UNIVERSIDADE FEDERAL DE UBERLÂNDIA
Instituto de Ciências Biomédicas
Programa de Pós-Graduação em Imunologia e Parasitologia Aplicadas

PESQUISA DO AGENTE DA FEBRE MACULOSA, *RICKETTSIA PARKERI* CEPA MATA ATLÂNTICA, NO ESTADO DE MINAS GERAIS.

Rodrigo da Costa Maia

Uberlândia, MG
Março, 2026

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PESQUISA DO AGENTE DA FEBRE MACULOSA, *RICKETTSIA PARKERI* CEPA MATA ATLÂNTICA, NO ESTADO DE MINAS GERAIS.

Tese apresentada ao Colegiado do Programa de Pós-Graduação em Imunologia e Parasitologia Aplicadas como parte de obtenção do título de Doutor.

Rodrigo da Costa Maia

Discente

Prof. Dr. Matias Pablo Juan Szabó

Orientador

Uberlândia, MG

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A Deus por sempre estar comigo e abençoar quem me rodeia.

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ATA DE DEFESA - PÓS-GRADUAÇÃO

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Projeto de Pesquisa de vinculação:	Detecção do agente zoonótico *Rickettsia Parkeri* em carrapatos no estado de Minas Gerais e avaliação do risco de exposição humana				

Reuniu-se dia vinte e seis de março de dois mil e vinte e seis, às quatorze horas, em formato híbrido na sala 2B213 e por Webconferência a Banca Examinadora, designada pelo Colegiado do Programa de Pós-graduação em Imunologia e Parasitologia Aplicadas (ColPPGIPA), assim composta: Prof. Dr. Marcelo Bahia Labruna - USP; Prof. Dr. Adriano Pinter dos Santos - USP; Prof. Dr. Hermes Ribeiro Luz - UFMA; Prof. Dr. Stefan Vilges de Oliveira - UFU e Prof. Dr. Matias Pablo Juan Szabó - UFU, presidente e orientador do candidato.

Iniciando os trabalhos o(a) presidente da mesa, Dr. Matias Szabó, apresentou a Comissão Examinadora e o candidato, agradeceu a presença do público, e concedeu ao Discente a palavra para a exposição do seu trabalho. A duração da apresentação do Discente e o tempo de arguição e resposta foram conforme as normas do Programa.

A seguir o senhor(presidente concedeu a palavra, pela ordem sucessivamente, aos examinadores, que passaram a arguir o candidato. Ultimada a arguição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu o resultado final, considerando o candidato:

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Carrapatos são ectoparasitas hematófagos responsáveis pela transmissão de doenças para humanos e animais. Dentre essas, a Febre Maculosa Brasileira causada por *Rickettsia rickettsii* que detêm destaque em função de sua alta letalidade quando tardiamente diagnosticada. Casos confirmados dessa doença possuem íntima relação com carrapatos do gênero *Amblyomma*, este, representante da maior diversidade de espécies de ixodídeos no Brasil. Por outro lado, outras espécies do gênero *Rickettsia*, como *Rickettsia parkeri* cepa Mata Atlântica, podem causar quadros clínicos mais brandos e frequentemente subdiagnosticados, embora mantenham importância epidemiológica. Minas Gerais é um estado com grandes proporções geográficas com áreas que favorecem a circulação de ambas as bactérias, assim como seus vetores, no entanto a *R parkeri* cepa Mata Atlântica associada ao seu principal vetor *Amblyomma ovale* nunca foi relatada neste estado. Neste trabalho, divido em dois artigos, avaliamos no Estado a exposição a antígenos em amostras de soro de cães, importantes sentinelas dessa doença, e material genético, em carrapatos, além da sazonalidade do *Amblyomma ovale*, o vetor da *R. parkeri* cepa Mata Atlântica. Essas observações permitiram concluir maior frequência e abundância do vetor *A. ovale* na primavera, com maior intensidade em novembro, além de evidenciar a exposição aos antígenos riquetsiais em cães de propriedades rurais de Minas Gerais. Além disso, foi constatada a presença de DNA de *R. parkeri* cepa Mata Atlântica em dois carrapatos, *A. ovale*¸ configurando o primeiro relato dessa bactéria associada a esse carrapato em Minas Gerais.

Palavras-Chave: *Amblyomma ovale*; cão; *Rickettsia parkeri*; Minas Gerais.

