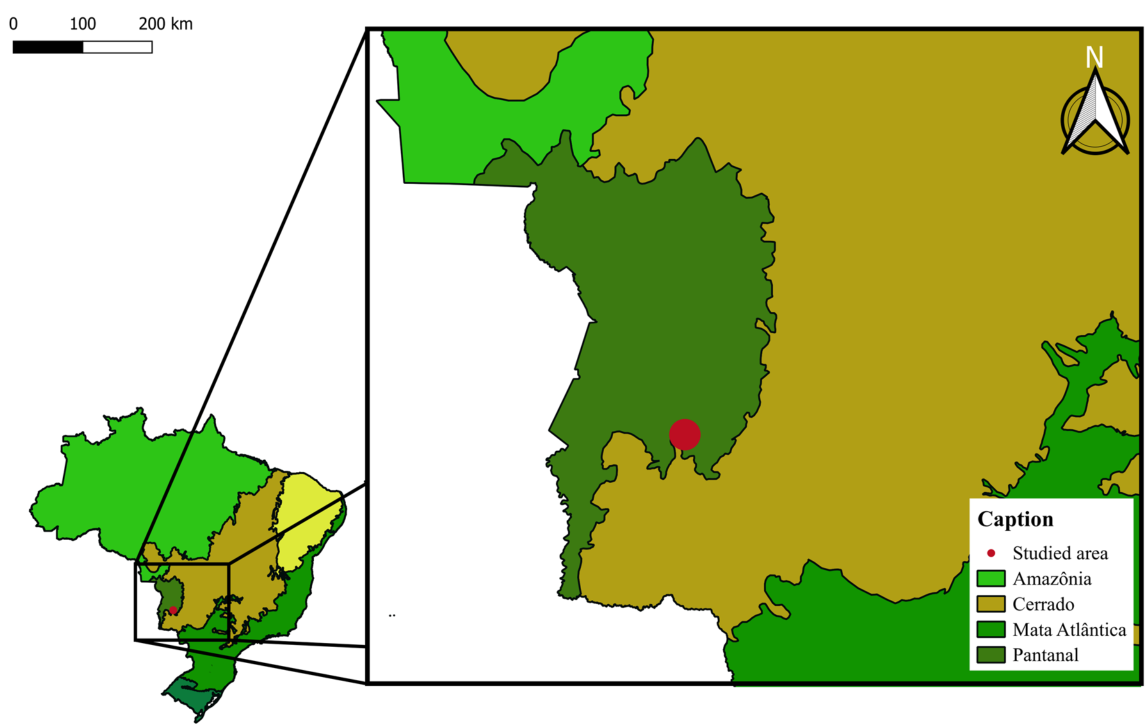

Fig. 1 Geographical location of the studied area in the Brazilian territory

Table 1 Captures and recaptures of *Trypanosoma evansi* positive free-ranging jaguars (*Panthera onca*)

Sample	Gender	Capture		Weight (Kg)	PCV (%)	Detection of *T. evansi*
		Month	Year			
bPon424	Female	September	2017	92	nt	+
bPon440	Female	June	2014	110	38	−
		January	2017	100	nt	−
		November	2018	86.4	38	+
bPon495	Male	July	2017	~ 120	nt	+
		June	2020	127	nt	−
bPon498	Female	September	2017	50.7	nt	+
bPon509	Female	November	2018	60	42	+
bPon522	Male	February	2021	96.2	40	+

Kg, kilogram; *PCV*, packed cell volume; %, percent; *nt*, not tested

evaluated sequence showed 100% nucleotide identity with *T. evansi* (Accession number: AB551922.1, AY912279.1, AF306775.1). The jaguars did not suffer for major clinical changes at the time of capture. However, two females (bPon 424 and bPon 440) had capillary refill in 2 (bPon 424) and 1.5 s (bPon 440) and presented a senile aspect with white hair and loss of muscle mass in their hind limbs, probably associated with aging, as bPon 424 was more than 10 years old and bPon 440, more than 12 years old. Jaguars maintained a regular PCV (Table 1), according to the parameters for the species in the studied region (40.7 ± 5.0—calculus made from 37 monitored jaguars—data not shown). All blood smears evaluated through optical microscopy were negative for *Trypanosoma* sp.

Discussion

In this study, we report the occurrence of *T. evansi* in free-ranging jaguars from the Pantanal wetland in Brazil. While the role of this animal species in the epidemiology of this parasite is unknown, the finding herein reported is of importance since their involvement in the maintenance of *T. evansi* in nature.

