BÁRBARA CRISTINA FÉLIX NOGUEIRA

ASPECTOS DO PARASITISMO POR CARRAPATOS: HOSPEDEIROS DIVERSOS, PATÓGENOS ASSOCIADOS, ALTERAÇÕES HISTOPATOLÓGICAS E OXIDATIVAS NO LOCAL DE FIXAÇÃO

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Medicina Veterinária, para obtenção do título de *Doctor Scientiae*.

Orientador: Artur Kanadani Campos
Coorientador: Thiago Fernandes Martins

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Autora

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Artur Kanadani Campos
Orientador

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RESUMO

NOGUEIRA, Bárbara Cristina Félix, D.Sc., Universidade Federal de Viçosa, fevereiro 2023. **Aspectos do parasitismo por carrapatos: hospedeiros diversos, patógenos associados, alterações histopatológicas e oxidativas no local de fixação**. Orientador: Artur Kanadani Campos. Coorientador: Thiago Fernandes Martins.

Os carrapatos apresentam uma infinidade de hospedeiros, no entanto a maioria dos estudos para desvendar as relações desse parasitismo se concentra em hospedeiros primários e de importância econômica. Mesmo assim, alguns aspectos ainda permanecem pouco abordados tanto para estes hospedeiros como para hospedeiros secundários e acidentais. Diante do exposto, esta tese foi organizada em três capítulos em formato de artigo científico. No primeiro capítulo é apresentada uma avaliação das alterações histopatológicas e as respostas do perfil oxidativo de equinos no local de fixação de *A. sculptum*. Nele foi observado aumento da celularidade, infiltrado inflamatório, mastócitos, núcleos picnóticos e alterações nos componentes fibrosos da matriz, o nível da enzima antioxidante SOD foi maior em Breton Postier, o que pode significar que esses animais tiveram maior atividade enzimática e, consequentemente, menos dano tecidual, enquanto a GST caiu nos locais de fixação em comparação ao controle, o que pode indicar que os animais estavam em estado de estresse oxidativo significativo ou a possível ocorrência de sequestro enzimático por carrapatos, além disso, foi observada correlação negativa entre a infestação por carrapatos e a resposta inflamatória indicando que animais com maior resposta inflamatória tendem a ter menos infestação por carrapatos. No segundo capítulo foi feita uma avaliação da diversidade de ixodídeos e detecção Rickettsia spp. em carrapatos que parasitam aves no bioma Mata Atlântica. Nele foram capturadas 773 aves, das quais 130 estavam parasitadas por carrapatos 479 carrapatos das espécies *Amblyomma longirostre*, *Amblyomma calcaratum*, *Amblyomma varium*, *Amblyomma sculptum* e larvas de *Amblyomma* spp., foi observada distribuição sazonal dos estágios de vida ao longo do ano e foram encontradas correlações negativas significativas entre temperatura e carrapatos e temperatura e aves infestadas. Foram positivas para Rickettsia spp. 43 amostras de carrapatos e a análise de sequência indicou alta identidade de nucleotídeos com *Rickettsia rhipicephali*, *R. massiliae*, *R. africae* e *R. honei marmionii*, o que chamou a atenção devido ao potencial de dispersão de carrapatos pelas aves somado à agressividade espécies do gênero *Amblyomma* e o potencial zoonótico de algumas espécies de *Rickettsia*. No terceiro capítulo foi realizado um compilado de dados publicados que relatam o parasitismo de carrapatos em humanos nos diferentes biomas do Brasil e sua relação com as alterações ambientais. Foram incluídos estudos publicados entre 1909 e 2022, que apresentavam nove espécies da família Argasidae e 32 espécies da família Ixodidae parasitando humanos nos seis biomas do território brasileiro. A espécie com maior número de registros de parasitismo humano foi *Amblyomma sculptum*, seguido por *Amblyomma coelebs*, *Amblyomma cajennense* sensu stricto e *Amblyomma brasiliense*. Além disso foram encontrados registros para *Amblyomma ovale*, vetor da *Rickettsia parkeri* no país, e *A. sculptum* e *A. aureolatum* que são as principais espécies que atuam como vetores de *Rickettsia rickettsii*, o que é bastante preocupante considerando que a ampla distribuição das espécies e fases de vida mais freqüentemente mencionados no parasitismo (ou seja, ninfas e adultos) são os que favorecem a transmissão do patógeno.

Palavras-chave: Doenças transmitidas por carrapatos. Ixodidae. Argasidae. parasitologia.

ABSTRACT

NOGUEIRA, Bárbara Cristina Félix, D.Sc., Universidade Federal de Viçosa, February, 2022. **Aspects of tick parasitism: diverse hosts, associated pathogens, histopathological and oxidative changes at the site of attachment**. Adviser: Artur Kanadani Campos. Co-adviser: Thiago Fernandes Martins.

Ticks have a wide hos rang; however, most studies to unravel the relationships of this parasitism focus on primary hosts and on those with economic importance. Nevertheless, some aspects still remain little addressed both for these hosts and for secondary and accidental hosts. Under the above circunstances, this thesis was organized into three chapters in the format of scientific articles. In the first chapter, an evaluation of the histopathological changes and responses of the oxidative profile of horses at the site of *Amblyomma sculptum* fixation is presented. An increase in cellularity, inflammatory infiltrate, mast cells, pyknotic nuclei and alterations in the fibrous components of the matrix were observed, the level of the antioxidant enzyme SOD was higher in Breton Postier, which may indicate that these animals had greater enzymatic activity and, consequently, less tissue damage, while GST dropped in the fixation sites compared to the control, which suggests that the animals were in a state of significant oxidative stress or the possible occurrence of enzymatic sequestration by ticks. In addition, a negative correlation was observed between infestation by ticks and the inflammatory response indicating that animals with a greater inflammatory response tend to have less tick infestation. In the second chapter, we performed an evaluation of the diversity of ixodids and detection of *Rickettsia* spp. in ticks that parasitize birds in the Atlantic Forest biome. For this, 773 birds were captured, of which 130 were parasitized by ticks (479 ticks of the species *Amblyomma longirostre*, *Amblyomma calcaratum*, *Amblyomma varium*, *Amblyomma sculptum* and larvae of *Amblyomma* spp.). A seasonal distribution of life stages was observed throughout the year and were found significantly negative correlations between temperature and ticks and temperature and infested birds. They were positive for *Rickettsia* spp. (43 tick samples) and the sequence analysis indicated a high nucleotide identity with *Rickettsia rhipicephali*, *R. massiliae*, *R. africae* and *R. honei marmionii*, which drew attention due to the potential for tick dispersion by birds added to the aggressiveness of the tick species of the genus *Amblyomma* and the zoonotic potential of some species of *Rickettsia*. In the third chapter, a compilation of published data was carried out about the parasitism of ticks on humans in the different biomes of Brazil and its relationship with environmental changes. Studies published between 1909 and 2022 were included, which presented nine species of the Argasidae family and 32 species of the Ixodidae family parasitizing humans in the six biomes of the Brazilian territory. The species with the highest number of records of human parasitism was *Amblyomma sculptum*, followed by *Amblyomma coelebs*, *Amblyomma cajennense* sensu stricto and *Amblyomma brasiliense*. In addition, records were found for *Amblyomma ovale*, the vector of *Rickettsia parkeri* in the country, and *A. sculptum* and *A. aureolatum*, which are the main species that act as vectors of *Rickettsia rickettsii*, which is quite worrying considering that the wide distribution of species and the most frequently mentioned life stages in parasitism (i.e., nymphs and adults) are those that favor the transmission of the pathogen.

Keywords: Tick-borne diseases. Ixodidae. Argasidae. parasitology.

SUMÁRIO

1. INTRODUÇÃO GERAL

O Brasil apresenta 76 espécies de carrapatos das quais 51 pertencem à família Ixodidae e 25 à família Argasidae (MARTINS et al., 2021; MUÑOZ-LEAL et al., 2020; MUÑOZ-LEAL et al., 2021; PACHECO ET AL., 2021). A família Ixodidae se destaca no conceito de saúde única, pois seus carrapatos apresentam amplo leque de hospedeiros e atuam como vetores de patógenos que causam doenças em animais e humanos, como bactérias, vírus, protozoários e helmintos (KRČMAR, 2019).

Entre os ixodídeos, o gênero *Amblyomma* é o mais importante para a saúde pública no Brasil (HIGA et al., 2020). Este gênero abriga o complexo *Amblyomma cajennense* que foi originado a partir de estudos aprofundados dos carrapatos anteriormente identificados apenas como *Amblyomma cajennense* e resultou na divisão destes carrapatos em seis espécies distintas, das quais apenas duas ocorrem no Brasil, *A. cajennense* no bioma Amazônia e *A. sculptum* que se distribui por Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Pernambuco, Piauí, Mato Grosso, Mato Grosso do Sul e Goiás (NAVA et al., 2014; MARTINS et al., 2016), com exceção do interior do bioma Mata Atlântica e áreas degradadas (SZABÓ et al., 2009; MARTINS et al., 2016).

Os carrapatos da espécie *A. sculptum* são conhecidos por atuarem como vetores de diversos patógenos, com destaque para *Rickettsia rickettsii* que é o agente causador da febre maculosa e o patógeno mais letal transmitido por carrapatos para humanos no Brasil (ESTEVES et al., 2019). Entre os hospedeiros que possibilitam o estabelecimento desta espécie de carrapato estão mamíferos, principalmente equídeos, antas, capivaras e porcos (LABRUNA et al., 2004; MARTINS et al., 2016).

Alguns hospedeiros oferecem condições mais favoráveis ao desenvolvimento dos carrapatos e devido a isso permitem que os carrapatos completem seu ciclo biológico com facilidade, entretanto, mesmo em hospedeiros adequados, a viabilidade deste ciclo pode ser prejudicada devido a alimentação em animais resistentes e/ou expostos a infestações sucessivas (LOULY et al., 2009). Este prejuízo para os carrapatos pode ser observado na redução das fases do ciclo biológico, maior tempo de ingurgitamento, menor taxa de conversão alimentar, menor quantidade de fêmeas ovipondo, menor quantidade de ovos e eclodibilidade larval (LOULY et al., 2009).

O aparelho bucal e a saliva dos carrapatos, que é composta por diversas moléculas biologicamente ativas, possibilitam os processos de fixação na pele do hospedeiro, hematofagia e transmissão de patógenos (ESTEVES et al., 2019). Estes processos prejudicam a hemostasia e provocam feridas cutâneas caracterizadas por alterações histopatológicas próximas ao local de fixação do carrapato (ENGRACIA FILHO et al., 2017). Consequentemente, estas alterações podem desencadear a ocorrência de estresse oxidativo, visto que células inflamatórias liberam espécies reativas de oxigênio na área inflamada e estas podem sinalizar a expressão gênica pró-inflamatória (BISWAS, 2016). Apesar disso, a saliva dos carrapatos apresenta atividade sobre moléculas imunorreguladoras, como a redução de citocinas e fatores de crescimento com ação pró-inflamatória e aumento das moléculas com ação anti-inflamatória (KOTÁL et al., 2015). Estas moléculas apresentam importante papel durante o processo de reparo cutâneo (BARRIENTOS et al., 2008) e são reguladas a fim de manter um ambiente favorável para a permanência do carrapato (KOTÁL et al., 2015).

Apesar do conhecimento já existente acerca das relações dos carrapatos com seus hospedeiros primários, existem poucos estudos que abordem as alterações histopatológicas e oxidativas ocasionadas pelo parasitismo, assim como o parasitismo em hospedeiros secundários e em humanos, principalmente quando consideramos espécies que ocorrem no Brasil. Diante do exposto, esta tese foi organizada em três capítulos em formato de artigo científico. No primeiro capítulo é apresentada uma avaliação das alterações histopatológicas e as respostas do perfil oxidativo de equinos no local de fixação de *A. sculptum*. O segundo capítulo aborda uma avaliação da diversidade de ixodídeos e detecção *Rickettsia* spp. em carrapatos que parasitam aves no bioma Mata Atlântica. Enquanto o terceiro capítulo engloba um compilado de dados publicados que relatam o parasitismo de carrapatos em humanos nos diferentes biomas do Brasil e sua relação com as alterações ambientais.

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2. CAPÍTULO 1:

Oxidative and local histopathological response on skin wound of horses due to *Amblyomma sculptum* tick parasitism

Bárbara Cristina Félix Nogueira[a], Artur Kanadani Campos[a], Raul Santos Alves[b], Rita de Cássia Vieira Faria[a], Mariáurea Matias Sarandy[b], Fabyano Fonseca e Silva[c], Reggiani Vilela Gonçalves[b,d]

[a]Department of Veterinary Medicine, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

[b]Department of General Biology, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

[c]Department of Animal Science, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

[d]Department of Animal Biology, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

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Abstract

Amblyomma sculptum is frequently observed parasitizing horses, responsible for economic losses, damage to the host's skin and transmission of pathogens. The oxidative stress profile and inflammatory mechanisms involved in this parasitism remain poorly studied. Thus, this study aimed to assess the histopathological changes and oxidative profile responses of horses in the attachment site of *A. sculptum* to find variations that indicate resistance and susceptibility between the breeds to this tick, based on the hypothesis that resistant animals have a greater inflammatory response and lesser number of attached ticks. We analysed female horses of two breeds, Mangalarga Marchador and Breton Postier, naturally infested by *Amblyomma sculptum*. The ticks were counted and full-thickness excisional skin wounds of 10 mm were made on the perineal region on the attachment site of partially engorged females for histological and biochemical analyses. The occurrence of the tick on the skin caused an increase in cellularity, inflammatory infiltrate, mast cells, pyknotic nuclei, and changes in the fibrous components of the matrix. The negative correlation observed between tick infestation and inflammatory response indicated that animals with greater inflammatory response tend to have less tick infestation. The oxidative stress markers, MDA, PCN and NO not present great variation; however, between the antioxidant enzymes levels, SOD was higher in tick attachment of Breton Postier skin, this may mean that these animals had higher oxidative enzymatic activity and consequently less tissue damage, while the GST dropped in the attachment sites compared to the control, which may indicate that animals were in a state of significant oxidative stress or raises the question of the possibility of enzymatic sequestration by ticks. No significant differences were found in the resistance of the two breeds since most of the analyses varied due to the presence or absence of the tick attached to the skin. We draw attention to the importance of studying characteristics of the animal's antioxidant responses to the tick and the action of tick saliva on antioxidant enzymes and ROS because these characteristics are interdependent with the inflammatory response.

Keywords: Inflammation; Oxidative stress; Resistance; Susceptibility; Ticks.

2.1. Introduction

Horses stand out as parasite hosts due to their potential to sustain intense parasite infestations and the high population density systems they are raised (Vieira et al., 2002). Among the important ectoparasites for equideoculture we can highlight *Amblyomma sculptum*, which frequently is found in horses (Keirans, 1992; Molento, 2005; Oliveira and Borges, 2011). This tick species act as the most important vector of pathogens such as *Rickettsia rickettsii* (Esteves et al., 2019), *Theileria equi* (Scoles and Ueti, 2013), and *Babesia caballi* (Sousa et al., 2018), which may cause injuries to the endothelial tissue and in the circulatory, digestive, nervous, urinary and respiratory systems, affecting development and leading to the death of the host (Fiol et al., 2010; Onyiche et al., 2019; Vieira et al., 2002). In addition, some humans and animals when infected with *R. rickettssii* have a rash with maculopapules on the skin (Fiol et al., 2010).

The harmful effects of this tick species occur due to the presence of salivary molecules and mechanisms of evasion of the host immune response represented by anti-inflammatory, immunosuppressive, antihemostatic, anti-angiogenic, and vasodilators molecules (Batista et al., 2010; Carvalho-Costa et al., 2015; Esteves et al., 2017; Moreira et al., 2017; Sá Junior et al., 2019). Therefore, the long-term attachment of ixodid ticks causes skin injuries characterized by inflammation, degeneration and necrosis (Allen et al., 1977; Engracia Filho et al., 2017; Gill, 1986; Gill and Walker, 1985; Latif et al., 1991; Schleger et al., 1976; Szabó and Bechara, 1995; Walker and Fletcher, 1986). These injuries are classified as primary lesions, which occur by the permanence of the tick's mouthparts with the release of salivary substances, and secondary lesions as a consequence of infections by pathogens (Haddad Jr. et al., 2018).

The skin repair process is divided into four stages: hemostasis, inflammation, proliferation, and remodeling (Oryan et al., 2016). The inflammatory phase is characterized by the presence of cells that release cytokines and growth factors that are responsible for mediating cell migration and differentiation in the proliferative phase (Landén et al., 2016). In vivo studies on the analysis of *A. sculptum* saliva in skin repair are not available. Thus, it is important to evaluate cell and fibrous components of the extracellular matrix analyses to understand the skin repair process since the stages of this process are affected by the tick's saliva properties (Wikel, 2013). In addition, studies addressing the oxidative action of tick saliva on the skin are scant. In this context, these analyses are important to quantify the release of reactive oxygen species (ROS) and to assess the body’s response through detoxification of antioxidant defense mechanism (Soomro, 2019).

Although it is known that tick species are responsible for large damages on the skin of the host and consequently also to great economic losses, the oxidative stress profile and consequently the histopathological changes involved in this type of parasitism is not yet known. Thus, this study aimed to assess the histopathological changes and oxidative profile responses of horses in the attachment site of *A. sculptum* to find variations that indicate resistance and susceptibility between the breeds to this tick, based on the hypothesis that resistant animals have a greater inflammatory response and lesser number of attached ticks.

2.2. Materials and methods

2.2.1. Study area

The study was carried out in the city of Viçosa (20◦45′14″ S, 42◦52′54″W), Minas Gerais, Brazil, where the climate is classified as monsoon-influenced humid subtropical climate (Köppen climate classification Cwa), mesothermic, with hot and rainy summers and cold and dry winters. The area is 650 m above sea level and presents an annual average temperature varying from 20 to 22 ◦C.

2.2.2. Study animals

A total of 12 *Equus caballus* females sensitized to ticks were randomly selected by a blinded collaborator of Teaching, Research, and Extension Unit in Equideoculture of the Federal University of Viçosa, Viçosa, Minas Gerais, Brazil, which is an endemic region for *A. sculptum* ticks. The animals were of two different breeds, being 6 Mangalarga Marchador (MM) and 6 Breton Postier (B), with approximately 450 kg and an average age of 6 years old. The animals were allocated on a pasture of *Cynodon* spp. and *Cynodon dactylon* to natural tick infestation and fed on ration composed of corn and soybean meal, mineral salt, and water *ad libitum*.

2.2.3. Clinical analyses for the detection of associated pathogens

Blood samples of the animals were collected by puncture of the jugular vein for posterior blood smear, complete blood count (CBC), and biochemical tests to ascertain the health of animals based on the parameters established by Weiss and Wardrop (2010).

The DNA of blood was extracted using a commercial kit for blood (Illustra tissue and cells genomic Prep Mini Spin Kit, GE Healthcare Life Sciences) following the manufacturer's instructions. The polymerase chain reaction (PCR) was performed on blood samples for the detection of tick-borne pathogens. For this, genomic DNA was screened for the presence of *Rickettsia* spp. with primers (5'GGGGGCCTGCTCACGGCGG3'and 5'ATTGCAAAAAGTACAGTGAACA3') that amplify from 300 to 380 bp product of the gltA gene (Regnery et al., 1991); and for *Babesia* and *Theileria* spp. with Primers (5'GTCTTGTAATTGGAATGATGG3 'and 5'TAGTTTATGGTTAGGACTACG3') that amplify a product from 411 to 452 bp of the 18S gene (Casati et al., 2006). In addition, parasitological examination with fecal egg counting was performed according to Gordon and Whitlock (1939).

2.2.4. Experimental design

The animals were preventively treated to ticks and helminths with moxidectin/ praziquantel oral gel, cypermethrin pour on and spraying with deltamethrin and were followed up daily for 55 days considering: 1) safety interval of the approximately 7 to 28 days (Pereira et al., 2008), 2) verification of the presence of ticks from the new infestation, 3) selection of the biopsy site based on the monitoring of the engorgement of adult females of *A. sculptum* which is approximately 7 to 10 days. All the experiments were approved by the Ethics Committee for the use of Production Animals of the Federal University of Viçosa (License number: 88/2019).

2.2.5. Excisional wounding procedures

After the 55-day period, the animals were locally anesthetized with 10% lidocaine and after disinfection with chlorhexidine. Two full thickness excisional skin wounds were generated by a veterinarian responsible for biopsies on the perineal region of each animal with a 10 mm sterile punch, one on the place of attachment of partially engorged *A. sculptum* females after 6 days of attachment, and other on the clean skin area in the same region. Afterward, the wounds were sutured and treated with antiseptic spray and repellents. The 10 mm skin samples were cut in half for histological and biochemical analysis. Thus, the Mangalarga Marchador group was identified as MM (fragment containing the attached tick) and its control (intact skin) as

MMc, and the Breton Postier group as B (fragment containing the attached tick) and its control (intact skin) as Bc.

2.2.6. Tick count on animal body, morphological identification and PCR analyses of ticks

The count of ticks was performed considering the left body antimere of the animals according to Wharton and Utech (1970) following the collection of skin fragments. The confirmation of the tick species attached in the collected skin fragments was performed in a stereoscopic microscope using the identification key described by Barros-Battesti et al. (2006), Nava et al. (2014) and Martins et al. (2016). In addition, PCR analysis can detect other tick-borne pathogens that could influence histological results. For this, tick DNA was extracted according to Ramos et al. (2015). PCR analysis was performed for *Rickettsia*, *Babesia*, and *Theileria* spp. using the primers previously described (Casati et al., 2006; Regnery et al., 1991).

2.2.7. Histological analysis

The samples collected from the wounds were fixed in formaldehyde solution buffered in 0.1 M sodium phosphate (pH 7.2) for 24 h, dehydrated in ethyl alcohol, diaphanized in xylol, and immersed in paraffin. Histological sections (4 μm) were obtained on a microtome (Leica Multicut® 2045, Reichert-Jung Products, Germany). We used 1 of every 10 sections to avoid repeating the analysis of the same histological area. These sections were mounted on a histological slide and stained with Hematoxylin and Eosin for the analysis of cellularity and blood vessels (Gonçalves et al., 2010a), Sirius red was used to differentiate collagen fibers under polarizing microscopy (Sigma, St. Louis, Mo, USA) (Dolber and Spach, 1993; Novaes et al., 2015) and Toluidine blue for evidence of mast cells (Junqueira and Carneiro, 2005). The slides were visualized and captured in a BX601 light microscope (Olympus, São Paulo, Brazil) coupled with a QColor-31 digital camera (Olympus, São Paulo, Brazil). Six images were selected using a 20 objective lens around the tick attachment site and in areas next of the skin appendix. The control slides (intact skin) were analysed in each histological section. Two blinded evaluators scanned images with a grid of 400 intersections associated with the Image Pro-plus 4.5 (Media Cybernetcs1, Silver Spring, USA). Each image represents a total area of 1.53 × 106 μm^2 was submitted to stereological analysis. The stereological parameters of volumetric density (Vv) were calculated by counting the points that occurred over cells, blood vessels, inflammatory infiltrate, nuclear pyknosis, type III and type I collagen fibers using the ratio: Vv = (PP/PT) × 100, where PP is the number of points occurring over the structure of interest and PT is the total number of grid points (Gonçalves et al., 2010a; Novaes et al., 2014). Collagen fibers were analysed according to the differential properties of birefringence since thick collagen fibers (type I) appear in shades of bright colors ranging from red to yellow whereas thin reticular fibers (type III collagen fibers) appear bright green under polarization (Cupertino et al., 2013; Gonçalves et al., 2010a). The thickness of collagen fibers (type I) was measured. The collagen maturation index (CMI) was calculated using the ratio: % collagen I/ % collagen III, where indexes close to or over 1 (CMI ≥ 1) indicating a higher proportion of type I collagen and a greater level of maturation of the collagen fibers (Gonçalves et al., 2010b; Novaes et al., 2015).

The mast cell counts were made in BX-60® light microscope (Olympus, Tokyo, Japan) with a 40 objective lens to obtain a total area (TA) of 1.96 mm^2. The number of mast cells per unit of histological area was calculated according to the relation QA = Σ mast cells/TA (Mandarim-de-Lacerda, 2003).

