UNIVERSIDADE ESTADUAL DE SANTA CRUZ
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA ANIMAL

FERNANDA COELHO SIMAS BERNARDES

SAÚDE E PREVALÊNCIA DE PARASITOS EM PREGUIÇA-DE-COLEIRA (*Bradypus torquatus)* ILLIGER, 1811

ILHÉUS-BAHIA
2022

FERNANDA COELHO SIMAS BERNARDES

SAÚDE E PREVALÊNCIA DE PARASITOS EM PREGUIÇA-DE-COLEIRA (*Bradypus torquatus)* ILLIGER, 1811

Dissertação apresentada à Universidade Estadual de Santa Cruz, como parte das exigências para obtenção do título de Mestre em Ciência Animal.

Linha de Pesquisa: Clínica e Sanidade Animal.
Sub-área: Medicina e Conservação de Animais Selvagens.

Orientadora: Flávia Regina Miranda

ILHÉUS-BAHIA
2022

B522 Bernardes, Fernanda Coelho Simas.
Saúde e prevalência de parasitos em preguiça-de-coleira (Bradypus torquatus) Illiger, 1811 / Fernanda Coelho Simas Bernardes. – Ilhéus, BA: UESC , 2022.
50f. : il.

Orientadora: Flávia Regina Miranda
Dissertação (Mestrado) – Universidade Estadual de Santa Cruz. Programa de Pós-Graduação em Ciência Animal – PPGCA.
Inclui referências.

1. Parasitologia veterinária. 2. Ectoparasitoses animal. 3. Hematologia veterinária. 4. Patologia clínica. 5. Saúde ambiental. 6. Xenarthra. I. Título.

CDD 636.089696

FERNANDA COELHO SIMAS BERNARDES

SAÚDE E PREVALÊNCIA DE PARASITOS EM PREGUIÇA-DE-COLEIRA (*Bradypus torquatus)* ILLIGER, 1811

Ilhéus – Bahia, 27/06/2022

_____

Flávia Regina Miranda – DSc.
UESC/PPGCA
(Orientadora)

_____

Hllytchaikra Ferraz Fehlberg – DSc.
UESC/DCB
(Co-orientadora)

_____

Anaiá da Paixão Sevá – DSc.
UESC/PPGCA

_____

Júlia Angélica Gonçalves da Silveira – DSc.
UFMG/DMVP

ILHÉUS-BAHIA
2022

AGRADECIMENTOS

À Universidade Estadual de Santa Cruz, seus professores, educadores, funcionários e alunos, pela oportunidade e ensinamentos ao longo da minha jornada.

À minha orientadora Flávia Regina Miranda, pela orientação e confiança. Para além dessas palavras, expresso minha admiração e respeito pela profissional e pessoa que tanto me inspira e instiga a ser a minha melhor versão - até agora.

À CAPES, pelo apoio financeiro imprescindível para que eu me dedicasse integralmente à pesquisa.

Ao Instituto Tamanduá, pela parceria. À equipe, Flávia Miranda, Karina Molina, Suelen Sanches Ferreira, Alexandre Martins e Beatris Felipe Rosa, por transbordar conhecimento e não hesitar ao passá-lo adiante. Ainda, por ser uma das razões para eu querer voltar pro campo o quanto antes, é um prazer o convívio e troca com cada um.

Ao Laboratório de Biodiversidade & Saúde da Universidade Estadual de Santa Cruz por tornar possível todas as análises laboratoriais. Obrigada em especial a médica veterinária Hllytchaikra Ferraz Fehlberg que me guiou no universo da biologia molecular do início ao fim.

Ao Laboratório de Parasitologia de São Paulo-USP, pela identificação dos carrapatos. Em especial, ao médico veterinário Thiago Fernandes Martins, pela disponibilidade e contribuição à pesquisa.

Aos meus pais, Cristina e Fernando, e minha irmã, Gabrielle, pelo amor, que me move e me faz perceber que sorte é sentir todo esse amor, que vai do sudeste ao nordeste, até o outro lado do mapa mundi. Faço tudo por e para vocês. Obrigada sempre e muito.

Ao meu marido, Zak, pelo apoio às minhas andanças, já há dois anos - e sabe lá quantos anos mais, sem previsão de parada. Agradeço imensamente por tornar minha vida muito mais significativa e feliz.

À Paula Valencise, pela feliz coincidência do nosso encontro no Pantanal. Só tenho a agradecer pela partilha e pelo abrigo - emocional e físico.

Aos amigos de Petrópolis, Uruguaiana, Vila Velha, Ilhéus e do mundo, por permanecerem. Sinto muita gratidão por sempre se fazerem presentes na minha vida tão simples, mas rara por tê-los.

Aos nomes e rostos que se cruzaram com o meu e somaram a este trabalho.

SAÚDE E PREVALÊNCIA DE PARASITOS EM PREGUIÇA-DE-COLEIRA (*Bradypus torquatus)* ILLIGER, 1811

RESUMO

A preguiça-de-coleira (*Bradypus torquatus*, Bradypodidae, Pilosa, Xenarthra) é uma espécie endêmica da Mata Atlântica, reconhecida atualmente como vulnerável (VU). Estudos envolvendo a espécie são escassos e dependendo da temática, inexistentes. O *Amblyomma varium* é um carrapato encontrado quase que exclusivamente em mamíferos da superordem Xenarthra, e apesar de existirem estudos significativos envolvendo a sua descrição, ainda há discordância entre autores sobre algumas características. Além disso, não há estudos publicados relatando doenças transmitidas por *A. varium* envolvendo a espécie *Bradypus torquatus*. A análise bioquímica e hematológica é considerada o método de avaliação de saúde mais comum em animais silvestres, sendo essencial para avaliar a condição de indivíduos e populações. Apesar disto, infelizmente ainda não há nenhum estudo publicado envolvendo valores bioquímicos em *B. torquatus* e há apenas um estudo sobre valores hematológicos com um número baixo de indivíduos de preguiça-de-coleira. Ademais, o contato de indivíduos com humanos e animais domésticos aumenta a possibilidade de disseminação de agentes parasitários para novos ambientes e espécies silvestres, tornando imprescindível o estudo da saúde e circulação de agentes em populações de animais silvestres, principalmente em espécies ameaçadas de extinção. Com isso, o objetivo deste estudo é relatar as novas descobertas do carrapato *Amblyomma varium* Koch, 1844 encontrado parasitando preguiças-de-coleira de vida livre da Mata Atlântica do nordeste e sudeste do Brasil (**Capítulo 1**), apresentar valores hematológicos de preguiças-de-coleira de vida livre e fornecer os primeiros valores bioquímicos publicados para *B. torquatus* da Mata Atlântica (**Capítulo 2**) e identificar pela primeira vez, hemoparasitos (*Hepatozoon* spp., *Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp., *Mycoplasma* spp. e *Rickettsia* spp.) em amostras de sangue de preguiças-de-coleira da Reserva Ecológica da Sapiranga, nordeste do Brasil. No **Capítulo 1**, observa-se que mais de 50% dos indivíduos capturados estavam parasitados por adultos ou ninfas de *A. varium*, e apenas um espécime de *B. torquatus* apresentou parasitismo simultâneo pelos dois estágios de *A. varium*. Ainda neste capítulo, a variação no comprimento dos espinhos na coxa IV em machos de *A. varium* é confirmada e esta característica é relatada pela primeira vez no nordeste do Brasil. No **Capítulo 2**, amostras de sangue de 30 indivíduos foram submetidas a análises hematológicas e 8 a análises bioquímicas. Os resultados somam aos dados divulgados anteriormente na literatura, permite comparações adicionais entre os resultados, auxilia na avaliação da saúde de preguiças-de-coleira de vida livre e fornece os primeiros valores bioquímicos publicados para *B. torquatus* da Mata Atlântica. No **Capítulo 3**, 29 amostras de preguiças-de-coleira de vida livre foram testadas para *Hepatozoon* spp., *Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp., *Mycoplasma* spp. e *Rickettsia* spp. por reação em cadeia da polimerase (PCR). Do total de amostras, 11 foram positivas para *Hepatozoon* spp. Não houve positividade para *Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp., *Mycoplasma* spp. e *Rickettsia* spp neste estudo. As informações aqui compartilhadas contribuem como dados importantes para o fortalecimento dos estudos voltados à Medicina da Conservação dos Xenarthras envolvendo a preguiça-de-coleira.

Palavras-chave: ectoparasito; hemopatógeno; medicina da conservação; patologia clínica; Xenarthra.

HEALTH AND PREVALENCE OF PARASITES IN MANED SLOTH (*Bradypus torquatus*) ILLIGER, 1811

ABSTRACT

The maned sloth (*Bradypus torquatus*, Bradypodidae, Pilosa, Xenarthra) is an endemic species of the Atlantic Forest and is currently recognized as vulnerable (VU). Studies involving the species are scarce and, depending on the topic, non-existent. The *Amblyomma varium* is a tick found almost exclusively in mammals of the superorder Xenarthra, and although there are significant studies involving its description, there is still a disagreement among authors about some of its characteristics. In addition, there are no published studies reporting diseases transmitted by *A. varium* involving the species *Bradypus torquatus*. Biochemical and hematological analysis are considered the most common health assessment methods in wild animals and are essential to assess the condition of individuals and populations. Unfortunately, there are no published studies about biochemical values in *B. torquatus* and there is only one study about hematological values, which studied a small number of individuals of *B. torquatus*. Furthermore, the contact of individuals with humans and domestic animals increases the possibility of dissemination of parasitic agents to new environments and wild species, making it essential to study the health and circulation of these agents in populations of wild animals, especially in endangered species. Thus, the aim of this study is to report new findings of the tick *Amblyomma varium* Koch, 1844 found parasitizing free-living maned sloths from the Atlantic Forest of Northeastern and Southeastern Brazil (**Chapter 1**), to present hematological values of free-living maned sloths and provide the first published biochemical values for *B. torquatus* from the Atlantic Forest (**Chapter 2**), and to identify for the first time hemoparasites (*Hepatozoon* spp., *Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp., *Mycoplasma* spp., and *Rickettsia* spp.) in blood samples from maned sloths from the Reserva Ecológica da Sapiranga, Northeastern Brazil. In **Chapter 1**, it is observed that more than 50% of the captured individuals were parasitized by adults or nymphs of *A. varium*, and only one specimen of *B. torquatus* presented simultaneous parasitism by the two stages of *A. varium*. Also in this chapter, the variation in the length of spurs on coxa IV in males of *A. varium* is confirmed and this characteristic is reported for the first time in Northeastern Brazil. In **Chapter 2**, blood samples from 30 individuals of *B. torquatus* were submitted to hematological analysis and 8 to biochemical analysis. The results add to the data previously published, allow additional comparisons between the results, assist in the health assessment of free-living maned sloths, and provide the first published biochemical values for *B. torquatus* from the Atlantic Forest. In **Chapter 3**, 29 free-living maned sloth samples were tested for *Hepatozoon* spp., *Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp., *Mycoplasma* spp., and *Rickettsia* spp. using Polymerase Chain Reaction (PCR). Of 29 samples, 11 were positive for *Hepatozoon* spp. There was no positivity for *Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp., *Mycoplasma* spp., or *Rickettsia* spp in this study. The information shared here contributes important data to strengthen Conservation Medicine studies of the maned sloth.

Keywords: clinical pathology; conservation medicine; ectoparasite; hemopathogens; Xenarthra.

SUMÁRIO

1 INTRODUÇÃO

Originalmente a Mata Atlântica ocupava cerca de 15% do território brasileiro (1,3 milhão de quilômetros quadrados) (MMA, 2010). Atualmente, os remanescentes de vegetação nativa estão reduzidos a cerca de 12,4% de sua cobertura original (INPE, 2019). Mesmo reduzida e muito fragmentada, a Mata Atlântica ainda abriga 321 espécies de mamíferos, sendo 89 endêmicas deste bioma e 39 ameaçadas de extinção a nível mundial (GRAIPEL *et. al.*, 2017 *apud* ROSA, 2020). A preguiça-de-coleira (*Bradypus torquatus*, Bradypodidae, Pilosa, Xenarthra) está inserida nas duas categorias, é endêmica da Mata Atlântica e é reconhecida atualmente como uma espécie Vulnerável (VU) pela IUCN, por meio dos critérios B2ab(ii, iii), segundo a última avaliação realizada por Chiarello e Barros (2014). Tal fato se deve por esta espécie enfrentar alto risco de extinção na natureza, possuir uma área de ocupação bastante fragmentada estimada em menos de 2.000 km², e por apresentar um declínio contínuo em sua área de ocupação. A preguiça-de-coleira ocorre nos estados de Sergipe, Rio de Janeiro, Bahia e Espírito Santo e há registros não confirmados em Minas Gerais e Pernambuco (CHIARELLO *et al*., 2018). Um estudo recente (FEIJÓ *et al*., 2022) revelou que as zonas com diversidade de Xenarthra que enfrentam os mais altos níveis de alteração de habitat são as menos protegidas e, portanto, são uma prioridade para a conservação e, consequentemente, a pesquisa, destacando a Região Nordeste do Brasil para tais fins.

A família Bradypodiae possui características morfológicas, genéticas e fisiológicas muito específicas e ainda são pouco estudadas. A preguiça-de-coleira é uma espécie arborícola, única do subgênero *Scaeopus*, que se alimenta estritamente de folhas e brotos (MUREB, 2022; CORK; FOLEY, 1991). Devido a esta natureza arbórea, torna-se uma tarefa difícil encontrar preguiças na floresta e técnicas complexas de escalada devem ser usadas para capturá-las. Elas raramente descem ao solo, apenas para defecar ou para se deslocar para outra árvore na ausência de conexão entre as copas (GINÉ *et al*., 2015). Tanto no estado da Bahia quanto no Espírito Santo observou-se que a preguiça-de-coleira requer áreas onde haja conectividade entre as árvores (CASSANO; KIERULFF; CHIARELLO, 2011), sendo necessário que a área tenha pelo menos 35% de cobertura florestal (SANTOS *et al*., 2019), tornando ainda mais difícil encontrar essa espécie na floresta tropical fragmentada. A perda e fragmentação de habitat oriundas do desmatamento, aumento da malha rodoviária e incêndios estão entre as principais ameaças já relatadas para esta espécie (CHIARELLO, 2008). Porém, é preciso considerar também a invasão de espécies exóticas (REZENDE *et al*., 2018) e as doenças parasitárias (PEDERSEN *et al*., 2018; WOODROFFE, 1999).