The absence of clinical signs compatible with *T. evansi* infection in animals herein evaluated contrasts with findings from captive jaguars (Sinha et al. 1971), and domestic cats, that shows clinical signs and positive in blood smears (Priyowidodo et al. 2023), suggesting that free-ranging individuals may exhibit a distinct response to this protozoan infection. Immunotolerance to trypanosomes in carnivores remains a complex and understudied phenomenon. However, extrinsic factors, such as the exposure to high challenges through predation of infected prey and invertebrate vectors, have been associated with resistance (Murray et al. 1982; Kasozi et al. 2021). In the context of our study population of free-ranging jaguars, it is likely that they exhibit trypanotolerance, mainly considering Pantanal region is an enzootic area for *T. evansi* (Herrera et al. 2004). In a similar scenario, Serengeti lions, which are frequently exposed to multiple trypanosome species through tsetse fly bites and the consumption of infected meat, have demonstrated cross-immunity to *Trypanosoma brucei*, enabling them to eliminate the infection after exposure (Welburn et al. 2008). Furthermore, it is worth noting that one male (bPon 495), initially tested positive upon capture, scored negative during a 3-year interval recapture. This observation suggests either the suppression of the infection or a reduction of trypanosomiasis to undetectable levels by PCR. This highlights the complexities of pathogen-host interactions and underscores the need for thorough species-specific investigations. In addition, the jaguar population of this study presents a high prevalence of infection by *Cytauxzoon* sp. (Fagundes-Moreira et al. 2022). The synergism between infections of distinct pathogens, associated with environmental changes, had important effects on the populations of African large felids (Munson et al. 2008).

One of the possible transmission routes to jaguars may be the ingestion of reservoir preys, once oral transmission was already suggested in the Indian zoo outbreak (Sinha et al. 1971, Khan et al., 2023) and proved in dogs and mice (Raina et al. 1985; Bazolli et al. 2002); additionally, leopards (*Panthera pardus*) are reservoirs to *T. brucei* and were also suspected to be orally infected by its prey (Anderson et al. 2011). For example, tapirs (*Tapirus terrestris*) and capybaras (*Hydrochaerus hydrochaeris*) are common prey for jaguars, being also considered reservoirs of *T. evansi* in the Pantanal wetlands, with high parasitemia recorded in capybaras (Franke et al. 1994; Herrera et al. 2004; Rademaker et al. 2009; Fundación Rewilding Argentina, 2020). The transmission of *T. evansi* to domestic animals is commonly associated with tabanid bites (Desquesnes et al. 2005, Kamidi et al., 2017), but it may also be the case for wildlife reservoirs. For example, previous studies demonstrated that tabanid species (e.g., *Tabanus importunus*, *Tabanus occidentalis*), associated with *T. evansi*, were more prevalent during the rainy season, from September to the first days of March (Silva et al., 1995; Barros, 2001), which also coincides with capybaras breeding period (Aldana-Domínguez et al. 2002). Accordingly, in this study, five out of six *T. evansi* positive jaguars were diagnosed during the rainy season. Therefore, the increase of vectors and the availability of "easier" prey (younger and susceptible capybaras) acting as reservoirs may represent an important factor in *T. evansi* transmission to jaguars.

The implications of these findings are far-reaching for wildlife conservation efforts, especially considering the critical role of jaguars in the ecological balance of the Pantanal wetland ecosystem. Importantly, our results should be incorporated into risk assessments for reintroduction or translocation programs of jaguars and other South American wildlife to prevent accidental pathogen introduction to a susceptible population and potential disease outbreaks (Viggers et al. 1993).

Conclusion

Our findings from the Pantanal demonstrate the presence of subclinical *T. evansi* in South American free-ranging jaguars. As our understanding of the relationships between pathogens, hosts, and the environment continues to grow, the importance of interdisciplinary research in developing effective conservation strategies remains undeniable. Therefore, further studies on the impact of *T. evansi* on jaguars are advocated to better understand the risks associated and guide the safeguarding of biodiversity and long-term survival of this endangered species.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00436-023-08101-0.

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Author contributions RFM: Conceptualization, Investigation, Methodology, Writing—original draft preparation. VBS: Formal Analysis, Methodology, Writing—review and editing. JAMJ: Investigation, Methodology, Writing—review and editing. LB: Conceptualization, Formal Analysis, Investigation, Methodology. LCB: Investigation, Methodology. AOR: Investigation, Methodology. LS: Investigation, Methodology. LER: Investigation, Methodology. MABS: Investigation, Methodology, Writing—review and editing. DO: Conceptualization, Methodology, Supervision, Writing— review and editing. JFS: Conceptualization, Investigation, Methodology, Project administration, Supervision, Writing—review and editing.

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Data availability No datasets were generated or analyzed during the current study.

Declarations

Ethics approval The procedures herein described were conducted in accordance with the Brazilian Institute of the Environment and Renewable Natural Resources-IBAMA (Authorization n. 42093-1) and the Research Committee of the Federal University of Rio Grande do Sul-Compesq (Authorization n. 38198).

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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