2.2.8. Biochemical analysis

For biochemical analysis, tissue samples were immediately frozen in liquid nitrogen (- 196 ◦C) and stored in a freezer at - 80 ◦C. The samples were homogenized in phosphate-buffered saline (PBS), 100 mg sample and 1 mL PBS, and centrifuged for 5 min at 10000g (12,000 rpm) under refrigeration at 4 °C (Sarandy et al., 2018). Biochemical data were normalized according to the total protein levels in the supernatant (Bradford, 1976). Lipid peroxidation (LPO) was estimated according to the total malondialdehyde levels (MDA) (Buege and Aust, 1978). The concentration of MDA was determined by using the standard curve of known concentrations of 1, 1, 3, 3-tetramethoxypropane (TMPO). The results were expressed as μmol x L^{-1} per mg of protein. Protein carbonyl (PCN) content was measured using 2, 4-dinitrophenylhydrazine (DNPH) (Levine et al., 1994), based on the carbonyl group's reaction with DNPH. The pellets resulting from the tissue homogenates from previous extractions were used for quantification. The results were expressed as nmol per mL of protein. Nitric oxide (NO) was indirectly quantified through the detection of nitrite/nitrate (NO_{2^-} /NO_{3^-}) levels by the standard Griess reaction (Tsikas, 2007). 50 μL of supernatants were incubated with an equal volume of Griess reagent, (1%

sulfanilamide, 0,1% N-(1-Naftil) etilenodiamina e 2,5% H_3PO_4) and kept at room temperature for 10 min. The conversion of absorbance into micromolar concentrations of NO was obtained from a sodium nitrite standard curve (0–125 µM) and expressed as NO concentrations (µmol x L^{-1}). The activity of superoxide dismutase (SOD) was determined by the method of the reduction of the superoxide (O_2-) and hydrogen peroxide, thereby decreasing the auto-oxidation of pyrogallol (Dieterich et al., 2000). SOD activity was calculated as units per milligram of protein, with one unit (U) of SOD defined as the amount that inhibited the rate of pyrogallol autoxidation by 50%. The catalase (CAT) activity was evaluated according to the method described by (Aebi, 1984), through measuring the rate of decomposition of hydrogen peroxide. One unit of CAT activity was calculated using the amount of enzymes that decompose one mmol H2O2 for 1 min. The results were expressed as units of catalase/mg of protein. The glutathione S-transferase (GST) activity was measured using the method of Habig et al. (1974). Glutathione S-transferase activity was analysed according to the formation of glutathione conjugated 2,4- dinitrochlorobenzene (CDNB). One unit of GST activity was defined as the amount of enzyme that catalyzed the formation of one µmol of product/min/mL. GST activity was expressed as U per milligram of protein.

2.2.9. Statistical analysis

The histological and biochemical analyses were investigated under a factorial essay based on a completely randomized design with six replications. Two factors were applied, including breed (Mangalarga Marchador and Breton Postier) and tick (presence or absence). Analyses were carried out under a two-way ANOVA framework. ANOVA's normality assumption was evaluated through Shapiro-Wilk test. The parametric comparisons were performed based on Student's t-Test, since ANOVA factors from with one degree of freedom is to equivalent ANOVA-based F-test. Non-parametric analyses for non-normal variables were analysed by using the Mann-Whitney Test. The correlation between the results of cell and tick counts was performed using Pearson's correlation. A significance level of 5% was considered for all reported analyses.

2.3. Results

2.3.1. Clinical analyses for the detection of associated pathogens

Based on CBC results and biochemical tests there were no hematologic changes of relevance in the horses. Fecal tests were negative for the presence of endoparasites eggs. In addition, no evidence of pathogens was found in the blood smear and the PCR analyses.

2.3.2. Correlation between the cells count and ticks count

The number of ticks counted in the body of each animal varied from 16 to 752 and no significant differences was observed between the infestation on the two breeds ($p > 0.05$, $p = 0.24$). The correlation observed between the cells count at the tick attachment site and ticks count was significantly negative ($r = - 0.88$, $p < 0.05$, $p = 0.00075$).

2.3.3. Tick species and detection of associated pathogens

The morphological identification of the ticks confirmed that the ticks attached at the biopsy sites were of the species *Amblyomma sculptum*. The PCR analyses made with the DNA extracted from the ticks were negative for the pathogens analysed.

2.3.4. Histopathological results

Severe disruption of the skin due to the tick's attachment and the presence of the cementum cone that have eosinophilic characteristics, inflammatory infiltrate, and areas with the absence of nucleus, characterizing necrosis areas were observed in the dermis (Figure 1A) when compared with intact skin (Figure 1B). The location selected in the images for the cell count can be seen in the representative diagram (Figure 1C).

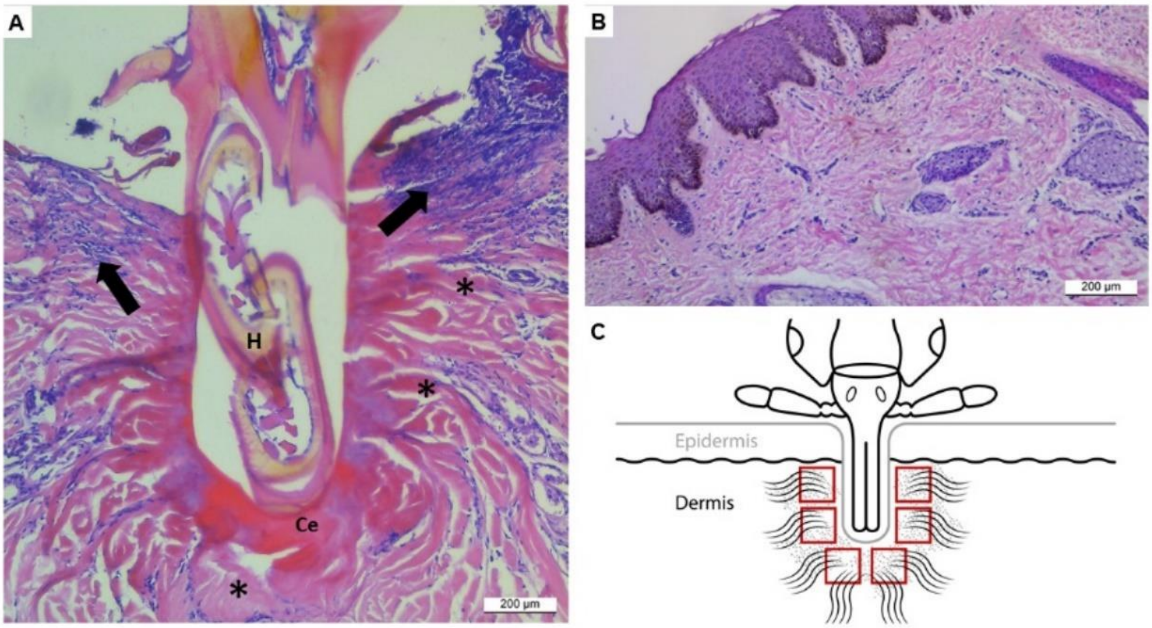

Figure 1. Photomicrographs of *Amblyomma sculptum* attachment site (A) and intact skin (B). Hematoxylin e Eosin staining. The cementum cone (Ce), hypostome (H), inflammatory infiltrate (arrows) and areas with an absence of nucleus (*) are shown (Objective lens: 10×). Diagram of tick attachment site at the skin of the host and the red squares indicating the location of the images for cell counting (C).

The proportion of cells (Figure 2A) and inflammatory infiltrate (Figure 2B) with the presence of the tick were significantly higher when compared to their respective intact skin (without tick). However, in relation to the proportion of blood vessels, no significant change was observed (Figure 2C). The distribution of cells, inflammatory infiltrate and blood vessels are shown in Figure 2 (respectively D, E, and F).

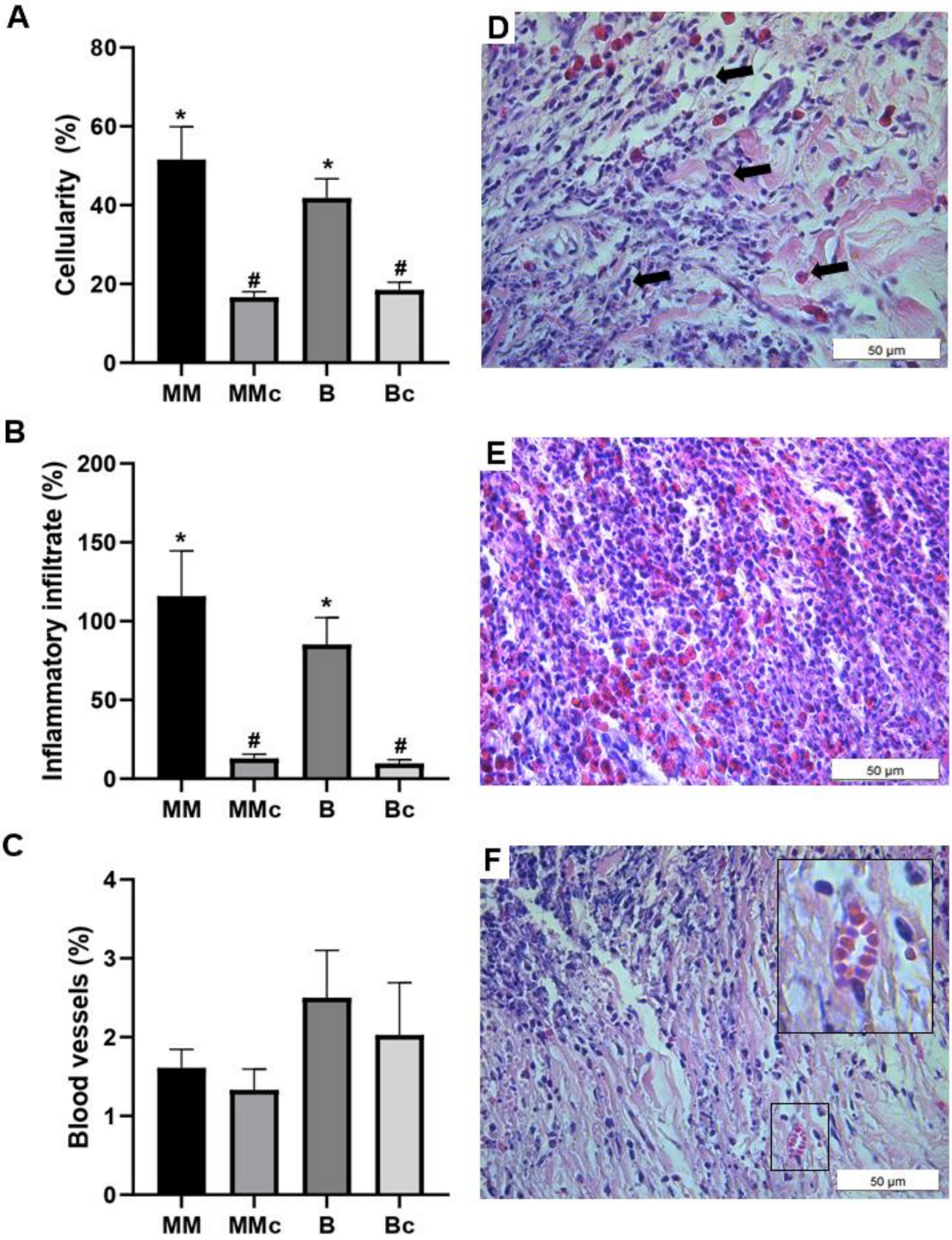

Figure 2. Percentual of cellularity (A), inflammatory infiltrate (B), and blood vessels (C). MM and B - *Amblyomma sculptum* attachment skin and MMc and Bc – skin intact tissue. Data represented as mean ± standard error of the mean. *, #p < 0.05, the statistical difference between treatments: MM and MMc; B and Bc (in cellularity and inflammatory infiltrate analyses). Representative photomicrographs showing cells (arrows) (D), inflammatory infiltrate in all its extension (E) and blood vessel in Hematoxylin and Eosin (Objective lens: 40×).

The number of mast cells (Figure 3A) and pyknotic nuclei (Figure 3B) was significantly higher in the skin with a tick, when compared to their respective control groups. The Photomicrograph of mast cells is represented in Figure 3C and pyknotic nuclei in Figure 3D.

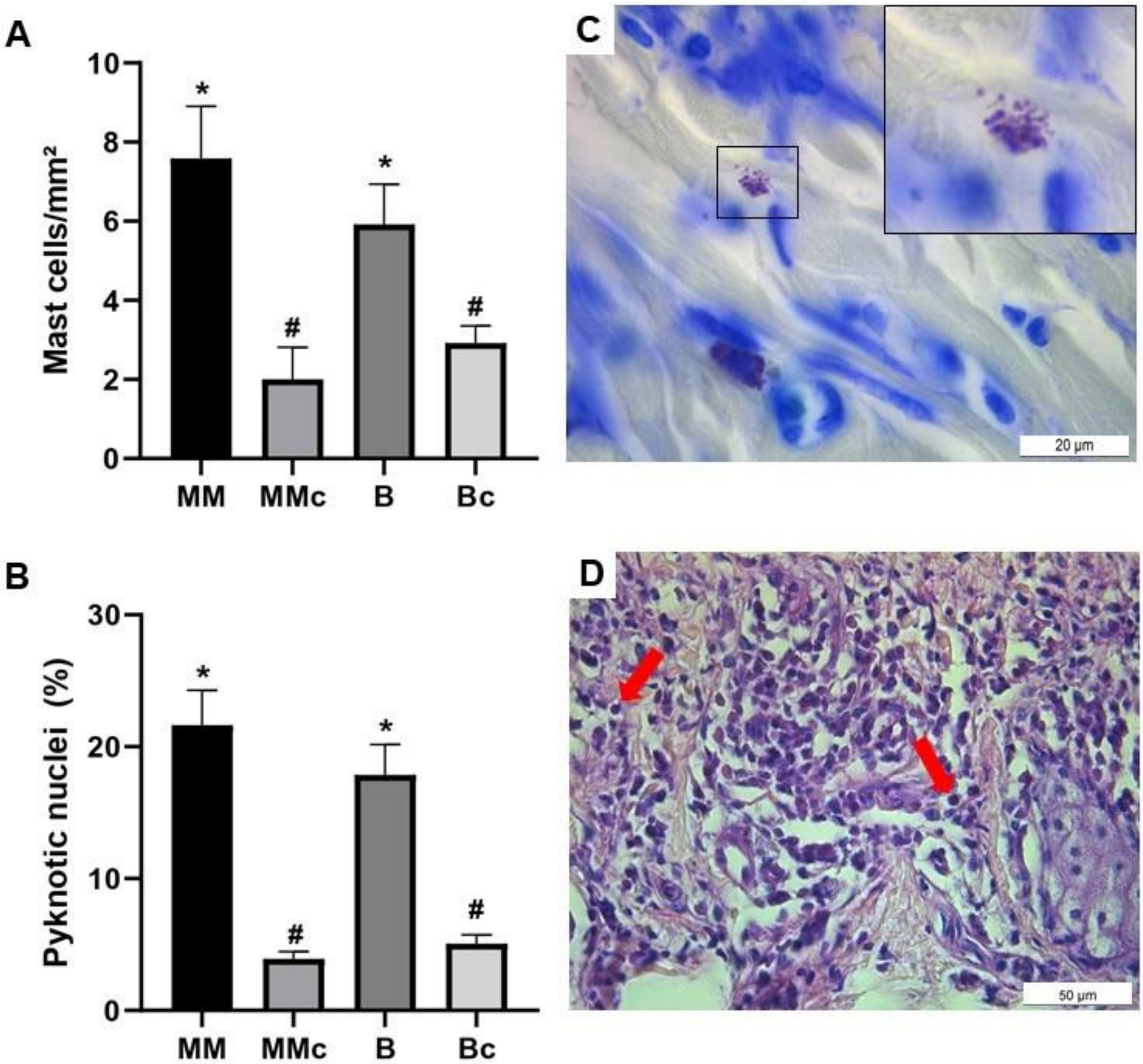

Figure 3. Percentual of mast cells (A) and pyknotic nuclei (B). MM and B - *Amblyomma sculptum* attachment skin and MMc and Bc - skin intact tissue. Data represented as mean ± standard error of the mean. *, #p < 0.05, the statistical difference between treatments: MM and MMc; B and Bc (in both analyses). B) Representative photomicrograph showing mast cells in toluidine blue (C) (Objective lens: 40× and 100×) and pyknotic nuclei (red arrows) in hematoxylin and eosin (D) (Objective lens: 40×).

The proportion of collagen fibers and collagen maturation varied with the presence of the tick. A higher proportion of type I collagen fibers were observed in the skin attachment *A. sculptum* tick of both breeds, when compared to the respective control groups, while type III collagen fibers only showed variation in Mangalarga Marchador, being higher in the control group (Figure 4A). The thickness of collagen fibers presents significant interaction between the breeds and the presence of the tick. The thickness of type I collagen fibers varied in the skin attachment *A. sculptum* tick when compared to the control of each breed and also when compared between breeds (Figure 4B). In addition, the CMI showed that collagen maturation was greater in the skin attachment *A. sculptum* tick of both breeds when compared to control groups (Figure 4C). The representative distribution of the type I and type III collagen fibers in the intact skin is shown in Figure 4D and tick attachment in Figure 4E. Figure 4F presents the photomicrograph under the grid mask used to collagen fibers.

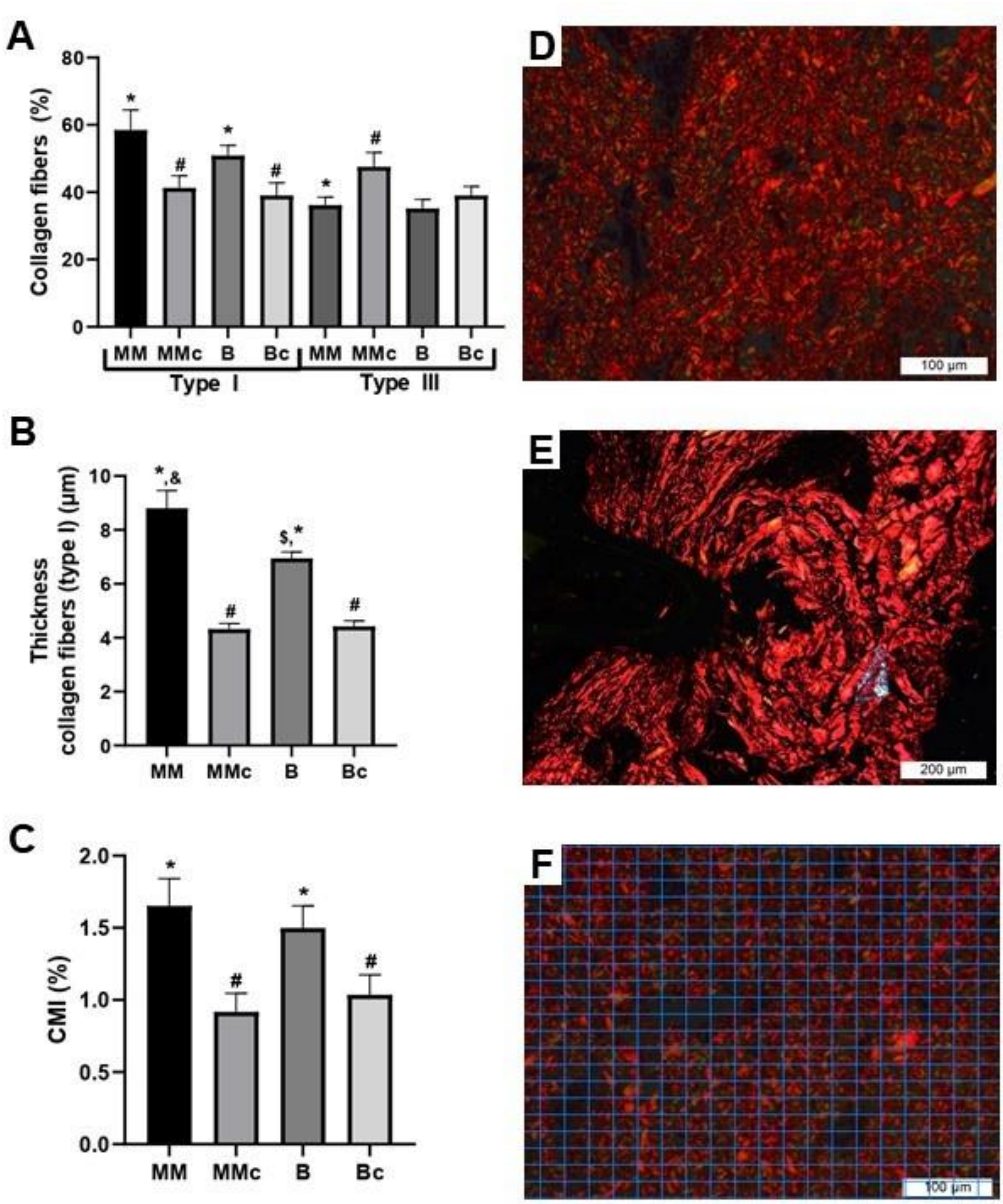

Figure 4. Percentual of collagen fibers I and III (A), the thickness of collagen fibers (type I) (B), and collagen maturation index (CMI) (C). MM and B – *Amblyomma sculptum* attachment skin and MMc and Bc - skin intact tissue. Data represented as mean ± standard error of the mean. *, #, &, $p < 0.05, the statistical difference between treatments: MM and MMc, B and Bc (in percentual of type I collagen fibers); MM and MMc (in percentual of type III collagen fibers); MM and MMc, B and Bc, MM and B (in thickness type I collagen fibers); MM and MMc, B and Bc (in CMI). Representative photomicrographs showing collagen fibers distribution in the intact skin (D) (Objective lens: 20×), tick attachment site (E) (Objective lens: 10×), and under the grid mask (F) (Objective lens: 20×) in Sirius red.

2.3.5. Biochemical results

2.3.5.1. Oxidative stress markers

The MDA presented a small increase in the control groups when compared with the tick attachment group but without significant difference (Figure 5A). The PCN values (Figure 5B) were close in all studied groups and higher NO values (Figure 5C) were found in the fragments that corresponded to the tick attachment site in both groups, however, these oxidative stress markers no showed significant differences.

2.3.5.2. Antioxidants enzymes

The antioxidant enzyme SOD was found significantly higher in the fragments with the tick of Breton Postier breed when compared with control (Figure 5D). The CAT no present significant changes between the groups analysed (Figure 5E). The GST showed higher values in the control groups with a significant difference when compared to both fragments that corresponded to the tick attachment site (Figure 5F).

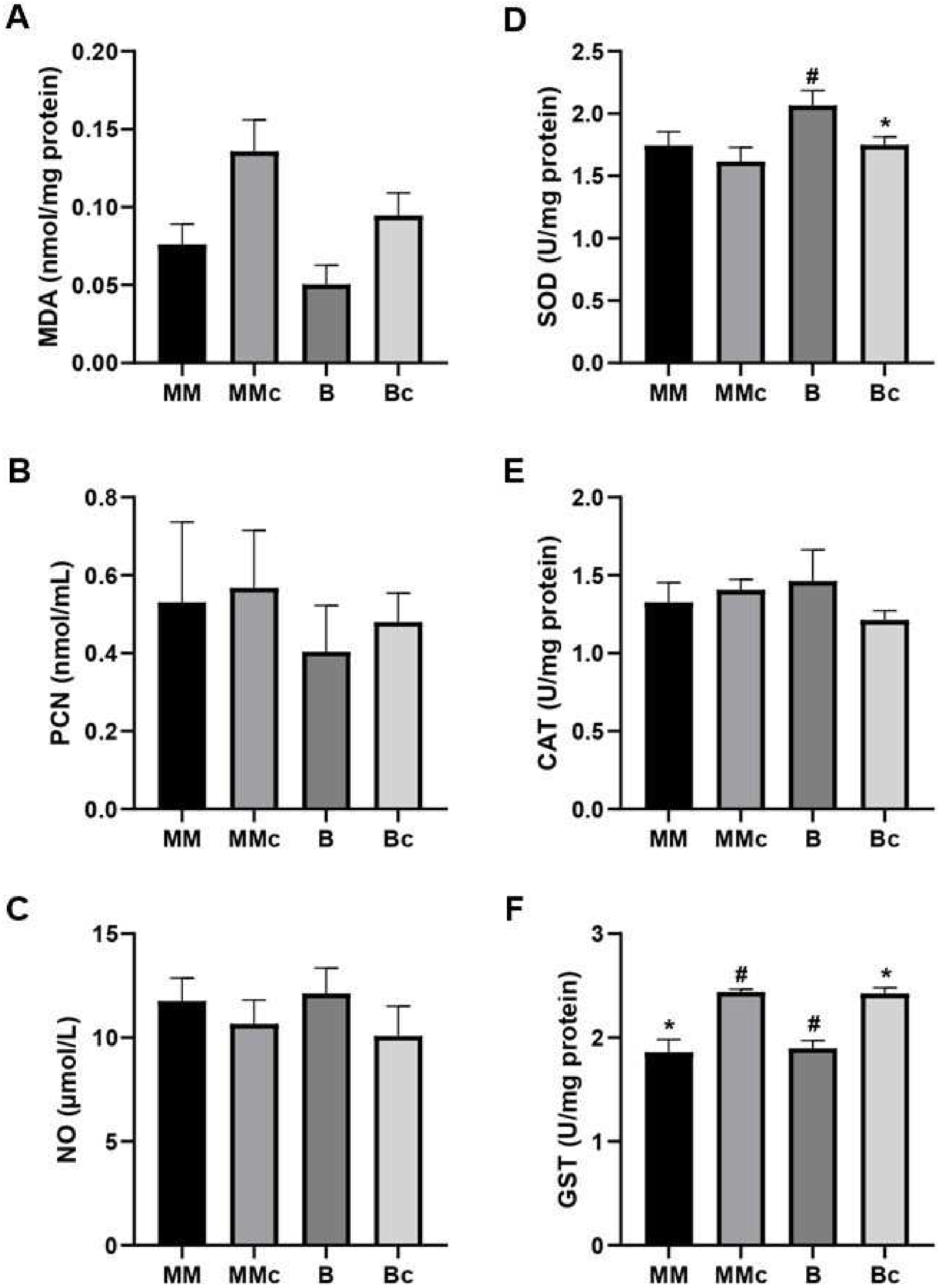

Figure 5. Levels of Malondialdehyde (MDA) (A), Carbonylated Proteins (PCN) (B), Nitric Oxide (NO) (C), Superoxide Dismutase (SOD) (D), Catalase (CAT) (E), and Glutathione S-Transferase (GST) (F). MM and B - *Amblyomma sculptum* attachment skin and MMc and Bc - skin intact tissue and MM. Data represented as mean ± standard deviation. *, #$p < 0.05$, the statistical difference between treatments: B and Bc (in SOD); MM and MMc (in GST); B and Bc (in GST).