A Medicina da Conservação, termo introduzido por Kock em 1996, foca no ponto de encontro entre três campos de atuação: 1. ambiente, 2. hospedeiros humanos e não humanos e 3. patógenos (SILVEIRA; D'ELIA, 2014). O estudo das doenças e a dinâmica da infecção, bem como relação entre os hospedeiros, e as suas associações com as variáveis ambientais pode resultar em ações de melhoria efetiva da saúde dos ecossistemas, e isto difere a Medicina da Conservação de outras disciplinas (BRANDÃO, 2007). As doenças podem ter impactos desastrosos por levar a uma redução de determinada população, podendo se tornar em algumas situações o principal fator de extinção local, regional e global (SEBASTIANI, 2018).

A presença de humanos e animais domésticos próximos a reservas naturais aumenta a possibilidade de transmissão de doenças para novos ambientes e espécies silvestres, tornando imprescindível o estudo da saúde, fatores de risco nos ecossistemas e a circulação de agentes em populações de animais silvestres (PAIVA, 2016).

Tendo em vista que a infecção por diferentes agentes infecciosos pode ocorrer devido ao contato com o solo, contato direto com o hospedeiro ou com a vegetação compartilhada por espécies (CHIARELLO, 1998), o comportamento da preguiça-de-coleira de descer ao solo pode levar a ocorrência de infecção parasitária. No caso das hemoparasitoses, com o avanço da biologia molecular, novas espécies, cepas, ou variantes genéticas de microorganismos estão sendo identificadas mundialmente, devido ao avanço da biologia molecular, assim como a lista de potenciais patógenos transmitidos por carrapatos está em constante aumento (SEBASTIANI, 2018).

Igualmente a preguiça-de-coleira, o carrapato *Amblyomma varium* é encontrado em florestas tropicais (ONOFRIO *et al*., 2008; GILMORE; DA-COSTA; DUARTE, 2000) e é popularmente conhecido como carrapato gigante da preguiça, pois durante a fase adulta é encontrado quase que exclusivamente em mamíferos das famílias Bradypodidae e Megalonychidae da superordem Xenarthra (ONOFRIO *et al*., 2008; MARQUES *et al*., 2002). Porém, ainda não há estudos relatando doenças transmitidas por carrapatos em preguiça-de-coleira (*Bradypus torquatus*).

Embora os agentes parasitários desempenhem um papel importante na manutenção da diversidade ecológica e ecossistêmica, eles também podem ameaçar a biodiversidade, levando ao declínio das populações naturais, reduzindo a sobrevivência ou mesmo a reprodução do hospedeiro (ALTIZER; FOUFOPOULOS; GAGER, 2001). Além disso, os parasitos podem ser responsáveis por extinções locais de espécies endêmicas, como já foi relatado em estudos com outras espécies silvestres (WARNER, 1968). Com isso, o estudo de parasitos torna-se fundamental, principalmente em espécies ameaçadas de extinção.

2 OBJETIVO GERAL

Avaliar o estado de saúde e valores hematológicos e bioquímicos, bem como verificar a ocorrência de carrapatos e hemopatógenos (*Hepatozoon* spp., *Babesia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Mycoplasma* spp. e *Rickettsia* spp.), identificados através de morfologia e análise molecular por Reação em Cadeia da Polimerase (PCR) na espécie *Bradypus torquatus* (preguiça-de-coleira).

3 OBJETIVOS ESPECÍFICOS

- Identificar a ocorrência de carrapatos em *Bradypus torquatus* na reserva de Sapiranga, região Norte da Bahia (**CAPÍTULO 1**);
- Identificar os carrapatos quanto ao gênero e espécie através dos aspectos morfológicos descritos na literatura (**CAPÍTULO 1**);
- Apresentar valores hematológicos e bioquímicos de *Bradypus torquatus* da Mata Atlântica (Bahia, Rio de Janeiro e Sergipe) (**CAPÍTULO 2**);
- Identificar por Reação em Cadeia da Polimerase (PCR) *Hepatozoon* spp., *Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp., *Mycoplasma* spp. e *Rickettsia* spp., em *Bradypus torquatus* na reserva de Sapiranga, região Norte da Bahia (**CAPÍTULO 3**).

4 REVISÃO DE LITERATURA

4.1. A preguiça-de-coleira (*Bradypus torquatus*)

A preguiça-de-coleira é pertencente à classe Mammalia, superordem Xenarthra, ordem Pilosa, família Bradypodidae e subgênero *Scaleopus*. É a única espécie de preguiça ameaçada de extinção no Brasil, classificada como vulnerável pela lista oficial da fauna brasileira ameaçada de extinção (CHIARELLO *et al*., 2018) e pela lista vermelha da União Internacional para a Conservação da Natureza e dos Recursos Naturais, por meio dos critérios B2ab(ii, iii) (CHIARELLO; BARROS, 2014), por enfrentar alto risco de extinção na natureza, possuir uma área de ocupação muito fragmentada estimada em menos de 2.000 km², e por apresentar um declínio contínuo em sua área de ocupação.

Esta espécie é endêmica ao Brasil, ocorrendo apenas nos estados Sergipe, Bahia, Rio de Janeiro e Espírito Santo, com registros não confirmados em Pernambuco e Minas Gerais (CHIARELLO *et al*., 2018). Ela está presente apenas no sul de Sergipe, no município de Estância (CHAGAS *et al*., 2009); ao centro-norte do Rio de Janeiro, nos municípios de Silva Jardim, Rio das Ostras, Macaé, Cachoeiras de Macacu, Nova Friburgo e Teresópolis (BOFFY *et al*., 2010); na Bahia, nos municípios de Ilhéus e Itabuna, até o extremo Sul, na região do Recôncavo Baiano (CHIARELLO *et al*., 2018); e no Espírito Santo, incluindo a região serrana e litorânea do centro-sul do estado, ao sul do Rio Doce. O registro em Minas Gerais foi feito no extremo nordeste do estado, no município de Bandeira, na divisa com a Bahia (VAZ, 2003), mas não é um registro confirmado por pesquisadores (HIRSCH; CHIARELLO, 2012). Apesar da espécie ter sido introduzida em Parques Nacionais do Rio de Janeiro (PARNA da Tijuca) e do Espírito Santo (PARNA do Caparaó) (CHIARELLO, 2008), não se sabe sobre o estabelecimento de populações em tais Parques. A extensão de ocorrência da preguiça-de-coleira é estimada em 71.427,8 km², baseando-se apenas em registros confirmados, e 116.170,9 km² considerando registros confirmados e não confirmados (HIRSCH; CHIARELLO, 2012).

A Mata Atlântica é o bioma de ocorrência da preguiça-de-coleira, que originalmente ocupava cerca de 15% do território nacional (1,3 milhão de quilômetros quadrados) e 17 estados brasileiros (MMA, 2010; IBGE, 2006). Atualmente, os remanescentes de vegetação nativa estão reduzidos a cerca de 12,4% (INPE, 2019) de sua cobertura original e encontram-se em diferentes estágios de regeneração. Tanto no estado da Bahia quanto no Espírito Santo foi observado que a preguiça-de-coleira necessita de áreas onde haja conectividade entre as árvores (CASSANO; KIERULFF; CHIARELLO, 2011). É necessário que a área tenha pelo menos 35% de cobertura florestal (SANTOS *et al*., 2019), tornando ainda mais difícil encontrar essa espécie

na Mata Atlântica severamente fragmentada. Com isso, sabe-se que a perda e fragmentação de habitat oriundas do desmatamento tem impacto direto na espécie (CHIARELLO, 2008).

Esta espécie possui hábito arborícola e é herbívora (CASSANO, 2006), se alimenta estritamente de folhas jovens e sempre verdes (MUREB, 2022; CORK; FOLEY, 1991). Com isso, estes animais dependem do estrato superior das florestas para realizar suas principais atividades, como locomoção e forrageio (BARRETO; CASSANO, 2007). Sua temperatura é baixa e variável, com capacidade de controlar a temperatura corpórea a partir das atividades desempenhadas (NAGY; MONTGOMERY, 1980). Como é uma espécie de baixo metabolismo e hábito arborícola, a preguiça-de-coleira passa a maior parte do tempo em repouso na copa das árvores e se desloca lentamente pelas extremidades dos galhos e cipós (CASSANO, 2006). Raramente desce ao solo, apenas para defecar ou para deslocar-se para outra árvore na ausência de conexão entre elas (GINÉ *et al*., 2015, CASSANO, 2006; CHIARELLO, 1998).

Dentre as principais ameaças às populações desta espécie estão: 1) destruição das florestas, 2) perda da variabilidade genética, decorrente do isolamento das populações pela fragmentação e descaracterização de habitats (LARA-RUIZ; CHIARELLO, 2008; CHIARELLO *et al*., 2004; FONSECA; AGUIAR, 2004), 3) aumento da malha rodoviária, e 4) incêndios (CHIARELLO, 2008). A caça é identificada como ameaça secundária, com relatos pontuais em algumas regiões, principalmente no sul da Bahia, onde se faz para venda ou consumo do animal (CHIARELLO, 2008). Ainda não há relatos ou estudos que abordem a questão sanitária como ameaça para a espécie.

4.2. Carrapato *Amblyomma varium* em preguiça-de-coleira (*Bradypus torquatus*)

Amblyomma varium Koch 1844, popularmente conhecido no Brasil como carrapato-gigante-da-preguiça, é a maior espécie de carrapato já registrada (MARQUES *et al*., 2002), sendo encontrado quase que exclusivamente em mamíferos das famílias Bradypodidae e Magalonychidae (ONOFRIO *et al*., 2007; MARQUES *et al*., 2002). Com exceção de registros de parasitismo relatados como provavelmente acidentais em um cão doméstico, um veado, um marsupial e um porco selvagem, sugere-se que os adultos de *A. varium* sejam específicos de preguiças (MARQUES *et al*., 2002).

Pouco se sabe sobre os hospedeiros das larvas de *A. varium* na natureza. Porém, um estudo (OGRZEWALSKA *et al*., 2012) relata que larvas de *A. varium* foram coletadas de aves selvagens no Peru, sugerindo que elas podem atuar como hospedeiras intermediárias para os estágios imaturos desta espécie de carrapato. Ainda, há registros de provável parasitismo de ninfas coletadas em *B. torquatus* (MARQUES *et al*., 2002). Desde 2010, há uma descrição

completa (MARTINS *et al*., 2010) de ninfas de *A. varium* coletadas de *B. torquatus*, indicando dentição 2/2 com 6–7 dentes por fileira, coxa I com 2 espinhos pontiagudos (o interno curto e triangular, o externo cerca de duas vezes maior), coxa II–IV com um pequeno espinho triangular (que gradualmente diminui de tamanho de II para IV) e trocanteres sem espinho.

O macho de *A. varium* foi originalmente descrito por Koch em 1844 como tendo um espinho curto na coxa IV. Esta característica chegou a ser confirmada futuramente por pesquisadores (ARAGÃO; JONES *et al*., 1972; FONSECA, 1961; ARAGÃO, 1936; ROBINSON, 1926; NEUMANN, 1899) e discordada ao ser relatado espinho longo ao invés de curto na coxa IV (GUIMARÃES, 2001; FAIRCHILD; KOHLS; TIPTON, 1966). Tais autores também definiram padrão 4/4 de dentição ao invés de 3/3. A redescrição mais recente (ONOFRIO *et al*., 2007) apresenta padrão dentário 3/3, 2 espinhos curtos e robustos na coxa I, 1 espinho curto nas coxas II-III e 1 espinho de comprimento variável na coxa IV.

A fêmea de *A. varium*, quando ingurgitada, é uma das maiores espécies de *Amblyomma* podendo atingir peso de quase 8g (MARQUES *et al*., 2002) e põe até quinze mil ovos (ARAGÃO, 1936). A redescrição mais recente (ONOFRIO *et al*., 2007) apresenta padrão dentário 4/4, 2 espinhos curtos e robustos na coxa I e 1 espinho curto nas coxas II-IV.

Há relatos de *A. varium* na Costa Rica, Panamá, Guatemala, Nicarágua, Guiana Francesa, Venezuela, Peru, Colômbia, Argentina e Brasil (GUGLIELMONE *et al*., 2003). No Brasil, o *A. varium* foi relatado nos estados Pará, Acre, Amazonas, Rondônia, Paraíba, Pernambuco, Alagoas, Bahia, Mato Grosso, Espírito Santo, Minas Gerais, Rio de Janeiro e São Paulo (GRUHN *et al*., 2019; ACOSTA *et al*., 2016; WITTER *et al*., 2016; SANCHES *et al*., 2014).

O gênero *Amblyomma* possui considerável importância veterinária na América do Sul, sendo que suas espécies são os principais vetores e reservatórios de patógenos de importância zoonótica (LABRUNA, 2009). Porém, ainda não há estudos com *A. varium* coletados de *B. torquatus* o indicando como vetor ou reservatório de alguma doença, ou relatos de preguiças-de-coleira diagnosticadas com alguma doença que pudesse ser transmitida por *A. varium*, nem sequer há evidência sobre a patogenicidade desta espécie para humanos ou animais (OGRZEWALSKA *et al*., 2012; LABRUNA *et al*., 2011). Apesar do interesse pelo estudo das características biológicas particulares do *A. varium*, como morfologia, pesquisas sobre outras características desta espécie são ainda escassos ou inexistentes na literatura.

4.3. Valores hematológicos e bioquímicos em preguiça-de-coleira (*Bradypus torquatus*)

A avaliação sanitária em animais selvagens de vida livre é fundamental para definir o estado de saúde de uma população, auxiliando na detecção de problemas ambientais e doenças e até mesmo na identificação de risco de declínio populacional devido a impactos antropogênicos em ambientes naturais (KOPHAMEL *et al*., 2022). Os dados obtidos a partir da avaliação podem ser interpretados tanto em nível individual quanto populacional, podendo ser valiosos para o monitoramento da saúde de espécies ameaçadas de extinção (BARNES; GOLDIZEN; COLEMAN, 2008).