Ticks are hematophagous ectoparasites responsible for transmitting diseases to humans and animals. Among these diseases, Brazilian Spotted Fever, caused by *Rickettsia rickettsii*, stands out due to its high lethality when diagnosed late. Confirmed cases of this disease are closely associated with ticks of the genus *Amblyomma*, which represents the greatest diversity of ixodid species in Brazil. On the other hand, other species of the genus *Rickettsia*, such as *Rickettsia parkeri* Atlantic rainforest strain (ARF), may cause milder and frequently underdiagnosed clinical manifestations, although they still hold epidemiological importance. Minas Gerais is a state of large geographic proportions, with areas that favor the circulation of both bacteria, as well as their vectors. However, *R. parkeri* ARF , associated with its main vector *Amblyomma ovale,* had never been reported in this state. In this study, divided into two articles, we evaluated in the state of Minas Gerais the exposure of dogs, important sentinels of this disease, to the rickettsial antigens, as well as the presence of genetic material in ticks, in addition to assessing the seasonality of *A. ovale*, the main vector of *R. parkeri* ARF. These observations allowed us to conclude that the vector *A. ovale* shows higher frequency and abundance in spring, with greater intensity in November. Furthermore, the results provided evidence of exposure to rickettsial antigens in dogs from rural properties in the state of Minas Gerais, Brazil. Furthermore, the presence of *R. parkeri* ARF DNA was detected in two *A. ovale* ticks, constituting the first report of this bacterium associated with this tick in Minas Gerais.

Keywords: *Amblyomma ovale*; cão; *Rickettsia parkeri*; Minas Gerais.

SUMÁRIO

1. INTRODUÇÃO

1.1 Apresentação

Nesta tese, desenvolvida em formato alternativo, são apresentados dois artigos científicos que, em conjunto, abordam a exposição de cães a *Rickettsia* spp. e a presença dessas bactérias em carrapatos, assim como aspectos ecológicos do carrapato vetor. Inicialmente é feita uma introdução geral que contextualiza a importância epidemiológica desse ectoparasita, especialmente aquelas associadas a carrapatos do gênero *Amblyomma*. Em seguida, são apresentados os objetivos dos dois trabalhos que orientaram a pesquisa. No primeiro descrevemos o padrão sazonal de ocorrência do vetor da *R. parkeri* cepa Mata Atlântica o carrapato *Amblyomma ovale*, em cães de propriedades rurais de Minas Gerais. No segundo descrevemos a composição das espécies de carrapatos que parasitam cães em diferentes municípios de Minas Gerais, bem como a sororreatividade canina para riquétsias e a identificação de *R. parkeri* cepa Mata Atlântica em sua associação com *A. ovale*.

1.2 Fundamentação Teórica

Carrapatos são ectoparasitas hematófagos que ganharam destaque, principalmente, devido ao seu papel como vetores de patógenos que infectam humanos e animais, além de resultarem na diminuição da produtividade de rebanhos (GRISI *et al.*, 2014; JONGEJAN; UILENBERG, 2004).

Tais problemas tem como fator fundamental a hematofagia, que permite a transmissão de doenças e espoliação de animais. Os carrapatos constituem um grupo monofilético, com ressalvas para o gênero *Amblyomma* Koch, 1844, e evidências filogenéticas sugerem que seus ancestrais eram ácaros de vida livre que se alimentavam de fluidos corporais de animais mortos ou debilitados. Entretanto, mecanismos fisiológicos dos hospedeiros, como a coagulação sanguínea e a agregação plaquetária, impediam a exploração de recurso alimentar mais nutritivo, como o sangue (BARKER; BURGER, 2018; WALTER; PROCTOR, 1998).

No entanto, ao longo do processo evolutivo, adaptações em sua saliva, incluindo moléculas com atividade anticoagulante, como inibidores de trombina, além de compostos capazes de inibir a agregação plaquetária, permitiram o estabelecimento da hematofagia, contribuindo para o estreitamento da relação ecológica e evolutiva entre carrapatos e seus hospedeiros(MANS; LOUW; NEITZ, 2002).

Assim que passíveis de se alimentar do sangue de animais, carrapatos adquiriram o potencial para se tornar um grande problema para saúde humana e animal. No entanto, a relação entre carrapatos e seus hospedeiros data de mais de 30 milhões de anos, muito antes do estabelecimento de humanos como espécie dominante, com achados de espécimes preservadas em âmbar, como exemplo *Ornithodoros antiquus* e fosseis atribuídos ao gênero *Amblyomma* (LANE; POINAR, 1986; POINAR, [S.d.]). Essa amplitude temporal, somada a variedade e a dispersão de suas espécies e hospedeiros são fatores que contribuíram para que os carrapatos estejam presentes em grande parte do globo (GUGLIELMONE; NAVA; ROBBINS, 2023).