2.4. Discussion

Our findings indicate that the lesion caused by the tick *A. sculptum* attachment on the skin the Mangalarga Marchador and Breton Postier horses, presented marked histopathology changes such as inflammatory infiltrate. In addition, it was possible observe nuclear modifications such as pyknotic nuclei with extensive areas of fibrosis, characterizing necrosis. Furthermore, we confirmed our hypothesis, since the resistance variation occurred between animals regardless of race, and these resistant animals showed greater inflammatory response and less attached ticks.

Despite the constant modifications of the antigens present in tick saliva to avoid the host's immune response (Engracia Filho et al., 2017), our results demonstrated important changes at cellular level in the horses evaluated (Figures 1, 2, 3 and 4). The tick saliva is considered the most complex among animals (Nuttall, 2019a) that have anti-inflammatory, immunosuppressive, antihemostatic, and vasodilators molecules (Bowman et al., 1997; Chmelar et al., 2012; Kazimírová and Stibrániová, 2013; Nuttall, 2019a; Valenzuela, 2004). The molecules present in the *A. sculptum* saliva affects the skin repair by inhibiting the production of proinflammatory cytokines, such as TNF-α, IL-6 and IL-12 (Esteves et al., 2019) and stimulating production of anti-inflammatory cytokines, like IL-10 (Carvalho-Costa et al., 2015). In this context, cell signaling is important for defense and consequently tissue repair, in addition to the increased cellularity in the injured tissue during the inflammatory process is important for the repair to occur (Jin et al., 2016; Kazemi-Darabadi et al., 2014).

Cellular responses and histopathological parameters in the skin of tick-infested animals have been progressively studied in recent decades (Allen et al., 1977; Engracia Filho et al., 2017; Gill, 1986; Gill and Walker, 1985; Latif et al., 1991; Schleger et al., 1976; Szabó and Bechara, 1995; Walker and Fletcher, 1986). The inflammatory process present negative effects for the tick (Kotál et al., 2015) and, according to the negative correlation found between the cell count at the attachment site and the tick count found in this study, we suggest that the animals with greater inflammatory response are resistant and tend to have less tick infestation, however, it is worth mentioning that this relationship had no difference between the studied breeds, because it was observed in animals of both breeds randomly. A similar situation was observed in a study that compared the inflammatory cell count in the animals' blood with the tick infestation, where the increase in inflammatory cells, such as eosinophils and mononuclear cells, reflects the acquisition of resistance, which increases from successive tick infestations in cattle (Rechav et al., 1990). The inflammation occurs in a well-located manner, that is, close to the tick's attachment site (Engracia Filho et al., 2017), however we need to consider that some animals have a large infestation with ticks (Pires et al., 2013) and in some cases tick species are organized in colonies (Bello et al., 2008), which probably causes damage and greater impacts on animal tissues, both histopathologically and biochemically. However, no significant differences were observed between the two breeds, although they are of genetic origin and different strains.

Some ways regulate the host's hemostasis are known like through the serine protease inhibitors (Blisnick et al., 2017; Chmelar et al., 2017; Esteves et al., 2017; Kotál et al., 2015) that have action anti-angiogenic inhibiting the formation of new vessels (Sá Junior et al., 2019). Probably due to this, our findings not presenting significant difference in blood vessels count in the horses with tick attachment on the skin compared to controls. In this study, we observed an increase in the number of mast cells in the skin of both breeds horses that had attached tick (Figures 3A and C). Mast cells are responsible for promoting the recruitment of neutrophils after an injury (Shiota et al., 2010), and produce various growth factors and proteases, that recruit cells to the injury site, leading to the formation of the inflammatory infiltrate, positively affecting the proliferative response and the vascular homeostasis (Shiota et al., 2010, 2005). In addition, these molecules produced by mast cells stimulates the organization of collagen and help in tissue regeneration and remodeling (Iba et al., 2004). Mast cells also act by releasing histamine which has a harmful function in tick feeding, as it stimulates itching and recruitment of proinflammatory cells, hindering the tick's attachment on the skin (Kemp and Bourne, 1980).

At the *A. sculptum* attachment site we can see the presence of the cementum cone located around the tick's hypostome in the dermis and intense histopathological changes due to deeper localization in the host's skin (Figure 1A), unlike most other tick species and genera, in which the mouthparts is inserted only in the epidermis (Suppan et al., 2018). The cementum cone of this species is composed of proteins, free lipid structures, carbohydrates, and have eosinophilic characteristics (Suppan et al., 2018). It has the functions of firmly anchoring the tick so that it can feed on the host's blood and also restraining the blood leakage (Kim et al., 2014; Suppan et al., 2018). In this study, we observed at the tick attachment site the presence of cell death by necrosis demonstrated through the identification of pyknotic nuclei (Figures 3B and D), followed by a fibrotic process, caused by the increase in the quantity and thickness of type I collagen fibers (Figure 4). The necrotic process has already been described in another study of the attachment site of two tick species *Amblyomma variegatum* and *Rhipicephalus appendiculatus* in cattle (Latif et al., 1991) and cattle and rabbits in response to *R. appendiculatus* (Walker and Fletcher, 1986). Changes such as the pyknotic nucleus and karyolysis are indicators of necrosis, and the high deposition of type I collagen fibers, that are fibers more thickness when compared to type III collagen fibers characterize tissue fibrosis (Aziz et al., 2016; D'Arcy, 2019; Hargis and Myers, 2017). However, some authors associate the deposition of collagen around the hypostome as a way to allow a firm attachment of the tick (Arthur, 1970; Whitwell, 1978).

When the tissue suffers an injury, inflammatory mediators are released and, consequently, occur inflammatory cells migration and the release of free radicals and reactive oxygen species (ROS) (Chapple, 1997; Fujiwara and Kobayashi, 2005; Kumar et al., 2017; Maier and Chan, 2002; Mates, 1999; Winrow et al., 1993). Most studies on oxidative stress and ticks are related to the oxidative damage in tick due to blood-feeding (Galay et al., 2014; Hernandez et al., 2018). Here, we performed for the first time the biochemical analyses of oxidative stress markers at the tick attachment site and observed that despite of the occurrence of the inflammation where normally we have an increase in ROS, the oxidative stress markers, MDA, PCN and NO showed no change (Figure 5), this can occur due to the modulation of molecules at the site of attachment by the tick to ensure a favorable environment for feeding. The ROS are produced by living organisms as a result of normal cell metabolism, but in high quantity due to imbalance with antioxidant enzymes, the ROS act on the tissue it is common observe changes in lipids, proteins and cell DNA, which result in oxidative stress (Birben et al., 2012; Guo and DiPietro, 2010). MDA is a bioproduct of lipid peroxidation considered the general biomarker of lipid oxidative damage (Martinez et al., 2015; Vasconcelos et al., 2007). Another important marker of oxidative stress is PCN that represent destruction of the proteins by ROS (Guo and DiPietro, 2010). The NO is a type of reactive nitrogen species generated during inflammation and could cause excessive vasodilation resulting in hypotension, and septic shock, in addition to being toxic and responsible for DNA mutations (Soomro, 2019). In the opposite direction to oxidative stress, we have antioxidant enzymes, which work to inhibit the damaging action of ROS (Case, 2017).

The tick's saliva is rich in molecules not yet investigated, which may be act on antioxidant enzymes and ROS, possibly masking or even reducing oxidative stress as they do in the body of ticks (Sabadin et al., 2019), in addition, these molecules guarantee an environment a more favorable environment for blood-feeding, as already described in other tick species (Adamson et al., 2014; Das et al., 2001; Kim et al., 2020; Kumar et al., 2016; Tirloni et al., 2014; Wu et al., 2010) and also as a facilitating mechanism for the transmission of pathogens (Nuttall, 2019b). Due to our study being the pioneer in these biochemical analyses with horses, we cannot compare the values found in the control groups with other studies, despite this, we see that in most analyses the control groups presented more homogeneous data than those present in the fragments of skin with ticks. We observed that the SOD enzyme present higher values in fragments of tick attachment skin in Breton Postier when compared to Mangalarga Marchador and when compared to your control group, this may mean that Breton Postier had higher oxidative enzymatic activity and consequently less tissue damage, while Mangalarga Marchador had apparently greater inflammation and areas of cell death. It is a very important result because SOD and CAT are primary enzymes for removing ROS, with SOD responsible for the dismutation of superoxide (O_{2-}) in hydrogen peroxide (H_2O_2) and CAT for the decomposition of H_2O_2 (Case, 2017), together these enzymes act on the harmful effects of free radicals and other ROS (Case, 2017). Besides this, is important point out that inflammation and oxidative stress process presented an interdependent relationship since inflammatory cells release ROS in the inflamed area and ROS can signal proinflammatory gene expression (Anderson et al., 1994; Biswas, 2016; Flohe et al., 1997).

On the other hand, the values for the antioxidant enzyme GST were significantly higher in the control groups when compared to the fragments of tick attachment skin of both breeds (Figure 5). The GST is an enzyme that is involved in the biotransformation of molecules and disrupts the production of lipid peroxidation formed by the production of ROS, contributing to the detoxification (Hayes et al., 2005; Sharma et al., 2004). Interestingly, GST is an enzyme that has been extensively studied in the tick detoxification system during the blood meal and for use in tick vaccines (Hernandez et al., 2018; Huercha et al., 2020; Perner et al., 2018; Sabadin et al., 2017). GST homologous molecules are found in mammals and experiments that used knockout ticks for this enzyme observed possible compensation with other GST (Hernandez et al., 2018). The changes observed in GST by our study suggest that the animals were in a state of significant oxidative stress, as observed by Mahajan et al. (2017) in circulating oxidative stress in buffaloes infested by *Psoroptes natalensis*, or even, that the ticks have some mechanism that promotes the sequestration of molecules, as observed in other hematophagous arthropods (Kim et al., 2018); however, in this situation, sequestration of this enzyme to use for their benefit. For understanding this mechanism is necessary more studies since the current evidence is scarce and fragmented.

Besides that, we can believe that there is variation in resistance between individuals regardless of breed since some analyses carried out in this study showed a high coefficient of variation so that the data were not homogeneous enough to clarify a variation in susceptibility and resistance between breeds. We draw attention to the importance of studying the animal's antioxidant responses to the tick, since as we mentioned inflammation and oxidative stress are interdependent and influencing processes. There are studies of the tick saliva transcriptome and proteome, despite that we emphasize that more studies are needed to analyse these molecules in order to define their specific functions, find antioxidant molecules and understand the methods used by the tick to maintain blood supply.

2.5. Conclusion

Although there are no significant differences in resistance and susceptibility between the breeds of horses in the study since most of the analyses varied due to the presence or absence of the tick attached to the skin, we herein confirm the hypothesis that animals with a higher inflammatory response have less attached *A. sculptum* ticks. The site of the tick's attachment on the horses' skin presented marked histopathology changes such as inflammatory infiltrate, and nuclear modifications such as pyknotic nuclei with extensive areas of fibrosis, characterizing necrosis. In addition, this study contributes to the knowledge of biochemical changes at the tick attached site, providing the basis for further studies that elucidate the relationship of these parasites with the host's antioxidant enzymes, the role of salivary substances on them and the development of efficient strategies of control.

Author's contributions

BCFN, RSA, RCVF, MMS, FFS, RVG, AKC conceived the study. BCFN, RSA, RCVF, FFS and MMS collected the data, analysed and interpreted the data. BCFN, MMS, RVG and AKC analysed, interpreted the data and writing. All authors reviewed and approved the final draft of the manuscript.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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3. CAPÍTULO 2:

Ixodid diversity and detection of spotted fever group *Rickettsia* spp. in ticks collected on birds in the Brazilian Atlantic Forest

Bárbara Cristina Félix Nogueira[a], Liara de Azevedo Cassiano[b], Thiago Fernandes Martins[c,d], Ricardo Seiti Yamatogi[a], Rômulo Ribon[b], Artur Kanadani Campos[a]

[a]Departamento de Medicina Veterinária, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil
[b]Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil
[c]Instituto Pasteur, Secretaria de Estado da Saúde de São Paulo, São Paulo, Brazil
[d]Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil

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Abstract

The Brazilian Atlantic Forest helds one of the most diverse and unique avifauna in the world. Many vertebrate species are reservoirs of tick-borne pathogens, and birds are an important group among them due to their mobility which facilitates the dispersion of ticks and the infectious agents they carry. This study brings data on the tick diversity parasitizing birds and the molecular detection of *Rickettsia* spp. in these arthropods. Birds (n = 773) were captured, identified, and banded at Mata do Paraíso Research, Training, and Environmental Education Center located in Viçosa, Minas Gerais, Brazil. Birds were checked for the presence of ticks, which were individually collected, identified, and molecularly processed through Polymerase Chain Reaction (PCR) for the detection of *Rickettsia* spp. A total of 130 individuals were infested by ticks, and 479 tick specimens were collected, showing a seasonal distribution of the life stages throughout the year. Ticks were identified as *Amblyomma longirostre* (59/479); *Amblyomma calcaratum* (20/479); *Amblyomma varium* (3/479); *Amblyomma sculptum* (2/479) and *Amblyomma* spp. larvae (395/479). Seasonal distribution of the life stages of ticks was observed along the year and significant negative correlations were found between temperature and collected ticks and temperature and infested birds. From the evaluated samples of ticks, 25.44% (n = 43/169) scored positive for *Rickettsia* spp., and sequence analysis indicated high nucleotide identity with *Rickettsia rhipicephali*, *R. massiliae*, *R. africae* and *R. honei marmionii*. The potential for dispersal of ticks by birds added to the aggressiveness of species of the genus *Amblyomma* and the zoonotic potential of some species of *Rickettsia* are quite worrying when we consider that the study area is widely attended by students, researchers, people from the city and neighboring municipalities.

Keywords: Amblyomma; One health; Passeriformes; Pathogens; Tick-borne diseases.

3.1. Introduction

The Atlantic Forest is a biome that covered roughly 17.4% of the Brazilian territory and now only 11.7% of the original forest remains (Ribeiro et al., 2009). This biome is one of the global biodiversity hotspots, given its extremely high numbers of endemic life forms and its current highly vanished and fragmented condition (Myers et al., 2000).

The Atlantic Forest presents 800 bird species, of which about 223 are endemic to it (Vale et al., 2018; Pizo and Tonetti, 2020). The original area covered by this biome is currently composed by secondary native forest scattered fragments and anthropized areas mainly used for agriculture, cattle ranching, silviculture, and urban areas (Myers et al., 2000).

Birds present great ecological and public health importance due to their ability to disseminate pathogens either directly or through arthropods vectors such as ticks. Pathogens of the genera *Babesia* spp., *Theileria* spp., *Borrelia* spp., *Anaplasma* spp., *Ehrlichia* spp. and *Rickettsia* spp. have been worldwide detected in ticks collected from these birds (Olsén et al., 1995; Bjöersdorff et al., 2001; Santos-Silva et al., 2006; Franke et al., 2010; Hildebrandt et al., 2010; Elfving et al., 2010; Ogrzewalska et al., 2011; Pacheco et al., 2012; Diakou et al., 2016; Luz et al., 2017; Abreu et al., 2019; Dolz et al., 2019; Muñoz-Leal et al., 2019; Pascucci et al., 2019). In Brazil, among these pathogens transmitted by ticks, *Rickettsia* spp. stand out because some species have zoonotic potential, often lethal to humans (Guimarães et al., 2001; Barros-Battesti et al., 2006). However, as Brazil has a significant territorial extension, there are few studies on tick infestation in Brazilian birds, which are mainly on the molecular analysis to detection of pathogens that use ticks as vectors (Labruna et al., 2007a; Ogrzewalska et al., 2009; Tolesano-Pascoli et al., 2010; Ogrzewalska et al., 2011; Luz et al., 2012; Santolin et al., 2012; Pascoal et al., 2013; Sanches et al., 2013; Torga et al., 2013; Martins et al., 2014; Maturano et al., 2015; Osava et al., 2016; Luz et al., 2017; Zering´ota et al., 2017; Ramirez et al., 2020). Thus, this study aimed to assess the ixodid diversity and to detect *Rickettsia* spp. on ticks parasitizing these avian hosts.

3.2. Materials and methods

3.2.1. Study area

The study was carried out at the Mata do Paraíso Research, Training, and Environmental Education Center (20◦46′ to 20◦ 50′ S and 42◦51′ to 42◦49′ W), a 194 ha reserve of Federal University of Viçosa (UFV), located in the city of Viçosa, at the “Zona da Mata” region, southeastern Minas Gerais, Brazil (Figure 1). The research center comprises the best-preserved part of a 384 ha Atlantic Forest, where the vegetation is composed of Submontane Semideciduous Forest (Veloso et al., 1991), mostly in advanced and moderate succession stage. The altitude of the reserve varies between 690 and 870 m (Ribon, 2005) and the climate of the region is Cwa (Köppen), mesothermal, with hot rainy summer and cold dry winters. The mean annual temperature is 21.8ºC and the mean annual rainfall is 1314.2 mm (Neto et al., 2012; Martins et al., 2018). The climatic data referring to the data collection period were obtained by consulting the National Institute of Meteorology.

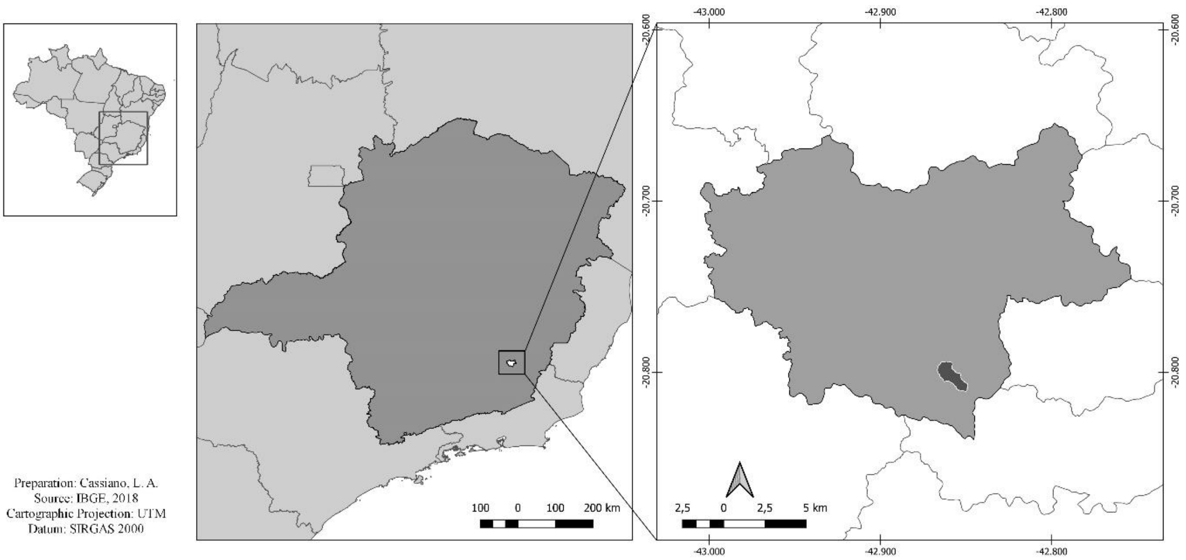

Figure 1. Location of Mata do Paraíso Research, Training, and Environmental Education Center in Viçosa, Minas Gerais, Brazil, where birds and ticks were captured and collected.

3.2.2. Capture and identification of birds

Birds were captured along 38 days of field expeditions between September 2018 to July 2020 (average of three days per month) with capture events starting at 6:00 am to 11:30 am. In each sampling, from 6 to 10 mist nets (12 m long, 2 m wide, 36 mm mesh) were used to trap the birds. The bird species captured were identified according to Sigrist (2015), examined for ticks, marked with numbered aluminum leg bands of the “Centro Nacional de Pesquisa e Conservação de Aves Silvestres” (CEMAVE) to allow the identification of recaptures and were subsequently released at the same capture site. Birds were classified according to the type of nest according to their architecture (open or closed) and location (branch attached or on the ground) (Hilty and Brown, 1986; Euler, 1900; Sick, 2001), and according to their diet as frugivorous, omnivorous, and insectivorous (Sick, 2001), to analyze the influence of these factors in the tick infestations (Table S1).

All the procedures were approved by the Ethics Committee for the Use of Animals of the Federal University of Viçosa (License numbers: 625/2017 and 908/2019) and CEMAVE (License number: 13066-3, Project: 1195).

3.2.3. Identification and PCR analyses of ticks

The captured birds were individually inspected for the presence of ticks. Ticks collected were stored in 70% alcohol in plastic vials and sent for taxonomic identification using the keys for genus and species (Barros-Battesti et al., 2006; Martins et al., 2010) at the "Laboratório de Parasitologia e Doenças Parasitárias" of the Department of Veterinary Medicine of the Federal University of Viçosa. Genomic DNA of ticks was extracted according to Ramos et al. (2015), being the larvae of the same bird processed in pools, totaling 169 DNA samples. The Polymerase Chain Reaction (PCR) was performed using previously published primers to amplify a fragment of approximately 460 bp of the tick mitochondrial 16S gene to confirm the morphological identification of some ticks that generated doubts (Mangold et al., 1998; Ramirez et al., 2020) and a fragment of approximately 300–380 bp of the *Rickettsia* spp. *gltA* gene (Regnery et al., 1991). Amplification was done using Taq Pol Master Mix Green 2X according to manufacturer's instructions, including 400 nmol of each primer, 5 µL of DNA sample, and nuclease free water until complete 25 µL volume.

The amplicons were purified using PCR Purification Kit (Cellco Biotec) according to the manufacturer's instructions. Sanger's method was performed for sequencing amplicons in both directions (Sanger et al., 1977) in an automated sequencer AB 3500 Genetic Analyzer. Sequences were aligned using Mega7 (Kumar et al., 2016) and compared to other sequences available in GenBank Database via Basic Local Alignment Search Tool analysis (BLAST) (Altschul et al., 1990).

3.2.4. Data analysis

Prevalence was defined as *(Number of birds infested / Number of birds examined) x 100*, Average Intensity was defined as *Total number of ticks / Number of* *infested birds* and Relative Intensity of infestation was defined as *Total number of ticks / Total number of birds*.

The proportion of collected ticks/ captured birds and infested birds/ captured birds, defined as non-parametric by Shapiro-Wilk test, were correlated with precipitation and temperature data captured through Spearman rank non-parametric test. These proportions were compared to the diet of the birds by a Kruskal-Wallis non-parametric test.

Two analyses were carried out under a two-way ANOVA framework, one applied for the analysis of the interaction between tick infestation on birds and the types of nests, and the other applied for the analysis of the prevalence of parasitized birds and the types of nests. The two factors of the types of nests, included architecture (open or closed) and location (branch attached or on the ground). ANOVA's normality assumption was evaluated through the Shapiro-Wilk test. Non-parametric analyses for non-normal variables were analyzed by using the Mann-Whitney Test. A significance level of 5% was considered for all reported analyzes.

3.3. Results

3.3.1. Tick infestation on Birds

Birds (n = 773) belonging to the orders Passeriformes (n = 737), Piciformes (n = 12), Apodiformes (n = 11), Columbiformes (n = 9), Accipitriformes (n = 2), and Coraciiformes (n = 2) were captured. Among them, we found 130 individuals (16.82%) infested by ticks (Tables 1 and 2), which were frequently found on the head (Figure 2A), throat (Figure 2B), eyering (Figure 2C) and neck (Figure 2D). In all, 479 (395 larvae, 83 nymphs and 1 adult) ticks were collected, and identified as *Amblyomma longirostre* (59/479), *Amblyomma calcaratum* (20/479), *Amblyomma varium* (3/479), *Amblyomma sculptum* (2/479), and *Amblyomma* spp. larvae (395/479) (Table 2). The bird species collected that did not have ticks can be seen in Table S2.