A análise hematológica e bioquímica já foi relatada como o método de avaliação de saúde mais comum em animais silvestres (KOPHAMEL *et al*., 2022), sendo essencial para avaliar a condição de indivíduos e populações, indicando doenças, estado nutricional, qualidade do habitat, entre outros estressores (HANKS, 1981).

Há na literatura publicações disponíveis envolvendo valores hematológicos e bioquímicos de preguiça-comum (*Bradypus variegatus*), preguiça-de-hoffmann (*Choloepus hoffmanni*), preguiça-real (*Choloepus didactylus*) e preguiça-bentinho (*Bradypus tridactylus*) (CHAGAS *et al*., 2022; TSCHÁ *et al*., 2021; PEREIRA *et. al*, 2020; GALVÃO *et al*., 2019; QUEIROZ *et al*., 2015; COSTA, 2014; KINNEY *et al*., 2013; RAMOS, 2006; XAVIER, 2006; VOGEL *et al*., 1999). Enquanto para preguiça-de-coleira (*B. torquatus*) há apenas um estudo (CATENACCI *et al*., 2017), entretanto envolvendo valores hematológicos em apenas 14 indivíduos de *B. torquatus*, sendo 07 de cativeiro e 07 de vida livre, não sendo possível determinação de intervalos de referência. Ainda, não há estudo publicado envolvendo valores bioquímicos em *B. torquatus*.

4.4. Hemoparasitoses em preguiça-de-coleira (*Bradypus torquatus*)

Hemopatógenos transmitidos por vetores, endêmicos ou introduzidos, podem comprometer tanto a saúde quanto o desempenho de animais silvestres em vida livre (SPRINGER *et al*., 2015).

Considerando que a infecção por diferentes agentes parasitários pode ocorrer por contato com o parasito no solo, contato direto com o animal ou contato com vegetação compartilhada por espécies (CHIARELLO, 1998), o fato da preguiça-de-coleira apresentar o comportamento de descer ao solo, mesmo não sendo frequente, pode levar a ocorrência de alguma infecção. Ainda, a presença de humanos e animais domésticos próximos a reservas naturais aumenta a possibilidade de disseminação de agentes parasitários para novos ambientes

e espécies silvestres, tornando imprescindível o estudo da saúde, fatores de risco nos ecossistemas e a circulação de agentes em populações de animais silvestres (PAIVA, 2016).

Apesar do amento do número de estudos envolvendo essas espécies, pesquisas envolvendo doenças infecciosas em populações de preguiças e as consequências clínicas associadas ainda são relativamente desconhecidas (SMITH; RUPLE, 2020) e no caso da preguiça-de-coleira, inexistentes. O foco dos estudos disponíveis na literatura ainda é, em sua maioria, na possibilidade de preguiças servirem como reservatórios para infecções características de humanos, sem avaliar o impacto da infecção na saúde da preguiça (SMITH; RUPLE, 2020; GENTILE *et al*., 1981; LAINSON *et al*., 1981).

Recentemente, foi publicado uma revisão sistemática (SMITH; RUPLE, 2020) que revisou toda literatura disponível desde 1809 sobre doenças infecciosas de diferentes espécies de preguiças. Tais autores não relataram estudos envolvendo preguiças e hemoparasitoses causando sinal clínico, enquanto há relatos de infecção por tripanosomatídeos, *Babesia*, *Anaplasma* e *Ehrlichia* (CALCHI *et al*., 2020; SMITH; RUPLE, 2020; SOARES *et al*., 2017), sem causar sinal clínico em preguiça. Ainda assim, nenhum estudo até o momento envolve indivíduos de *Bradypus torquatus*.

5 CAPÍTULO 1

SLOTH'S GIANT TICK (*Amblyomma varium*) PARASITIZING FREE-RANGING MANED SLOTH (*Bradypus torquatus*) IN THE ATLANTIC FOREST BIOME, BRAZIL

(Publicado: *Brazilian Journal of Veterinary Medicine*)

SLOTH'S GIANT TICK (*Amblyomma varium*) PARASITIZING FREE-RANGING MANED SLOTH (*Bradypus torquatus*) IN THE ATLANTIC FOREST BIOME, BRAZIL

5.1 ABSTRACT

Amblyomma varium is a neotropical tick popularly known as the sloth's giant tick, during the adult stage is found almost exclusively on mammals of the Bradypodidae and Megalonychidae families of the superorder Xenarthra. The maned sloth (*Bradypus torquatus*) is the only sloth species in danger of extinction in Brazil. This species is arboreal and descends to the ground only to perform defecation behavior. This behavior can be the determining factor for infestation and infection by different parasitic agents, including *A. varium*. This study reported the occurrence of *A. varium* found parasitizing free-living maned sloths (*B. torquatus*) in the Atlantic Forest biome of Northeast and Southeast Brazil. A total of 36 individuals were evaluated and more than 50% of the individuals were parasitized by ticks of the species *A. varium*. This study confirmed the importance of *B. torquatus* as a host for adults and nymphs of *A. varium* and reported the variation in length of the spurs on coxa IV of *A. varium* males.

Keywords: *Amblyomma*; *Bradypus torquatus*; giant tick; parasite; sloth.

5.2 INTRODUCTION

The species *Amblyomma varium* was first described by Koch (1844), in a male specimen, without data on host and locality. Over the years, several other researchers have published information (e.g. morphology, distribution, and hosts) that corroborates or adds to what was found by Koch (GUIMARÃES, 2001; ARAGÃO; FONSECA, 1961; NEUMANN, 1899). The redescription of the adults of *A. varium*, including a complete revision of the bibiography and clarification of the taxonomic status, was published by, Onofrio *et al.* (2008) published a redescription of both sexes of *A. varium*, clarifying the taxonomy and establishing the most recent and complete bibliograph by Onofrio *et al.* (2008). Additionally, immature stages (larva and nymph) were described by Amorim *et al.* (1996) and Martins *et al.* (2010), respectively.

Amblyomma varium is a neotropical tick, distributed in the following Brazilian states: Pará, Acre, Amazonas, Rondônia, Paraíba, Pernambuco, Alagoas, Bahia, Mato Grosso, Espírito Santo, Minas Gerais, Rio de Janeiro, and São Paulo (GRUHN *et al.*, 2019; ACOSTA *et al.*,

2016; WITTER *et al*., 2016; SANCHES *et al*., 2014). Popularly known as the sloth's giant tick, during the adult stage is found almost exclusively on mammals of the Bradypodidae and Megalonychidae families of the superorder Xenarthra (ONOFRIO *et al*., 2008; MARQUES *et al*., 2002).

Similarly to *A. varium*, sloths are found in tropical forests (ONOFRIO *et al*., 2008; GILMORE; DA-COSTA; DUARTE, 2000). The maned sloth (*Bradypus torquatus*) is the only species of sloth that is in danger of extinction in Brazil, and is listed as vulnerable by both the official list of Brazilian fauna in danger of extinction (CHIARELLO *et al*., 2018) and the International Union for Conservation of Nature and Natural Resources (CHIARELLO; MORAES-BARROS, 2014). Its geographic distribution is limited to the Atlantic Forest coast (CHIARELLO *et al*., 2018), a biome that has already lost 72% of its area, mainly due to predatory hunting, illegal logging, invasion of exotic species (REZENDE *et al*., 2018), and also infectious diseases, which could lead to the decline of a wild population or even lead to an increase in the risk of extinction of a wildlife (PEDERSEN *et al*., 2007; WOODROFFE, 1999).

The species *B. torquatus* is arboreal and descends to the ground only to perform defecation behavior (VOIRIN *et al*., 2013). However, when considering the extensive deforestation and fragmentation of its inhabited area, the animal's behavior of descending to the ground may also be related to the absence of connection between trees. Therefore, this behavior can be the determining factor for infestation and infection by different parasitic agents, including *A. varium*, either through contact with the soil, direct contact with the animal, or contact with vegetation shared among species (PINTO; GRISARD; ISHIDA, 2011; CHIARELLO, 1998).

This study reports the new findings of the tick *A. varium* Koch, 1844 found parasitizing free-living maned sloths (*B. torquatus*) in the Atlantic Forest biome of Northeast and Southeast Brazil.

5.3 MATERIAL AND METHODS

The study was carried out in three different locations (Figure 1), all with species of fauna and flora in danger of extinction: 1. Reserva Sapiranga, in the municipality of Mata de São João, state of Bahia, which was named an Environmental Protection Area (APA) through the State Decree 1.046 of March 17, 1992 and consists predominantly of 600 hectares of preserved secondary Atlantic Forest; 2. APA of Bacia do Rio São João/Mico Leão Dourado, which covers seven municipalities in the state of Rio de Janeiro. Among these municipalities are Silva Jardim and Rio das Ostras, which were created by the decree of June 27, 2002 and consist of 150,000

hectares of total area, including anthropized areas and preserved forest fragments, such as the 5,000 hectares referred to as the Reserva Biológica de Poço das Antas (Decree nº 73.791/1974 and Decree nº 76.534/1975); 3. Reserva Biológica União, in the state of Rio de Janeiro, which covers the municipalities of Macaé, Rio das Ostras, and Casimiro de Abreu, an Integral Protection Conservation Unit containing more than 7,000 hectares (Decree of April 22, 1998 and Decree of June 05, 2017).

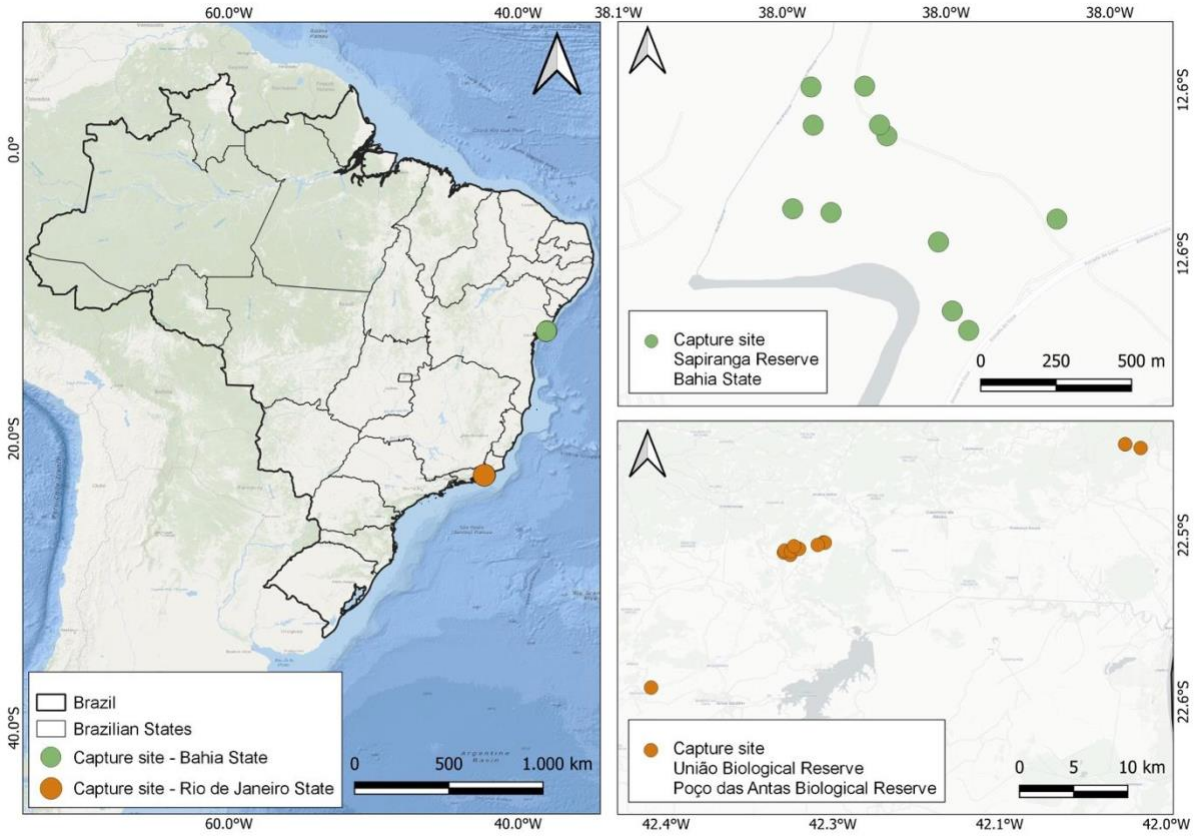

Figure 1 - Map of the study areas showing the capture site.

The animals were captured/recaptured between the months of June 2019 and May 2021 under the approval and legal consent of the Brazilian Federal Authority. Authorization for activities with scientific purpose under numbers 67274-8 and 64635-5 of the Ministério do Meio Ambiente - MMA through Instituto Chico Mendes de Conservação da Biodiversidade – ICMBIO. These captures were completed in different locations and were georeferenced with a Global Positioning System (GPS).

The captures were conducted during an active search. Once an individual was spotted, a trained climber accessed the tree canopy to hand catch the animal. All of the animals' claws were wrapped with Velcro tape to keep them closed during physical restraint. Thereafter, the animal was placed in a cotton bag and was lowered to the forest floor using a rope.

A combination of anesthetics (Ketamine Hydrochloride 4.0mg/kg and Medetomidine Hydrochloride 0.03mg/kg, intramuscularly, and Atipamezole Hydrochloride 0.1mg/kg, intravenously, as anesthetic antagonist) was used for sedation. These doses allowed safe handling of the animals for up to 60 minutes. During the anesthetic procedure heart rate, respiratory rate, and temperature were monitored every ten minutes. All information regarding chemical restraint was recorded on a pre-made anesthetic form.

The age of the maned sloths was established at both capture and recapture, and sexing was performed according to collar morphology, weight, and especially the morphology of the genitalia (LARA-RUIZ; CHIARELLO, 2005). The physical examination of the sloths was performed by a veterinarian and for the new captures, a nanochip was inserted subcutaneously for animal identification. The ticks found were collected, individually packaged in bottles containing 70% ethyl alcohol and sent to the Laboratory of Parasitic Diseases of the Department of Veterinary Preventive Medicine and Animal Health of the School of Veterinary Medicine and Animal Science of the University of São Paulo (FMVZ–USP).

