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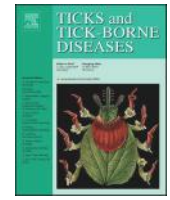

Short communication

Epidemiological compatibility of *Amblyomma sculptum* as possible vector and *Panthera onca* as reservoir of *Cytauxzoon* spp. in Midwestern Brazil

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ABSTRACT

Cytauxzoonosis is an acute and highly lethal tick-borne disease of wild and domestic cats, and is widely distributed in Africa, Asia, Europe, the USA and Brazil. So far, only two tick species present on the USA are experimentally confirmed in *Cytauxzoon* transmission however, in Brazil and other continents, the epidemiology of the disease remains unknown. Evidences points to *Panthera onca* as a possible reservoir, but there is no evidence to point the vector. Therefore, this study evaluates the presence of *Cytauxzoon* spp. in wild felids from areas with and without records of *Amblyomma sculptum* this ixodid for comparison. Overall, 53 blood samples of *P. onca, Puma concolor*, and *Leopardus pardalis* from the Midwest region (MR; region with *A. sculptum*) and 143 blood and/or spleen samples from *Leopardus geoffroyi, Leopardus wiedii, Leopardus munoai, Leopardus guttulus, Herpailurus yagouaroundi*, L. *pardalis*, and *P. concolor* from Rio Grande do Sul State (RS; without *A. sculptum*). Only one feline sample was negative for *Cytauxzoon* sp. from MR; no samples from RS were positive. In total, 507 ticks were identified from MR felids, with predominance of *A. sculptum* (69.23%). In RS, there were 93 ixodids, of which 90.32% were *Amblyomma aureolatum*. The difference in the tick fauna of the two regions studied (presence/absence of *A. sculptum*) reflects the results found. This study highlighted *A. sculptum* as a possible vector since this hemoparasite was abundantly observed in areas where it occurs, also, there was no evidence of *Cytauxzoon* spp. where it was absent. Additionally, the study supported the suggestion that *P. onca* is the reservoir for the agent in MR.

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1. Introduction

Feline tick-borne diseases such as babesiosis and cytauxzoonosis are of great importance as they are widely distributed, evolve acutely and present high lethality (Wagner, 1976; Greene, 2006; Birkenheuer et al., 2006). In Brazil, studies have evaluated the presence of *Cytauxzoon* spp. in *Panthera onca* and other free-living and captive felids, suggesting them as reservoirs (André et al., 2009; Furtado et al., 2017) as they do not manifest clinical changes. Still, more studies are needed in different areas inhabited by wild felids.

The genus *Cytauxzoon* is reported in Africa, Asia, Europe, the USA, and Brazil (Karaca et al., 2007; Millán et al., 2007; Peixoto et al., 2008; André et al., 2009; Gallusová et al., 2016; Furtado et al., 2017; Hodžić et al., 2018; Zou et al., 2019; Moghaddam et al., 2020; Panait et al., 2021). *Amblyomma americanum* and *Dermacentor variabilis* are the only known vectors, however, they only occur in the USA (Blouin et al., 1984; Reichard et al., 2009). *Ixodes ricinus* is believed to be the vector in Europe since it is the predominant tick on domestic and wild animals and is present where the genus *Cytauxzoon* circulates (Gallusová et al., 2016; D'Amico et al., 2017; Davies et al., 2017; Geurden et al., 2018), but the evidence to support this suggestion is weak. Therefore, the vector(s) of *Cytauxzoon* spp. is still unknown in other continents. Furthermore, the vector(s) of the agent in Brazil remain unknown since the tick vectors of *Cytauxzoon felis* in the USA do not occur in Brazil (Blouin et al., 1984; Reichard et al., 2009). There are several records of *Amblyomma cajennense* sensu lato, *Amblyomma sculptum, Amblyomma ovale, Amblyomma tigrinum, Amblyomma triste, Amblyomma aureolatum* and *Rhipicephalus microplus* infesting wild jaguars in different biomes such as the Amazon, Cerrado, Pantanal, and the Atlantic Forest (Aragão and Fonseca, 1961; Sinkoc et al., 1998; Labruna et al., 2005; Furtado, 2010; Martins, 2014).