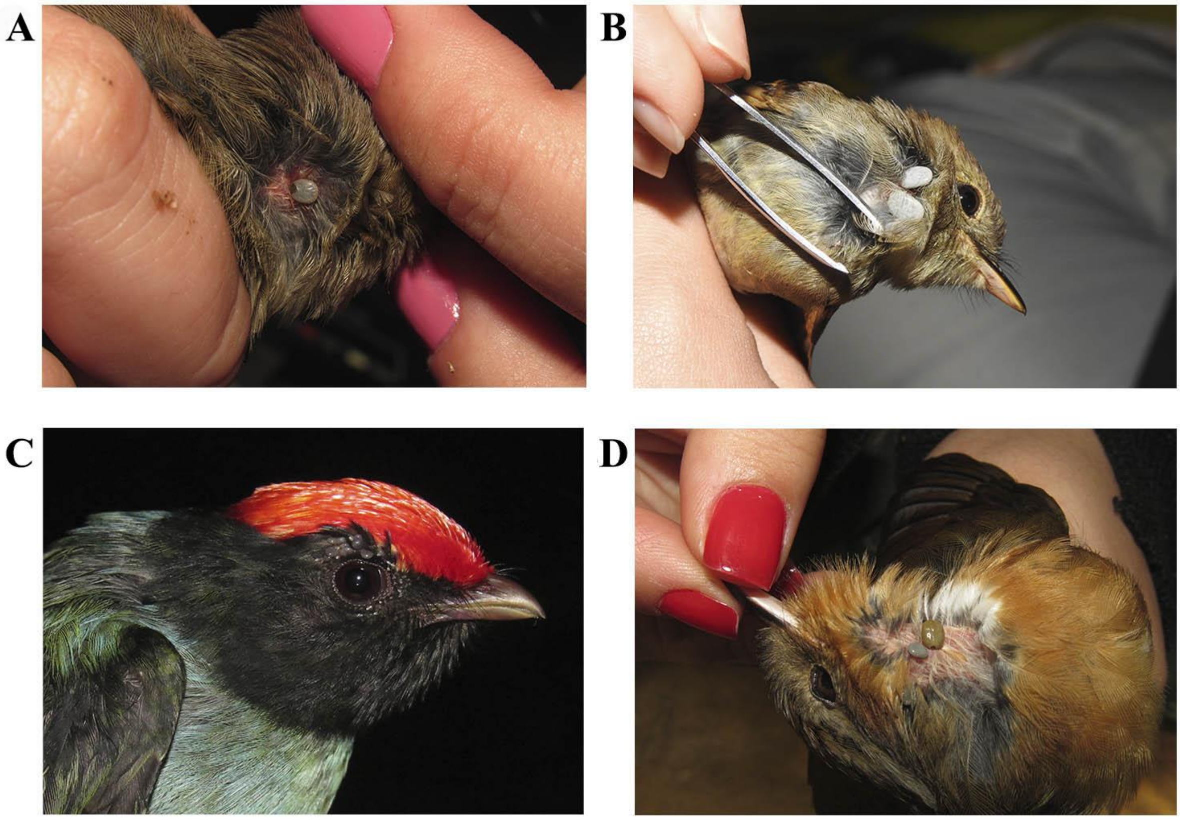

Figure 2. Parts of the bodies of Atlantic Forest birds captured in Viçosa, Minas Gerais, Brazil, where ticks were most frequently found. (A) Nymphs of *Amblyomma longirostre* attached to the neck of *Lathrotriccus euleri*; (B) Nymph of *Amblyomma longirostre* attached to the throat of *Lathrotriccus euleri*; (C) Larvae of *Amblyomma* spp. attached to the eye of *Chiroxiphia caudata* and (D) Nymph of *Amblyomma calcaratum* attached to the neck of *Conopophaga lineata*.

Table 1. Tick species collected from birds captured in the Mata do Paraíso Research, Training, and Environmental Education Center in Viçosa, Minas Gerais, Brazil, between September 2018 to July 2020.

Bird family	Bird species	English names	Nb	Aca	Alo	Asc	Ava	Larvae	Total
Accipitridae	*Rupornis magnirostris*	Roadside Hawk	1			1ª			1
Thamnophilidae	*Dysithamnus mentalis*	Plain Antvireo	2		3			2	5
	Thamnophilus caerulescens	Variable Antshrike	1		2				2
	Pyriglena leucoptera	White-shouldered Fire-eye	6		3*			25	28
Conopophagidae	*Conopophaga lineata*	Rufous Gnateater	8	16	2	1ª		4	23
Dendrocolaptidae	*Xiphorhynchus fuscus*	Lesser Woodcreeper	1					1	1
	Campylorhamphus falcularius	Black-billed Scythebill	1				1ª		1
Furnariidae	*Anabazenops fuscus*	White-collared Foliage-gleaner	3		2			1	3
	Automolus leucophtalmus	White-eyed Foliage-gleaner	6					46	46
	Synallaxis ruficapilla	Rufous-capped Spinetail	2					6	6
Pipridae	*Chiroxiphia caudata*	Swallow-tailed Manakin	5		2			40	42
	Manacus manacus	White-bearded Manakin	21		4			69	73
Platyrinchidae	*Platyrinchus mystaceus*	White-throated Spadebill	20		3			48	51
Rhynchocyclidae	*Mionectes rufiventris*	Grey-hooded Flycatcher	4					14	14
	Leptopogon amaurocephalus	Sepia-capped Flycatcher	9					72	72
	Corythopis delalandi	Southern Antpipit	2					3	3
	Tolmomyias sulphurescens	Yellow-olive Flycatcher	4		4			1	5
Tyrannidae	*Lathrotriccus euleri*	Euler's Flycatcher	9	1	9			5	15
Turdidae	*Turdus rufiventris*	Rufous-bellied Thrush	1					2	2
Parulidae	*Turdus albicollis*	White-necked Thrush	4		2			9	11
	Basileuterus culicivorus	Golden-crowned Warbler	3	1	1			3	5
Cardinalidae	*Habia rubica*	Red-crowned Ant Tanager	5	1ª	5		2ª	5	13
Thraupidae	*Trichothraupis melanops*	Black-goggled Tanager	4		5			35	40
	Coryphospingus pileatus	Grey Pileated Finch	1		1ª				1
	Tachyphonus coronatus	Ruby-crowned Tanager	5	1	11			2	14
	Tangara cyanoventris	Gilt-edged Tanager	2					2	2

Table 1. Tick species collected from birds captured in the Mata do Paraíso Research, Training, and Environmental Education Center in Viçosa, Minas Gerais, Brazil, between September 2018 to July 2020.

Bird family	Bird species	English names	Nb	Aca	Alo	Asc	Ava	Larvae	Total
			130	20	59	2	3	395	**479**

No: Number of birds with ticks; Aca: *Amblyomma calcaratum*; Alo: *Amblyomma longirostre*; Asc: *Amblyomma sculptum*; Ava: *Amblyomma varium.* *Alo: an adult female. [a]First record.

Table 2. Average Intensity, Prevalence, and Relative Intensity of ticks on birds Atlantic Forest captured in the Mata do Paraíso Research, Training, and Environmental Education Center in Viçosa, Minas Gerais, Brazil, between September 2018 to July 2020.

Bird family	Bird species	B/b	P (%)	AI	RI	Ticks
Accipitridae	*Rupornis magnirostris*	1/2	50.00	1.00	0.50	N: Asc
Thamnophilidae	*Dysithamnus mentalis*	2/41*	4.88	2.50	0.12	N: Alo L: Asp
	Thamnophilus caerulescens	1/17*	5.88	2.00	0.12	N: Alo
	Pyriglena leucoptera	6/28*	21.43	4.67	1.00	A: Alo N: Alo L: Asp
Conopophagidae	*Conopophaga lineata*	8/28*	28.57	2.88	0.82	N: Aca Alo Asc L: Asp
Dendrocolaptidae	*Xiphorhynchus fuscus*	1/4	25.00	1.00	0.25	L: Asp
	Campylorhamphus falcularius	1/5	20.00	1.00	0.20	N: Ava
Furnariidae	*Anabazenops fuscus*	3/5	60.00	1.00	0.60	N: Alo, L: Asp
	Automolus leucophthalmus	6/15*	40.00	7.67	3.07	L: Asp
	Synallaxis ruficapilla	2/31*	6.45	3.00	0.19	L: Asp
Pipridae	*Chiroxiphia caudata*	5/37	13.51	8.40	1.14	N: Alo L: Asp
	Manacus manacus	21/84*	25.00	3.48	0.87	N: Alo L: Asp
Platyrinchidae	*Platyrinchus mystaceus*	20/50*	40.00	2.55	1.02	N: Alo L: Asp
Rhynchocyclidae	*Mionectes rufiventris*	4/38	10.53	3.50	0.37	L: Asp
	Leptopogon amaurocephalus	9/38*	23.68	8.00	1.89	L: Asp
	Corythopis delalandi	2/50	4.00	1.50	0.06	L: Asp
	Tolmomyias sulphurescens	4/24	16.67	1.25	0.21	N: Alo, L: Asp
Tyrannidae	*Lathrotriccus euleri*	9/25*	36.00	1.67	0.60	N: Alo Aca L: Asp
Turdidae	*Turdus rufiventris*	1/6	16.67	2.00	0.33	L: Asp
	Turdus albicollis	4/16	25.00	2.75	0.69	N: Alo L: Asp
Parulidae	*Basileuterus culicivorus*	3/31*	9.68	1.67	0.16	N: Aca Alo Asp
Cardinalidae	*Habia rubica*	5/20*	25.00	2.60	0.65	N: Aca Alo Ava
Thraupidae	*Trichothraupis melanops*	4/38	10.53	10.00	1.05	N: Alo L: Asp
	Coryphospingus pileatus	1/2	50.00	1.00	0.50	N: Alo
	Tachyphonus coronatus	5/28*	17.86	2.80	0.50	N: Alo Aca L: Asp
	Tangara cyanoventris	2/3	66.67	1.00	0.67	L: Asp

L: Larvae; N: Nymph; A: Adult; B: Infested birds; b: Captured birds; AI: Average intensity; P: Prevalence; RI: Relative intensity of infestation. Aca: *Amblyomma calcaratum*; Alo: *Amblyomma longirostre*; Asc: *Amblyomma sculptum*; Ava: *Amblyomma varium*; Asp: *Amblyomma* sp. * Individuals capture twice or more times and infested with ticks.

3.3.2. Influence of seasonality and ecological parameters of birds on tick infestation

Seasonal distribution of the life stages of ticks was observed along the year with larger numbers of larvae being collected between March and May followed by a slight increase in July. Nymphs peaked in August and November while the number of adults remained constant along the year (Figure 3).

Significant negative correlations were found between temperature and collected ticks (*r* = - 0.4692, *p* = 0.0276) (Figure 4A) and temperature and infested birds (*r* = - 0.5268, *p* = 0.0118) (Figure 4B), while precipitation did not show significant correlation with collected ticks (*p* = 0.0554) (Figure 4C) and infested birds (*p* = 0.1811) (Figure 4D). No significant differences were found between the type of nests and bird diet that justify the tick infestation on birds.

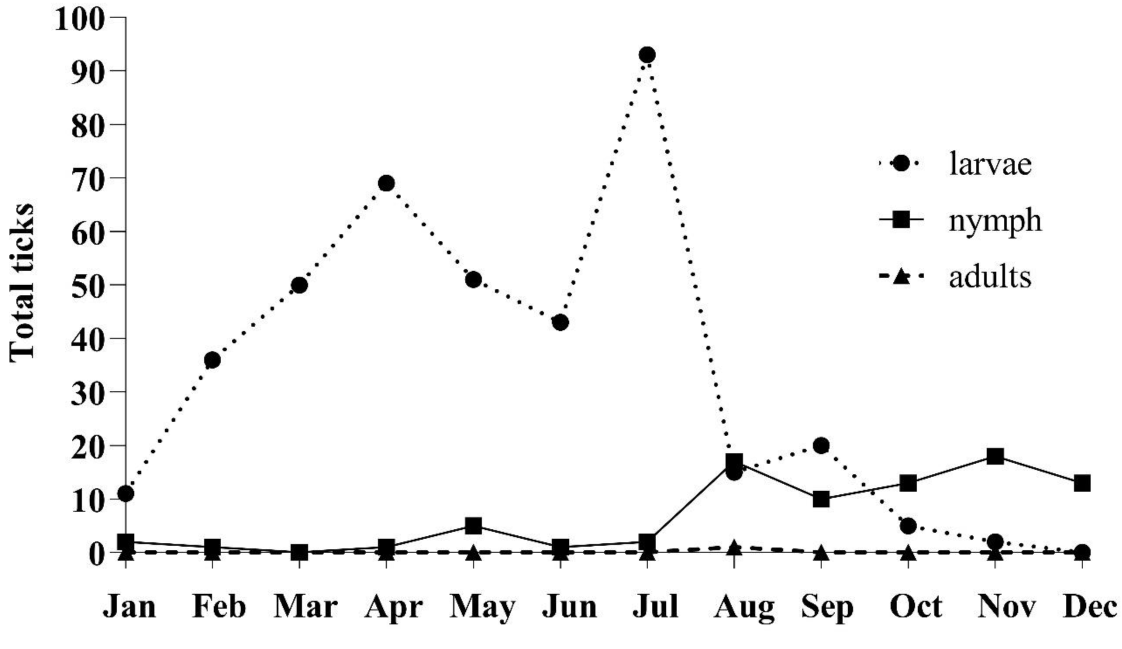

Figure 3. Distribution of the life stages of ticks along a year based in bird captures from September 2018 to July 2020 in the Mata do Paraíso Research, Training, and Environmental Education Center in Viçosa, Minas Gerais, Brazil.

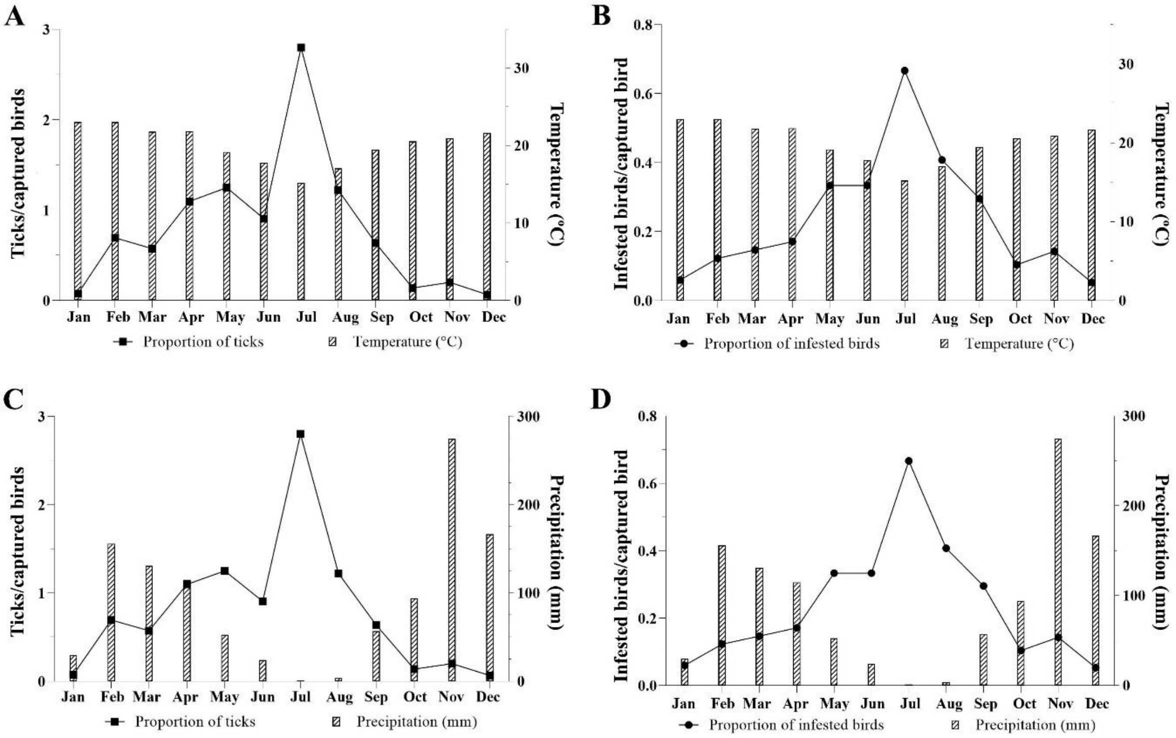

Figure 4. Tick and bird parameters according to climatic data along a year based on bird captures taken from September 2018 to July 2020 in the Mata do Paraíso Research, Training, and Environmental Education Center in Viçosa, Minas Gerais, Brazil. (A) Influence of temperature data on the proportion of ticks; (B) Influence of temperature data on proportion of infested birds; (C) Influence of precipitation data on the proportion of ticks; (D) Influence of precipitation data on the proportion of infested birds.

3.3.3. Molecular analysis

The sequence analysis of the tick mitochondrial 16S gene and comparisons with BLAST results in the GenBank database revealed *A. longirostre, A. calcaratum* and *A. varium* (Table S2). Among the 169 tick samples searched for *Rickettsia* spp., 43 (25.44%) samples were positive, being 30 samples of *Amblyomma* spp. larvae, 12 samples of *A. longirostre* nymphs and, 1 sample of *A. calcaratum* nymph. Sequencing was only done on 16 of these samples due to the quality of the material. For these samples, the sequence analysis of the *gltA* gene and comparisons with BLAST results in the GenBank database revealed six samples of *Amblyomma* sp. positive for *Rickettsia rhipicephali*, three samples of *Amblyomma* sp. positive for *R. rhipicephali* or *Rickettsia massiliae*, two samples of *Amblyomma* sp. positive for *Rickettsia africae* or *Rickettsia honei marmionii*, and five samples positive just for *Rickettsia* sp. due to the size of the sequenced fragment, being four samples from larvae and one from *A. longirostre* nymph (Table S3).

3.4. Discussion

This study assessed the occurrence of ixodid diversity as well as the detection of *Rickettsia* spp. infecting these ticks collected on Atlantic Forest birds. All the ticks collected belonged to the genus *Amblyomma.* The predominance of this hard tick genus contrasts with the proximity of birds with soft ticks, but can be justified by this genus being the most common found in the Neotropical region (Nava et al., 2017) and collected frequently among wild birds in Brazil, mainly in the larval stage (Figueiredo et al., 1999; Labruna et al., 2007a; Ogrzewalska et al., 2009, 2011; Luz et al., 2012; Santolin et al., 2012; Pascoal et al., 2013; Martins et al., 2014; Maturano et al., 2015; Luz et al., 2017). These probably occur, due to the different habits of the tick families, since soft ticks have a nidicular habit and quickly feed on their hosts, which makes it difficult to disperse them in the environment (Rataud et al., 2020).

The *A. longirostre,* species in which adults commonly parasitize rodents of the genera *Sphiggurus* and *Coendou* (Arzua et al., 2005), is the most abundant parasitizing birds, as shown in other studies (Labruna et al., 2007a; Ogrzewalska et al., 2008; Pacheco et al., 2012). Just as observed by Maturano et al. (2015), in our study, this tick species was found parasitizing several bird species. Here, we also record for the first time an adult parasitizing *Pyriglena leucoptera* and we include *Coryphospingus pileatus* as a new host species, accounting for eleven of thirteen families found, which supports the idea that this species has wild birds as important primary hosts (Arzua et al., 2005; Labruna et al., 2007a). In contrast, *A. calcaratum* is commonly associated with anteaters during the adult stage, but birds are considered important hosts for the immature stages (Ogrzewalska et al., 2010; Martins et al., 2014). In our study, we found *Habia rubica* as a new host record for this tick species. The *A. varium* is a species known as sloth's giant tick, due to its relationship with mammals of the families Bradypodidae and Magalonychidae (Marques et al., 2002). Little is known about the immature stages of the species and Ogrzewalska et al. (2012) speculates that birds can play that role. In our study, nymph stages of these species were also detected supporting the idea that birds can act as hosts for *A. varium* immature stages (Luz et al., 2017; Rocha et al. 2021). This tick species was herein detected for the first time parasitizing *Habia rubica* and *Conopophaga lineata*. Lastly, *A. sculptum* is commonly detected in medium and large mammals (Guimarães, 2001). In birds, its occurrence seems to be more recurrent among Non-Passeriformes (Luz et al., 2016; Ogrzewalska and Pinter, 2016; Araújo et al., 2019), but it was also recorded in two passerines, *Dendrocincla turdina* and *Tachyphonus coronatus* (Luz et al., 2017), and, in our study, this tick species was found for the first time parasitizing *Conopophaga lineata*, another species of Passeriformes.

The bird species more commonly found infested by ticks are *Manacus manacus* (15.24%), *Leptopogon amaurocephalus* (15.03%), *Platyrinchus mystaceus* (10.64%) and *Automolus leucophtalmus* (9.60%). In addition, the prevalence and infestation varied between the bird families (Table S4), with the families Platyrinchidae, Conopophagidae and Cardinalidae presenting the highest prevalence, while the average intensity was higher in families Turdidae, Rhynchocyclidae and Furnariidae. In contrast to the observed by Marini et al. (1996) and Maturano et al. (2015), no high prevalence was found in the family Dendrocolaptidae, and such as observed by Marini et al. (1996), Ogrzewalska et al. (2009) and Maturano et al. (2015), *Platyrinchus mystaceus* stood out for its high prevalence, while *Basileuterus culicivorus* had low rates.

The climatic conditions and latitude are important variables regulating the biological cycle and seasonality of ticks, since temperature influences the duration of each developmental phase outside the host while the photoperiod, influenced by latitude, affects the induction of diapause, allowing synchronization with favorable climatic conditions, and allowing them to survive without a host for a long time (Barros-Battesti et al., 2006). The genus *Amblyomma* tends to have a generation during the year with well-defined stages in the study region. The ticks collected have shown a pattern similar to other species of *Amblyomma* which commonly occurs in this region (i.e. larvae are predominant in the drier seasons, with a peak between April and July) (Labruna et al., 2002; Guedes et al., 2005; Barros-Battesti et al., 2006). Nymphs and adults were collected in a smaller quantity, although the nymphs showed the expected population pattern, occurring in greater quantity between July and November, and being more frequent in winter, adults tend to occur throughout the year, with greater activity in the hot and humid months, from October to March (Labruna et al., 2002; Guedes et al., 2005; Barros-Battesti et al., 2006), but in our study this stage was not as graphically representative as the others, probably because adult ticks are not frequently found infesting avian hosts.

In addition to the influence of weather conditions, we evaluated the tick distribution pattern on birds according to food guilds and nest types. We expected insectivorous birds to have greater prevalence and infestation, as some of these species forage in the soil in search of insects, which would make them easier for ectoparasites to access. However, in our study, no significant differences were found between food guilds, contrary to what was observed by Santolin et al. (2012), who found a higher prevalence among omnivores, and Marini et al. (1996), who found a higher prevalence among insectivores, especially among those who took food from tree bark. We also found no relationship between tick prevalence and infestation between different types of bird nests, although Maturano et al. (2015) have observed that birds with nests in trees are more susceptible to infestation by *A. longirostre* and that the parasite intensity is low for above-ground nests. However, the largest amount of ticks collected in this study occurred between April and August, months when the birds are finishing their breeding period at the study area (Cassiano, L. A., Personal communication), making the infestation unlikely to occur in nests. Furthermore, for hard ticks, the association of tick infestations is not yet defined, while for soft ticks which commonly infest these animals, this is widely discussed (Keirans et al., 1973; Maturano et al., 2015; Rataud et al., 2020). Although the guild and type of nest do not seem to be good indicators to understand the parasitism by ticks in wild birds, the birds' sleeping place and resting behavior could be a factor influencing tick infestation (Marini et al., 1996), as it is known that some bird species continue to use the nest as a sleeping place after reproduction (i.e., the Furnariidae and Dendrocolaptidae families) (Oniki, 1970), others are grouped in mixed colonies or not (Hagan and Walters, 1990; Beauchamp, 1999; Koli et al., 2019), but for many birds this resting behavior is still not well understood.

The potential for dispersal of ticks by birds added to the aggressiveness of species of the genus *Amblyomma* and the zoonotic potential of some species of *Rickettsia* spp. are quite worrying when we consider that the study area is widely attended by students, researchers, people from the city and neighboring municipalities. Furthermore, in our study, we collected larvae and nymphs of *Amblyomma* species positive for this pathogen on Atlantic Forest birds. However, we cannot consider only birds as responsible for this infection, because although they are reservoirs of several pathogens, they are not multipliers of these bacteria (Elfving et al., 2010), due to this, the infection of these ticks was probably caused by transovarian transmission, transstadial perpetuation (Horta et al., 2006) and/or co-feeding transmission (Voordouw, 2015).