The ticks were identified by stereomicroscope using taxonomic keys (MARTINS *et al*., 2010; BARROS-BATTESTI *et al*., 2006; GUIMARÃES, 2001; ARAGÃO; FONSECA, 1961). Some specimens were deposited at the tick collection "Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva" of the FMVZ–USP, under the accession numbers CNC-4357 and 4358.

5.4 RESULTS

Between 2019 and 2021, 36 specimens of *B. torquatus* were captured or recaptured, of which 64% (n=23) were parasitized by *A. varium* ticks. Of the 23 parasitized maned sloths, 13 and 8 were females and males, respectively. Two specimens were of undetermined sex. The ages stipulated on the different capture dates along with the data for each individual are shown in table 1.

A total of 70 ticks were collected, 4 nymphs, 12 females, and 54 males. The length of the spurs on coxa IV can be seen in table 1. Among the 54 males of the species *A. varium* collected in this study, 37 specimens were from the State of Rio de Janeiro (municipalities of Silva Jardim and Rio das Ostras) in the Southeast region of Brazil. A total of 8 and 29 males had the spur on coxa IV short and long, respectively. Of the three individuals of *B. torquatus* captured in the municipality of Rio das Ostras, only one had two specimens of *A. varium* with spurs of both sizes on coxa IV, while the other two individuals had three ticks with long spurs. The same variation in the length of the spur of coxa IV was observed in individuals of *B. torquatus* captured in the municipality of Silva Jardim.

The remaining 17 male specimens of *A. varium* collected were from the state of Bahia (municipality of Mata de São João), Northeast region of Brazil. Of these males, 8 specimens had a short spur and 9 had a long spur on coxa IV.

Of the 38 ticks with long spurs, 76% (n=29) come from the state of Rio de Janeiro. In addition, of the 37 ticks from Rio de Janeiro, 78% had long spurs, while in Bahia the distribution between ticks with long and those with short spurs was closer, 53% and 47% respectively.

Table 1 - *Amblyomma varium* ticks collected on free-ranging *Bradypus torquatus* from the Atlantic Forest biome between 2019 and 2021

Bradypus torquatus data					*Amblyomma varium* data		
Individual Identification	Capture Date	Municipality (Federative Unit)	Age	Sex	Number of ticks collected per host	Number of ticks per stage and sex	Length of the spurs on coxa IV of males
BT 03	July/2019	SJ (RJ)	Adult	Male	6	6 ♂	Long
BT 03	November/2019	SJ (RJ)	Adult	Male	3	1 ♂ 2 ♀	Long
BT 04	June/2019	RO (RJ)	Adult	Female	2	2 ♂	Long
BT 04	July/2019	RO (RJ)	Adult	Female	1	1 ♂	Long
BT 05	June/2019	SJ (RJ)	Subadult	Male	2	2 ♂	Long
BT 05	July/2019	SJ (RJ)	Subadult	Male	1	1 ♂	Short
BT 05	November/2019	SJ (RJ)	Adult	Male	2	1 ♂ 1 ♀	Long
BT 07	July/2019	SJ (RJ)	Adult	Female	3	3 ♂	2 Long 1 Short
BT 07	January/2021	SJ (RJ)	Adult	Female	2	2 ♂	Long
BT 08	July/2019	SJ (RJ)	Adult	Female	1	1 ♂	Long
BT 09	July/2019	SJ (RJ)	Adult	Female	3	3 ♂	2 Long 1 Short
BT 09	January/2021	SJ (RJ)	Adult	Female	2	2 ♂	Long
BT 10	January/2021	SJ (RJ)	Adult	Female	6	3 ♂ 2 ♀ 1 N	2 Long 1 Short
BT 11	December/2019	RO (RJ)	Adult	Female	2	2 ♂	1 Long 1 Short
BT 12	December/2019	SJ (RJ)	Subadult	Female	5	3 ♂ 2 ♀	Long

BT 12	January/2021	SJ (RJ)	Subadult	Female	1	1 ♂	Long
BT 13	January/2021	SJ (RJ)	Adult	Female	1	1 ♀	-
BT 14	January/2021	SJ (RJ)	Adult	Female	2	2 ♂	Short
BT 15	January/2021	SJ (RJ)	Subadult	Female	1	1 ♂	Short
BTPF 02	July/2020	MSJ (BA)	Adult	Male	4	3 ♂ 1 ♀	Short
BTPF 02	May/2021	MSJ (BA)	Adult	Male	2	2 ♂	2 Long
BTPF 04	May/2021	MSJ (BA)	Adult	Female	2	1 ♂ 1 ♀	Long
BTPF 10	March/2021	MSJ (BA)	Adult	Male	1	1 ♂	Long
BTPF 11	March/2020	MSJ (BA)	Adult	Male	1	1 ♂	Short
BTPF 13	March/2020	MSJ (BA)	Subadult	Male	2	2 ♂	1 Long 1 Short
BTPF 15	March/2021	MSJ (BA)	Subadult	Male	1	1 N	-
BTPF 30	March/2021	MSJ (BA)	Adult	Male	2	2 N	-
BTPF 31	May/2021	MSJ (BA)	Adult	Female	2	1 ♂ 1 ♀	Long
BTPF 35	May/2021	MSJ (BA)	Adult	Undetermined	1	1 ♀	-
BTPF 36	May/2021	MSJ (BA)	Adult	Female	4	4 ♂	3 Long 1 Short
BTPF 37	May/2021	MSJ (BA)	Adult	Undetermined	2	2 ♂	Short

SJ (RJ): Silva Jardim (Rio de Janeiro); RO (RJ): Rio das Ostras (Rio de Janeiro); MSJ (BA): Mata de São João (Bahia); N: nymph; ♂: male; ♀: female.

5.5 DISCUSSION

In this study, adults and nymphs of *A. varium* parasitizing *B. torquatus* were found in the Southeast and Northeast regions of Brazil, corroborating Marques *et al*. (2002)'s study which also found the same tick-host association.

Of the 54 males of *A. varium* collected, 16 had short spurs while 38 had long spurs on coxa IV. The difference in length between spurs on coxa IV has already been described in Brazil by Onofrio *et al*. (2008), who studied this intraspecific polymorphic characteristic in populations from the North (states of Amazonas, Pará, and Rondônia), Southeast (states of Espírito Santo, Minas Gerais, Rio de Janeiro, and São Paulo), and Northeast (states not reported). The authors reported long length of the spur on coxa IV only in the states of Amazonas, Rio de Janeiro, and São Paulo, making this the first study to report this feature in the state of Bahia.

Since the spurs primary function is to assist in attaching to the host hair, perhaps the longer spurs can fixate deeper or more securely in the host hair, which could support the fact that more animals are parasitized in Rio de Janeiro than in Bahia. However, further studies are strongly recommended for any conclusive statement on this topic.

5.6 CONCLUSION

In the present study, more than 50% of the captured individuals were parasitized by adults or nymphs of *A. varium*, and only one specimen of *B. torquatus* showed simultaneous parasitism by both stages of this tick species. This study confirms the importance of *B. torquatus* as a host for adults of *A. varium*. Additionally, the results report the variation in the length of the spurs on coxa IV in male specimens of the species *A. varium*, confirming previous findings in the literature of this intraspecific polymorphic characteristic in populations from the Southeast region, and recording this characteristic for the first time in Northeast Brazil.

6 CAPÍTULO 2

WHAT TO KNOW BEFORE CLINICAL PRACTICE: HEMATOLOGIC AND BIOCHEMICAL VALUES OF FREE-LIVING MANED SLOTHS (*Bradypus torquatus*) FROM THE ATLANTIC FOREST - BRAZIL.

(Submetido: *Acta Veterinaria Brasilica*)

WHAT TO KNOW BEFORE CLINICAL PRACTICE: HEMATOLOGIC AND BIOCHEMICAL VALUES OF FREE-LIVING MANED SLOTHS (*Bradypus torquatus*) FROM THE ATLANTIC FOREST - BRAZIL.

6.1 ABSTRACT

The maned sloth (*Bradypus torquatus*) is the only species of sloth that is in danger of extinction in Brazil, it inhabits a severely fragmented area. Because of this species' arboreal nature, it is a difficult task to find sloths in the forest and complex techniques must be used to capture them. The specific morphological and physiological characteristics of this species are still seldom studied. There are several studies on the physiology and health of other species of the genus *Bradypus*, but there is a lack of scientific data related to the health of maned sloths. Given the importance of blood tests for the assessment of animal health status and as an indicator of changing health status of individuals over time as well as between groups, this study presents hematological and biochemical values of maned sloths from the Atlantic Forest. A total of 30 clinically healthy individuals were captured and assessed for total leukocyte count, differential leukocyte count, red blood cell count, hematocrit, total solids, buffy coat, mean corpuscular volume, urea, creatinine, ALP, and ALT. The hematological values in this study adds to previous released data, allows further comparisons between results, and helps in assessing the health of free-living maned sloths. Furthermore, this study provides the first published biochemical values of *B. torquatus*.

Keywords: biochemistry; clinical pathology; hematology; sloth.

6.2 INTRODUCTION

The Atlantic Forest is a biome that occupies a vast area of Brazil and is made up of several sets of ecosystems, from dense forests to mangroves. It suffers an intense threat related to forest fragmentation and until 2013 has been left 28% (32 Mha) of its native forest, of which only 30% are protected areas (REZENDE *et al*., 2018). This biome shelters more than 50% of Brazilian threatened wild species (CHIARELLO *et al*., 2018), including an arboreal species that exists only in Brazil: the maned sloth (*Bradypus torquatus*, Bradypodidae, Pilosa, Xenarthra), both threatened (CHIARELLO; MORAES-BARROS, 2014) and endemic (OLIVER; SANTOS, 1991; WETZEL; ÁVILA-PIRES, 1980).

The maned sloth distribution has been confirmed in only four Brazilian states: Rio de Janeiro, Espírito Santo, Bahia, and Sergipe (HIRSCH; CHIARELLO, 2012). It is the only species of sloth that is in danger of extinction in Brazil and is listed as vulnerable by the official list of Brazilian fauna in danger of extinction (CHIARELLO *et al*., 2018) and by The International Union for Conservation of Nature and Natural Resources (CHIARELLO; MORAES-BARROS, 2014). This species was identified as occupying an area of less than $10km^2$ and inhabiting a severely fragmented area (CHIARELLO; MORAES-BARROS, 2014). Meanwhile, Schetino, Coimbra, and Santos (2017) observed a factor that must be considered: the populations of the four Brazilian states are genetically different.

The family Bradypodidae have very specific morphological and physiological characteristics and are still seldom studied. They are arboreal, solitary, and folivores. They have a low metabolism (GINÉ *et al*., 2015) due in part to a low-calorie diet, spending most of their time at rest in treetops, and moving slowly through branches and when crossing between trees. Sloths descend to the ground to defecate once a week. However, they can also be found on the ground because of the lack of trees in the area (CHIARELLO, 1998; QUEIROZ, 1995). Nonetheless, because of this species' arboreal nature, it is a difficult task to find sloths in the forest and complex climbing techniques must be used to capture them.

Both in the state of Bahia and Espírito Santo it was observed that the maned sloth needs areas where there is connectivity between trees (CASSANO; KIERULFF; CHIARELLO, 2011). It is necessary for the area to have at least 35% of forest cover (SANTOS *et al*., 2019), making it even harder to find this species in the fragmented rainforest. Furthermore, there are no maned sloths in captivity, since their maintenance is difficult, as well as its venipuncture, due to the thick skin, deep vessels, and *rete mirabile* present in the limbs (JOHANSEN *et al*, 1966 *apud* DUARTE; COSTA; HUGGINS, 1982). All these factors contribute to the lack of data on the health of this species.

There are several studies on the physiology and health of other species of the genus *Bradypus*, like blood pressure assessments related to animal posture in *B. tridactylus* (DUARTE; COSTA; HUGGINS, 1982), circadian rhythm of blood pressure in *B. variegatus* (DUARTE *et al*., 2004), data related to the presence of ticks, commensal moths, intestinal parasites, other parasites, and even hematological data of *B. variegatus* (CHAGAS *et al*., 2022; PEREIRA *et al*., 2020; MIRANDA; MESSIAS-COSTA, 2007; VOGEL *et al*., 1999; GILLESPIE, 1993; EBERHARD, 1978).

There is a lack of scientific data related to the health of maned sloths. Given the importance of hematological values and biochemical values for the assessment of animal health

status and as an indicator of change in the health of others over time and between groups (DEEM; KARESH; WEISMAN, 2001), the objective of this study is to present hematological values of free-living maned sloths (*Bradypus torquatus*) and to provide the first published biochemical values in *B. torquatus* from the Atlantic Forest, Brazil.

6.3 MATERIAL AND METHODS

The study took place at Reserva Ecológica da Sapiranga, at Bacia do Rio São João/Mico Leão Dourado, and at private properties. The Reserva Ecológica da Sapiranga is located in the municipality of Mata de São João, on the North Coast of the state of Bahia. There, the expeditions occurred during the months of September 2020, October 2020, May 2021, and October 2021. The reserve was named as Environmental Protection Area (APA) through the State Decree 1.046 of March 17, 1992. One of the characteristics of the Reserva Ecológica da Sapiranga is the predominance of 600 hectares of preserved secondary Atlantic Forest, with species of fauna and flora in danger of extinction. In addition to being an important tourist attraction for the region, it is also the focus of research carried out by public and private higher education institutions. The Bacia do Rio São João/Mico Leão Dourado is also an APA but located in the municipality of Silva Jardim, in the state of Rio de Janeiro. There, the expedition occurred during the month of January 2021. The private properties were located in six cities in the state of Sergipe: Estância, Arauá, Santa Luzia do Itanhy, Salgado, Indiaroba, and Umbaúba. There, the expedition occurred during the month of October 2021.

The animal captures were made at different locations, which were georeferenced with Global Positioning System (GPS) (eTrex 10®). Once a team member spotted an individual during an active search, a trained climber accessed the tree canopy to hand catch the animal. During physical restraint, all the claws were wrapped with a Velcro® tape to keep them closed. Subsequently, the animal was placed in a cotton bag and was lowered to the forest floor using a rope. A total of 30 individuals were captured, 18 in Bahia, 08 in Rio de Janeiro, and 04 in Sergipe. The maned sloths were aged based on body mass and sexed according to the pelage and genitalia, which can suffer changes with the reproductive period (LARA-RUIZ; CHIARELLO, 2005). All the animals were weighed using a scale (Pesola AG®).