The Brazilian fauna of wild felids is composed by 10 species, two large felids (*P. onca* and *Puma concolor*) and eight small neotropical felids, (*Leopardus pardalis, Leopardus wiedii, Leopardus tigrinus, Leopardus guttulus, Leopardus geoffroyi, Leopardus munoai, Leopardus braccatus*, and *Herpailurus yagouaroundi*) (Reis et al. 2006; Trigo et al. 2013a, 2013b, 2013c; Nascimento et al., 2020). All species show some degree of vulnerability and are threatened in the Brazilian territory or in the Neotropical region.

Based on this, the present study aims to evaluate the presence of *Cytauxzoon* spp. in different wild felid populations distributed in two distinct Brazilian regions, and identify the tick species found parasitizing the studied hosts. The understanding of the epidemiology of *Cytauxzoon* in different parts of the world can contribute to a global understanding of the disease.

2. Materials and methods

2.1. Sampling

Ministry of Environment (MMA) approved the study protocol through the permit SISBIO 42,093–1 and COMPESQ UFRGS 38,198.

2.2. The Midwest region

From 2013 to 2021, 53 blood samples of *P. onca* ($n = 45$), *P. concolor* ($n = 3$), and *L. pardalis* ($n = 5$) were collected from the Pantanal biome in Brazil (Midwest region = MR). Captures occurred randomly and as described by May-Junior et al. (2021). Details of sampling and tick identification are available in Supplementary Material 1.

2.3. Rio grande do Sul state

Between 2014 and 2022, blood samples from live captures or spleen samples from road kills of 143 wild felids from Rio Grande do Sul State (RS) were collected and deposited in the Laboratório de Protozoologia e Rickettsioses Vetoriais at the Federal University of Rio Grande do Sul. Captures occurred randomly, as described by Souza et al. (2020, 2021). Among the sampled species were L. *geoffroyi* ($n = 70$), *L. wiedii* ($n = 34$), *L. munoai* ($n = 6$), *L. guttulus* ($n = 12$), *H. yagouaroundi* ($n = 13$), *L. pardalis* ($n = 3$), and *P. concolor* ($n = 5$). Further details are available in Supplementary Material 1.

2.4. Molecular detection of Cytauxzoon spp

Blood samples were collected in tubes with ethylenediamine tetraacetic acid (EDTA), and spleen samples were collected and then frozen at −20 °C until processing. Part of the samples was extracted with a commercial PureLink® Genomic DNA Mini Kit (Invitrogen ™, Carlsbad, CA, USA) and another part by the phenol/chloroform protocol (Sambrook and Russell, 2006); in both cases, 200 μL of blood and 10 mg of spleen were used for DNA extraction. This distinction of extraction processes was necessary due to part of the material was from a sample bank.

The samples were analysed by PCR with specific primers for *Cytauxzoon* spp., namely forward: 5′ GCGAATCGCATTGCTTTATGCT-3′ and reverse: 5′ CCAATTGATACTCCGGAAAGAG-3′, which amplifies a 284-bp fragment of the 18S rRNA gene, as described by Birkenheuer et al. (2006).

The quantity and quality of the extracted DNA were evaluated using a NanoDrop™ spectrophotometer at an absorbance of 260/280 nm. Additionally, samples that were negative in these studies or in previous studies that used the same genetic material (Souza et al., 2020, 2021), were tested using the endogenous mammalian cytochrome B gene (*cytb*) (Steuber et al., 2005) to evaluate the success of DNA extraction in the two used protocols.

Two random samples were submitted to sequencing to confirm the specificity previously confirmed by Birkenheuer et al. (2006). For this, amplicons of the expected size were purified with Invitrogen™ PureLink™ Quick PCR Purification Kit (Thermo Fisher Scientific Corporation, Carlsbad, California, USA), and sequenced in an automated sequencer (Sanger).