Although we did not find in our study *Rickettsia rickettsii* and *Rickettsia parkeri*, which are the causative agents vectors of spotted fever in Brazil (Labruna, 2009; Oliveira et al., 2016; Paddock et al., 2017), our results are important because we found for the second time *R. rhipicephali* associated with ticks of the genus *Amblyomma*, in this way we confirm the precedent of this association and expand the knowledge of the distribution of this bacterium throughout the country by confirming that this association is not restricted to the Botanic Garden of the Federal University of Juiz de Fora, where it was registered for the first time, but also in other areas of the Atlantic Forest (Zeringóta et al., 2017). This bacterium already has three of the main genera of the Ixodidae as hosts and is commonly reported in ticks of the genus *Rhipicephalus* in Africa and Europe (Parola et al., 2013), *Rhipicephalus sanguineus* sensu lato and *Rhipicephalus haemaphysaloides* in Taiwan (Hsu et al., 2011), and *R. sanguineus* (s. l.) (Burgdorfer et al., 1975) and *Dermacentor* spp. in the United States of America (Philip et al., 1981; Philip and Casper, 1981; Shapiro et al., 2010; Mitchell et al., 2016). In Brazil, in addition to the record in *Amblyomma* sp. in Juiz de Fora, Minas Gerais (Zeringóta et al., 2017), it was also reported in *Haemaphysalis juxtakochi* in Rondônia (Labruna et al., 2005), São Paulo (Labruna et al., 2007b) and Mato Grosso (Soares et al., 2015). Further studies are needed to explore this bacterium, since its pathogenic potential remains undetermined and there is still no record of infection in humans that could indicate that it is important in the context of one health (Zeringóta et al., 2017).

More studies are needed to define the other *Rickettsia* species whose sequencing result was incomplete due to the great similarity between the species of *Rickettsia*, however, the samples that appeared to be closely related to *R. rhipicephali* and *R. massiliae*, probably have a high chance of being *R. rhipicephali*, since the other has no record of occurrence in Brazil. *Rickettsia massiliae* has as main vectors ticks of the genus *Rhipicephalus* (Matsumoto et al., 2005) that were not found in our study, but recorded in *R. sanguineus* (s. l.) (Eremeeva et al., 2006; Beeler et al., 2011), *Rhipicephalus microplus* (Ali et al., 2021), *R. haemaphysaloides* (Ali et al., 2021), and *Rhipicephalus turanicus* (Wei et al., 2015; Bezerra-Santos et al., 2021). In addition, this species is known as a human pathogen in Europe (Labruna et al., 2007c) causing the SENLAT Syndrome (Zaharia et al., 2016) and Mediterranean Spotted Fever (Portillo et al., 2015). *Rickettsia africae* is a species whose main vectors are

Amblyomma variegatum and *Amblyomma hebraeum*, and also has zoonotic potential, being responsible for the African tick-bite fever (Parola et al., 2013), which is a disease already reported in Brazil, but in one victim that was bitten by ticks in its passage through Africa (Angerami et al., 2018). Other studies carried out in Brazil reported the appearance of this species in their genetic sequencing, however in none of them there was confirmation of *R. africae*, but the identification of species closely related to it (Labruna et al., 2004; Ramirez et al., 2020). In addition, the other result found appears to be a new strain of *Rickettsia honei*, the subspecies *R. honei marmionii*, which normally occurs in Australia, having as vectors *Haemaphysalis novaeguineae* and being responsible for causing the zoonosis Australian spotted fever (Dehhaghi et al., 2019).

The identification of this bacterium in ticks collected from birds in our study contributes to the knowledge of these interactions, since in the state of Minas Gerais there were only records of *Rickettsia* spp. in bird ticks in Juiz de Fora and in the Ibitipoca State Park (Zeringóta et al. al., 2017; Ramirez et al., 2020). While in other animals such as dogs, horses and humans they are known in other regions such as Belo Horizonte (Brasileiro et al., 2010), Caratinga (Cardoso et al., 2006), Pingo d' Água (Milagres et al., 2010), Santa Cruz do Escalvado (de Lemos et al., 1997) and Coronel Fabriciano (Galvão et al., 2003). In addition, in Coronel Fabriciano and Caratinga there are also records of *Rickettsia* in fleas (Oliveira et al., 2002; Cardoso et al., 2006) and in Pingo d' Água and Santa Cruz do Escalvado in small rodents (Pena et al., 2009; Milagres et al., 2013). Considering the global scope, birds have been acting as important dispersers of ticks with pathogens including *Rickettsia* spp., in several parts of the world, such as Europe (Santos-Silva et al., 2006, Graham et al., 2010, Wallménius et al., 2014, Berthová et al., 2016, Palomar et al., 2016), South America (Ogrzewalska et al., 2012, 2014, Cardona-Romero et al., 2020), Central America (Ogrzewalska et al., 2015, Dolz et al., 2019, Bermúdez et al., 2020) and North America (Mukherjee et al., 2014). Special attention should be given to migratory birds that can act as vectors for various microorganisms (Elfving et al., 2010). Although our study included captures of resident species, with the exception of *Myiodynastes maculatus* and *Elaenia chilensis*, it is known that the forest under study also harbors migratory species, such as *Turdus subalaris* which could act as dispersers over long distances.

Finally, our results demonstrate the importance of exploring pathogens associated with bird ticks, which are often neglected as they are not multipliers of pathogens such as *Rickettsia* spp. In our study, ticks associated with birds were infected by species of this genus, and although our results showed that birds in the studied area do not play a role in the ecology of those responsible for Brazilian spotted fever, *R. rickettsii* and *R. parkeri*, we do not yet know the pathogenic potential of the species *R. rhipicephali* and other spotted fever group *Rickettsia* spp. herein reported.

Author contributions

LAC and RR captured and identified the birds and collected the ticks. BCFN, TFM and AKC identified the ticks. BCFN and LAC extracted tick DNA and performed PCRs. AKC and RSY provided the genetic sequencing and analysis of its results. BCFN, LAC, TFM, RSY, RR and AKC analyzed, interpreted the data and writing. All authors reviewed and approved the final draft of the manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Table S1. Types of bird nests are classified according to architecture (open/closed) and location (branch attached or on the ground) and eating habits.

	Types of nest		
Bird species	**Architecture**	**Location**	**Eating habit**
Dysithamnus mentalis	Open	branch attached	insectivorous
Pyriglena leucoptera	Closed	ground	insectivorous
Thamnophilus caerulescens	Open	branch attached	insectivorous
Conopophaga lineata	Open	branch attached	insectivorous
Sittasomus griseicapillus	Closed	branch attached	insectivorous
Xiphorhynchus fuscus	Closed	branch attached	insectivorous
Lepidocolaptes squamatus	Closed	branch attached	insectivorous
Campylorhamphus falcularius	Closed	branch attached	insectivorous
Automolus leucophtalmus	Closed	ground	insectivorous
Synallaxis cinerascens	Closed	ground	insectivorous
Lochmias nematura	Closed	ground	insectivorous
Anabazenops fuscus	Closed	branch attached	insectivorous
Synallaxis ruficapilla	Closed	branch attached	insectivorous
Ilicura militaris	Open	branch attached	frugivore
Manacus manacus	Open	branch attached	frugivore
Chiroxiphia caudata	Open	branch attached	frugivore
Pachyramphus polychopterus	Open	branch attached	frugivore
Platyrinchus mystaceus	Open	branch attached	insectivorous
Poecilotriccus plumbeiceps	Closed	branch attached	insectivorous
Mionectes rufiventris	Closed	branch attached	insectivorous
Leptopogon amaurocephalus	Closed	ground	insectivorous
Corythopis delalandi	Closed	ground	insectivorous
Tolmomyias sulphurescens	Closed	branch attached	insectivorous
Elaenia chilensis	Open	branch attached	insectivorous
Fluvicula nengeta	Open	branch attached	insectivorous
Lathrotriccus euleri	Open	ground	omnivorous
Stelgidopteryx ruficollis	Closed	ground	insectivorous
Turdus leucomelas	Open	branch attached	omnivorous
Turdus rufiventris	Open	branch attached	omnivorous
Turdus albicollis	Open	branch attached	omnivorous
Zonotrichia capensis	Open	branch attached	omnivorous
Basileuterus culicivorus	Closed	ground	insectivorous
Troglodytes musculus	Closed	branch attached	insectivorous
Coereba flaveola	Closed	branch attached	omnivorous
Saltator similis	Open	branch attached	omnivorous
Trichothraupis melanopis	Open	branch attached	omnivorous
Tachyphonus coronatus	Open	branch attached	omnivorous
Habia rubica	Open	branch attached	insectivorous

Table S2. Bird species captured without ticks.

Bird family	Bird species	English name	n
Columbidae	*Geotrigon montana*	Ruddy Quail-Dove	2
	Leptotila rufaxilla	Grey-fronted Dove	6
	Leptotila verreauxi	White-tipped Dove	1
Trochilidae	*Phaethornis pretrei*	Planalto Hermit	2
	Chlorostilbon lucidus	Glittering-bellied Emerald	2
	Thalurania glaucopis	Violet-capped Woodnymph	6
	Chrysuronia versicolor	Versicolored Emerald	1
Momotidae	*Baryphthengus ruficapillus*	Rufous-capped Motmot	2
Ramphastidae	*Pteroglossus aracari*	Black-necked Aracari	2
Picidae	*Picumnus cirratus*	White-barred Piculet	7
	Veniliornis maculifrons	Yellow-eared Woodpecker	3
Thamnophilidae	*Formicivora serrana*	Serra Antwren	2
	Sittasomus griseicapillus	Olivaceous Woodcreeper	2
Dendrocolaptidae	*Lepidocolaptes squamatus*	Scaled Woodcreeper	2
Xenopidae	*Xenops rutilans*	Streaked Xenops	2
Furnariidae	*Lochmias nematura*	Sharp-tailed Streamcreeper	1
	Synallaxis cinerascens	Gray-bellied Spinetail	6
	Synallaxis frontalis	Sooty-fronted Spinetail	1
Pipridae	*Ilicura militaris*	Pin-tailed Manakin	1
Tityridae	*Pachyramphus polychopterus*	White-winged Becard	1
	Pachyramphus validus	Crested Becard	1
Rhynchocyclidae	*Poecilotriccus plumbeiceps*	Ochre-faced Tody-Flycatcher	2
	Hemitriccus nidipendulus	Hangnest Tody-Tyrant	2
Tyrannidae	*Elaenia chilensis*	Chilean Elaenia	1
	Myiopagis viridicata	Greenish Elaenia	1
	Pitangus sulphuratus	Great Kiskadee	2
	Myiodynastes maculatus	Streaked Flycatcher	1
	Myiozetetes similis	Social Flycatcher	3
	Fluvicola nengeta	Masked Water-Tyrant	1
	Myiarchus tyrannulus	Brown-crested Flycatcher	10
Hirundinidae	*Stelgidopteryx ruficollis*	Southern Rough-winged Swallow	6
Troglodytidae	*Troglodytes musculus*	Southern House-Wre	1
Turdidae	*Turdus leucomelas*	Pale-breasted Thrush	5
	Turdus amaurochalinus	Creamy-bellied Thrush	1
Passerellidae	*Arremon semitorquatus*	Half-collared Sparrow	2
	Zonotrichia capensis	Rufous-collared Sparrow	3
Thaupidae	*Saltator similis*	Green-winged Saltator	1
	Coereba flaveola	Yellowbreast	1
	Sporophila caerulescens	Double-collared Seedeater	1
	Sicalis luteola	Grassland Yellow Finch	1
	Thraupis sayaca	Sayaca Tanager	2
	Stilpnia cayana	Burnished-buff Tanager	1
			101

Table S3. Result of the genetic sequencing of *Amblyomma* species.

***Amblyomma* species**	**Query code**	**Id**	**Acession number**
A. longirostre	99.00%	97.69%	MT275642.1
A. calcaratum	100.00%	99.43%	KU953952.1
A. varium	98.00%	98.27%	KX544818.1

Id: Identity.

Table S4. Result of genetic the sequencing of positive samples for *Rickettsia* sp.

Ticks samples	*Rickettsia* species	Query code	Id	Acession number
Ambylomma sp. (7L)	*R. rhipicephali*	100.00%	100.00%	MN477897.1
Ambylomma sp. (1L)	*R. rhipicephali*	100.00%	100.00%	MN477897.1
Ambylomma sp. (1L)	*R. rhipicephali*	100.00%	100.00%	MN477897.1
Ambylomma sp. (10L)	*R. rhipicephali*	100.00%	100.00%	MN477897.1
Ambylomma sp. (1L)	*R. rhipicephali*	100.00%	100.00%	MN477897.1
Ambylomma sp. (1L)	*R. rhipicephali*	100.00%	100.00%	MN477897.1
Ambylomma sp. (3L)	*R. rhipicephali or R. massiliae*	100.00%	99.48%	KX018048.1 and KY640405.1
Ambylomma sp. (17L)	*R. rhipicephali or R. massiliae*	100.00%	99.25%	MN477897.1 and MN223696.1
Ambylomma sp. (1L)	*R. rhipicephali or R. massiliae*	100.00%	99.48%	KX018048.1 and KY640405.1
Ambylomma sp. (1L)	*R. africae* or *R. honei marmionii*	100.00%	99.74%	LC565701.1 and AY737684.1
Ambylomma sp. (1L)	*R. africae* or *R. honei marmionii*	100.00%	99.74%	LC565701.1 and AY737684.1
Ambylomma sp. (1L)	*Rickettsia* sp.	100.00%	98.80%	EU274654.1
Ambylomma sp. (1L)	*Rickettsia* sp.	100.00%	94.22%	EU274654.1
Ambylomma sp. (4L)	*Rickettsia* sp.	99.00%	89.34%	JF803886.1
Ambylomma sp. (7L)	*Rickettsia* sp.	100.00%	99.21%	EU274654.1
Ambylomma longirostre (1N)	*Rickettsia* sp.	100.00%	99.41%	EU274654.1

L: Larvae; N: Nymph; Id: Identity.

Table S5. Average Intensity, Prevalence, and Relative Intensity of ticks on the birds families captured in the Mata do Paraíso Research, Training, and Environmental Education Center in Viçosa, Minas Gerais, Brazil, between September 2018 to July 2020.

Bird family	B/b	N ticks	P (%)	AI	RI
Accipitridae	1/2	1	50.00	0.50	1.00
Thamnophilidae	8/86*	35	10.47	0.41	3.89
Conopophagidae	8/28*	23	28.57	0.82	2.88
Dendrocolaptidae	2/9	2	22.22	0.22	1.00
Furnariidae	11/51	55	21.57	1.08	5.00
Pipridae	26/121	115	21.49	0.95	4.42
Platyrinchidae	20/50*	51	40.00	1.02	2.55
Rhynchocyclidae	18/150*	94	12.67	0.63	4.95
Tyrannidae	9/25*	15	36.00	0.60	1.67
Turdidae	5/22	13	22.73	0.59	2.60
Parulidae	3/31*	5	9.68	0.16	1.67
Cardinalidae	5/20*	13	25.00	0.65	2.60
Thraupidae	12/71*	57	16.90	0.80	4.75
Total	128/666	479	19.52	0.72	3.68

AI: Average intensity; P: Prevalence; RI: Relative intensity of infestation. * Individuals capture twice or more times and infested with ticks.

4. CAPÍTULO 3:

Soft and hard ticks (Parasitiformes: Ixodida) on humans: A review of Brazilian biomes and the impact of environmental change

Bárbara Cristina Félix Nogueira[a], Artur Kanadani Campos[a], Sebastián Muñoz-Leal[b], Adriano Pinter[c], Thiago Fernandes Martins[c,d]

[a]Departamento de Medicina Veterinária, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil
[b]Departamento de Ciencia Animal, Facultad de Ciencias Veterinarias, Universidad de Concepción, Chillán, Chile
[c]Departamento de Laboratórios Especializados, Superintendência de Controle de Endemias, Secretaria de Estado da Saúde de São Paulo, São Paulo, São Paulo, Brasil
[d]Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, São Paulo, Brasil

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Abstract

Records of accidental parasitism by ticks in humans from Brazil are scarce, with most being reported by researchers who are parasitized during their research and by professionals who work with animals. In order to compile these records, an extensive literature review was carried out. Our revision includes studies published between 1909 and 2022, including nine species of the Argasidae family and 32 species of the Ixodidae family that were reported biting humans in the six biomes of the Brazilian territory. The species with the highest number of records of human parasitism was *Amblyomma sculptum*, followed by *Amblyomma coelebs, Amblyomma cajennense* sensu stricto, and *Amblyomma brasiliense.* The Atlantic Forest was the most frequent biome where human parasitism occurred, probably due to the greater number of inhabitants, universities, and researchers in the region; however, this does not mean that this biome is more conducive to the development of ticks and their parasitism in humans. In addition to *Amblyomma ovale*, a vector of *Rickettsia parkeri* in the country*,* two of the main species that act as vectors of *Rickettsia rickettsii, A. sculptum*, and *Amblyomma aureolatum*, have been reported, which is quite worrying considering that the wide distribution of the species and life stages most frequently mentioned in parasitism (i.e., nymphs and adults) are the ones that favour pathogen transmission. This research provides a significant contribution to the knowledge of tick species associated with human parasitism in Brazil; however, due to environmental change potentiated by deforestation and fires, it is expected that there will be a geographic expansion of some tick species and the pathogens that use them as a vector and an increase in human parasitism.

Keywords: *Amblyomma*; *Dermacentor*; *Haemaphysalis*; *Ixodes*; *Ornithodoros*; *Rhipicephalus*.

4.1. Introduction

Brazil has a large biological richness of animal and plant species that are distributed across six biomes that occur along its wide territorial extension (MMA, 2017). Among this wide variety of species, a group that stands out are ticks with 76 described species, 25 belonging to the Argasidae family (soft ticks) and 51 belonging to the Ixodidae family (hard ticks) (Martins et al., 2021; Muñoz-Leal et al., 2020; Muñoz-Leal et al., 2021b; Pacheco et al., 2021).

Some species of ticks are often found parasitizing domestic, pet, and production animals and, consequently, are often found in environments frequented by humans (Louly et al., 2006). Due to the environmental changes caused by the urbanization process and agricultural practices, the habitat of several species of wild animals has been invaded, leading humans to encounter different tick species and the zoonotic pathogens they transmit (Myers et al., 2000; Gardner, 2008). Consequently, tick-borne diseases for humans are emerging in the Neotropical region (Oliveira et al., 2016).

Despite this, there are few published studies that report accidental parasitism by ticks on humans in Brazil. Since humans are not primary who are parasitized during their research and professionals who work with animals (Louly et al., 2006; Aguirre et al., 2019). This probably occurs because people who do not know the potential of these ectoparasites as vectors of zoonotic pathogens, quickly remove the ticks attached to their skin and discard them. A different behaviour is seen in other countries, where published studies show that people tend to seek medical care when they notice ticks fixed on their skin, so health professionals can withdraw them and provide guidance (Orkun et al., 2014; Briciu et al., 2016; Blanda et al., 2017; Karasartova et al., 2018). In this study, we provide a compilation of published data reporting tick parasitism in the different biomes of the country to contribute to the knowledge of human parasitism by soft and hard ticks.

4.2. Materials and methods

The search for scientific articles on accidental tick parasitism on humans in Brazil was performed and finished on May 26, 2022 and conducted using the following databases: PubMed/Medline (https: //www.ncbi.nlm.nih.gov/pubmed), Scopus (https://www.scopus.com /home.uri), Web of Science (https://www.webofknowledge.com), LILACS (https://lilacs.bvsalud.org/), and Google Scholar (https://scholar. google.com.br/). The descriptors were structured based on search filters built for two domains: (i) tick parasitism on humans and (ii) Brazil. We also used articles that presented records of tick parasitism in humans that did not appear in searches but were known by the authors. In addition, the reference list of every selected manuscript was also added to our literature survey.

After analysis of the studies, only records of ticks identified to the species level were used. Information about each reference, stage of tick life, number of attached ticks, and year and location of registration were collected (when informed). The biome was obtained according to the geographic location. The status was defined as confirmed or doubtful according to conventional occurrence of the species, information regarding the stage and the number of ticks parasitizing humans (Guglielmone and Robbins, 2018). Since humans are not usual hosts too many ticks parasitizing humans can be considered misleading, mainly in the nymphal and adult stage. Identification of immature life stages was also considered doubtful if an identification key was not available at the time of publication of the studies. The retrieved data were tabulated, separating soft and hard ticks, in the software Microsoft Word (2019). The records of parasitism were mapped when the locality allowed the determination of the biome. To this effect, the geographic coordinates of each municipality or locality defined in the records were tabulated in the software Microsoft Excel (2019) as input data for the software QGIS version 3.16.7 LTR that was subsequently employed for spatial distribution analysis. The records were geocoded and plotted on the cartographic mesh of the biome map of the Brazil available at the IBGE website.

The occurrence of tick parasitism on humans was analysed according to the most frequent biome, life stage, and species, using only records composed of defined stages, specific locations, and confirmed data.

4.3. Results

In our analysis, studies published between the years of 1909 and 2022 were addressed. The records of parasitism by ticks in humans reported in these studies occurred between the years of 1933 and 2021, with more frequent reports and publications concentrated in the 21st century. Based on the records of ticks parasitizing humans in Brazil found in the literature, we found records for 39 tick species in addition to two taxonomic groups identified as sensu lato, with 22% soft ticks (Table 1) and 78% hard ticks (Table 2), distributed among the six biomes found in the country (Amazon, Atlantic Forest, Caatinga, Cerrado, Pampa, and Pantanal).

Table 1. Literature records for soft ticks on humans in Brazil as published in studies between the years 1923 and 2022.

Tick species / Number and stages	**Municipalities or localities / States**	**Years**	**References**	**Biomes / Status**
Ornithodoros brasiliensis / Un	São Francisco de Paula / RS	Un	(Aragão, 1923)	Atlantic Forest / Confirmed
O. brasiliensis / Un	São Francisco de Paula / RS	Un	(Evans et al., 2000)	Atlantic Forest / Confirmed
O. brasiliensis / Un	São Francisco de Paula / RS	2007	(Martins et al., 2011)	Atlantic Forest / Confirmed
O. brasiliensis / Un	São Francisco de Paula / RS	2009 to 2010	(Reck et al., 2013)	Atlantic Forest / Confirmed
O. brasiliensis / Un	Jaquirana / RS	2009 to 2010	(Reck et al., 2013)	Atlantic Forest / Confirmed
O. brasiliensis / 1 N, 2 F	São Francisco de Paula / RS	2009 / 2010	(Reck et al., 2018)	Atlantic Forest / Confirmed
O. brasiliensis / 4 N	Jaquirana / RS	2010 / 2011	(Reck et al., 2018)	Atlantic Forest / Confirmed
O. brasiliensis / 2 M, 67 Un	Caxias do Sul / RS	2017	(Dall'Agnol et al., 2019)	Atlantic Forest / Confirmed
Ornithodoros fonsecai / 1 F (published as *Carios fonsecai*)	Bonito / MS	2005	(Labruna and Venzal, 2009)	Cerrado / Confirmed
Ornithodoros hasei / Un	Un / CE	2018 to 2019	(Muñoz-Leal et al., 2021)	Un / Confirmed
Ornithodoros marinkellei / 1 A	Porto Velho / RO	2007 to 2009	(Labruna et al., 2011)	Amazon / Confirmed
Ornithodoros mimon / Un (published as *Carios mimon*)	Araraquara / SP	Un	(Barros-Battesti et al., 2011)	Cerrado and Atlantic Forest / Confirmed
O. mimon / Un	Tiradentes / MG	Un	(Labruna et al., 2014)	Atlantic Forest/ Confirmed
Ornithodoros tabajara / Un (published as *Ornithodoros* sp.)	Serra das Almas / CE	2019	(Muñoz-Leal et al., 2021)	Caatinga / Confirmed
*Ornithodoros talaje** / Un	Un / MT	Un	(Aragão, 1936)	Un / Doubtful
*O. talaje** / Un L, Un N	Carmo do Rio Claro / MG	Un	(Carvalho, 1942)	Cerrado and Atlantic Forest / Doubtful
*O. talaje** / 4 Un	Paragominas / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
*O. talaje** / 18 Un	Santarém / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful

Table 1. Literature records for soft ticks on humans in Brazil as published in studies between the years 1923 and 2022.

Tick species / Number and stages	**Municipalities or localities / States**	**Years**	**References**	**Biomes / Status**
Ornithodoros rietcorreai / 2M, 3F	Jaguaribe / CE	2017	(Oliveira et al., 2018)	Caatinga / Confirmed
O. rietcorreai / 1F	Chapadinha / MA	2019	(Muñoz-Leal et al., 2019a)	Cerrado / Confirmed
O. rietcorreai / 1F	Sobral/ CE	2019 to 2021	(Jorge et al., 2022)	Caatinga/ Confirmed
Ornithodoros rostratus / Un	São Paulo / SP	Un	(Aragão, 1936)	Atlantic Forest/ Confirmed

N: Nymph; M: Male; F: Female; Un: Unclear; CE: Ceará; MA: Maranhão; MG: Minas Gerais; MS: Mato Grosso do Sul; MT: Mato Grosso; PA: Pará; RO: Rondônia; RS: Rio Grande do Sul; SP: São Paulo. *Should be considered *Ornithodoros* sp., misidentified as *O. talaje* (see Discussion topic).