An anesthetic protocol was instituted after the physical examination performed by a veterinarian in order to withdraw blood and collect body measurements. The protocol consisted of a combination of ketamine (Cetamin®) (4.0mg/kg) and dexmedetomidine hydrochloride (Dexdomitor®) (0.03mg/kg), intramuscularly, as well as atipamazole hydrochloride (Antisedan®) (0.1mg/kg) intramuscularly, as an anesthetic antagonist. The average sedation

time was 50 minutes for each animal. A form was filled out for each animal containing the animal's number, date, time of collection, species, weight, sex, age, body measurements, rectal temperature, heart, and respiratory rates (checked every 10 minutes), activity level during the procedure, and any other observations made during the physical examination, such as presence of scars.

Immediately after the animals were anesthetized, blood samples were collected from the cephalic vein, as was done by Pereira *et al*., (2020) and Galvão *et al*., (2019) in *Bradypus variegatus* and Catenacci *et al*. (2017) in *B. torquatus*. The blood for hematological analysis was stored in 0,5 mL EDTA microtubes and for biochemical analysis in 1.5ml Eppendorf® tubes. EDTA microtubes were kept refrigerated (2-8°C) until the exams were performed, which did not exceed 15 hours, and the Eppendorf® tubes were immediately sent to a private laboratory in town for sample processing. The volume of blood collected corresponded to a maximum of 1% of the animal's weight. 3mL syringes (Descarpack®) and 25x7-gauge needles (Descarpack®) were used for adult animals and 3mL syringes (Descarpack®) and 20x5.5-gauge needles (Descarpack®) for subadults. After the animals were fully recovered from the anesthesia, they were released at the base of the tree where they were captured or in the vines of the nearest tree.

Sample processing for hematological analysis was conducted by two different experts at a laboratory structure set up nearby after the animals were released. The blood was centrifuged (15,800g/5 minutes) in microcapillaries tubes to obtain the microhematocrit (%) and buffy coat (%). For red blood cell count, 10µL of blood was diluted in 2mL of 0.9% saline solution (1:201) and for total leukocyte count, 10µL of blood was diluted in 0.2mL of Turk's solution (1:21 dilution). Erythrocytes and leukocytes were manually counted in a Neubauer chamber (Brand®) using an optical microscope (Zeiss®) (zoom rate of 400X). The differential leukocyte count (based on 100 cells) was performed on a blood smear stained with fast panoptic stains using an optical microscope (zoom rate of 1000X). The measurement of total solids was performed using a portable Atago® model 301/311 refractometer previously calibrated with deionized water.

For biochemical analysis, the blood was centrifuged for serum release and the samples were processed at PKL® PPC 125 fully automatic chemistry analyzer.

Of the 30 animals studied: 03 were assessed for total solids and total leukocyte count; 04 were assessed for total solids, total leukocyte count, and differential leukocyte count; 01 was assessed for total leukocyte count and differential leukocyte count; 06 were assessed for total leukocyte count, differential leukocyte count, red blood cell count, hematocrit, buffy coat, and

mean corpuscular volume (MCV); 04 were assessed for total leukocyte count, differential leukocyte count, red blood cell count, hematocrit, mean corpuscular volume (MCV), and total solids; 02 were assessed for total leukocyte count, red blood cell count, hematocrit, buffy coat, and MCV; 01 was assessed for total leukocyte count, red blood cell count, hematocrit, and MCV; 01 was assessed for total leukocyte count, differential leukocyte count, red blood cell count, hematocrit, buffy coat, MCV, and total solids; 07 were assessed for hematocrit, buffy coat, total solids, urea, creatinine, ALP, and ALT; and 01 was assessed for total solids urea, creatinine, ALP, and ALT.

The research was carried out under the legal approval and authorization for activities with scientific purpose under the Sistema de Autorização e Informação em Biodiversidade (SISBIO) nº 67274-2 and nº 64635 issued by Ministério do Meio Ambiente (MMA) through Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), and by Ethics Commission for the Use of Animals under protocol CEUA/IOC-036/2020.

6.4 RESULTS

Of the 30 animals captured in this study, 21 were adults, 06 were subadults, and 03 were juveniles. Of these, 07 animals were males, 15 were females, and 08 were classified as undetermined. Based on the physical exams performed, all the animals captured were considered as clinically healthy. The average weight of the 30 individuals was 4.8kg.

The hematological values of the maned sloths are shown in Table 1 and the biochemical values of the maned sloths are shown in Table 2.

Table 1 - Minimum, maximum, mean, standard deviation, and median of hematologic parameters of free living *Bradypus torquatus*

Parameters	Unit	Minimum	Maximum	Mean and Standard Deviation	Median
Red blood cell (n=14)	$x10^6/\mu l$	1,680	2,800	2,44 ± 0.37	2,525
Hematocrit (n=21)	%	27	42	33.85 ± 3.94	33
MCV (n=14)	fl	123.07	238.09	148.0 ± 29.89	137.5
Total solids (n=20)	g/dL	8.5	13	11.1 ± 1.19	11
Buffy coat (n=16)	%	0.5	2	1.21 ± 0.48	1

Leukocytes (n=22)	Total/µl	5,800	14,000	9,209 ± 2.23	9,050
Band Neutrophils (n=16)	%	0	4	0.93 ± 1.38	0
Segmented Neutrophils (n=16)	%	4	45	23.1 ± 12.06	22
Lymphocytes (n=16)	%	46	88	70.93 ± 11.95	71
Monocytes (n=16)	%	0	11	2.31 ± 3.11	1
Eosinophils (n=16)	%	0	5	1.87 ± 1.50	2
Basophils (n=16)	%	0	4	0.50 ± 1.09	0

MCV = Mean corpuscular volume; n = Number of individuals.

Table 2 - Minimum, maximum, mean, standard deviation, and median for biochemical parameters for *Bradypus torquatus*

Parameters	Unit	Minimum	Maximum	Mean and Standard Deviation	Median
Urea (n=8)	mg/dL	30	172	74.12 ± 44.14	61
Creatinine (n=8)	mg/dL	0.3	3.2	1.36 ± 1.15	1.1
ALP (n=8)	mg/dL	62	147	105.5 ± 26.98	103
ALT (n=8)	mg/dL	1	17	4.5 ± 5.34	3

ALP = Alkaline phosphatase; ALT = Alanine aminotransferase; n = Number of individuals.

6.5 DISCUSSION

The mean hematocrit count were quite similar to what has been observed in *Bradypus torquatus* (CATENACCI *et al*., 2017) and even *Bradypus variegatus* (CHAGAS *et al*., 2022; PEREIRA *et al*., 2020; GALVÃO *et al*., 2019). Similarities were also noticed when comparing total leukocyte count, band neutrophil count, basophil count, and red blood cell count to Catenacci *et al*. (2017). Despite the similarities, the results of eosinophils, monocytes, and segmented neutrophils counts were quite higher and the results of lymphocyte count and total solids quite lower in Catenacci *et al*. (2017).

While Catenacci *et al*. (2017) provided the first description of hematological parameters for this species, the study did not differentiate the results between free-living animals and captive animals when presenting its findings. This comparison could have been interesting to evaluate if there are differences between results of free-living and captive animals due to differences in diet, food availability, physical activities, and environmental stimuli that could interfere with such hematological parameters. Additionally, the maned sloths in Catenacci *et al*. (2017) were unanesthetized and in this study the animals were under the effect of anesthetic drugs, which both can alter hematological values (BUSH; SMITH, 1980). Further, it is important to consider that the populations studied in this study and in Catenacci *et al*. (2017) were geographically separated millions of years ago (SCHETINO; COIMBRA; SANTOS, 2017), which may explain some similarities − and differences − between the populations.

Due to equipment availability and in addition to Catenacci *et al*. (2017), this study also used the manual cell count method instead of using an automated hematology analyzer. This method can lead to lower sensitivity, lower diagnostic accuracy, and higher variability (PIERRE, 2002) and can also interfere with results.

This is the first publication of biochemical values in *B. torquatus*. When compared to free living (PEREIRA *et al*., 2020) and captive (CHAGAS *et al*., 2022) *B. variegatus*, the results for urea and creatinine are significantly lower, while alkaline phosphatase (ALP) and alanine aminotransferase (ALT) results are substantially higher. However, due to the distinct habitats and biologic and physiologic particularities of each species, comparisons between them must be made narrowly.

Considering the small number of samples in this study, the results are valuable by contributing to the baseline hematological values of a rare species but not for determining reference intervals (FRIEDRICHS *et al*., 2012).

Finding clear explanations for the results is a challenge considering the species studied is very rare, there is a lack of medical information, and there are now only two studies involving hematological values of *B. torquatus*.

6.6 CONCLUSION

The hematological values herein can add to previous released data, allow further comparisons between results, and help in assessing the health of free-living maned sloths. This study generates data to assist in the laboratory monitoring and diagnosis of diseases and contributes to veterinary medicine applied to the conservation of the species.

7 CAPÍTULO 3

INVESTIGATION OF *Hepatozoon* spp., *Babesia* spp., *Anaplasma* spp., , *Ehrlichia* spp., *Rickettsia* spp , and *Mycoplasma* spp. IN FREE-RANGING MANED SLOTH (*Bradypus torquatus*) FROM NORTHEASTERN BRAZIL

INVESTIGATION OF *Hepatozoon* spp., *Babesia* spp., *Anaplasma* spp., , *Ehrlichia* spp., *Rickettsia* spp , and *Mycoplasma* spp. IN MANED SLOTH (*Bradypus torquatus*) FROM NORTHEASTERN BRAZIL

7.1 ABSTRACT

The maned sloth (*Bradypus torquatus*) is the only sloth species threatened by extinction in the world and is classified as vulnerable. This species is a restricted arboreal, spending much of its time resting in the treetop and descending to the ground only to defecate. The contact with the soil, direct contact with other animals, contact with vegetation shared by species, and the presence of humans and domestic animals near natural reserves raises the possibility of dissemination of infectious agents. There are no publications related to infectious diseases in *B. torquatus*. Due to the scarcity of data and health studies in populations of *B. torquatus* in Brazil, the aim of this study is to identify hemoparasites in blood samples of maned sloths from Northeastern Brazil. Out of 29 samples collected from maned sloths, 11 tested positive for *Hepatozoon* spp. and all 29 samples tested negative for *Babesia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Mycoplasma* spp., and *Rickettsia* spp. This study provides the first identification of *Hepatozoon* spp. in maned sloths and is a pioneer study of hemoparasites in *B. torquatus*.

Keywords: hemopathogens; parasitology; PCR; sloth.

7.2 INTRODUCTION

The Atlantic Forest is one of the world's biodiversity hotspots but only has 12.4% of its original area (INPE, 2019), and has been reduced to several small forest fragments (REZENDE *et al*., 2018). This is mainly due to predatory hunting, illegal logging, and invasion of exotic species (REZENDE *et al*., 2018), as well as parasitic diseases, which can lead to the decline or even increase the risk of extinction of a wild population (PEDERSEN *et al*., 2018; WOODROFFE, 1999).

This biome shelters more than 50% of Brazilian wild species threatened by extinction (CHIARELLO *et al*., 2018), including the maned sloth (*Bradypus torquatus*, Bradypodidae, Pilosa, Xenarthra), an arboreal species that exists only in Brazil. *B. torquatus* is the only sloth species threatened by extinction in Brazil and is classified as vulnerable B2ab(ii,iii) (CHIARELLO *et al*., 2018; CHIARELLO; MORAES-BARROS, 2014) as it faces a high risk of extinction in the wild, has a very fragmented area of occupation estimated at less than 2,000

km², and shows a continuous decline in its area of occupation. This species occurs in the states of Sergipe, Rio de Janeiro, Bahia, and Espírito Santo, and there are unconfirmed records in Minas Gerais and Pernambuco (CHIARELLO *et al*., 2018).

This species is a restricted arboreal, spending much of its time resting in the treetops, moving slowly between branches, and rarely descends to the ground, except to defecate (VOIRIN; WIKELSKI; LOWMAN, 2013). Considering the extensive deforestation and fragmentation of the inhabited area, this behavior may also be related to the lack of connection between trees (GINÉ *et al*., 2015). Bearing in mind that infection by different parasitic agents can occur through contact with the soil, direct contact with the animal, or contact with vegetation shared between species (CHIARELLO, 1998), the behavior of descending to the ground could be the determinative factor for infection.

The presence of humans and domestic animals near natural reserves raises the possibility of dissemination of parasitic agents in new environments and wild species, making it essential to study health, risk factors in ecosystems, and the circulation of agents in wild animal populations (PAIVA, 2016). Although parasitic agents play an important role in maintaining ecological and ecosystem diversity, they can also threaten biodiversity, leading to a decline in natural populations, reducing the survival or even reproduction of the host (ALTIZER; FOUFOPOULOS; GAGER, 2001). Furthermore, they may be responsible for local extinctions of endemic species, as has been reported in studies with other wild species (WARNER, 1968). Therefore, the study of parasites becomes fundamental, especially in endangered species.

Most recently, a review (SMITH; RUPLE, 2020) of all the literature related to infectious diseases in sloths was published. There are no published studies related to hemoparasitosis causing a clinical sign in different species of sloths. However, there are reports of trypanosomatids, *Babesia, Anaplasma*, and *Ehrlichia* (CALCHI *et al*., 2020; SMITH; RUPLE, 2020; SOARES *et al*., 2017) infecting sloths without causing a clinical sign. Nonetheless, there are no published studies related to *B. torquatus*.

Due to the scarcity of data and health studies in populations of *Bradypus torquatus* in Brazil, it is necessary to conduct and deepen analyses on this topic. The objective of this study is to identify for the first time, through a molecular technique at the level of genotypes, *Hepatozoon* spp., *Babesia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Rickettsia* spp. and *Mycoplasma* spp. in blood samples of maned sloths from the Reserva Ecológica da Sapiranga, Northeastern Brazil.