2.5. Ticks collection and identification

During the capture or collection of the carcasses, the animals were carefully inspected for the presence of ticks. Ticks were removed manually, packed in collection tubes containing 70% alcohol to preserve the material and sent to the Laboratório de Protozoologia e Rickettsioses Vetoriais at the Federal University of Rio Grande do Sul for genus and species identification by stereomicroscopy, and confirmation using morphological characteristics described in Barros-Battesti (2006), Martins et al. (2010) and Dantas-Torres et al. (2019).

3. Results

Cytauxzoon spp. genetic material was detected in the majority (98.11%) of the samples from the MR. All *P. onca* ($n = 45$) and *P. concolor* ($n = 3$) were positive for *Cytauxzoon* spp. Eight *P. onca* recaptured in different years remained positive, with capture intervals ranging from 1 to 4 years, one of which was recaptured three times; all of them tested positive. Of the five L. *pardalis* sampled, four were positive.

All felid samples from RS were negative in PCR. We highlight three *L. geoffroyi* recaptured in different years and which remained negative throughout the years. All samples tested by the *cytb* protocol were positive, confirming the success of DNA extraction.

All captured animals were healthy according to physiological data obtained from patterns evaluated in samplings.

Overall, 600 ixodid ticks were collected. In the MR, 507 ticks were identified and *A. sculptum* was the most prevalent (69.23% - $n = 351$ specimens), followed by *A. ovale, A. triste,* and *Amblyomma* spp. larvae. Ninety-three ticks were found in the RS region and *A. aureolatum* was the