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
Amblyomma aureolatum / Un L, Un A (published as *Amblyomma striatum*)	São Paulo / SP	1933	(Fonseca, 1935)	Atlantic Forest / Confirmed
A. aureolatum / Un (published as *A. striatum*)	Un / Um	Un	(Aragão, 1936)	Un / Confirmed
A. aureolatum / Un A	Un / SP	Un	(Aragão and Fonseca, 1961a)	Un / Confirmed
A. aureolatum / 1 M, 2 F (published as *A. striatum*)	Guarulhos / SP	1993	(Figueiredo et al., 1999)	Atlantic Forest / Confirmed
A. aureolatum / 1 F	Mogi das Cruzes / SP	2000 to 2002	(Pinter et al., 2004)	Atlantic Forest / Confirmed
A. aureolatum / 1 M	São José dos Pinhais / PR	1993	(Arzua et al., 2005)	Atlantic Forest / Confirmed
A. aureolatum / 1 M	Almirante Tamandaré / PR	1995	(Arzua et al., 2005)	Atlantic Forest / Confirmed
A. aureolatum / 1 F	Curitiba / PR	2003	(Arzua et al., 2005)	Atlantic Forest / Confirmed
A. aureolatum / 1 M, 1 F	Itapecerica da Serra / SP	1996	(Guglielmone et al., 2006)	Atlantic Forest / Confirmed
A. aureolatum / 1 M	Mogi das Cruzes / SP	2002	(Guglielmone et al., 2006)	Atlantic Forest / Confirmed
A. aureolatum / 1F	São Paulo / SP	2003	(Guglielmone et al., 2006)	Atlantic Forest / Confirmed
A. aureolatum / 1 F	Embu das Artes / SP	2005	(Guglielmone et al., 2006)	Atlantic Forest / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. aureolatum / 1 F	Mairiporã / SP	2005	(Guglielmone et al., 2006)	Atlantic Forest / Confirmed
A. aureolatum / 1F	São Lourenço da Serra / SP	2005	(Guglielmone et al., 2006)	Atlantic Forest / Confirmed
A. aureolatum / 2 A	Santo André / SP	2005	(Moraes-Filho et al., 2009)	Atlantic Forest / Confirmed
A. aureolatum / 2 Un	Cachoeira do Arari / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
A. aureolatum / 14 Un	Paragominas / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
A. aureolatum / 49 Un	Santarém / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
A. aureolatum / 176 Un	Rio de Janeiro / RJ	1997 to 2007	(Serra-Freire et al., 2011)	Atlantic Forest / Doubtful
A. aureolatum / 2 A	Volta Redonda / RJ	2009 to 2011	(Borsoi and Serra-Freire, 2012)	Atlantic Forest / Confirmed
A. aureolatum / Un A	Un / SP	Un	(Moraes-Filho, 2017)	Un / Confirmed
A. aureolatum / 1 N, 1 M	Eldorado do Sul / RS	2004 / 2012	(Reck et al., 2018)	Pampa / Confirmed
A. aureolatum / 1 F	Gramado / RS	2005	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. aureolatum / 1 F	Nova Prata / RS	2005	(Reck et al., 2018)	Atlantic Forest / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. aureolatum / 1 F	Veranópolis / RS	2005	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. aureolatum / 1 M	Chuvisca / RS	2006	(Reck et al., 2018)	Pampa / Confirmed
A. aureolatum / 1 M	Erechim / RS	2006	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. aureolatum / 1 F	Caxias do Sul / RS	2007	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. aureolatum / 1 M	Nova Petrópolis / RS	2007	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. aureolatum / 1 N	Cerro Largo / RS	2010	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. aureolatum / 1 M	Viamão / RS	2014	(Reck et al., 2018)	Pampa / Confirmed
A. aureolatum / 1 F	São Paulo / SP	2013	(Savani et al., 2019)	Atlantic Forest / Confirmed
A. aureolatum / 1 F	São Mateus do Sul / PR	2017	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. aureolatum / 1 F	Tijucas do Sul / PR	2017	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. aureolatum / 1 M, 1 F	São José dos Pinhais / PR	2017 / 2018	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. aureolatum / 1 M	Campo Largo / PR	2018	(Valente et al., 2020)	Atlantic Forest / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. aureolatum / 1 M	Mandirituba / PR	2018	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. aureolatum / 1 N	Paulo Frontin / PR	2018	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. aureolatum / 1 M	Campo Largo / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. aureolatum / 1 Un	Araucária / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. aureolatum / 2 M, 1 F	São José dos Pinhais / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. aureolatum / Un	Lapa / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. aureolatum / 1 M	Mandirituba / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. aureolatum / Un	Tijucas do Sul/ PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. aureolatum / 1 F	Irati / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. aureolatum / Un	Paulo Frontin / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
Amblyomma auricularium / Un	Un / PB	2009 to 2010	(Serra-Freire, 2014)	Atlantic Forest / Doubtful
A. auricularium / 1 N	Natal / RN	2012 / 2013	(Lopes et al., 2018)	Atlantic Forest / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. auricularium / 3 N	Parque Nacional Grande Sertão Veredas / MG	2012 to 2014	(Szabó et al., 2020)	Cerrado / Confirmed
Amblyomma brasiliense / 1 M	Un / Um	Un	(Aragão, 1911)	Un / Confirmed
A. brasiliense / Un N	Alto Rio Doce / MG	Un	(Aragão, 1936)	Atlantic Forest / Confirmed
A. brasiliense / Un N	South of state / ES	Un	(Aragão, 1936)	Atlantic Forest / Confirmed
A. brasiliense / Un	Belo Horizonte / MG	Un	Magalhães (1950)	Atlantic Forest and Cerrado / Confirmed
A. brasiliense / 1 M	Adrianópolis / PR	2000	(Arzua et al., 2005)	Atlantic Forest / Confirmed
A. brasiliense / 1 M	Serra da Cantareira / SP	1937	(Guglielmone et al., 2006)	Atlantic Forest / Confirmed
A. brasiliense / 1 F	Itanhaém / SP	1944	(Guglielmone et al., 2006)	Atlantic Forest / Confirmed
A. brasiliense / 1 F	Parque Nacional de Itatiaia / RJ	2001	(Guglielmone et al., 2006)	Atlantic Forest / Confirmed
A. brasiliense / 1 N	Ribeirão Grande / SP	2003	(Guglielmone et al., 2006)	Atlantic Forest / Confirmed
A. brasiliense / 1 M, 1 F	Londrina / PR	2004	(Guglielmone et al., 2006)	Atlantic Forest / Confirmed
A. brasiliense / 1 N, 1 A	Parque Estadual de Intervales / SP	2002 / 2003	(Szabó et al., 2006)	Atlantic Forest / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. brasiliense / Un N	Linhares / ES	2006	(Ogrzewalska et al., 2007)	Atlantic Forest / Doubtful
A. brasiliense / 1 Un	Cachoeira do Arari / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
A. brasiliense / 16 Un	Paragominas / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
A. brasiliense / 5 Un	Santarém / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
A. brasiliense / 2 N	Santa Teresa / ES	2012	(Acosta et al., 2016)	Atlantic Forest / Confirmed
A. brasiliense / 38 N, 4 F	Parque Nacional do Itatiaia / MG	2014 to 2015	(Luz et al., 2018)	Atlantic Forest / Confirmed
A. brasiliense / 1 N	Guaraqueçaba / PR	1994	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. brasiliense / 1 N	Campina Grande do Sul / PR	2000	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. brasiliense / 1 N	Colombo / PR	2000	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. brasiliense / 1 F	Parque Nacional do Iguaçu / PR	2015 to 2017	(Santos et al., 2020)	Atlantic Forest / Confirmed
A. brasiliense / 2 A, 43 N	Parque Nacional do Iguaçu / PR	2014 to 2020	(Suzin et al., 2022)	Atlantic Forest / Confirmed
Amblyomma calcaratum / 1 N	Guaraqueçaba / PR	1994	(Valente et al., 2020)	Atlantic Forest / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. cajennense sensu stricto / Un M, Un F (published as *A. cajennense*)	North of state / PA	Un	(Aragão and Fonseca, 1961b)	Amazon / Confirmed
A. cajennense s. s. / Un (published as *A. cajennense*)	Un / RO	Un	(Labruna et al., 2005a)	Amazon / Confirmed
A. cajennense s. s. / 812 Un (published as *A. cajennense*)	Cachoeira do Arari / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
A. cajennense s. s. / 3394 Un (published as *A. cajennense*)	Paragominas / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
A. cajennense s. s. / 912 Un (published as *A. cajennense*)	Santarém / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
A. cajennense s. s. / 33 M, 24 F (published as *A. cajennense*)	Monte Alegre / PA	1957	(Martins et al., 2016)	Amazon / Confirmed
A. cajennense s. s. / 2 M (published as *A. cajennense* s. l.)	Tucuruí / PA	1984	(Martins et al., 2016)	Amazon / Confirmed
A. cajennense s. s. / 1 M (published as *A. cajennense* s. l.)	Monte Alegre / PA	2007	(Martins et al., 2016)	Amazon / Confirmed
A. cajennense s. s. / 3 M (published as *A. cajennense* s. l.)	Sinop / MT	2010	(Martins et al., 2016)	Amazon / Confirmed
A. cajennense s. s. / 1 F	Urbano Santos / MA	2011	(Martins et al., 2016)	Amazon / Confirmed
A. cajennense s. s. / 21 N	Porto de Moz / PA	2013	(Pacheco et al., 2021)	Amazon / Confirmed
A. cajennense s. s. / 39 N, 2 M, 1 F	Marabá / PA	2015	(Pacheco et al., 2021)	Amazon / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. cajennense s. s. / 8 M, 22 F	Novo Progresso / PA	2017	(Pacheco et al., 2021)	Amazon / Confirmed
A. cajennense s. s. / 30 N, 3 F	Pacajá / PA	2017	(Pacheco et al., 2021)	Amazon / Confirmed
A. cajennense s. s. / 11 N, 1 M	Parauapebas / PA	2018	(Pacheco et al., 2021)	Amazon / Confirmed
A. cajennense s. s. / 1 M	Brasiléia / AC	2021	(Souza et al., 2022a)	Amazon / Confirmed
A. cajennense sensu lato / Un (published as *A. cayennense*)	Un / Un	Un	(Aragão, 1911)	Un / Confirmed
A. cajennense s. l. / 1 M, 1 F (published as *A. cajennense*)	Ponta da Pedra / MT	Un	(Aragão, 1913)	Un / Confirmed
A. cajennense s. l. / Un L, Un N, Un M, Un F (published as *A. cajennense*)	Un/ Un	Un	(Aragão, 1936)	Un / Confirmed
A. cajennense s. l. / Un (published as *A. cajennense*)	Un / MA	Un	(Fonseca, 1958a)	Un / Confirmed
A. cajennense s. l. / 1 N (published as *A. cajennense*)	Un / Un	Un	(Serra-Freire, 2009)	Un / Doubtful
A. cajennense s. l. / Un N, Un L (published as *A. cajennense*)	Un / Um	Un	(Serra-Freire, 2009)	Un / Doubtful
A. cajennense s. l. / 8 M, 23 F (published as *A. cajennense*)	Caxias / MA	2005 to 2011	(Reis et al., 2013)	Cerrado / Confirmed
A. cajennense s. l. / 1 M	Jauru / MT	2002	(Martins et al., 2016)	Amazon and Cerrado / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
Amblyomma coelebs / Un N, Un A	Un / RO	Un	(Labruna et al., 2005a)	Amazon / Confirmed
A. coelebs / 1 F	Caroebe / RR	2000	(Guglielmone et al., 2006)	Amazon / Confirmed
A. coelebs / 1 F	Teodoro Sampaio / SP	2000	(Guglielmone et al., 2006)	Atlantic Forest / Confirmed
A. coelebs / 1 N	Bonito / MS	2005	(Guglielmone et al., 2006)	Cerrado / Confirmed
A. coelebs / 1 Un	Un / PB	2009 to 2010	(Serra-Freire, 2014)	Un / Doubtful
A. coelebs / 1 N	Aquidauana / MS	2013	(Garcia et al., 2015)	Cerrado and Pantanal / Confirmed
A. coelebs / 1 N	Terenos / MS	2014	(Garcia et al., 2015)	Cerrado / Confirmed
A. coelebs / 1 N	Presidente Figueiredo / AM	2015	(Silva et al., 2016)	Amazon / Confirmed
A. coelebs / 1 N, 1 F	Coari / AM	1992 / 1996	(Gianizella et al., 2018)	Amazon / Confirmed
A. coelebs / 1 N	Santa Isabel do Rio Negro / AM	2012	(Gianizella et al., 2018)	Amazon / Confirmed
A. coelebs / 1 N	Fonte Boa / AM	2015	(Gianizella et al., 2018)	Amazon / Confirmed
A. coelebs / 18 N	Parque Nacional do Iguaçu / PR	2015 to 2017	(Santos et al., 2020)	Alantic Forest / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. coelebs / 1 N	Juruti / PA	2013	(Pacheco et al., 2021)	Amazon / Confirmed
A. coelebs / 1 L, 18 N	Porto de Moz / PA	2013	(Pacheco et al., 2021)	Amazon / Confirmed
A. coelebs / 21 N	Marabá / PA	2015	(Pacheco et al., 2021)	Amazon / Confirmed
A. coelebs / 1 N	Trairão / PA	2015	(Pacheco et al., 2021)	Amazon / Confirmed
A. coelebs / 12 N	Pacajá / PA	2017	(Pacheco et al., 2021)	Amazon / Confirmed
A. coelebs / 2 A, 177 N	Parque Nacional do Iguaçu / PR	2014 to 2020	(Suzin et al., 2022)	Atlantic Forest / Confirmed
A. coelebs / 1 N	Parauapebas / PA	2019	(Souza et al., 2022b)	Amazon / Confirmed
Amblyomma dissimile / 1 F	Rio Maicuru / PA	1955	(Guglielmone et al., 2006)	Amazon / Confirmed
A. dissimile / 1 F (published as *A. bibroni*)	Un / Um	Un	(Serra-Freire, 2009)	Un / Doubtful
Amblyomma dubitatum / 1A	Pedreira / SP	Un	(Famadas et al., 1997)	Atlantic Forest / Confirmed
A. dubitatum / 1 L, 2 N, 1 M	Itu / SP	2006	(Labruna et al., 2007)	Atlantic Forest / Confirmed
A. dubitatum / 1 M	Ribeirão Grande / SP	2005	(Labruna et al., 2007)	Atlantic Forest / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. dubitatum / 201 Un	Rio de Janeiro / RJ	1997 to 2007	(Serra-Freire et al., 2011)	Atlantic Forest / Doubtful
A. dubitatum / Un L, Un N, Un A	Americana / SP	Un	(Brites-Neto et al., 2015)	Atlantic Forest and Cerrado / Confirmed
A. dubitatum / 1 N	Pinhais / PR	2017	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. dubitatum / 1 N	Rio Bom / PR	2018	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. dubitatum / 1 N	Eldorado do Sul / RS	2008	(Reck et al., 2018)	Pampa / Confirmed
A. dubitatum / 1 N	Cerro Largo / RS	2016	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. dubitatum / 15 Un	Cachoeira do Arari / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
A. dubitatum / 268 Un	Paragominas / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
A. dubitatum / 94 Un	Santarém / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
A. dubitatum / 1 N	Rio Bom / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. dubitatum / Un	Inajá / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
Amblyomma fuscum / 1 F	Guarujá / SP	2005	(Marques et al., 2006)	Atlantic Forest / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. fuscum / 1 F	Florianópolis / SC	2006	(Marques et al., 2006)	Atlantic Forest / Confirmed
A. fuscum / 1 M	Palmares do Sul / RS	2005	(Reck et al., 2018)	Pampa / Confirmed
A. fuscum / 1 F	Veranópolis / RS	2006	(Reck et al., 2018)	Atlantic Forest / Confirmed
Amblyomma humerale / 2 L	Porto de Moz / PA	2013	(Pacheco et al., 2021)	Amazon / Confirmed
A. humerale / 1 N	Marabá / PA	2015	(Pacheco et al., 2021)	Amazon / Confirmed
Amblyomma incisum / 1 N	Ribeirão Grande / SP	2004	(Guglielmone et al., 2006)	Atlantic Forest / Confirmed
A. incisum / 6 N, 1 A	Parque Estadual de Intervales / SP	2003 / 2004 / 2005	(Szabó et al., 2006)	Atlantic Forest / Confirmed
A. incisum / 1 M, 2 F	Derrubadas / RS	2004 / 2017	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. incisum / 1 N	Ponta Grossa / PR	1999	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. incisum / 1 N	Fazenda Rio Grande / PR	2000	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. incisum / 4 F	Parque Nacional do Iguaçu / PR	2015 to 2017	(Santos et al., 2020)	Atlantic Forest / Confirmed
A. incisum / Un	Japurá / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. incisum / 10 N	Parque Nacional do Iguaçu / PR	2014 to 2020	(Suzin et al., 2022)	Atlantic Forest / Confirmed
Amblyomma latepunctatum / 1 F	Coari / AM	1993	(Gianizella et al., 2018)	Amazon / Confirmed
A. latepunctatum / 1 M	Santa Isabel do Rio Negro / AM	2012	(Gianizella et al., 2018)	Amazon / Confirmed
A. latepunctatum / 1 N	Juruti / PA	2013	(Pacheco et al., 2021)	Amazon / Confirmed
A. latepunctatum / 2 N	Porto de Moz / PA	2013	(Pacheco et al., 2021)	Amazon / Confirmed
Amblyomma longirostre / 1 N	Fênix / PR	1988	(Arzua et al., 2005)	Atlantic Forest / Confirmed
A. longirostre / 1 F	São Paulo / SP	Un	(Arzua et al., 2005)	Atlantic Forest / Confirmed
A. longirostre / 1 F	São Paulo / SP	Un	(Guglielmone et al., 2006)	Atlantic Forest / Confirmed
A. longirostre / 39 Un	Cachoeira do Arari / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
A. longirostre / 205 Un	Paragominas / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
A. longirostre / 56 Un	Santarém / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
A. longirostre / 1 M	Arroio do Sal / RS	2005	(Reck et al., 2018)	Pampa / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. longirostre / 1 N	Almirante Tamandaré / PR	2002	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. longirostre / 1 N	Porto Vitória / PR	2018	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. longirostre / 1 F	Colombo / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. longirostre / Un	Rio Negro / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. longirostre / 1 F	Fernandes Pinheiro / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. longirostre / 1 N	Porto Vitória / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
Amblyomma naponense / Un M (published as *Amblyomma mantiquirense*)	North of state / PA	Un	(Aragão and Fonseca, 1961b)	Amazon / Confirmed
A. naponense / 1 N	Un / RO	Un	(Labruna et al., 2005a)	Amazon / Confirmed
A. naponense / 4 M	Rio Maicuru / PA	1955	(Guglielmone et al., 2006)	Amazon / Confirmed
A. naponense / 1 N	Guajará Mirim / RO	2004	(Guglielmone et al., 2006)	Amazon / Confirmed
A. naponense / Un N	Linhares / ES	2006	(Ogrzewalska et al., 2007)	Atlantic Forest / Doubtful
A. naponense / 2 N	Coari / AM	1992	(Gianizella et al., 2018)	Amazon / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. naponense / 1 N	Santa Isabel do Rio Negro / AM	2012	(Gianizella et al., 2018)	Amazon / Confirmed
A. naponense / 2 N	Juruti / PA	2013	(Pacheco et al., 2021)	Amazon / Confirmed
A. naponense / 5 N	Porto de Moz / PA	2013	(Pacheco et al., 2021)	Amazon / Confirmed
A. naponense / 3 N	Marabá / PA	2015	(Pacheco et al., 2021)	Amazon / Confirmed
A. naponense / 1 N	Pacajá / PA	2017	(Pacheco et al., 2021)	Amazon / Confirmed
A. naponense / 2 N	Parauapebas / PA	2018	(Pacheco et al., 2021)	Amazon / Confirmed
Amblyomma oblongoguttatum / Un	Alto Rio Doce / MG	Un	(Aragão, 1936)	Atlantic Forest / Confirmed
A. oblongoguttatum / Un F	North of state / PA	Un	(Aragão and Fonseca, 1961b)	Amazon / Confirmed
A. oblongoguttatum / 1 M	Uruará / PA	1998	(Labruna et al., 2000)	Amazon / Confirmed
A. oblongoguttatum / 1 M	Londrina / PR	Un	(Arzua et al., 2005)	Atlantic Forest / Doubtful
A. oblongoguttatum / 2 N[a]	Un / RO	Un	(Labruna et al., 2005a)	Amazon / Confirmed
A. oblongoguttatum / 2 F	Praia do Areaio / Un	1936	(Guglielmone et al., 2006)	Un / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. oblongoguttatum / 2 F	Rio Maicuru / PA	1955	(Guglielmone et al., 2006)	Amazon / Confirmed
A. oblongoguttatum / 1 N, 1 M	Manacapuru / AM	1957	(Guglielmone et al., 2006)	Amazon / Confirmed
A. oblongoguttatum / 1 M, 2 F	Estação Ecológica de Maracá / RR	1982	(Guglielmone et al., 2006)	Amazon / Confirmed
A. oblongoguttatum / 1 F	Caroebe / RR	2000	(Guglielmone et al., 2006)	Amazon / Confirmed
A. oblongoguttatum / 1 N	Monte Negro / RO	2004	(Guglielmone et al., 2006)	Amazon / Confirmed
A. oblongoguttatum / Un N	Linhares / ES	2006	(Ogrzewalska et al., 2007)	Atlantic Forest / Doubtful
A. oblongoguttatum / 1 F	Presidente Figueiredo / AM	2015	(Silva et al., 2016)	Amazon / Confirmed
A. oblongoguttatum / 2 N, 1 M	Manacapuru / AM	1957	(Gianizella et al., 2018)	Amazon / Confirmed
A. oblongoguttatum / 1 N	Santa Isabel do Rio Negro / AM	2012	(Gianizella et al., 2018)	Amazon / Confirmed
A. oblongoguttatum / Un	Estreito / MA	Un	(Costa et al., 2020)	Cerrado/ Confirmed
A. oblongoguttatum / 1 F	Juruti / PA	2013	(Pacheco et al., 2021)	Amazon / Confirmed
A. oblongoguttatum / 11 N, 3 M	Porto de Moz / PA	2013	(Pacheco et al., 2021)	Amazon / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. oblongoguttatum / 3 N, 6 M, 9 F	Pacajá / PA	2017	(Pacheco et al., 2021)	Amazon / Confirmed
A. oblongoguttatum / 1 N, 1 F	Parauapebas / PA	2018	(Pacheco et al., 2021)	Amazon / Confirmed
Amblyomma ovale / Un (published as *Amblyomma fossum*)	Alto Rio Doce / MG	Un	(Aragão, 1936)	Atlantic Forest / Confirmed
A. ovale / 1 F	Morretes / PR	1994	(Arzua et al., 2005)	Atlantic Forest / Confirmed
A. ovale / Un	Un / RO	Un	(Labruna et al., 2005a)	Amazon / Confirmed
A. ovale / 1 M	Ribeirão Grande / SP	2005	(Guglielmone et al., 2006)	Atlantic Forest / Confirmed
A. ovale / 3 F	Ubatuba / SP	2005	(Guglielmone et al., 2006)	Atlantic Forest / Confirmed
A. ovale / 1 A	Parque Estadual de Intervales / SP	2005	(Szabó et al., 2006)	Atlantic Forest / Confirmed
A. ovale / 52 Un	Rio de Janeiro / RJ	1997 to 2007	(Serra-Freire et al., 2011)	Atlantic Forest / Doubtful
A. ovale / 1 A	Volta Redonda / RJ	2009 to 2011	(Borsoi and Serra-Freire, 2012)	Atlantic Forest / Confirmed
A. ovale / 4 F	Ubatuba / SP	2014	(Luz et al., 2016)	Atlantic Forest / Confirmed
A. ovale / 1 Un	Aratuba / CE	2011 to 2013	(Moerbeck et al., 2016)	Atlantic Forest / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. ovale / 1 Un	Guaramiranga / CE	2011 to 2013	(Moerbeck et al., 2016)	Atlantic Forest / Confirmed
A. ovale / 1 F	Lindolfo Collor / RS	2013	(Vizzoni et al., 2016)	Atlantic Forest / Confirmed
A. ovale / 1 F	Mondaí / SC	Un	(Jaguezeski et al., 2017)	Atlantic Forest / Confirmed
A. ovale / 2 M	Cerro Largo / RS	2005	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. ovale / 1 M	Linha Nova / RS	2005	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. ovale / 1 F	Maquiné / RS	2005	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. ovale / 1 F	Campo Bom / RS	2006	(Reck et al., 2018)	Atlantic Forest and Pampa / Confirmed
A. ovale / 1 F	Cândido Godoi / RS	2006	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. ovale / 2 M	Porto Mauá / RS	2006	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. ovale / 1 M	Rolador / RS	2006	(Reck et al., 2018)	Atlantic Forest and Pampa / Confirmed
A. ovale / 1 F	Rio Claro / SP	1997	(Oniki-Willis and Willis, 2018)	Atlantic Forest and Cerrado / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. ovale / 1 F	Un / BA	2018	(Sevá et al., 2019)	Atlantic Forest / Confirmed
A. ovale / 1 F	Ilhéus / BA	2019	(Sevá et al., 2019)	Atlantic Forest / Confirmed
A. ovale / 1 F	Guaramiranga / CE	2012	(Bitencourth et al., 2019)	Atlantic Forest / Confirmed
A. ovale / 1 F	Lindolfo Collor / RS	2017	(Bitencourth et al., 2019)	Atlantic Forest / Confirmed
A. ovale / 1 M, 1 F	Morretes / PR	2006 / 2019	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. ovale / 2 M, 1 F	Antonina / PR	2019	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. ovale / 1 M	Porto Barreiro / PR	2019	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. ovale / 1 M	Juruti / PA	2013	(Pacheco et al., 2021)	Amazon / Confirmed
A. ovale / 1 A	Parque Nacional do Iguaçu / PR	2014 to 2020	(Suzin et al., 2022)	Atlantic Forest / Confirmed
Amblyomma parkeri / 1 N	Cotia / SP	2012	(Martins et al., 2013)	Atlantic Forest / Confirmed
A. parkeri / 12 N	Un / PR	2013 to 2018	(Borsoi et al., 2019)	Un / Confirmed
A. parkeri / 12 N	Un / RS	2013 to 2018	(Borsoi et al., 2019)	Un / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. parkeri / 12 N	Un / SC	2013 to 2018	(Borsoi et al., 2019)	Atlantic Forest / Confirmed
A. parkeri / 1 N	Farroupilha / RS	2005	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. parkeri / 1 N	Novo Hamburgo / RS	2005	(Reck et al., 2018)	Atlantic Forest and Pampa / Confirmed
A. parkeri / 1 N	Santa Rosa / RS	2005	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. parkeri / 1 N	Sapiranga / RS	2005	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. parkeri / 3 N	Caxias do Sul / RS	2005 / 2006	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. parkeri / 2 N	Nova Prata / RS	2005 / 2006	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. parkeri / 1 N	Bento Gonçalves / RS	2006	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. parkeri / 1 N	Flores da Cunha / RS	2006	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. parkeri / 1 N	Ipiranga do Sul / RS	2006	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. parkeri / 1 N	Eldorado do Sul / RS	2009	(Reck et al., 2018)	Pampa / Confirmed
A. parkeri / 1 N	Gramado / RS	2009	(Reck et al., 2018)	Atlantic Forest / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. parkeri / 2 N	Cerro Largo / RS	2010 / 2016	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. parkeri / 1 N	Nova Bassano / RS	2014	(Reck et al., 2018)	Atlantic Forest / Confirmed
A. parkeri / 1 N	Curitiba / PR	2006	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. parkeri / 2 N	São José dos Pinhais / PR	2017	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. parkeri / 1 N	Lapa / PR	2018	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. parkeri / 1 N	Campo Largo / PR	2019	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. parkeri / 1 N	Curitiba / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. parkeri / 1 N	Pinhais / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. parkeri / 2 N	São José dos Pinhais / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. parkeri / 1 N	Lapa / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. parkeri / 2 N	Paulo Frontin / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. parkeri / 1 N	São Mateus do Sul / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
Amblyomma parvum / Un	Un / MT	Un	(Fonseca, 1958b)	Un / Confirmed
A. parvum / Un	Un / BA	Un	(Guimarães et al., 2001)	Un / Confirmed
A. parvum / 2 M	Santa Isabel / GO	1948	(Guglielmone et al., 2006)	Cerrado / Confirmed
A. parvum / 1 F	Três Lagoas / MS	1953	(Guglielmone et al., 2006)	Atlantic Forest and Cerrado / Confirmed
A. parvum / 1 M, 1 F	Aldeia do Porto / MA	2005	(Guglielmone et al., 2006)	Un / Confirmed
A. parvum / 2 M, 2 F	Gilbués / PI	2005	(Guglielmone et al., 2006)	Cerrado / Confirmed
A. parvum / 1 F	Araguapaz / GO	2006	(Guglielmone et al., 2006)	Cerrado / Confirmed
A. parvum / 1 F	Pau dos Ferros / RN	2008	(Ferreira et al., 2008)	Caatinga / Confirmed
A. parvum / 2 N, 1 F	Caxias / MA	2005 to 2011	(Reis et al., 2013)	Cerrado / Confirmed
A. parvum / 10 M, 9 F	Corumbá / MS	2012 to 2013	(Ramos et al., 2014)	Pantanal / Confirmed
A. parvum / 3 N, 23 M, 38 F	Parque Nacional Grande Sertão Veredas / MG	2012 to 2014	(Szabó et al., 2020)	Cerrado / Confirmed
A. parvum / 3 N	Un/ Un	2014 / 2016	(Oliveira et al., 2020)	Caatinga/ Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
Amblyomma romitii / 1 M (published as *Amblyomma tasquei*)	North of state / PA	Un	(Aragão and Fonseca, 1961b)	Amazon / Confirmed
A. romitii / Un L, Un A	Rurópolis / PA	2009	(Sampaio et al., 2010)	Amazon / Confirmed
Amblyomma rotundatum / 1 F	Belém / PA	Un	(Serra-Freire et al., 1995)	Amazon / Confirmed
A. rotundatum / 1 F	Itaboraí / RJ	Un	(Serra-Freire et al., 1995)	Atlantic Forest / Confirmed
Amblyomma scalpturatum / Un M, Un F	North of state / PA	Un	(Aragão and Fonseca, 1961b)	Amazon / Confirmed
A. scalpturatum / 2 M, 3 F	Rio Maicuru / PA	1955	(Labruna et al., 2005b)	Amazon / Confirmed
A. scalpturatum / 1 N	Jauru / MT	2001	(Labruna et al., 2005b)	Amazon and Cerrado / Confirmed
A. scalpturatum / Un A	Un / RO	Un	(Labruna et al., 2005a)	Amazon / Confirmed
A. scalpturatum / 3 M, 3 F	Rio Maicuru / PA	1955	(Guglielmone et al., 2006)	Amazon / Confirmed
A. scalpturatum / 1 M	Londrina / PR	2010	(Onofrio et al., 2010)	Atlantic Forest / Confirmed
A. scalpturatum / 1 N	Presidente Figueiredo / AM	2015	(Silva et al., 2016)	Amazon / Confirmed
A. scalpturatum / 1 M, 2 N	Coari / AM	1992	(Gianizella et al., 2018)	Amazon / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. scalpturatum / 1 N	Santa Isabel do Rio Negro / AM	2012	(Gianizella et al., 2018)	Amazon / Confirmed
A. scalpturatum / 1 N	Jutaí / AM	2014	(Gianizella et al., 2018)	Amazon / Confirmed
A. scalpturatum / 1 N	Fonte Boa / AM	2015	(Gianizella et al., 2018)	Amazon / Confirmed
A. scalpturatum / 1 N	Porto Velho / RO	2017	(Aguirre et al., 2019)	Amazon / Confirmed
A. scalpturatum / 1 L, 1 N	Porto de Moz / PA	2013	(Pacheco et al., 2021)	Amazon / Confirmed
A. scalpturatum / 1 N	Marabá / PA	2015	(Pacheco et al., 2021)	Amazon / Confirmed
A. scalpturatum / 4 N	Pacajá / PA	2017	(Pacheco et al., 2021)	Amazon / Confirmed
Amblyomma sculptum / Un (published as *A. cajennense*)	Pedreira / SP	1987 to 1988	(Lima et al., 1995)	Atlantic Forest / Confirmed
A. sculptum / Un N (published as *A. cajennense*)	Santa Cruz do Escalvado / MG	1989 to 1990	(Lemos et al., 1997)	Atlantic Forest / Confirmed
A. sculptum / 14 N, 6 A (published as *A. cajennense*)	Pedreira / SP	1993 to 1994	(Lemos et al., 1997)	Atlantic Forest / Confirmed
A. sculptum / 1 F (published as *A. cajennense*)	Itu / SP	1987	(Arzua et al., 2005)	Atlantic Forest / Confirmed
A. sculptum / 2 N (published as *A. cajennense*)	Pinhão / PR	1991	(Arzua et al., 2005)	Atlantic Forest / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. sculptum / 2 N, 8 M, 2 F (published as *A. cajennense*)	Adrianópolis / PR	1992 / 1993	(Arzua et al., 2005)	Atlantic Forest / Confirmed
A. sculptum / 1 F (published as *A. cajennense*)	Guaraqueçaba / PR	1994	(Arzua et al., 2005)	Atlantic Forest / Confirmed
A. sculptum / 1 M, 15 N (published as *A. cajennense*)	Indianópolis / PR	1995	(Arzua et al., 2005)	Atlantic Forest / Confirmed
A. sculptum / 1 M (published as *A. cajennense*)	Aquidauana / MS	1998	(Arzua et al., 2005)	Cerrado and Pantanal / Confirmed
A. sculptum / 1 N (published as *A. cajennense*)	Itu / SP	2006	(Labruna et al., 2007)	Atlantic Forest / Confirmed
A. sculptum / Un (published as *A. cajennense*)	Cotia / SP	2000 to 2001	(Sangioni et al., 2005)	Atlantic Forest / Confirmed
A. sculptum / Un (published as *A. cajennense*)	Pedreira / SP	2000 to 2001	(Sangioni et al., 2005)	Atlantic Forest / Confirmed
A. sculptum / Un (published as *A. cajennense*)	Pirassununga / SP	2000 to 2001	(Sangioni et al., 2005)	Atlantic Forest and Cerrado / Confirmed
A. sculptum / Un (published as *A. cajennense*)	Porto Feliz / SP	2000 to 2001	(Sangioni et al., 2005)	Atlantic Forest / Confirmed
A. sculptum / 8668 Un (published as *A. cajennense*)	Rio de Janeiro / RJ	1997 to 2007	(Serra-Freire et al., 2011)	Atlantic Forest / Doubtful
A. sculptum / 51 Un (published as *A. cajennense*)	Volta Redonda / RJ	2009 to 2011	(Borsoi and Serra-Freire, 2012)	Atlantic Forest / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. sculptum / 1 M, 1 F	Jauru / MT	Un	(Nava et al., 2014)	Amazon and Cerrado / Confirmed
A. sculptum / 5 M, 2 F	Pedreira / SP	Un	(Nava et al., 2014)	Atlantic Forest / Confirmed
A. sculptum / 1 M, 1 F	Três Lagoas / MS	Un	(Nava et al., 2014)	Atlantic Forest and Cerrado / Confirmed
A. sculptum / 93 N, 5 M, 4 F (published as *A. cajennense* s. l.)	Corumbá / MS	2012 to 2013	(Ramos et al., 2014)	Pantanal / Confirmed
A. sculptum / 97 Un (published as *A. cajennense*)	Un / PB	2009 to 2010	(Serra-Freire, 2014)	Un / Doubtful
A. sculptum / Un L, Un N, Un A	Americana / SP	Un	(Brites-Neto et al., 2015)	Atlantic Forest and Cerrado / Confirmed
A. sculptum / 1 N	Santa Teresa / ES	2012	(Acosta et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 N, 10 M (published as *A. cajennense* s. l.)	São Paulo / SP	1932 / 1937 / 1940 / 2000 / 2005 / 2006	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 F	Ceres / GO	1934	(Martins et al., 2016)	Cerrado / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. sculptum / 7 F	São Paulo / SP	1940 / 2005 / 2006	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 3 M, 1 F	Resende / RJ	1950	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 M, 1 F	Teodoro Sampaio / SP	1951	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 F	Brasília / DF	1998	(Martins et al., 2016)	Cerrado / Confirmed
A. sculptum / 1 M (published as *A. cajennense* s. l.)	Brasília / DF	1999	(Martins et al., 2016)	Cerrado / Confirmed
A. sculptum / 1 M, 1 F	Três Lagoas / MS	1999	(Martins et al., 2016)	Atlantic Forest and Cerrado / Confirmed
A. sculptum / 1 N (published as *A. cajennense* s. l.)	Atibaia / SP	2000	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 7 N	Pedreira / SP	2000	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 N (published as *A. cajennense* s. l.)	Teodoro Sampaio / SP	2000	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 F	Atibaia / SP	2001	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 M, 1 F	Jauru / MT	2001	(Martins et al., 2016)	Amazon and Cerrado / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. sculptum / 1 M, 1 F	Nobres / MT	2001	(Martins et al., 2016)	Cerrado / Confirmed
A. sculptum / 1 F	Osasco / SP	2001	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 N (published as *A. cajennense* s. l.)	Campinas / SP	2002	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 M (published as *A. cajennense* s. l.)	Juatuba / MG	2002	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 8 M, 12 F	Poconé / MT	2002	(Martins et al., 2016)	Cerrado and Pantanal / Confirmed
A. sculptum / 6 M, 6 F	Reserva do Cabaçal / MT	2002	(Martins et al., 2016)	Amazon and Cerrado / Confirmed
A. sculptum / 1 F	Águas da Prata / SP	2004	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 M (published as *A. cajennense* s. l.)	Bragança Paulista / SP	2005	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 F	Caieiras / SP	2005	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 M (published as *A. cajennense* s. l.)	Caieiras / SP	2005	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 F	Carapicuíba / SP	2005	(Martins et al., 2016)	Atlantic Forest / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. sculptum / 1 F	Catanduva / SP	2005	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 M (published as *A. cajennense* s. l.)	Corumbá / MS	2005	(Martins et al., 2016)	Cerrado / Confirmed
A. sculptum / 3 F	Francisco Morato / SP	2005	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 4 F	Ibiúna / SP	2005	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 M (published as *A. cajennense* s. l.)	Paulicéia / SP	2005	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 2 M, 6 F	São Lourenço da Serra / SP	2005	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 2 M (published as *A. cajennense* s. l.)	São Lourenço da Serra / SP	2005	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 17 M, 33 F	Ubatuba / SP	2005	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 M (published as *A. cajennense* s. l.)	Vargem Grande Paulista / SP	2005	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 3 M, 11 F	Caraguatatuba / SP	2005 / 2006	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum/ 2 M, 4 F	Gilbués / PI	2005 / 2009	(Martins et al., 2016)	Cerrado / Confirmed
A. sculptum / 1 F	Itapetininga / SP	2006	(Martins et al., 2016)	Atlantic Forest and Cerrado / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. sculptum / 1 N	Itu / SP	2006	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 M (published as *A. cajennense* s. l.)	Santana do Riacho / MG	2006	(Martins et al., 2016)	Cerrado / Confirmed
A. sculptum / 1 F	Taubaté / SP	2007	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 N	Colatina / ES	2007	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 N (published as *A. cajennense* s. l.)	São Roque de Minas / SP	2007	(Martins et al., 2016)	Cerrado / Confirmed
A. sculptum / 1 M, 4 F	Chapada Gaúcha / MG	2007 / 2012	(Martins et al., 2016)	Cerrado / Confirmed
A. sculptum / 6 M, 15 F	Corumbá / MS	2008 / 2009	(Martins et al., 2016)	Pantanal / Confirmed
A. sculptum / 1 F	Jarinu / SP	2009	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 M (published as *A. cajennense* s. l.)	Angatuba / SP	2010	(Martins et al., 2016)	Atlantic Forest and Cerrado / Confirmed
A. sculptum / 1 F	Embu-Guaçu / SP	2010	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 N (published as *A. cajennense* s. l.)	Americana / SP	2011	(Martins et al., 2016)	Atlantic Forest and Cerrado / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. sculptum / 2 M, 2 F	Cumari / GO	2012	(Martins et al., 2016)	Atlantic Forest and Cerrado / Confirmed
A. sculptum / 2 M, 4 F	Marliéria / MG	2012	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 M (published as *A. cajennense* s. l.)	Marliéria / MG	2012	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 F	Miranda / MS	2012	(Martins et al., 2016)	Cerrado and Pantanal / Confirmed
A. sculptum/ 1 F	Londrina / PR	2013	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 1 M (published as *A. cajennense* s. l.)	Sorocaba / SP	2013	(Martins et al., 2016)	Atlantic Forest / Confirmed
A. sculptum / 6 M, 5 F	Três Marias / MG	2013	(Martins et al., 2016)	Cerrado / Confirmed
A. sculptum / Un	Un / RJ	2005 to 2009	(Montenegro et al., 2017)	Atlantic Forest / Confirmed
A. sculptum / Un (published as *A. cajennense*)	Un / SP	Un	(Moraes-Filho, 2017)	Un / Confirmed
A. sculptum / 1 N	Sorocaba / SP	2015	(Teixeira et al., 2017)	Atlantic Forest / Confirmed
A. sculptum / 2 N, 5 M, 4 F	Parque Nacional Serra da Canastra / MG	2007 / 2009	(Szabó et al., 2018)	Cerrado / Confirmed
A. sculptum / 19 M, 3 F	Aporé / GO	2016 / 2018	(Kmetiuk et al., 2019)	Cerrado / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. sculptum / 2 L, 271 N, 31 M, 27 F	Parque Nacional Grande Sertão Veredas / MG	2012 to 2014	(Szabó et al., 2020)	Cerrado / Confirmed
A. sculptum / 5 N	Paranaguá / PR	2018 / 2019	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. sculptum / 3 N	Rio Bom / PR	2018 / 2019	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. sculptum / 1 N	Paulo Frontin / PR	2019	(Valente et al., 2020)	Atlantic Forest / Confirmed
A. sculptum / 1 M, 1 F	Dueré / TO	2013	(Bitencourth et al., 2017)	Cerrado / Confirmed
A. sculptum / 1 F, 1 N	Paranaguá / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. sculptum / 1 F	Curitiba / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. sculptum / 1 N	União da Vitória / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. sculptum / Un	Cianorte / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. sculptum / 2 N	Japurá / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. sculptum / 1 N	Doutor Camargo / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
A. sculptum / 6 N	Rio Bom / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
A. sculptum / Un	Ribeirão Claro / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
Amblyomma tigrinum / 1 M	Guaíba / RS	1983	(Evans et al., 2000)	Pampa / Confirmed
A. tigrinum / 1 N, 1 F	Rio Araguaia / Un	1935	(Guglielmone et al., 2006)	Un / Confirmed
A. tigrinum / 1 Un	Redenção / CE	2011 to 2013	(Moerbeck et al., 2016)	Caatinga / Confirmed
Amblyomma triste / Un L, Un N, Un A	Poconé / MT	2003 / 2005 / 2007	Serra-Freire and Leal (2009)	Cerrado and Pantanal / Doubtful
Amblyomma varium / 1 N	Un / Un	Un	Serra-Freire (2009)	Un / Doubtful
A. varium / 54 Un	Cachoeira do Arari / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
A. varium / 26 Un	Paragominas / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
A. varium / 31 Un	Santarém / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
A. varium / 675 Un	Rio de Janeiro / RJ	1997 to 2007	(Serra-Freire et al., 2011)	Atlantic Forest / Doubtful
A. varium / 1 N	Mandirituba / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
Dermacentor nitens / 1 Un (published as *Anocentor nitens*)	Un / Un	Un	(Serra-Freire, 2009)	Un / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
D. nitens / 227 Un (published as *A. nitens*)	Cachoeira do Arari / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
D. nitens / 158 Un (published as *A. nitens*)	Paragominas / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
D. nitens / 96 Un (published as *A. nitens*)	Santarém / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
D. nitens / 1 Un (published as *A. nitens*)	Un / PB	2009 to 2010	(Serra-Freire, 2014)	Un / Confirmed
D. nitens / 1 M	Parque Nacional Grande Sertão Veredas / MG	2012 to 2014	(Szabó et al., 2020)	Cerrado / Confirmed
Haemaphysalis juxtakochi / 1 M, 1 F	Curitiba / PR	1992 / 1996	(Arzua et al., 2005)	Atlantic Forest / Confirmed
H. juxtakochi / Un N	Un / RO	Un	(Labruna et al., 2005a)	Amazon / Confirmed
H. juxtakochi / 1 N	Itapeví / SP	1999	(Guglielmone et al., 2006)	Atlantic Forest / Confirmed
H. juxtakochi / 1 N	Santa Isabel do Rio Negro / AM	2012	(Gianizella et al., 2018)	Amazon / Confirmed
H. juxtakochi / 1 N	Herval / RS	2014	(Reck et al., 2018)	Pampa / Confirmed
Ixodes loricatus / 9 Un	Rio de Janeiro / RJ	1997 to 2007	(Serra-Freire et al., 2011)	Atlantic Forest / Doubtful
Rhipicephalus microplus / Un L (published as *Margaropus annulatus microplus*)	Un / Un	Un	(Rohr, 1909)	Un / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
R. microplus / Un L (published as *M. microplus*)	Un / Un	Un	(Aragão, 1911)	Un / Confirmed
R. microplus / Un M (published as *Boophilus microplus*)	Un / RO	Un	(Labruna et al., 2005a)	Amazon / Confirmed
R. microplus / 2 M (published as *B. microplus*)	Santa Maria / RS	2006	(Soares et al., 2007)	Atlantic Forest and Pampa / Confirmed
R. microplus / 12 A (published as *B. microplus*)	Volta Redonda / RJ	2009 to 2011	(Borsoi and Serra-Freire, 2012)	Atlantic Forest / Confirmed
R. microplus / 3 Un (published as *B. microplus*)	Un / PB	2009 to 2010	(Serra-Freire, 2014)	Un / Confirmed
R. microplus / Un	Un / RJ	2005 to 2009	(Montenegro et al., 2017)	Atlantic Forest / Confirmed
R. microplus / 1 M	São Gabriel / RS	2009	(Reck et al., 2018)	Pampa / Confirmed
R. microplus / 1 M	Lavras do Sul / RS	2010	(Reck et al., 2018)	Pampa / Confirmed
R. microplus / 1 M, 1 F	Eldorado do Sul / RS	2012 / 2017	(Reck et al., 2018)	Pampa / Confirmed
R. microplus / 1 M	Alegrete / RS	2013	(Reck et al., 2018)	Pampa / Confirmed
R. microplus / 2 M	Parque Nacional Grande Sertão Veredas / MG	2012 to 2014	(Szabó et al., 2020)	Cerrado / Confirmed
R. microplus / Un	Rio Bom / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
Rhipicephalus sanguineus sensu stricto / 1 M (published as *R. sanguineus*)	Cachoeira do Sul / RS	2005	(Guglielmone et al., 2006)	Pampa / Confirmed
R. sanguineus s. s. / 1 F (published as *R. sanguineus*)	Porto Alegre / RS	Un	(Mentz et al., 2016))	Pampa / Confirmed
R. sanguineus s. s. / 3 M, 3 F (published as *R. sanguineus* s. l.)	Caçapava do Sul / RS	2006	(Reck et al., 2018)	Pampa / Confirmed
R. sanguineus s. s. / 1 M, 1 F (published as *R. sanguineus* s. l.)	Capão da Canoa / RS	2006	(Reck et al., 2018)	Pampa / Confirmed
R. sanguineus s. s. / 1 M (published as *R. sanguineus* s. l.)	Caxias do Sul / RS	2006	(Reck et al., 2018)	Atlantic Forest / Confirmed
R. sanguineus s. s. / 1 M, 1 F (published as *R. sanguineus* s. l.)	Estância Velha / RS	2006	(Reck et al., 2018)	Atlantic Forest and Pampa / Confirmed
R. sanguineus s. s. / 1 N (published as *R. sanguineus* s. l.)	Esteio / RS	2006	(Reck et al., 2018)	Pampa / Confirmed
R. sanguineus s. s. / 1 M (published as *R. sanguineus* s. l.)	Santo Antônio da Patrulha / RS	2006	(Reck et al., 2018)	Atlantic Forest and Pampa / Confirmed
R. sanguineus s. s. / 1 N (published as *R. sanguineus* s. l.)	Uruguaiana / RS	2007	(Reck et al., 2018)	Pampa / Confirmed
R. sanguineus s. s. / 1 M, 2 F (published as *R. sanguineus* s. l.)	Porto Alegre/ RS	2007 / 2014 / 2015	(Reck et al., 2018)	Pampa / Confirmed
R. sanguineus s. s. / 1 M (published as *R. sanguineus* s. l.)	Viamão / RS	2010	(Reck et al., 2018)	Pampa / Confirmed