Em se tratando desses parasitas, a família Ixodidae, os carrapatos duros, têm papel de maior importância e é dividida em dois grupos principais: Prostriata, representada pelo gênero, *Ixodes* Latreille, definido pela curvatura do sulco anal anterior ao ânus; e Metastriata, que contém 17 gêneros e com a curvatura do sulco anal posterior ao ânus ou sulco anal ausente. No Brasil o grupo Metastriata possui as espécies de maior relevância econômica e zoonótica (ANDERSON; AMMERMAN; NORRIS, 2004; BARROS-BATTESTI; MACHADO; ANDRÉ, 2024)

O gênero *Rhipicephalus* possui maior importância veterinária. O carrapato do boi, *Rhipicephalus microplus*, por sua capacidade espoliativa em rebanhos e transmissão de doenças, gera prejuízo estimado em mais de 3 bilhões de dólares ao ano, grande parte devido à dificuldade no seu controle em função da resistência a uma ampla gama de carrapaticidas (DA SILVA RODRIGUES *et al.*, 2025; GRISI *et al.*, 2014) . As espécies do complexo *Rhipicephalus sanguineus sensu lato*, também conhecidos como carrapatos marrons do cão, estão entre os principais ectoparasitas de cães transmitindo doenças como a erliquiose e anaplasmose (AGUIAR *et al.*, 2007)

Já o gênero *Amblyomma* se destaca pela importância zoonótica, espécies desse gênero são os principais vetores em casos relatados de Febre Maculosa Brasileira (FMB), doença com altos índices de letalidade quando não tratada adequadamente (SZABÓ; PINTER; LABRUNA, 2013).

Tendo em vista essa importância, estudos que envolvem os carrapatos e doenças transmitidas por carrapatos (DTC) tiveram significativo aumento, nos últimos 10 anos, foi possível notar um crescimento de mais de 70% no número de publicações, de 893 trabalhos em 2015 para 1.590 em 2024. Desse montante destacam-se, numericamente, estudos envolvendo essas doenças transmitidas por carrapatos e estudos sobre espécies de carrapatos relacionadas a cães e bovinos (SU *et al.*, 2025).

Ainda assim, se considerada a ampla gama de espécies e suas particularidades, a necessidade de entender a ecologia desses vetores permanece, principalmente em locais como o Brasil, por sua extensão territorial que permite que albergue rica diversidade de espécies de carrapatos, hospedeiros e biomas aos quais pertencem. Nesse contexto, é importante ressaltar a interface criada entre humanos, vida selvagem e animais de companhia e produção, principalmente em propriedades rurais (FOLEY *et al.*, 2025).

Essa composição leva em conta diversas características, algumas delas relacionadas aos estágios de vida do carrapato. Larvas e ninfas daquelas espécies que fazem ciclo de vida de dois ou três hospedeiros (dioxenas e trioxenas, respectivamente) se alimentam, em geral, em pequenos vertebrados, esses estágios são menos específicos que os adultos, podendo parasitar uma maior diversidade de hospedeiros enquanto os adultos preferem animais de médio e grande porte, comportamento notado em várias espécies do gênero *Amblyomma* (SONENSHINE; ROE, 2014).

Carrapatos que participam dessa interface, também possuem diferentes estratégias para encontrar seus hospedeiros, espécies passivas, como a maioria dos carrapatos nidícolas encontrados preferencialmente em ninhos e tocas dependem do contato com animais vertebrados que o invadem ou utilizam e espécies não nidícolas, que usam a estratégia de emboscada ou busca ativa, quando os carrapatos deixam seu local de repouso e procuram ou aguardam ativamente seus hospedeiros (SONENSHINE; ROE, 2014; SUZIN *et al.*, 2020).