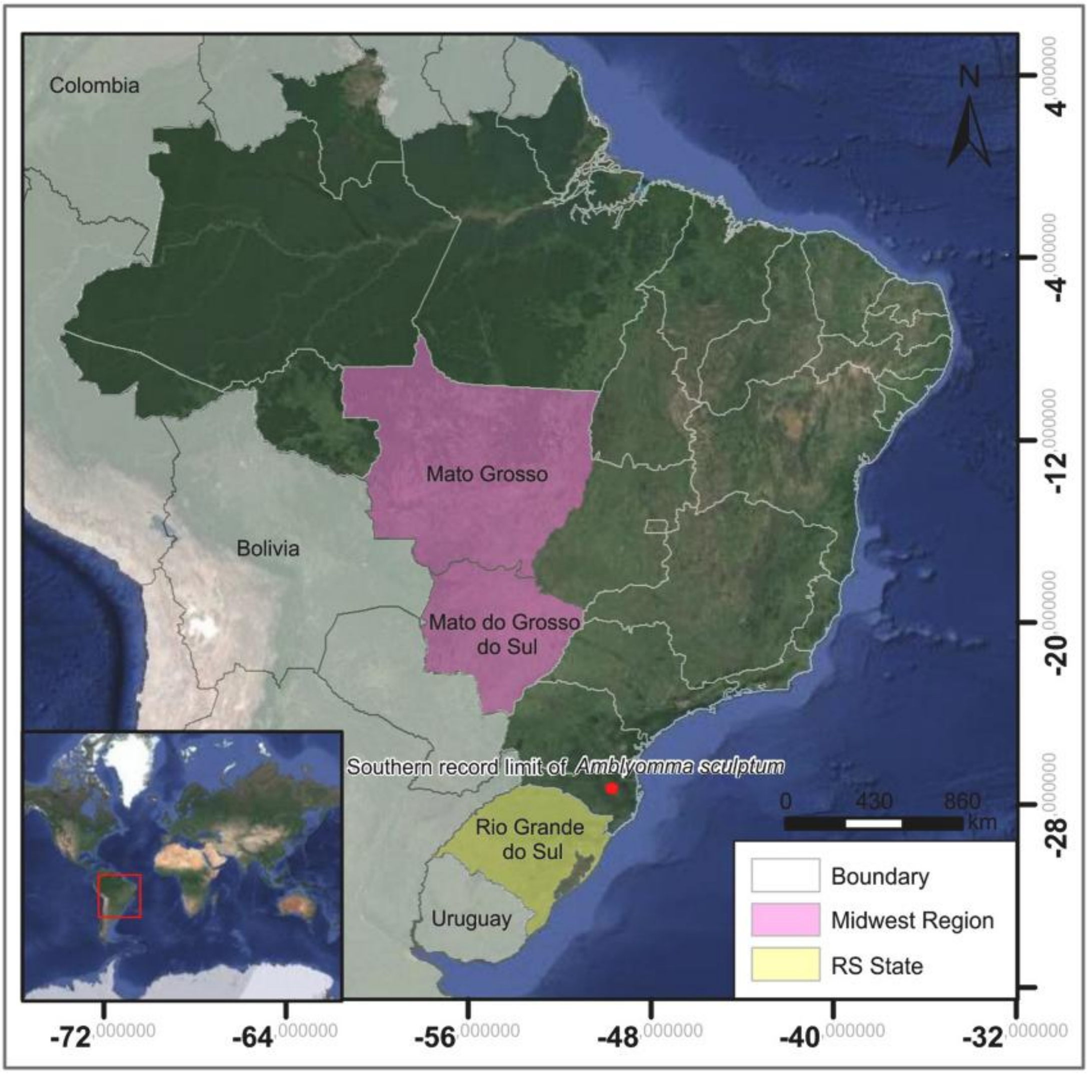

Fig. 1. Location between the Midwest Region (pink) and the Rio Grande do Sul State-RS (yellow) in the Brazilian territory. The southernmost report of Amblyomma sculptum in the municipality of Presidente Getúlio - State of Santa Catarina is shown in the red dot.

most commonly found species (90.32% - $n = 84$), along with *Amblyomma longirostre, Amblyomma dubitatum, Amblyomma* spp. larvae and *R. microplus* as the other identified species.

Generated sequence consensus was submitted to BLAST ® analysis (Altschul et al., 1990) to determine similarities in GenBank®. The sequences from this study were also deposited in GenBank® under the accession numbers: ON024311 and ON024312. The samples obtained 100% similarity with sequences from *Cytauxzoon* spp. deposited with GenBank® (MG705546, KY450746).

4. Discussion

The hypothesis that Brazilian wild felids act as reservoirs of *Cytauxzoon* spp. is already cited in the literature (André et al., 2009; Furtado et al., 2017). Regarding to the hypothesis of *P. onca* as reservoir, our study supports it based on: (1) the absence of clinical signs, (2) the maintenance of the infection for long periods (or constant reinfection) and (3) the high occurrence of the agent in the individuals studied. Additionally, the detection in *P. concolor* and *L. pardalis* of MR highlights that these two felids could also play such a role, however our sampling is insufficient for a solid conclusion regarding the potential reservoir status of these two species. The recaptures data in different years indicate constant reinfection of individuals in the environment or a long period infection (1 to 4 years), characteristic of the Theileriidae family (Barnet, 1968). Studies show that infected *Lynx rufus*, and *C. felis* and *Felis* catus that survive the infection, become chronic carriers (Zieman et al., 2018). Individuals of L. *pardalis* and *P. concolor* also infected by *Cytauxzoon* spp. showed no clinical signs of parasitism, but their potential as reservoirs still requires further studies.

In contrast to the high occurrence in MR, the 143 samples from RS were negative for *Cytauxzoon* spp. These included samples from different cities, species, years of collection, recaptures, and even individuals of the species *L. pardalis* and *P. concolor*, contrasting with the results found on the MR region. In the literature, there are two records of *L. guttulus* infected by *Cytauxzoon* in Rio de Janeiro-RJ, which is an area that *A. sculptum* occurs (Amaral, 2006; Peixoto et al., 2007). This felid species was sampled in the present study in RS, but *Cytauxzoon* spp. were not detected. So far, there is no evidence of the agent circulation among wild felids in RS. This can be the first step to stablish the epidemiology of *Cytauxzoon* in Brazil, although more studies are suggested.

Knowledge of the occurrence of agents, vectors, and the ecology of diseases in certain populations or areas is a key factor for conservation measures for wild species (Lyles and Dobson, 1993; Macdonald, 1996; Murray et al., 1999). This can be challenging for conservation action plans, since vertebrates adapted to different biomes with a wide geographic distribution are exposed to different vectors and agents.