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
R. sanguineus s. s. / 2 M (published as *R. sanguineus* s. l.)	Eldorado do Sul / RS	2012 / 2014	(Reck et al., 2018)	Pampa / Confirmed
R. sanguineus s. s. / 1 M, 1 F (published as *R. sanguineus* s. l.)	Guaíba / RS	2013	(Reck et al., 2018)	Pampa / Confirmed
R. sanguineus s. s. / 1 M (published as *R. sanguineus* s. l.)	Taquara / RS	2016	(Reck et al., 2018)	Atlantic Forest / Confirmed
Rhipicephalus sanguineus sensu lato / Un (published as *R. sanguineus*)	Pedreira / SP	1987 to 1988	(Lima et al., 1995)	Atlantic Forest / Confirmed
R. sanguineus s. l. / 3 M (published as *R. sanguineus*)	Olinda / PE	Un	(Dantas-Torres et al., 2006)	Atlantic Forest / Confirmed
R. sanguineus s. l. / 1 M (published as *R. sanguineus*)	Recife / PE	Un	(Dantas-Torres et al., 2006)	Atlantic Forest / Confirmed
R. sanguineus s. l. / 1 F (published as *R. sanguineus*)	São Paulo / SP	2002	(Guglielmone et al., 2006)	Atlantic Forest / Confirmed
R. sanguineus s. l. / 1 F (published as *R. sanguineus*)	São Paulo / SP	2002	(Guglielmone et al., 2006)	Atlantic Forest / Confirmed
R. sanguineus s. l. / 3 L, 1 N, 3 M, 1 F (published as *R. sanguineus*)	Goiânia / GO	2005	(Louly et al., 2006)	Cerrado / Confirmed
R. sanguineus s. l. / 1 F (published as *R. sanguineus*)	Un / Un	Un	(Serra-Freire, 2009)	Un / Confirmed
R. sanguineus s. l. / 292 Un (published as *R. sanguineus*)	Cachoeira do Arari / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
R. sanguineus s. l. / 1838 Un (published as *R. sanguineus*)	Paragominas / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful

Table 2. Literature records for hard ticks on humans in Brazil as published in studies between the years 1909 and 2022.

