FULL ARTICLE

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Molecular survey of *Leishmania* spp. in skin samples of capybaras (*Hydrochoerus hydrochaeris*) from different areas of Brazil

Investigação molecular de *Leishmania* spp. em amostras de pele de capivaras (*Hydrochoerus hydrochaeris*) de diferentes regiões do Brasil

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ABSTRACT

Leishmaniases comprise a spectrum of diseases caused by protozoan parasites of the genus *Leishmania*, with some species of rodents being incriminated as reservoirs. The capybara is the largest extant rodent species in the world and is widely distributed in South America. The occurrence of infection by *Leishmania* spp. was investigated in capybaras captured in Brazil during 2015–2019 from established populations in five highly anthropic areas of the state of São Paulo and two natural areas of the states of Mato Grosso and Mato Grosso do Sul. A total of 186 individuals were captured and subjected to abdominal skin biopsy. All skin samples were *Leishmania* kDNA-negative, suggesting that capybaras have no role in the transmission cycles of *Leishmania* species in the studied areas despite the well-known role of other rodents in the life cycle of *Leishmania* spp.

Keywords: *Leishmania* spp. Capybara. PCR. Rodents. Reservoirs.

RESUMO

As leishmanioses compreendem um espectro de doenças causadas por protozoários do gênero *Leishmania* e algumas espécies de roedores são incriminadas como reservatórios de *Leishmania* spp. As capivaras compreendem a maior espécie de roedores existentes e são amplamente distribuídas na América do Sul. Para investigar a ocorrência de infecção por *Leishmania* spp. em capivaras, durante os anos de 2015-2019 capivaras foram capturadas em cinco áreas antrópicas do estado de São Paulo e em duas áreas naturais dos estados do Mato Grosso e do Mato Grosso do Sul, todos esses ambientes com populações de capivaras estabelecidas. Um total de 186 indivíduos foram capturados e submetidos à biópsia de pele abdominal. Todas as amostras de pele foram negativas para o alvo kDNA, assim, os dados sugerem que nas áreas estudadas as capivaras não têm papel no ciclo de transmissão de espécies de *Leishmania* spp., apesar do papel bem conhecido de outros roedores no ciclo de vida de *Leishmania* spp.

Palavras-chave: *Leishmania* spp. Capivara. PCR. Roedores. Reservatórios.

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Introduction

Leishmaniases comprise a spectrum of diseases caused by protozoan parasites of the genus *Leishmania*. Out of 200 countries and territories reported to WHO, 98 countries and territories are endemic to leishmaniasis in 2020 (World Health Organization, 2021). Although some studies have described dozens of species infected with these parasites, only a minority have related their findings to the ecological scenario to indicate a possible role of that host in parasite maintenance and transmission (Roque & Jansen, 2014).

Several studies at the beginning of the twentieth century searched for natural wild reservoirs of the etiological agents of cutaneous and visceral leishmaniases. As a result, some species of rodents were incriminated as reservoirs of *Leishmania* spp. (Guimarães et al., 1968) and subsequently recognized as hosts of more than 60 zoonotic diseases that pose serious threats to human health (Meerburg et al., 2009; Luis et al., 2013).

The capybara (*Hydrochoerus hydrochaeris*, Linnaeus, 1766) is widely distributed in Brazil and other countries of South America, where individuals live in social groups of 10 to more than 100 (Moreira et al., 2013). Capybara populations have undergone great expansions in recent decades within human-modified landscapes, with their reproduction in rural, peri-urban and urban areas being favored by high food availability from crops, the creation of artificial water bodies, and declines in natural predators (Bovo et al., 2016; Luz et al., 2019). The proximity between high capybara densities and human settlements has resulted in the emergence of several conflicts related to crop damage, increased vehicle collisions, and the spread of infectious diseases (Bovo et al., 2016; Farikoski et al., 2019; Luz et al. 2019).

While several small rodents are known to play some role as reservoirs of species of *Leishmania* in different countries (Roque & Jansen, 2014), the role of capybaras in the natural history of leishmaniasis has not been elucidated. Therefore, the present study aimed to investigate the occurrence of infection by *Leishmania* spp. among capybaras from different areas of Brazil.

Materials and Methods

Ethical statements

Capybara sampling was primarily performed for another study related to the epidemiology of Brazilian spotted fever, caused by the bacterium *Rickettsia rickettsii*, for which capybaras are considered to play a role as amplifying hosts of the bacterium to the capybara tick, *Amblyomma sculptum* (Luz et al., 2019). The study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine of the University of São Paulo (approval number 5948070314), per the regulations/guidelines of the Brazilian National Council of Animal Experimentation (CONCEA). Field capture of capybaras and collections of ticks were authorized by the Brazilian Ministry of the Environment (permit SISBIO Nos. 43259–6) and by the São Paulo Forestry Institute (Cotec permit 260108–000.409/2015).

Study areas

Study areas and the captured animals were part of a doctoral thesis (Benatti, 2020). Capybaras were captured from 2015 to 2019 from within established populations in seven areas among three different states of Brazil. Five of the study areas were human-modified landscapes of the Cerrado biome or Cerrado-Atlantic Forest transition zones in the state of São Paulo, Southeast Brazil. The two other areas were in the Pantanal biome, one in the state of Mato Grosso and another in the state of Mato Grosso do Sul, Center-West Brazil. These two areas were characterized by minimal anthropic changes during the last few decades and the absence of artificial pastures or crops. Therefore, they were considered natural areas.