7.3 MATERIAL AND METHODS

The study was conducted in the Reserva Ecológica da Sapiranga, which is located in the municipality of Mata de São João, on the north coast of the state of Bahia. This area was named an Environmental Protection Area (APA) through the State Decree 1.046 of March 17, 1992 and consists predominantly of 600 hectares of preserved secondary Atlantic Forest.

The maned sloths were captured during the months of September 2020, October 2020, May 2021, and October 2021 under the approval and legal consent of the Brazilian Federal Authority. Authorization for activities with scientific purpose under numbers 67274-8 and 64635-5 of the Ministério do Meio Ambiente - MMA through Instituto Chico Mendes de Conservação da Biodiversidade – ICMBIO and case number 036/2020, license L-003/2021 of the Council for the Ethical Use of Animals of Oswaldo Cruz Institute (CEUA/IOC-FIOCRUZ).

Animal captures were made at different locations, which were georeferenced with a Global Positioning System (GPS). The capture was carried out during an active search of the animal. When spotted in the treetops, a trained climber accessed the tree canopy to hand catch the animal. During physical restraint, all the claws were wrapped with Velcro tape to keep them closed. Subsequently, the animal was placed in a cotton bag and was lowered to the forest floor using a rope.

An anesthetic protocol (Ketamine Hydrochloride 4.0mg/kg and Medetomidine Hydrochloride 0.03mg/kg, intramuscularly, and Atipamezole Hydrochloride 0.1mg/kg, intravenously, as anesthetic antagonist) was established, allowing safe handling of the animals for up to 60 minutes. During the anesthetic procedure, heart rate, respiratory rate, and temperature were monitored every ten minutes. All information regarding chemical restraint was recorded on a pre-made anesthetic form.

Immediately after the animals were anesthetized, blood samples were collected, blood smears prepared, and a physical examination of the maned sloths was performed by a veterinarian. Blood was drawn from the cephalic vein, stored in 0,5 mL microtubes with EDTA anticoagulant, and blood smears were done. The volume of blood collected corresponded to a maximum of 1% of the animal's weight. The samples were kept refrigerated and sent to the Biodiversity and Health Laboratory of the State University of Santa Cruz. Upon arrival, all microtubes were kept frozen at –20 °C until DNA extraction.

The DNA was extracted from each blood sample using the Easy-DNA™ Kit, according to the manufacturer’s instructions. The amount of extracted genomic DNA was established using a Nano-Drop 2000 (Thermo Scientific, USA), stored in microtubes, and placed in a freezer at -20℃. In order to detect the presence of the hemoparasites, 3µL microliters of

extracted DNA were used for polymerase chain reaction (PCR) amplification. PCRs were carried out in a total of 25µL water-solution containing 1x PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 1.0 U of Platinum Taq DNA Polymerase, and 0.2 µM of each primer. The *primers* used are shown in Table 1.

Table 1 - List of all *primers* used.

Target gene and *primer* pairs	Specificity	Sequence of primers	Amplified fragment (bp)	Reference
18S HEP-144-169 HEP-743-718	Genus *Hepatozoon*	F - GGTAATTCTAGAGCTAATACATGAGC R - ACAATAAAGTAAAAAACAYTTCAAAG	574bp	Almeida, 2011
18S BAB-143-167 BAB-694-667	Genus *Babesia*	F - CCGTGCTAATTGTAGGGCTAATACA R - GCTTGAAACACTCTARTTTTCTCAAAG	551bp	Almeida, 2011
16S GE2 HE3	Genus *Anaplasma* and *Ehrlichia*	F - GTTAGTGGCAGACGGGTGAGT R - ATAGGTACCGTCATTATCTTCCCTAT	360bp	Breitschwerdt; Hegarty; Hancoc, 1998
gltA CS-78 CS-323	Genus *Rickettsia*	F – GCAAGTATCGGTGAGGATGTAAT R - GCTTCCTTAAAATTCAATAAATCAGGAT	401bp	Labruna *et al*., 2004
OmpA Rr190.70 Rr190.602	Spotted Fever Group	F – ATGGCGAATATTTCTCCAAAA R - AGTGCAGCATTCGCTCCCCCT	532bp	Regnery; Spruil; Plikaytis, 1991
16S	Genus *Mycoplasma*	F - AGAGTTTGATCCTGGCTCAG R - CTCAAAACTGAAAGYCATCCGC	1000–1035 bp	Volokhov *et al*., 2017

F = Forward; R = Reverse.

Subsequently, the analyses of PCR amplifications were performed in a thermocycler of the Proflex PCR system (Applied Biosystems). The cycles used for target amplification are shown in Table 1. Ultrapure water was used as negative control and positive blood samples of each agent were used as positive controls. The PCR products were subjected to 1% agarose gel

electrophoresis, stained with SYBR® Safe, and examined by UV transillumination. Thereafter, the PCR products positive for *Hepatozoon* spp. were purified using the PureLink PCR Purification kit (Invitrogen) and sent for sequencing to the Fiocruz-Bahia Technology Platform Network. Sequencing was performed by capillary electrophoresis (modified Sanger sequencing) on the ABI 3500XL Genetic Analyzer platform (Applied Biosystems), chromatogram analysis was performed using FinchTV 1.4.0 software, and amplicons were Sanger sequenced in both directions.

7.4 RESULTS

All individuals were classified as healthy after a physical exam performed by a veterinarian. Out of 29 samples collected from maned sloths, 37% (11/29) tested positive for *Hepatozoon* spp. All 29 samples tested negative for *Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp., *Mycoplasma* spp., and *Rickettsia* spp. No hemoparasites were found in blood smears. The analysis of the sequences produced from the 18S rDNA gene using sense and antisense primers HEP had no genetic similarity to *Hepatozoon* spp. One of the causes of the error was insertions and deletions due to the presence of unspecific regions of the primer sequences, which projected sequences not corresponding to the agent studied. New sequences with other primers are being carried out by Fiocruz-Bahia Technology Platform Network.

7.5 DISCUSSION

This is the first study investigating the presence of *Hepatozoon* spp., *Babesia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Rickettsia* spp. and *Mycoplasma* spp. in *B. torquatus*. Even though all samples were negative for all of these agents except *Hepatozoon* spp., it is essential to consider that the Reserva Ecológica da Sapiranga is an area with a high level of anthropization and a considerable quantity of domestic animals. While the maned sloths studied here are not yet infected with many of these parasites, they do live in close proximity to humans and domestic animals, and share vegetation with other species. Conceivably, this fact leaves open the possibility that individuals may become infected in the future.

A few studies with other wild mammals (KHOSHNEGAH *et al.*, 2012; EAST *et al.*, 2008; DAVIDSON; CALPIN, 1976) report *Hepatozoon* spp. as a potential pathogen since animals presented clinical signs compatible with *Hepatozoon* infections, but clarifications of whether it represents a threat for wildlife are still necessary. Two of those studies (EAST *et al.*, 2008; DAVIDSON; CALPIN, 1976) determined that the species of *Hepatozoon* detected were specific to the species in the study, but further studies are needed to know whether animals

infected with other *Hepatozoon* species would be asymptomatic or would present clinical signs as well.

Considering the biological cycle of *Hepatozoon* spp., the only arthropod that can act as a definitive host that was found par asitizing the maned sloths was *Amblyomma varium*, popularly known as the sloth's giant tick, which is found almost exclusively on Xenarthrans. This indicates that the transmission probably occurred by *A. varium*, but could have occurred by another ectoparasite not found on the animal on the day of the capture. However, researchers have reported that maned sloths from Reserva Ecológica da Sapiranga spend a lot of time scratching themselves (L. S. MUREB, personal communication, June 01, 2022), which could make ticks fall off onto the leaves around where the animal is located. Engorged female ticks also fall off the maned sloths on their own. In either case, the maned sloths may ingest the ticks later while eating. Even though the *A. varium* life cycle is unknown, it is known that species of *Amblyomma* need three hosts to complete its life cycle and must be close to the host (SILVA, 2017). Furthermore, a hypothesis could be created: *Amblyomma varium* may be a transmitter of *Hepatozoon*.

Local researchers (L. S. MUREB, personal communication, June 01, 2022) have also noted that while collecting samples of leaves for maned sloth diet studies purposes at Reserva Ecológica da Sapiranga, immature *Amblyomma* spp. ticks were found in large quantities in the leaves. Nonetheless, immature *Amblyomma* spp. ticks has been described as having little or no importance in the transmission of *Hepatozoon canis* in Brazil (DEMONER *et al*., 2013), but new studies are required to verify the vectorial capacity of this ectoparasite, especially related to other species of *Hepatozoon*.

Determining which species of *Hepatozoon* infected the maned sloths would be helpful in ascertaining which species of *Hepatozoon* may have an asymptomatic role on them, making *B. torquatus* a potential reservoir host. The possibility of the maned sloth developing clinical signs are not discarded since the individuals were not monitored after capture.

7.6 CONCLUSION

The results herein show pioneer *Hepatozoon* spp. identification in maned sloth (*Bradypus torquatus*) from Northeastern Brazil. Further studies are strongly recommended to better understand the relationship between maned sloths and *Amblyomma varium*, and maned sloths and hemoparasites.

REFERÊNCIAS

ACOSTA, I. C. L,; MARTINS, T. F;, MARCILI, A.; SOARES, H. S.; KRAWCZAK, F. S.; VIEIRA, F. T.; LABRUNA, M. B. Ticks (Acari: Ixodidae, Argasidae) from humans, domestic, and wild animals in the State of Espírito Santo, Brazil, with notes on rickettsial infection. **Veterinary Parasitology: Regional Studies and Reports**, v. 3, p. 66-69, 2016.

ALMEIDA, A. P. **Pesquisa de Rickettsia, Ehrlichia, Anaplasma, Babesia, Hepatozoon e Leishmania em cachorro-do-mato (*Cerdocyon thous*) de vida livre do estado do Espírito Santo**. 2011. Tese (Mestrado em Ciências) - Universidade de São Paulo, São Paulo, 2011.

ALTIZER, S., FOUFOPOULOS, J.; GAGER, A. **Conservation and diseases**. 2 ed. San Diego: Academic Press, 2011

AMORIM, M.; SERRA-FREIRE, N. M. Morphological description of tick larval stage (Acari: Ixodidae). 7. *Amblyomma auricularium* (Conil, 1878). **Entomologia y vectores**, v. 7, n. 3, p. 297-309, 2000.

ARAGÃO, H. D. B. (1936). Ixodidas brasileiros e de alguns paizes limitrophes. **Memórias do Instituto Oswaldo Cruz**, v. 31, n. 4, p. 759-843, 1936.

ARAGÃO, H. B.; FONSECA, F. Notas de ixodologia: VIII. Lista e chave para os representantes da fauna ixodológica brasileira: notas de ixolodologia. **Memórias do Instituto Oswaldo Cruz**, v. 59, p. 115-129, 1961.

ARENALES, A.; SILVA, F. L.; MIRANDA, F.; GUEDES, P. E. B.; WERTHER, K.; COSTA, M. E. L. T.; SANTOS, R. L. Pathologic findings in 36 sloths from Brazil. **Journal of Zoo and Wildlife Medicine**, v. 51, n. 3, p. 672-677, 2020.

BARNES, T. S.; GOLDIZEN, A. W.; COLEMAN, G. T. Hematology and serum biochemistry of the brush-tailed rock-wallaby (*Petrogale penicillata*). **Journal of Wildlife Diseases**, v. 44, n. 2, p. 295-303, 2008.

BARRETO, R. M. F.; CASSANO, C. R. Uso do habitat por uma preguiça-de-coleira (*Bradypus torquatus*) em plantação de cacau (*Theobroma cacao*) sombreada por árvores nativas no sul da Bahia. **I Simpósio sobre Paisagem Cacaueira e Biodiversidade no Sudeste da Bahia, State University of Santa Cruz, Ilhéus,** p. 8-9, 2007.

BARROS-BATTESTI, D. M.; ARZUA, M.; BECHARA, G. H. **Carrapatos de importância médico-veterinária da região neotropical: um guia ilustrado para identificação de espécies**. ICTTD-3; Instituto Butantan, 2006.

BOFFY, A. C. M.; NOVAES, R. L. M.; MELLO, G. S.; SANT'ANNA, C. Registro da preguiça-de-coleira *Bradypus torquatus* (Pilosa, Bradypodidae) em três localidades do estado do Rio de Janeiro: Nova Friburgo, Cachoeiras de Macacu e Teresópolis. **Edentata**, v. 11, n. 1, p. 78-80, 2010.

BRANDÃO, R. M. L. Medicina da Conservação - Introdução e Conceitos Básicos. *In*: Aldeia.org documentos. [Gouveia, Portugal], Abril 2007. Site. Disponível em:

https://www.aldeia.org/portal/user/documentos/RBrandao_MedCons_intro_conceitos.pdf. Acesso em: 02 de jun. 2022.

BREITSCHWERDT, E. B.; HEGARTY, B. C.; HANCOCK, S. I. Sequential evaluation of dogs naturally infected with *Ehrlichia canis*, *Ehrlichia chaffeensis*, *Ehrlichia equi*, *Ehrlichia ewingii*, or *Bartonella vinsonii*. **Journal of Clinical Microbiology**, v. 36, n. 9, p. 2645-2651, 1998.

BUSH, M.; GILROY, B. A. A bleeding technique from nonpalpable vessels in anesthetized two-toed sloths (*Choloepus didactylus*): plus hematologic data. **The Journal of Zoo Animal Medicine**, v. 10, n. 1, p. 26-27, 1979.

BUSH, R. M.; SMITH, E. E. Clinical chemistry and hematology as diagnostic aids in zoological medicine. **The Comparative Pathology of Zoo Animals**, 1980.

CALCHI, A. C.; VULTAO, J. G.; ALVES, M. H.; YOGUI, D. R.; DESBIEZ, A. L. J.; SANTI, M., SANTANA, M. S.; SILVA, T. M. V.; WERTHER, K.; TEIXEIRA, M. M. G.; MACHADO, R. Z.; ANDRE, M. R. Ehrlichia spp. and Anaplasma spp. in Xenarthra mammals from Brazil, with evidence of novel 'Candidatus Anaplasma spp.'. **Scientific reports**, v. 10, n. 1, p. 1-15, 2020.