Tick species / Number and stages	Municipalities or localities / States	Years	References	Biomes / Status
R. sanguineus s. l. / 1890 Un (published as *R. sanguineus*)	Santarém / PA	2006 to 2008	(Serra-Freire, 2010)	Amazon / Doubtful
R. sanguineus s. l. / 2570 Un (published as *R. sanguineus*)	Rio de Janeiro / RJ	1997 to 2007	(Serra-Freire et al., 2011)	Atlantic Forest / Doubtful
R. sanguineus s. l. / 380 Un (published as *R. sanguineus*)	Volta Redonda / RJ	2009 to 2011	(Borsoi and Serra-Freire, 2012)	Atlantic Forest / Doubtful
R. sanguineus s. l. / 86 Un (published as *R. sanguineus*)	Un / PB	2009 to 2010	Serra-Freire (2014)	Un / Doubtful
R. sanguineus s. l. / 1 Un (published as *R. sanguineus*)	Baturité / CE	2011 to 2013	(Moerbeck et al., 2016)	Caatinga / Confirmed
R. sanguineus s. l. / 1 M	Campo Grande / MS	2016	(Acosta et al., 2017)	Cerrado / Confirmed
R. sanguineus s. l. / 1 M	Pinhais / PR	2018	(Valente et al., 2020)	Atlantic Forest / Confirmed
R. sanguineus s. l. / Un	Pinhais / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
R. sanguineus s. l. / Un	São José dos Pinhais / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed
R. sanguineus s. l. / 3 M, 2 F	Moreira Sales / PR	2006 to 2017	(Durães et al., 2021)	Atlantic Forest / Confirmed

L: Larvae; N: Nymph; M: Male; F: Female; Un: Unclear; AM: Amazonas; CE: Ceará; DF: Distrito Federal; ES: Espírito Santo; GO: Goiás; MA: Maranhão; MG: Minas Gerais; MS: Mato Grosso do Sul; MT: Mato Grosso; PA: Pará; PB: Paraíba; PE: Pernambuco; PI: Piauí; PR: Paraná; RN: Rio Grande do Norte; RO: Rondônia; RR: Roraima; SC: Santa Catarina; SP: São Paulo.

Among the nine species of soft ticks found, adults of *Ornithodoros* with most records being concentrated in the Atlantic Forest biome (Figure 1A). This tick species and *Ornithodoros rostratus* were found only in the Atlantic Forest in the states of Rio Grande do Sul and São Paulo, respectively, as well as *Ornithodoros mimon*, which was found in the states of Minas Gerais and São Paulo, and *O.* cf. *mimon*, which was found in the state of Rio de Janeiro.

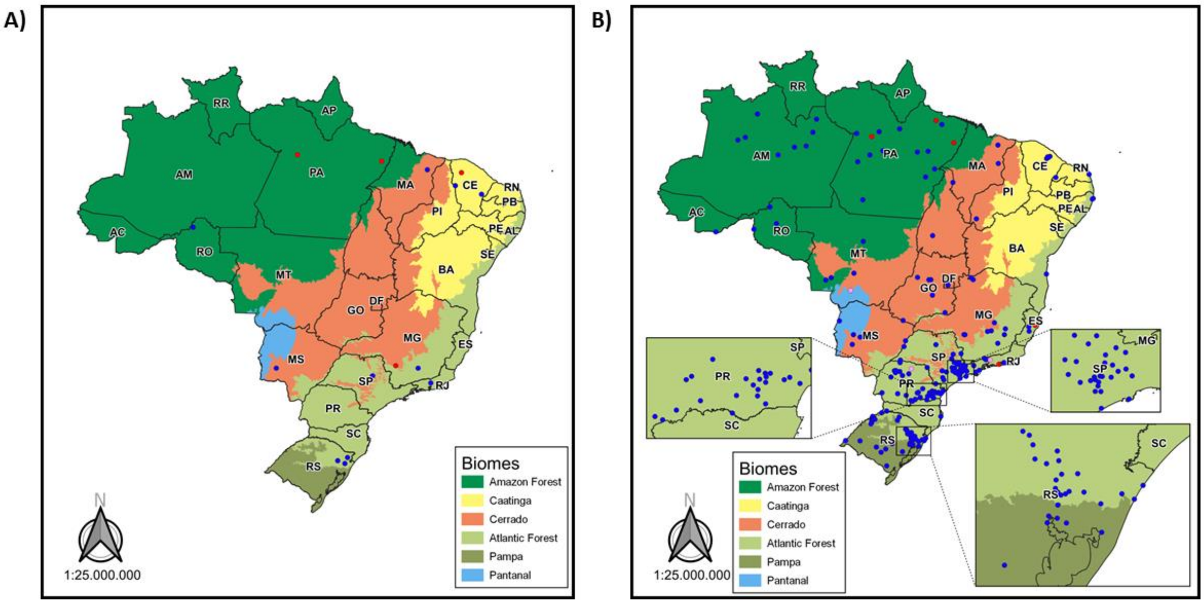

Figure 1. Geopolitical map of Brazil, showing the municipalities of occurrence of human parasitism by soft ticks (A) and hard ticks (B) according to the six major biomes that compose the Brazilian landscape. Blue circles indicate confirmed parasitism, pink circles indicate municipalities with confirmed and doubtful data and red circles indicate doubtful data.

The ticks *Ornithodoros fonsecai* and *Ornithodoros marinkellei* were found exclusively in the Cerrado and Amazon in the states of Mato Grosso do Sul and Rondônia, respectively. On the other hand, *Ornithodoros rietcorreai* was found in the Caatinga in the state of Ceará and in the Cerrado in the state of Maranhão. The tick *Ornithodoros tabajara* was found only in the Caatinga in the state of Ceará. *Ornithodoros hasei* was only found in the state of Ceará, but the municipality was not reported, precluding determination of the biome of origin. Finally, *Ornithodoros talaje* was found in Carmo do Rio Claro (municipality of transition between the Cerrado and Atlantic Forest biomes) located in the state of Minas Gerais; the tick was also found in the state of Mato Grosso (municipality not reported), but it was not possible to determine the biome. The reports of *O. talaje* for the municipalities of Paragominas and Santarém both located in the state of Pará (Amazon biome), are considered dubious.

Twenty-nine hard tick species were found parasitizing humans in the six Brazilian biomes (Table 2). The ticks *Ixodes loricatus, Amblyomma calcaratum* and *Amblyomma incisum* were found only in the Atlantic Forest. *Amblyomma varium* was also found in the Atlantic Forest and in the Amazon; however, the encounter in the Amazon region was considered doubtful due to the number of ticks found and the lack of information regarding the life stage. The tick *Amblyomma rotundatum* was observed parasitizing humans in both the Atlantic Forest and in the Amazon. The species *Amblyomma brasiliense* was observed on humans mainly in the Atlantic Forest, with only one Atlantic Forest-Cerrado transition site (Table 2); therefore, the reports of human parasitism of this species in the Amazon biome are doubtful due to the lack of information regarding the life stage. The ticks *Amblyomma aureolatum, Amblyomma fuscum, Amblyomma longirostre*, and *Amblyomma parkeri* were found in the Atlantic Forest and Pampa biomes, but *A. aureolatum* and *A. longirostre* were also reported in the Amazon biome. Reports of *A. aureolatum* and *A. longirostre* human parasitism are doubtful because it is outside the known range of the first species (Pinter et al., 2004; Szabó, et al., 2013), high number of specimens collected and lack of life stage information in both species in the Amazon region.

The species *Amblyomma cajennense* sensu stricto (s. s.), *Amblyomma dissimile, Amblyomma humerale, Amblyomma latepunctatum* and *Amblyomma romitii* were found exclusively in the Amazon. *Amblyomma naponense* was also found in the Amazon and the Atlantic Forest, but the reports of the species in the Atlantic Forest biome lacked information whether ticks were attached or only crawling on humans. On the other hand, the tick *Amblyomma scalpturatum* was reported mainly in the Amazon, with a single record in a transition area with the Cerrado and a single locality located in the Atlantic Forest (Table 2). The tick *Amblyomma tigrinum* was observed on humans in the Pampa and Caatinga biomes, and the species *Amblyomma triste* was reported in a single Cerrado-Pantanal transition area (Table 2). However, this report is doubtful since larvae, nymphs and adults of the tick were found at the same time and this does not match the known seasonal distribution of these life stages of the species (Nava et al., 2011). Finally, the other tick species *Amblyomma coelebs, Amblyomma dubitatum, Amblyomma oblongoguttatum, Amblyomma ovale, Amblyomma parvum,* *Amblyomma sculptum, Haemaphysalis juxtakochi* and *Rhipicephalus microplus* were found in several biomes (Table 2).

Among the hard tick species, the highest number of records were of *A. sculptum* followed by *A. coelebs, A. cajennense* s. s., and *A. brasiliense* (Table 2), which were more frequent in the Atlantic Forest, Amazon, and Cerrado biomes (Figure 1B). Among the tick species found in this study, *A. sculptum* stands out as a vector of zoonotic pathogens, in addition to *A. ovale* and *A. aureolatum*, which presented the lowest number of records of human parasitism. The tick *A. sculptum* presented the highest number of records for nymphs, followed by females and males. The records were mainly concentrated in the states of São Paulo, Paraná, and Minas Gerais, with the highest occurrence in the degraded Atlantic Forest biome followed by the Cerrado (Figure 2A). Most records of *A. ovale* and *A. aureolatum* on humans were of adults and associated with preserved Atlantic Forest in São Paulo, Paraná, and Rio Grande do Sul states (Figure 2B and C). Some records for these species were considered doubtful, such as the record of *A. aureolatum* in the Pará state, since this species does not occur in the Amazon biome and is morphologically very close to *A. ovale* that occurs in the Amazon region.

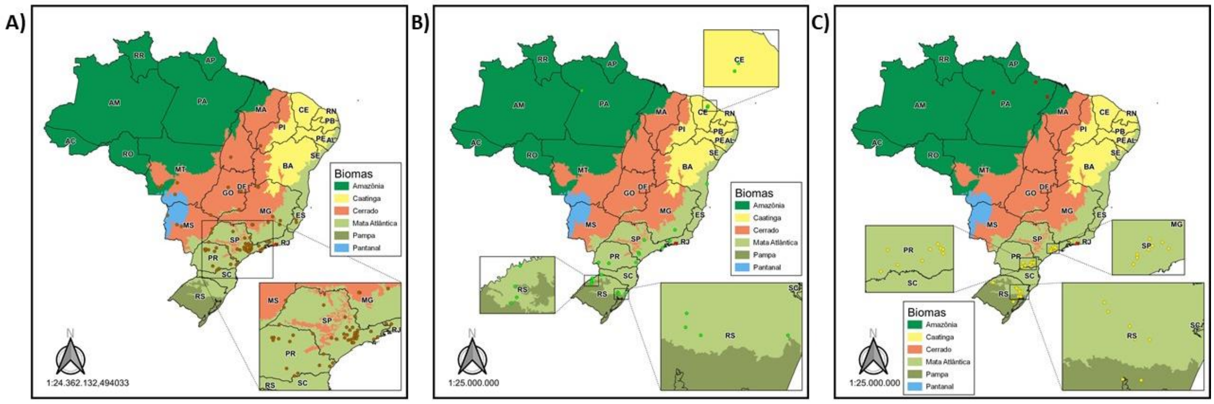

Figure 2. Geopolitical map of Brazil, showing the municipalities of occurrence of human parasitism by *Amblyomma sculptum* (brown circles indicate confirmed parasitism and red circles indicate doubtful data) (A), *Amblyomma ovale* (green circles indicate confirmed parasitism and red circles indicate doubtful data) (B) and *Amblyomma aureolatum* (yellow circles indicate confirmed parasitism and red circles indicate doubtful data) (C) according to the six major biomes that compose the Brazilian landscape.

Despite these important findings, our results have limitations resulting from flaws found in the compiled studies. We observed that many studies omitted important information, such as the number of ticks collected, life stages and precise collection sites, or yet provided doubtful information.

4.4. Discussion

4.4.1. Human parasitism by ticks in Brazilian biomes

Data on human parasitism by ticks in Brazil are scarce and fragmented. One of the reasons for this is that people do not seek medical care for the removal of ticks and post-bite guidance, unlike what occurs in other countries (Orkun et al., 2014; Briciu et al., 2016; Blanda et al., 2017; Karasartova et al., 2018). This behaviour of the population makes it difficult for us to have access to secure data on the epidemiology of ticks and diseases caused by zoonotic pathogens that use ticks as a vector; this also makes treatment difficult in the case of infection, which can lead to death, if not treated early (e.g., Brazilian spotted fever). On the other hand, this could be circumvented if there were greater interest from researchers and public health agencies to educate the population about the role of ticks in public health and if there were researchers to collect ticks on citizens in areas of interest and endemic. Another point that can be considered a limitation is the lack of complete and clear information found in the studies, as some do not mention the exact location, the stage of life, the number of fixed ticks, or the date of occurrence of parasitism.

In addition, there are studies that were not included or were partially included in this review as they only identified the genus. This was common when it came to the larval stage, as we still do not have keys to identify tick larvae of the genus *Amblyomma* for example. However human parasitism with larvae species is given in some reports without explanation of how identification was achieved (Serra-Freire and Leal, 2009), while others performed molecular analysis (Pacheco et al., 2021) or even circumvented the absence of identification for nymphs by rearing them in laboratory until the next stage (Labruna et al., 2005a).

Also, the reports of *O. talaje* in Brazil are probable misidentifications (Labruna et al., 2016). Therefore, the records of this species made for the Amazon biome and Atlantic Forest-Cerrado transition area are likely confusions with other *Ornithodoros* spp., such as *O. rietcorreai* and others that are still pending formal description. Despite these unconvincing data and the few records of human parasitism by soft ticks in Brazil, infestations by these ticks can be considered worrying, as we already have records of infection by zoonotic pathogens of the genus *Borrelia*, which are agents of tick-borne relapsing fever, and the use of ticks of the genus *Ornithodoros* as a vector (Muñoz-Leal et al., 2018; Muñoz-Leal et al., 2021a).

In addition, some data raised doubts about reliability. In some cases, these doubts were due to the absence of additional information, such as municipalities or exact localities, the life stage, quantity, and the occurrence of a tick in a region where there is no record, as well as exorbitant quantities of ticks parasitizing humans in the same report or even if they were fixed or walking on human (Arzua et al., 2005; Labruna et al., 2005a; Ogrzewalska et al., 2007; Serra-Freire and Leal, 2009; Serra-Freire, 2009, 2010, 2014; Serra-Freire et al., 2011, Borsoi and Serra-Freire, 2012).

However, among the studies found, it was possible to observe that the publication of scientific articles involving research with ticks, addressing human parasitism, showed a great increase starting in 2005. This is probably due to increased investment in research in Brazil (Chiarini et al., 2020) and greater concern with emerging diseases, since 75% of these diseases are caused by zoonotic pathogens (Taylor et al., 2001; Gebreyes et al., 2014). In addition, when compared to the territorial extension of the country, most records of human parasitism by ticks in Brazil are concentrated in regions corresponding to the Atlantic Forest biome, probably due to the greater number of inhabitants, universities, and researchers in these regions (Hogan, 2001); however, this does not mean that this biome is more conducive to the development of ticks and their parasitism in humans.

According to published data, most cases of human parasitism by ticks in Brazil are caused by hard ticks. This is probably due to their different habits when compared to soft ticks. Soft ticks parasitize rodents, bats, and birds with nidicolous habits and feed quickly on their hosts, which makes it difficult to disperse them in the environment (Rataud et al., 2020). Hard ticks, mainly of the *Amblyomma* genus, have a wide range of hosts and can feed for several days (Sonenshine and Roe, 2014), remaining fixed to skin of the host while it moves, thus having a wide dispersion and easier contact with humans.

Currently, 76 species of ticks are distributed throughout Brazil; in this study, we were able to report human parasitism by 9 (25/76) soft ticks and 29 (51/76) hard ticks (Barros-Battesti et al., 2006; Dantas-Torres et al., 2019; Martins et al., 2019; Muñoz-Leal et al., 2019a, 2019b; Muñoz-Leal et al., 2020; Labruna et al., 2020; Onofrio et al., 2020). In addition to the records of human parasitism by *Amblyomma cajennense* sensu lato (s. l.) and *Rhipicephalus sanguineus* sensu lato, which do not have an exact identification. In the first case, due to the overlap of the species, two species of the *A. cajennense* complex that occur in Brazil in the Amazon-Cerrado transition areas, *A. cajennense* s. s. and *A. sculptum* (Martins et al., 2016). Although we know that *R. sanguineus* s. l. is a different species, it still lacks a description, so we can only consider *Rhipicephalus sanguineus* sensu stricto as a temperate lineage and *R. sanguineus* s. l. as a tropical lineage (Moraes-Filho et al., 2011; Nava et al., 2018).

The large number of records of humans *A. sculptum* parasitism is quite alarming for public health issues as it is the main vector of the bacterium *Rickettsia rickettsii*, responsible for Brazilian spotted fever (Krawczak et al., 2014; Martins et al., 2016), and its wide distribution, occurring in Argentina, Bolivia, Paraguay, and the southeast and midwest regions of Brazil, in addition to the states of Rondônia, Pará, Tocantins, Maranhão, Piauí, Pernambuco, and Bahia (Martins et al., 2016). The species also has a wide range of hosts, including hosts that have a close relationship with humans (Labruna, 2009; Del Fiol et al., 2010; Szabó et al., 2013; Nava et al., 2014; Martins et al., 2016, 2017).

Although less involved with human parasitism, *A. ovale* also demands attention, as it is distributed throughout the Americas and in all regions from north to south Brazil (Guglielmone et al., 2003; Nieri-Bastos et al., 2016) and is a vector of the zoonotic pathogen *Rickettsia parkeri* strain Atlantic Forest (Sabatini et al., 2010). The species *A. aureolatum* occurs in South America, Uruguay, northeastern Argentina, part of Paraguay, French Guiana, and southern and eastern Brazil (Guglielmone et al., 2003) and stands out as a vector of *R. rickettsii* in urban areas close to fragments of the Atlantic Forest biome in the metropolitan region of the state of São Paulo (Ogrzewalska et al., 2012; Saraiva et al., 2014).

In general, the most frequent life stages of ticks parasitizing humans were nymphs, followed by females and males. This finding is quite worrying when we consider that they are the stages that most commonly act as vectors of pathogens. For example, adults in the species *A. aureolatum* (*R. rickettsii*) and *A. ovale* (*R. parkeri*) and nymphs of *A. sculptum* that are the most common vectors of *R. rickettsii* in humans (Pinter et al., 2021).

4.4.2. Impact of environmental change on human parasitism by ticks: "Walking" through Brazilian biomes

Knowing the distribution of tick species across the country's biomes is extremely important both to understand their parasitic relationships and to estimate the prevalence of pathogens that use ticks as a vector, however, this requires active surveillance, which is unfeasible (Dowling et al., 2021), especially in a country as large as Brazil. In this way, we are basically at the mercy of the few existing studies and the few records of accidental parasitism during the development of scientific research to determine the distribution of tick parasitism, since community (population) participation in the country is not common.

Although we have some knowledge of the distribution of ticks across Brazil, this knowledge may be partially invalidated as environmental change advances, as environmental change can interfere both in the biological cycle of the tick and in the existence and distribution of its hosts (Ogden and Lindsay, 2016). In this context, community participation can be an efficient and economical alternative, as observed in a study carried out in Arkansas (Dowling et al., 2021). Through community contributions, we can continue to monitor the distribution of tick species in the country and also identify possible atypical occurrences that require greater attention to understand their causes (Faccini-Martínez et al., 2021; Martins and Pinter, 2022).

There is evidence that ecosystems in South America are more vulnerable to environmental change, which can be extremely harmful to biodiversity, transforming more vulnerable forest ecosystems into ecosystems with less vegetation cover, such as savannah and pastures (Anjos and de Toledo, 2018). In Brazil, environmental change is enhanced by anthropic actions, such as deforestation and burning, and is already being felt in all biomes through the increase in air temperature, lower humidity, and changes in the hydrological cycle, which impact the existence of plants and animals in the affected areas (Lemes et al., 2014; Ribeiro et al., 2016; Torres et al., 2017; Cavalcante et al., 2020; Lázaro et al., 2020; Rocha et al., 2021; Hofmann et al., 2021).

The ticks have excellent survival conditions, as they can cope well with environmental change compared to other arthropods (Ogden and Lindsay, 2016). This is because they can take refuge to avoid the influence of temperature and humidity (Ogden and Lindsay, 2016) and can go a long time without feeding, avoiding unnecessary exposure to inadequate conditions for their survival (Labruna et al., 2003;

Vieira et al., 2004). Also, they are not fully exposed while feeding, due to contact with the host's body (Schwan and Piesman, 2002; Ogden and Lindsay, 2016), and are able to rehydrate with the humidity of their refuge or using their own saliva (Bowman and Sauer, 2004; Benoit et al., 2010). However, some factors are out of their control, such as floods in their shelters (Luz et al., 2020) and temperature changes that can inhibit their search for food (Ogden and Lindsay, 2016), reducing their life span.

In addition, environmental change can impact the biology of hosts in general, reducing their density in the affected area, which can make the presence of the tick unfeasible, causing it to migrate in search of favourable conditions (Ogden et al., 2008; Hasle et al., 2011; Ogden and Lindsay, 2016). In Brazil, we can exemplify the migration of ticks through the occurrence and expansion of the species *A. sculptum*, which is not commonly found in areas of dense forest, but widely found in Cerrado, Pantanal and in the degraded fragments of the Atlantic Forest. This species also occurs in transition areas between the Cerrado and the Amazon, and its distribution may increase as the replacement of the dense Amazon Forest by the "Cerrado" occurs due to degradation and the loss of humid areas (Martins et al., 2016). This has already been noticed when we consider that between 2000 and 2005 this species was not found in Rondônia (Labruna et al., 2005a). It was not found in this state until seven years later (Martins et al., 2016), after the state is characterized by high rates of deforestation and forest degradation (Souza et al., 2013).

This fact was also previously observed in the state of São Paulo, where the tick *A. sculptum* was not found within the preserved and humid areas of the Atlantic Forest but seen in the drier and degraded areas of this biome (Szabó et al., 2006). In addition, the advancement of humans occupying nearby or forest areas, whether for leisure, economic purposes or housing, facilitates their exposure to ticks, which contributes to a greater occurrence of parasitism cases (Szabó et al., 2013; Confalonieri et al., 2014; Saraiva et al., 2014; Ellwanger et al., 2020; Guégan et al., 2020).

Thus, in response to the effects of environmental change, we may have an expansion of the geographic area in which tick species and the pathogens that use them as a vector occur (Ogden and Lindsay, 2016) and increased parasitism in humans in the Brazilian biomes, due to ticks being generalists (McCoy et al., 2013).

Author contributions

B.C.F.N., T.F.M. and S.M.L. reviewed scientific articles and collected data. B.C.F.N., T.F.M., S.M.L., A.P. and A.K.C. analyzed, interpreted the data and writing. All authors reviewed and approved the final draft of the manuscript.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Apêndice 1: Outros artigos produzidos durante o período de Doutorado

What is the impact of depletion of immunoregulatory genes on wound healing? A systematic review of preclinical evidence. Oxidative medicine and cellular longevity. v. 2020, p. 1-19, 2020.

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Autores: Bárbara Cristina Félix Nogueira; Marcos Antônio Bezerra-Santos; Ricardo Seiti Yamatogi; Artur Kanadani Campos.

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Autores: Bárbara Cristina Félix Nogueira; José Eduardo Garcia Campos, Liara Azevedo Cassiano; Artur Kanadani Campos; Rômulo Ribon.

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