Capybara sampling

Capybaras were captured using 16 to 20-m^2 corrals baited with sugar cane and green corn at least 10 days before capture attempts. Once in the corrals, the animals were physically restrained by a net catcher and anesthetized with an intramuscular injection of a combination of ketamine (10 mg/kg) and xylazine (0.2 mg/kg). Corrals were not effective in Mato Grosso do Sul, thus capybaras were subdued by anesthetic darting via a CO_2-injection rifle (Dan-Inject model JM Standard, Denmark) using the same chemicals described above (Luz et al., 2019). Under anesthesia, animals were subjected to biometrical measures and identified with a subcutaneous microchip (Alflex model P/N 860005–001, Capalaba, Australia). A skin biopsy of the groin area (approximately 4 cm^2) was performed on each animal and the tissue was immediately frozen at -20°C until laboratory analysis. After recovering from anesthesia, the capybaras were released at the capture site in an attempt to minimize interference in animal health.

Molecular diagnosis

Isolation of DNA from skin biopsies involved DNA extraction and purification using the PureLink Genomic

Table 1 – Capybaras were captured in seven areas from 2015 to 2019

Location	Total captured	Gender			Age			
		Female	Male	Not identified	Young (<10kg)	Juvenile (10-35kg)	Adult (>35kg)	Not identified
Americana - SP	21	10	11	0	0	8	13	0
Araras - SP	32	20	7	5	6	3	18	5
Corumbá - MS	31	23	8	0	4	1	26	0
Piracicaba - SP	29	25	4	0	2	8	19	0
Pirassununga - SP	30	28	2	0	3	1	26	0
Poconé - MT	23	15	8	0	0	6	17	0
Ribeirão Preto - SP	20	14	6	0	2	15	3	0
Total	186	135	46	5	17	42	122	5

DNA Mini Kit, according to the manufacturer's instructions. Amplification by polymerase chain reaction (PCR) targeted a fragment of kDNA (*Leishmania* kinetoplast DNA) (Degrave et al., 1994), with amplified sequences being visualized by agarose gel electrophoresis using a UV transilluminator. Every assay included negative and positive controls. The interphotoreceptor retinoid-binding protein (IRBP) gene, which has a well-conserved sequence among different mammal species, was used as an endogenous control of PCR (Ferreira et al., 2015).

Results and Discussion

A total of 186 capybaras were captured and biopsied during the four consecutive years of the study (2015–2019). Some individuals were captured more than once but recaptures were not included in the total number captured as the unit chosen represents the total number of different individuals sampled. Forty-six individuals were male (24.7%) and 135 were female (72.5%), with 17 young (9.1%), 42 juveniles (22.5%), and 122 adults (65.6%). The sex and age of five animals (2.7%) were not recorded (Table 1).

All skin samples of the 186 individuals were kDNA-negative. Positive and negative controls behaved as expected. Furthermore, a strong 227 bp amplicon from the IRBP gene representing the control of DNA quality was, as expected, amplified from all capybara skin DNA samples. In this study, biopsies of the groin region were used, which may have limited the sensitivity of the PCR. It has been suggested that ear tissue should be used as the primary site for parasitological confirmation in dogs. In addition, it was pointed out as the better anatomical region from the skin to perform biopsies and PCR assays for detection of *Leishmania* infection (Ferreira et al., 2013).

This study encompassed the largest sample of capybaras, either numerically or geographically, in a survey of *Leishmania* infection. It has been postulated that 60 to 70% of emerging infectious disease events in humans are of animal origin (Cleaveland et al., 2001; Taylor et al., 2001) among which are leishmaniases. The roles played by mammalian hosts in the transmission of most species of *Leishmania* remain poorly understood, which reinforces the need for further studies of the occurrence of these parasites in other mammal species, in addition to those classically described as reservoirs (Cássia-Pires et al., 2014).

Richini-Pereira et al. (2014) proposed that the capybara might serve as an important reservoir for the transmission of *Leishmania* spp. based on a lung tissue sample from one road-killed female that was subjected to PCR and sequencing with a positive result for *Leishmania* sp. In contrast, previous studies employing molecular and serological tests have failed to detect evidence of *Leishmania* exposure or infection in several capybaras from Brazil (Valadas et al., 2010; Chiacchio et al., 2014), which is consistent with the present results.

PCR and other amplification techniques are highly sensitive and vulnerable to contamination. Despite technological improvements, false-positive findings have been reported, indicating that previously generated amplicons can cause carryover contamination of reaction tubes (Aslanzadeh, 2004). Therefore, we conclude that capybaras have no epidemiological importance for *Leishmania* spp. in the sampled areas, despite the well-known role of other rodents in their life cycles.

Conclusion

It seems unlikely that the capybara acts as a major reservoir for *Leishmania* spp. in Brazil, as demonstrated for areas belonging to different Brazilian biomes in the present study.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Statement

The study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of

Veterinary Medicine of the University of São Paulo (approval number 5948070314), following the regulations/guidelines of the Brazilian National Council of Animal Experimentation (CONCEA). Field capture of capybaras and collections of ticks were authorized by the Brazilian Ministry of the Environment (permit SISBIO Nos. 43259–6) and by the São Paulo Forestry Institute (Cotec permit 260108–000.409/2015).

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