Adicionalmente pode ser observada uma variação sazonal da ocorrência de espécies e estágios determinada pelo hospedeiro e por fatores abióticos, como temperatura, fotoperíodo e umidade relativa, que atuam regulando o ciclo biológico do carrapato. Esses fatores têm um papel importante na duração de cada fase de desenvolvimento fora do hospedeiro (por exemplo, oviposição, incubação de ovos, eclosão de larvas e ecdise), além de influência direta na indução da diapausa, principalmente em regiões não equatoriais, modulando os ciclos em ritmos sazonais que garantem aos carrapatos a sincronização de suas atividades com as condições climáticas adequadas para sua sobrevivência e encontro com hospedeiro (DA SILVA RODRIGUES *et al.*, 2026; SONENSHINE; ROE, 2014; SUZIN *et al.*, 2020).

Acompanhada de sua complexidade fisiológica e comportamental tem-se a associação de carrapatos com patógenos, que implicam em problemas na saúde humana. A Febre Maculosa Brasileira (FMB) é uma doença causada por uma bactéria intracelular obrigatória que leva a uma condição febril que pode ser fatal se não tratada prontamente. A bactéria intracelular *Rickettsia rickettsii,* no Brasil, é a espécie de maior importância zoonótica se consideradas as

DTCs e tem como principais vetores carrapatos como *Amblyomma sculptum* e *Amblyomma aureolatum* (SZABÓ; PINTER; LABRUNA, 2013).

A capivara (*Hydrochoerus hydrochaeris)* está intimamente relacionada a ecoepidemiologia da FMB, principalmente por ser presente em ambientes antropizados e servir como hospedeiro amplificador de carrapatos vetores e da bactéria. Estágios imaturos, para *A. sculptum* são principais vetores dessa doença para humanos. Porém cães também participam da ecoepidemiologia da FMB através do transporte de um outro vetor da bactéria, adultos do carrapato *A. aureolatum*, para domicílios (DONALISIO *et al.*, 2020; *SZABÓ; PINTER; LABRUNA, 2013*).

Ainda assim, outros mamíferos desempenham papel fundamental na transmissão de FMB para seres humanos. Cães domésticos (*Canis lupus familiaris)*, são conhecidos pela sua intima relação com seres humanos, essa relação é antiga e o condicionamento desses canídeos é evidente ao receber reforço direto. Nesse sentido os cães podem adentrar ambientes considerados de maior risco, em relação ao parasitismo por carrapatos, sem percepção de perigo, funcionando como importantes pontes epidemiológicas entre ambientes silvestres e humanos (BRÄUER; SCHÖNEFELD; CALL, 2013; FOLEY *et al.*, 2025).

Minas Gerais é um estado brasileiro, localizado no Sudeste do Brasil, região responsável por grande parte dos casos de FMB (DONALISIO *et al.*, 2020; SZABÓ; PINTER; LABRUNA, 2013). A abrangência territorial desse estado faz com que biomas diferentes o componham, incluindo a Mata Atlântica, com biodiversidade tão rica quanto ameaçada (RIBEIRO *et al.*, 2009). No entanto, tal bioma tem relação mais próxima a outra bactéria, também causadora de doença febril, no entanto mais branda, a *Rickettsia parkeri* cepa Mata Atlântica (CPM), tal patógeno tem como vetor o *Amblyomma ovale*, carrapato que parasita, preferencialmente, pequenos roedores em seus estágios imaturos e carnívoros, incluindo os cães, quando adulto (BARROS-BATTESTI; MACHADO; ANDRÉ, 2024).

Embora o vetor, ambiente e hospedeiro estejam presentes nesse estado o diagnóstico de febre maculosa causada por *R. parkeri* CPM ainda não foi notificado. No entanto, é possível que essa ausência se deva a escassez de estudos que tenham como foco esse patógeno. Em vista disso, nesse documento, apresentaremos dois trabalhos. No primeiro exploramos a sazonalidade do vetor dessa doença o *A. ovale¸* no estado de MG e, no segundo, avaliamos a ixodofauna, presença do patógeno *R. parkeri* CPM e a soroconversão de cães para essa doença.