CASSANO, C. R. **Ecologia e conservação da preguiça-de-coleira (Bradypus torquatus Illiger, 1811) no sul da Bahia**. 2006. 106 f. Dissertação (Mestrado em Zoologia) – Programa de Pós-graduação em Zoologia, Universidade Federal de Santa Cruz, Ilhéus, 2006.

CASSANO, C. R.; KIERULFF, M. C. M.; CHIARELLO, A. G. The cacao agroforests of the Brazilian Atlantic Forest as habitat for the endangered maned sloth *Bradypus torquatus*. **Mammalian Biology**, v. 76, n. 3, p. 243-250, 2011.

CATENACCI, L. S.; NASCIMENTO, A.; MUNIZ-NETA, E. S.; CASSANO, C. R.; DEEM, S. L.; DA ROSA, E. S. T.; MUNHOZ, A. D. First record of hematologic values in free-living and captive maned sloths (*Bradypus torquatus*; Xenartha, Bradypodidae). **Journal of Zoo and Wildlife Medicine**, v. 48, n. 2, p. 312-318, 2017.

CHAGAS, C. R. F.; LIMA, C. F. D. M.; GONZALEZ, I H. L.; SALGADO, P. A. B.; MONTICELLI, C.; RAMOS, P. L. Hematologic and biochemical reference intervals of brown-throated sloths (*Bradypus variegatus*). **Veterinary Clinical Pathology**, v. 51, n. 1, p. 126-133, 2022.

CHAGAS, R. R. D.; SOUZA-ALVES, J. P.; JERUSALINSKY, L.; FERRARI, S. F. New records of *Bradypus torquatus* (Pilosa: Bradypodidae) from southern Sergipe, Brazil. **Edentata**, v. 2009, n. 10, p. 21-24, 2009.

CHIARELLO, A. G. Diet of the Atlantic Forest Maned Sloth *Bradypus torquatus* (Xenarthra: Bradypodidae). **Journal of Zoology**. v. 246, n. 1, p. 11-19, 1998.

CHIARELLO, A. G. Sloth ecology: an overview of field studies. **The biology of the Xenarthra**, p. 269-280, 2008.

CHIARELLO, A. G.; CHIVERS, D. J.; BASSI, C.; MACIEL, M. A. F.; MOREIRA, L. S.; BAZZALO, M. A translocation experiment for the conservation of maned sloths, Bradypus torquatus (Xenarthra, Bradypodidae). **Biological Conservation**, v. 118, n. 4, p. 421-430, 2004.

CHIARELLO, A. G.; MORAES-BARROS, N. 2014. *Bradypus torquatus*. **The IUCN Red List of Threatened Species**. Disponível em: https://www.iucnredlist.org/species/3036/47436575. Acesso em: 03 jan. 2022.

CHIARELLO, A.; MIRANDA, F. R.; XAVIER, G. A. A.; MORAES-BARROS, N.; VAZ, S. M. *Bradypus torquatus* Illiger, 1811. *In*: ICMBio/MMA. **Livro Vermelho da Fauna Brasileira Ameaçada de Extinção**. 1 ed. Brasília: ICMBio/MMA, v. 1, p. 34-40, 2018.

CORK, S. J.; FOLEY, W. J. Digestive and metabolic strategies of arboreal mammalian folivores in relation to chemical defenses in temperate and tropical forests. **Plant Defenses Against Mammalian Herbivory**, p. 133-166, 1991.

COSTA, A. B. F. **Morfometria corporal e hemograma de populações simpátricas de preguiça-real e de preguiça-bentinho em Manaus, Brasil**. 2014. Relatório de pesquisa (Programa PIBIC) - Universidade Federal do Amazonas, Manaus, 2014.

DAVIDSON, W. R.; CALPIN, J. P. *Hepatozoon griseisciuri* infection in gray squirrels of the southeastern United States. **Journal of Wildlife Diseases**, v. 12, n. 1, p. 72-76, 1976.

DEEM, S. L.; KARESH, W. B.; WEISMAN, W. Putting theory into practice: wildlife health in conservation. **Conservation biology**, v. 15, n. 5, p. 1224-1233, 2001.

DEMONER, L. C.; RUBINI, A. S.; PADUAN, K. S.; METZGER, B.; ANTUNES, J. M. A. P.; MARTINS, T. F.; MATHIAS, M. I. C.; O'DWYER, L. H. Investigation of tick vectors of Hepatozoon canis in Brazil. **Ticks and tick-borne diseases**, v. 4, n. 6, p. 542-546, 2013.

DUARTE, D. P. F.; COSTA C.P.; HUGGINS S.E. The effects of posture on blood pressure and heart rate in the three-toed sloth. **Comparative Biochemistry and Physiology**, v. 73, n. 4, p. 697-702, 1982.

DUARTE, D. P. F.; JAGUARIBE, A. M.; PEDROSA, M. A. C.; CLEMENTINO, A. C. C. R.; BARBOSA, A. A.; SILVA, A. F. V.; GILMORE, D. P.; COSTA, C.P. Cardiovascular responses to locomotor activity and feeding in unrestrained three-toed sloths, *Bradypus variegatus*. **Brazilian Journal of Medical and Biological Research**, v. 37, n. 2, p. 1557-1561, 2004.

EAST, M. L.; WIBBELT, G.; LIECKFELDT, D.; LUDWIG, A.; GOLLER, K.; WILHELM, K.; SCHARES, G.; THIERER, D.; HOFER, H. A Hepatozoon species genetically distinct from *H. canis* infecting spotted hyenas in the Serengeti ecosystem, Tanzania. **Journal of Wildlife Diseases**, v. 44, n. 1, p. 45-52, 2008.

EBERHARD, M. L. Dirofilaria macrodemus and D. Panamensis spp.n. (nematoda: Filarioidea) from central and south american sloths. **The Journal of Parasitology**, v. 64, n. 2, p. 198-203, 1978.

FAIRCHILD, G. B.; KOHLS, G. M.; TIPTON, V. J. The ticks of Panama (Acarina: Ixodoidea). *In*: WENZEL, W. R.; TIPTON, V. J. **Ectoparasites of Panama**. 1 ed. Chicago: Field Museum of Natural History, 1966.

FEIJÓ, A.; GE, D.; WEN, Z.; XIA, L.; YANG, Q. Identifying hotspots and priority areas for xenarthran research and conservation. **Diversity and Distributions**, 2022.

FONSECA, G. A. B.; AGUIAR, J. M. The 2004 Edentate Species Assessment Workshop. **Edentata**, v. 6, p. 1–26, 2004.

FRIEDRICHS, K. R.; HARR, K. E.; FREEMAN, K. P.; SZLADOVITS, B.; WALTON, R. M.; BARNHART, K. F.; BLANCO-CHAVEZ, J. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. **Veterinary clinical pathology**, v. 41, n. 4, p. 441-453, 2012.

GALVÃO, G. R.; GIESE, E. G.; GALVÃO, G. R.; MOREIRA, L. F. M.; DAVID, M. B. M.; AZEVEDO, E. F. S.; SANTANA, M. A. C.; MAIA, B. K. S. Hematological values in *Bradypus variegatus* (Schinz, 1825) from creatories of the Belém metropolitan region. **Revista Ibero Americana de Ciências Ambientais**, v. 10, n. 5, p. 82-88, 2019.

GENTILE, B.; LE PONT, F.; PAJOT, F. X.; BESNARD, R. Dermal leishmaniasis in French Guiana: the sloth (*Choloepus didactylus* as a reservoir host. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, v. 75, n. 4, p. 612-613, 1981.

GILLESPIE, D. S. Edentata: Diseases. In: FOWLER, M. E. **Zoo & Wild Animal Medicine Current Therapy**. 1 ed. Philadelphia: WB Saunders; 1993.

GILMORE, D. P.; DA-COSTA, C, P.; DUARTE, D. P. F. An update on the physiology of two-and three-toed sloths. **Brazilian Journal of Medical and Biological Research**, v. 33, n. 2, p. 129-146, 2000.

GINÉ, G. A. F.; CASSANO, C. R.; DE ALMEIDA, S. S.; FARIA, D. Activity budget, pattern and rhythm of maned sloths (*Bradypus torquatus*): responses to variations in ambient temperature. **Mammalian Biology**, v. 80, n. 6, p. 459-467, 2015.

GRUHN, K. D.; OGRZEWALSKA, M.; ROZENTAL, T.; FARIKOSKI, I. O.; BLANCO, C.; FREITAS, L. S.; LEMOS, E. R. S.; RIBEIRO, V. M. F. Evaluation of rickettsial infection in free-range capybaras (*Hydrochoerus hydrochaeris* Linnaeus, 1766) (Rodentia: Caviidae) and ticks (Acari: Ixodidae) in the Western Amazon, Brazil. **Ticks and Tick-borne Diseases**, v. 10, n. 5, p. 981-986, 2019.

GUGLIELMONE, A. E. A.; ESTRADA-PEÑA, A.; MANGOLD, A. J.; BARROS-BATTESTI, D. M.; LABRUNA, M. B.; MARTINS, J. R.; VENZAL, J. M.; ARZUA, M.; KEIRANS, J. E. *Amblyomma aureolatum* (Pallas, 1772) and *Amblyomma ovale* Koch, 1844 (Acari: Ixodidae): hosts, distribution and 16S rDNA sequences. **Veterinary Parasitology**, v. 113, n. 3-4, p. 273-288, 2003.

GUIMARÃES, J.H. **Ectoparasitos de importância veterinária**. 1 ed. São Paulo:Plêiade/Fapesp, 2001.

HANKS, J. Characterisation of population condition. *In*: FOWLER, C. W.; SMITH, T. D. **Dynamics of Large Mammal Populations**. 1 ed. New York: John Wiley and Sons, 1981.

HIRSCH, A.; CHIARELLO, A. G. The endangered maned sloth *Bradypus torquatus* of the Brazilian Atlantic Forest: a review and update of geographical distribution and habitat preferences. **Mammal Review**, v. 42, n. 1, p. 35-54, 2012.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA (IBGE). **Mapa da Área de Aplicação da Lei 11.428 de 2006. Escala 1: 5.000.000.** Dezembro de 2006. Disponível em: https://antigo.mma.gov.br/biomas/mata-atlântica_emdesenvolvimento/mapas-da-mata-atlântica.html. Acesso em: 01 fev. 2022.

INSTITUTO NACIONAL DE PESQUISAS ESPACIAIS (INPE). **SOS Mata Atlântica e INPE lançam novos dados do Atlas do bioma**. Maio de 2019. Site. Disponível em: http://www.inpe.br/noticias/noticia.php?Cod_Noticia=5115. Acesso em: 01 jun. 2022.

JONES, E. K.; CLIFFORD, C. M.; KEIRANS, J. E.; KOHLS, G. M. Ticks of Venezuela (Acarina: Ixodoidea) with a key to the species of Amblyomma in the Western Hemisphere. **Brigham Young University Science Bulletin, Biological Series**, v. 17, n. 4, p. 1, 1972.

KHOSHNEGAH, J.; MOHRI, M.; MIRSHAHI, A.; MOUSAVI, S. J. Detection of Hepatozoon sp. in a Persian leopard (*Panthera pardus ciscaucasica*). **Journal of Wildlife Diseases**, v. 48, n. 3, p. 776-780, 2012.

KINNEY, M. E.; COLE, G. A.; VAUGHAN, C.; SLADKY, K. K. Physiologic and serum biochemistry values in free-ranging Hoffmann's two-toed (Choloepus hoffmanni) and brown-throated three-toed (Bradypus variegatus) sloths immobilized using dexmedetomidine and ketamine. **Journal of Zoo and Wildlife Medicine**, p. 570-580, 2013.

KOCAN, A. A.; BRESHEARS, M. A.; CUMMINGS, C. A.; PANCIERA, R. J.; EWING, S. A.; BARKER, R. W. Naturally occurring hepatozoonosis in coyotes from Oklahoma. Journal of Wildlife Diseases, v. 35, n. 1, p. 86-89, 1999.

KOCH, C. L. Systematische übersicht über die Ordnung der Zecken [Systematic overview of the order of ticks]. **Archiv fur Naturgeschichte**, v. 10, p. 217–239, 1844.

KOCK, M. D. Wildlife, people and development: veterinary contributions to wildlife health and resource management in Africa. **Tropical Animal Health and Production**, v. 28, n. 1, p. 68-80, 1996.

KOPHAMEL, S.; ILLING, B.; ARIEL, E.; DIFALCO, M.; SKERRATT, L. F.; HAMANN, M.; WARD, L. C.; MÉNDEZ, D.; MUNNS, S. L. Importance of health assessments for conservation in noncaptive wildlife. **Conservation Biology**, v. 36, n. 1, p. e13724, 2022.

LABRUNA, M. B. Ecology of rickettsia in South America. **Annals of the New York Academy of Sciences**, v. 1166, n. 1, p. 156-166, 2009.

LABRUNA, M. B.; MATTAR, S.; NAVA, S.; BERMUDEZ, S.; VENZAL, J. M.; DOLZ, G.; ABARCA, K.; ROMERO, L.; SOUSA, R.; OTEO, J.; ZAVALA-CASTRO, J. Rickettsioses in Latin America, Caribbean, Spain and Portugal. **Revista MVZ Córdoba**, v. 16, n. 2, p.

2435-2457, 2011.

LABRUNA, M. B.; WHITWORTH, T.; HORTA, M.; BOUYER, D. H.; MCBRIDE, J. W.; PINTER, A.; POPOV, V.; GENNARI, S. M.; WALKER, D. H. *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the State of São Paulo, Brazil, where Brazilian spotted fever is endemic. **Journal of Clinical Microbiology**, v. 42, n. 1, p. 90-98, 2004.

LAINSON, R.; BRAGA, R. R.; SOUZA, A. A. A.; POVOA, M. M.; ISHIKAWA, E. A. Y.; SILVEIRA, F. T. Leishmania (Viannia) shawi sp. n., a parasite of monkeys, sloths and procyonids in Amazonian Brazil. **Annales de parasitologie humaine et comparée**, v. 64, n. 3, p. 200-207, 1989.

LARA-RUIZ, P.; CHIARELLO, A. G. Life-history traits and sexual dimorphism of the Atlantic Forest maned sloth *Bradypus torquatus* (Xenarthra: Bradypodidae). **Journal of Zoology**, v. 267, n. 1, p. 63-73, 2005.