In the USA, the species *D. variabilis* and *A. americanum* are vectors of *Cytauxzoon felis* (Blouin et al., 1984; Reichard et al., 2009). However, these tick species do not occur in South America, a continent where the cytauxzoonosis vector is unknown. The studied region tick fauna include species such as *A. ovale, A. triste, A. longirostre, A. dubitatum, A. tigrinum*, and *R. microplus.* Specifically *A. sculptum*, which is widely found in MR felids (locality of high occurrence of *Cytauxzoon* spp.), does not occur in RS (Martins et al., 2016). Could this fact explain the differences in positivity for *Cytauxzoon* spp. in the two regions? Previous studies have detected *A. sculptum* (formerly *Amblyomma cajennense*) in positive *P. onca* (Furtado et al., 2017), suggesting that this ixodid can play a role in the transmission of *Cytauxzoon* spp. The records of *Cytauxzoon* spp. in Brazilian *F. catus* coincide with regions where *A. sculptum* occurs, such as Rio de Janeiro-RJ and cities in MR (Mendes-De-Almeida et al., 2007; Maia et al., 2013; André et al., 2015; Peixoto et al., 2007; Raimundo et al., 2021).

The southernmost report of *A. sculptum* is from the municipality of Presidente Getúlio (Santa Catarina State), approximately 500 km north of the RS border. It was a male specimen infesting a domestic dog in 1936 (Martins, 2014), suggesting that this species is not included among the tick fauna of the state of RS and that the regions studied, despite having several species in common, do not share *A. sculptum* (Fig. 1).

A. sculptum is adapted to the transmission of different agents and proven to transmit *Rickettsia rickettsii* (Soares et al., 2012). The literature is divergent regarding to the vectorial capacity of *A. sculptum* about another important theilerid: *Theileria equi*. Ribeiro et al. (2011) mentions failure to transmit, whereas Kerber et al. (2009) demonstrate an association between *T. equi* seropositivity and *A. sculptum* infestation.

In the USA, a tick of the genus *Amblyomma* transmits *C. felis* to felids (Reichard et al., 2009). The data presented here do not prove the transmission of *Cytauxzoon* spp. by *A. sculptum*, which must be evaluated through experimental studies. However, our study provides epidemiological information about the possible transmission of *Cytauxzoon* spp. in Brazil. Moreover, the lack of *C. felis* in the sampled felids of RS could also be related to the absence or scarcity of *P. onca* in this state. This study supports the hypothesis that *P. onca* acts as a reservoir of *Cytauxzoon* spp. in Brazil and provides important epidemiological information about the vector potential of *A. sculptum* in the transmission of this hemoparasite to felines.

CRediT authorship contribution statement

Renata Fagundes-Moreira: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Project administration. **Ugo Araújo Souza:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Project administration. **Joares Adenilson May-Junior:** Methodology, Investigation, Resources. **Vinícius Baggio-Souza:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Laura Berger:** Conceptualization, Methodology, Investigation. **Paulo Guilherme Carniel Wagner:** Methodology, Investigation, Resources. **Fabio Dias Mazim:** Methodology, Investigation, Resources. **Felipe Bortolotto Peters:** Investigation, Resources. **Marina Ochoa Favarini:** Investigation, Resources. **Marcos Adriano Tortato:** Investigation, Resources. **Ana Paula N. Albano:** Investigation, Resources. **Darwin Dias Fagundes:** Methodology, Resources. **Mario B. Haberfeld:** Investigation, Resources. **Leonardo R. Sartorelo:** Investigation, Resources. **Lilian Elaine Ranpim:** Investigation, Resources. **Carlos Eduardo Fragoso:** Investigation, Resources. **Aline Girotto-Soares:** Conceptualization, Methodology, Investigation, Resources. **Thiago F. Martins:** Investigation, Resources, Supervision. **Stella de Faria Valle:** Conceptualization, Methodology, Investigation, Resources, Supervision, Funding acquisition. **João Fabio Soares:** Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

None.

Data availability

The study data were shared in supplemental data provided in file attachments.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2022.102021.

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