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3. OBJETIVOS

2.1 Objetivo Geral

Prospectar evidências sorológicas, ecológicas vetoriais e moleculares da circulação do patógeno *Rickettsia parkeri* cepa Mata Atlântica no estado de Minas Gerais

2.2 Objetivos Específicos

- Identificar a ixodofauna em cães de propriedades rurais com acesso a áreas naturais no estado de Minas Gerais;
- Avaliar a presença de anticorpos anti-riquetsia em amostras de soro de cães de propriedades rurais com acesso a áreas naturais no estado de Minas Gerais;
- Avaliar a dinâmica sazonal da ocorrência de carrapatos em cães de propriedades rurais com acesso a áreas de naturais no estado de Minas Gerais;
- Avaliar presença de DNA de riquetsias em carrapatos de cães de propriedades rurais com acesso a áreas de mata no estado de Minas Gerais;

4. MANUSCRITOS

3.1 Seasonality of the Rickettsia vectors Amblyomma ovale and Amblyomma sculptum on dogs in Atlantic Forest and Cerrado Biomes of Minas Gerais, Brazil and its potential association with human Rickettsia parkeri rickettsioses

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Abstract

Free-ranging rural dogs in Brazil are parasitized by ticks from indigenous ixodofauna including *Amblyomma sculptum* and *Amblyomma ovale*, vectors of *Rickettsia rickettsii* and *Rickettsia parkeri* strain Atlantic Rainforest (ARF), respectively. Therefore, dogs are important sentinels and hosts for ticks and tick-borne diseases of public health relevance. This study evaluated abundance, and seasonal dynamics of these tick species infesting free-ranging rural dogs in two municipalities of Minas Gerais State, southeastern Brazil: Araguari (Cerrado biome remnants) and Guaxupé (Atlantic Forest remnants). Tick collections were conducted monthly between 2022 and 2024 through systematic examinations of dogs as well as environmental sampling in Guaxupé. In Araguari, canine infestations were dominated in spring by nymphs of *A. sculptum* an important vector of *R. rickettsii* while adults of this tick were rarely detected. This pattern reflects the limited suitability of dogs as hosts for adult *A. sculptum* and suggests that canine exposure is dependent on the presence of primary hosts, such as capybaras and equines. In contrast, *Rhipicephalus linnaei* was the most prevalent and abundant species on dogs in Guaxupé, particularly among animals more confined to household environments, reinforcing its synanthropic and endophilic behavior. Infestations by *R. linnaei* showed no clear seasonality, supporting a continuous life cycle without diapause. *Amblyomma ovale* exhibited high prevalence and abundance on dogs in Guaxupé, likely associated with humid Atlantic Forest fragments that favor its life cycle. Only adult *A. ovale* ticks were found on dogs, and they showed marked seasonal aggregation during spring, with peak abundance in November and persistence into summer. This seasonal pattern closely mirrors the temporal distribution of human rickettsiosis cases reported in Santa Catarina State, southern Brazil, the only state where *A. ovale* infected with *R. parkeri* ARF strain is recognized as the sole source of human infection. In conclusion, this study provides the first characterization of the seasonal pattern of adult *A. ovale* on dogs. A potential relationship between vector seasonality and human rickettsiosis is suggested and deserves further investigation.

Keywords: Ticks, Dogs, Cerrado, Atlantic rainforest, Rickettsiosis

1 Introduction

Understanding the seasonal prevalence of vector-borne infectious diseases allows for better planning and implementation of preventive measures. Additionally, it aids in distinguishing between diseases with similar clinical presentations but different seasonal patterns of occurrence. The life cycle of vectors such as ticks is directly related to the seasonality of vector-borne diseases. This is particularly prominent in temperate countries of the Northern Hemisphere, where pathogens responsible for severe illnesses are transmitted during the peak activity period of ticks following winter (Eisen et al., 2017). However, seasonality also characterizes the transmission of tick-borne diseases in tropical and subtropical regions. In Brazil, the transmission of *Rickettsia rickettsii* to humans occurs more frequently during spring, period of increased activity of nymphs the primary vector, the tick *Amblyomma sculptum* (Angerami et al., 2009; Szabó et al., 2013a).

Rickettsioses are infectious diseases caused by intracellular bacteria of the genus *Rickettsia*, which are globally distributed and transmitted by vectors such as fleas, lice, mites and ticks (Parola et al., 2013). In Brazil, rickettsioses represent the most significant tick-borne illnesses for public health. Among them, Brazilian spotted fever, caused by *R. rickettsii*, is the most severe. This disease in Brazil is mainly transmitted by *A. sculptum*, but geographically more restricted cases have also been linked to *Amblyomma aureolatum*, and potentially to *Rhipicephalus sanguineus* sensu lato (Szabó et al., 2013a). More recently, a novel human rickettsiosis caused by *Rickettsia parkeri* Atlantic Forest strain (*R. parkeri* AR) has been described in Brazil (Spolidorio et al., 2010). This pathogen is transmitted by adult *Amblyomma ovale* ticks and leads to a milder, non-lethal illness. The clinical manifestations are nonspecific, including fever, headache, and myalgia, and may also involve lymphadenopathy and a necrotic lesion at the tick bite site. However, these signs can be easily mistaken for other infectious diseases (Rodrigues et al., 2023; Silva-Ramos et al., 2021).