MARQUES, S.; BARROS-BATTESTI, D. M.; FACCINI, J. L. H.; ONOFRIO, V. C. Brazilian distribution of Amblyomma varium Koch, 1844 (Acari: Ixodidae), a common parasite of sloths (Mammalia: Xenarthra). **Memórias do Instituto Oswaldo Cruz**, v. 97, n. 8, p. 1141-1146, 2002.

MARTINS, T. F.; ONOFRIO, V. C.; BARROS-BATTESTI, D. M.; LABRUNA, M. B. Nymphs of the genus Amblyomma (Acari: Ixodidae) of Brazil: descriptions, redescriptions, and identification key. **Ticks and Tick-borne Diseases**, v. 1, n. 2, p. 75-99, 2010.

MERCER, S. H.; JONES, L. P.; RAPPOLE, J. H.; TWEDT, D.; LAACK, L. L.; CRAIG, T. M. Hepatozoon sp. in wild carnivores in Texas. **Journal of Wildlife Diseases**, v. 24, n. 3, p. 574-576, 1988.

MIRANDA, F.; MESSIAS-COSTA, A. Xenarthra (Tamanduá, Tatu, Preguiça). *In*: CUBAS, Z.; SILVA, J. C. R.; CATÃO-DIAS, J.L. **Tratado de Animais Selvagens: Medicina Veterinária**. 1 ed. São Paulo: Roca, 2007.

MINISTÉRIO DO MEIO AMBIENTE (MMA). **Mata Atlântica: patrimônio nacional dos brasileiros**. [Brasília, Brasil], 2010. Site. Disponível em: https://1library.co/document/zxldp2dz-mata-atlantica-patrimonio-nacional-dos-brasileiros.html. Acesso em: 03 fev. 2022.

MUREB, L. S. **Composição da dieta e seleção alimentar da preguiça-de-coleira (*Bradypus torquatus*) no norte e sul de sua distribuição: similaridades interindividual e interpopulacional**. 2022. Dissertação (Mestrado em Ecologia e Conservação) – Programa de Pós-graduação em Ecologia e Conservação, Universidade Federal de Santa Cruz, Ilhéus, 2022.

NAGY, K. A.; MONTGOMERY, G. G. Field metabolic rate, water flux, and food consumption in three-toed sloths (*Bradypus variegatus*). **Journal of Mammalogy**, v. 61, n. 3, p. 465-472, 1980.

NEUMANN, G. Révision de la famille des ixodidés [Review of the ixodidae family]. **Mémoires de la Société Zoologique de France**, v. 12, p. 107–294, 1899.

OGRZEWALSKA, M.; LITERAK, I.; CARDENAS-CALLIRGOS, J. M.; CAPEK, M.; LABRUNA, M. B. *Rickettsia bellii* in ticks *Amblyomma varium* Koch, 1844, from birds in Peru. **Ticks and Tick-borne Diseases**, v. 3, n. 4, p. 254-256, 2012.

OLIVER, W. L. R.; SANTOS, I. B. **Threatened endemic mammals of the Atlantic Forest region of south-east Brazil**. 1 ed. Jersey, Channel Islands: Jersey Wildlife Preservation Trust, 1991.

ONOFRIO, V. C.; BARROS-BATTESTI, D. M.; MARQUES, S;, FACCINI, J. L. H.; LABRUNA, M. B.; BEATI, L.; GUGLIELMONE, A. A. Redescription of *Amblyomma varium* Koch, 1844 (Acari: Ixodidae) based on light and scanning electron microscopy. **Systematic parasitology**, v. 69, n. 2, p. 137-144, 2008.

PAIVA, K. A. R. ***Rickettsia* spp. em roedores e marsupiais silvestres do Rio Grande do Norte, Brasil**. 2016. Dissertação (Mestrado em Ciência Animal) – Universidade Federal Rural do Semi-Árido, Mossoró, 2016.

PEDERSEN, A. B.; JONES, K. E.; NUNN, C. L.; ALTIZER, S. Infectious diseases and extinction risk in wild mammals. **Conservation Biology**, v. 21, n. 5, p. 1269-1279, 2007.

PEREIRA, K. F.; E SILVA, I. D. O.; SILVA, F. D. F. R.; E SILVA, V. H. D.; DE CASTRO, C. S. S.; BOERE, V. Hematology and serum biochemistry of free-range brown-throated sloths in two urban areas in Brazil. **Veterinary World**, v. 13, n. 9, p. 2033, 2020.

PIERRE, R. V. Peripheral blood film review. The demise of the eyecount leukocyte differential. **Clinics in Laboratory Medicine**, v. 22, n. 1, p. 279–297, 2002.
PINTO, C. J. C.; GRISARD, E. C.; ISHIDA, M.M.I. **Parasitologia**. Florianópolis: CCB/EAD/UFSC, 2011.

PINTO, C. M.; HELGEN, K. M.; FLEISCHER, R. C.; PERKINS, S. L. Hepatozoon parasites (Apicomplexa: Adeleorina) in bats. **The Journal of parasitology**, v. 99, n. 4, p. 722-724, 2013.

QUEIROZ, C.; RODRIGUES, J. L.; BOMFETI, C. A.; GONÇALVES, A. M. P. M. Hematologia de preguiças de três dedos *Bradypus variegatus* (Bradypodidae, Xenarthra) da praça Tiradentes. **Rev. Vozes Vales**, v. 4, p. 1-11, 2015.

QUEIROZ, H. L. **Preguiças e Guaribas: os Mamíferos Folívoros Arborícolas do** Mamirauá. 2 ed. Rio de Janeiro: CNPq e Sociedade Civil Mamirauá, 1995.

RAMOS, F. F. **Perfil hematimétrico e identificação da hemoglobina do bicho preguiça *Bradypus variegatus***. 2006. Dissertação (Mestrado em Ciências Biológicas) - Universidade Federal de Pernambuco, Recife, 2006.

REGNERY, R. L.; SPRUIL, C. L.; PLIKAYTIS, B. D. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portion of two Rickettsial gene. **Journal of Bacteriology**, v. 173, p. 1576-1589, 1991.

REZENDE, C. L.; SCARANO, F. R.; ASSAD, E. D.; JOLY, C. A.; METZGER, J. P.; STRASSBURG, B. B. N.; TABARELLI, M.; FONSECA, G. A.; MITTERMEIER, R. A.

From hotspot to hopespot: An opportunity for the Brazilian Atlantic Forest. **Perspectives in Ecology and Conservation**, v. 16, n. 4, p. 208-214, 2018.

ROBINSON, L. E. **The Genus Amblyomma.-Ticks: A Monograph of the Ixodoidea, Fart IV**. Cambridge: Cambridge University Press, 1926.

ROSA, B. F. **Pequenos Mamíferos Não-voadores (didelphimorphia, Rodentia) em Remanescentes de Floresta Semidecidual e Pastagens do Centro-sul da Bahia**. 2020. Dissertação (Mestrado em Zoologia) - Universidade Estadual de Santa Cruz, Ilhéus, 2020.

SANCHES, G. S.; ANDRÉ, M. R.; PRADO, A. P.; ALLEGRETTI, S. M.; REMEDIO, R. N.; NUNES, P. H.; MACHADO, R. Z.; BECHARA, G. H.; CAMARGO-MATHIAS, M. I. Oocyte maturation in the sloth's giant tick Amblyomma varium (Acari: Ixodidae) in an ecological context. **Experimental and Applied Acarology**, v. 64, n. 4, p. 519-531, 2014.

SANTOS, P. M.; BAILEY, L. L.; RIBEIRO, M. C.; CHIARELLO, A. G.; PAGLIA, A. P. Living on the edge: Forest cover threshold effect on endangered maned sloth occurrence in Atlantic Forest. **Biological Conservation**, v. 240, 108264, 2019.

SCHETINO, M. A. A.; COIMBRA, R. T. F.; SANTOS, F. R. Time scaled phylogeography and demography of Bradypus torquatus (Pilosa: Bradypodidae). **Global Ecology and Conservation**, v. 11, p. 224-235, 2017.

SEBASTIANI, M. C. **Detecção de hemopatógenos em Xenarthras de vida livre dos estados de Minas Gerais, Mato Grosso e Mato Grosso do Sul.** 2018. Monografia (Especialista - Residência em Medicina Veterinária) - Universidade Federal de Minas Gerais, Belo Horizonte, 2018.

SILVA, A. A. B. **Ixodofauna Em Fragmentos de Mata Atlântica no Estado da Bahia: Relação Parasito-hospedeiro e Detecção Molecular de Patógenos Associados**. 2017. Dissertação (Mestrado em Biotecnologia) - Universidade Federal da Bahia, Salvador, 2017.

SILVA, M. R. L.; FORNAZARI, F.; DEMONER, L. C.; TEIXEIRA, C. R.; LANGONI, H.; O'DWYER, L. H. *Didelphis albiventris* naturally infected with *Hepatozoon canis* in southeastern Brazil. **Ticks and Tick-borne Diseases**, v. 8, n. 6, p. 878-881, 2017.

SILVEIRA, J. A. G.; D'ELIA, M. L. Medicina da conservação: a ciência da saúde do ecossistema. *In*: Cadernos Técnicos de Veterinária e Zootecnia. [Belo Horizonte, Brasil], Fevereiro 2014. Site. Disponível em: https://vet.ufmg.br/ARQUIVOS/FCK/file/editora/caderno%20tecnico%2072%20epidemiologia%20e%20conservavao%20animais%20silvestres.pdf. Acesso em: 31 de mai. 2022.

SMITH, L. H.; RUPLE, A. Idly infected: a review of infectious agents in populations of two- and three-toed sloths (choloepus species and bradypus species). **Journal of Zoo and Wildlife Medicine**, v. 51, n. 4, p. 789-798, 2021.

SOARES, H. S.; MARCILI, A; BARBIERI, A. R.; MINERVINO, A. H.; MALHEIROS, A. F.; GENNARI, S. M.; LABRUNA, M. B. Novel Anaplasma and Ehrlichia organisms infecting the wildlife of two regions of the Brazilian Amazon. **Acta tropica**, v. 174, p. 82-87, 2017.

SPRINGER, A.; FICHTEL, C.; CALVIGNAC-SPENCER, S.; LEENDERTZ, F. H.; KAPPELER, P. M. Hemoparasites in a wild primate: Infection patterns suggest interaction of Plasmodium and Babesia in a lemur species. **International Journal for Parasitology: Parasites and Wildlife**, v. 4, n. 3, p. 385-395, 2015.

TABARELLI, M.; PINTO, L. P.; SILVA, J. M. C.; HIROTA, M. M.; BEDE, L. C. Desafios e oportunidades para a conservação da biodiversidade na Mata Atlântica brasileira. **Megadiversidade**, v. 1, n. 1, p. 132-138, 2005.

THRALL, M. A.; WEISER, G.; ALLISON, R.; CAMPBELL, T. W. Hematology of common non domestic mammals. **Veterinary hematology and clinical chemistry**, v. 2, p. 221-224, 2004.

TSCHÁ, M. C.; ANDRADE, G. P.; ALBUQUERQUE, P. V.; TSCHÁ, A. R.; DIMECH, G. S.; SILVA, C. J. F. L.; FARIAS, E. T. N.; AMORIM, M. J. A. A. L. Perfil hematológico e bioquímico da preguiça-de-garganta-marrom *Bradypus variegatus*, Schinz 1825, em cativeiro alimentando-se de embaúba Cecropia pachystachya Trécul 1847. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 73, p. 877-884, 2021.

VAZ, S. M. A localidade tipo da preguiça-de-coleira, *Bradypus torquatus* Illiger, 1811 (Xenarthra, Bradypodidae). **Edentata**, v. 5, p. 1-4, 2003.

VOGEL, I.; VIÉ, J. C.; DE THOISY, B.; MOREAU, B. Hematological and serum chemistry profiles of free-ranging southern two-toed sloths in French Guiana. **Journal of Wildlife Diseases**, v. 35, n. 3, p. 531-535, 1999.

VOIRIN, B.; KAYS, R.; WIKELSKI, M.; LOWMAN, M. Why do sloths poop on the ground?. *In*: DEVY, S.; GANESH, T.; LOWMAN, M. **Treetops at Risk Challenges Of Global Canopy Ecology And Conservation**. New York: Springer, 2013.

VOLOKHOV, D. V.; BECKER, D. J.; BERGNER, L. M.; CAMUS, M. S.; ORTON, R. J.; CHIZHIKOV, V. E.; ALTIZER, S. M.; STREICKER, D. G. Novel hemotropic mycoplasmas are widespread and genetically diverse in vampire bats. **Epidemiology & Infection**, v. 145, n. 15, p. 3154-3167, 2017.

WARNER, R. E. The role of introduced diseases in the extinction of the endemic Hawaiian aviafauna. **The Condor**, v. 70, n. 2, p. 101–120, 1968.

WETZEL, R. M.; AVILA-PIRES, F. D. Identification and distribution of the recent sloths of Brazil (Edentata). **Revista Brasileira de Biologia**, v. 40, n. 4, p. 831-836, 1980.

WITTER R.; MARTINS T. F.; CAMPOS A. K.; MELO, A. L. T.; CORREA, S. H. R.; MORGADO, T. O.; WOLF, R. W.; MAY-JÚNIOR, J. A.; SINKOC, A. L.; STRUSSMANN, C.; AGUIAR, D. M.; ROSSI, R. V.; SEMEDO, T. B. F.; CAMPOS, Z.; DESBIEZ, A. L. J.; LABRUNA, M. B.; PACHECO, R. C. Rickettsial infection in ticks (Acari: Ixodidae) of wild animals in midwestern Brazil. **Ticks and Tick-borne Diseases**, v. 7, n. 3, p. 415-423, 2016.

WOODROFFE, R. Managing disease threats to wild mammals. **Animal conservation**, v. 2, n. 3, p. 185-193, 1999.

XAVIER, G. A. A. **Aspectos clínicos e de manejo de preguiça-de-garganta-marrom Bradypus variegatus (Schinz, 1825) de vida livre na Mesorregião Metropolitana do Recife, Pernambuco, Brasil**. 2006. Tese (Doutorado em Ciência Veterinária) - Universidade Federal Rural de Pernambuco, Recife, 2006.