Even though few human cases have been unequivocally confirmed, human rickettsiosis caused by *R. parkeri* ARF appears to be highly prevalent in areas where the infected vector is present, as supported by retrospective case analyses in Santa Catarina State (Angerami et al., 2009; Barbieri et al., 2014; Rodrigues et al., 2023). At the same time, the tick *A. ovale* infected with *R. parkeri* ARF is known to have a broad geographic distribution, primarily, but not exclusively, associated with the Atlantic Forest biome in Brazil, as well as in other countries in South and Central America, with infection rates ranging from 0% to 27% (Rodrigues et al., 2023). Therefore, human infection is potentially underdiagnosed and likely misidentified as other prevalent febrile illnesses in Brazil, such as dengue and other viral infections (Szabó et al., 2013b).

Larvae and nymphs of *A. ovale* parasitize small mammals and birds, while adults are common parasites of tapirs and wild carnivores (Guglielmone et al., 2021). In these settings, the dog emerges as an alternative host for adult ticks and may develop substantial infestations, provided it has access to forested habitats. Therefore, understanding the seasonal prevalence of adult *A. ovale* ticks on dogs would enable more active surveillance and more accurate diagnosis of human rickettsiosis caused by *R. parkeri* ARF.

Previous studies have indicated that many tick species of the *Amblyomma* genus in Brazil exhibit an annual life cycle, with distinct periods of peak occurrence for each developmental stage (Labruna et al., 2009). In the specific case of *A. ovale*, a previous observation on tick seasonality in Iguaçu National Park reported a peak in the occurrence of adult *A. ovale* ticks along forest trails in spring, specifically in November (Suzin et al., 2020).

Regarding the main vector of *R. rickettsii* in Brazil, the tick *A. sculptum*, is considered a characteristic tick species of the tropical savannah biome (Cerrado), where it represents the dominant tick species (Martins et al., 2016; Barbieri et al., 2019; Szabó et al., 2018). In Brazil, horses, capybaras, tapirs, wild pigs, and giant anteaters are regarded as primary hosts for adult *A. sculptum* (De Paula et al., 2022; Ramos et al., 2014; Szabó et al., 2019). In contrast, immature stages of this species parasitize a wide range of mammals, including dogs (Guglielmone et al., 2021). In fact, dogs are considered amplifier hosts that increase the infection rate of *R. rickettsii* in vector populations at least for *A. aureolatum* and *R. sanguineus* sensu lato ticks (Binder et al., 2021; Foley et al., 2025). However, the involvement, especially the quantitative and temporal assessment of infestation of domestic dogs with *A. sculptum* across different Brazilian biomes remains poorly characterized. Given the close association between humans and dogs, this potential host–parasite interaction may have important implications for human exposure to *A. sculptum*. Therefore, the aim of this study was to characterize the seasonality of *A. ovale* and *A. sculptum* ticks infesting rural dogs with access to, respectively, areas of the Atlantic Forest and Cerrado biome remnants in the state of Minas Gerais. These findings may be relevant to understanding the epidemiology of human rickettsioses in Brazil.

2 Material and Methods

2.1 Study site

The study was conducted in rural areas of the municipalities of Araguari and Guaxupé, Minas Gerais, Brazil (Figure 1). Araguari located in the Cerrado Biome has a tropical savanna climate (Aw) according to the Köppen–Geiger classification, with mean annual temperatures of 20–25 °C and approximately 1006 mm of annual precipitation. The hottest months are January, September, and October (mean 24–25 °C), whereas the coolest months, May to July, show mean temperatures of 20–21 °C. Local elevations range from 500 to 1000 m (Nomad Season, 2023a; Infosanbas, 2023a).

Guaxupé has a humid subtropical climate (Cwa), with temperatures generally ranging from 17–23 °C and an average annual precipitation of 1500 mm. The warmest period also occurs in January, September, and October (mean ~23 °C), while the coldest months (May–July) range from 17–18 °C. The municipality lies at higher elevations (700–1200 m) and represents a transition zone between the Atlantic Forest and Cerrado biomes (Nomad Season, 2023b; Infosanbas, 2023b).

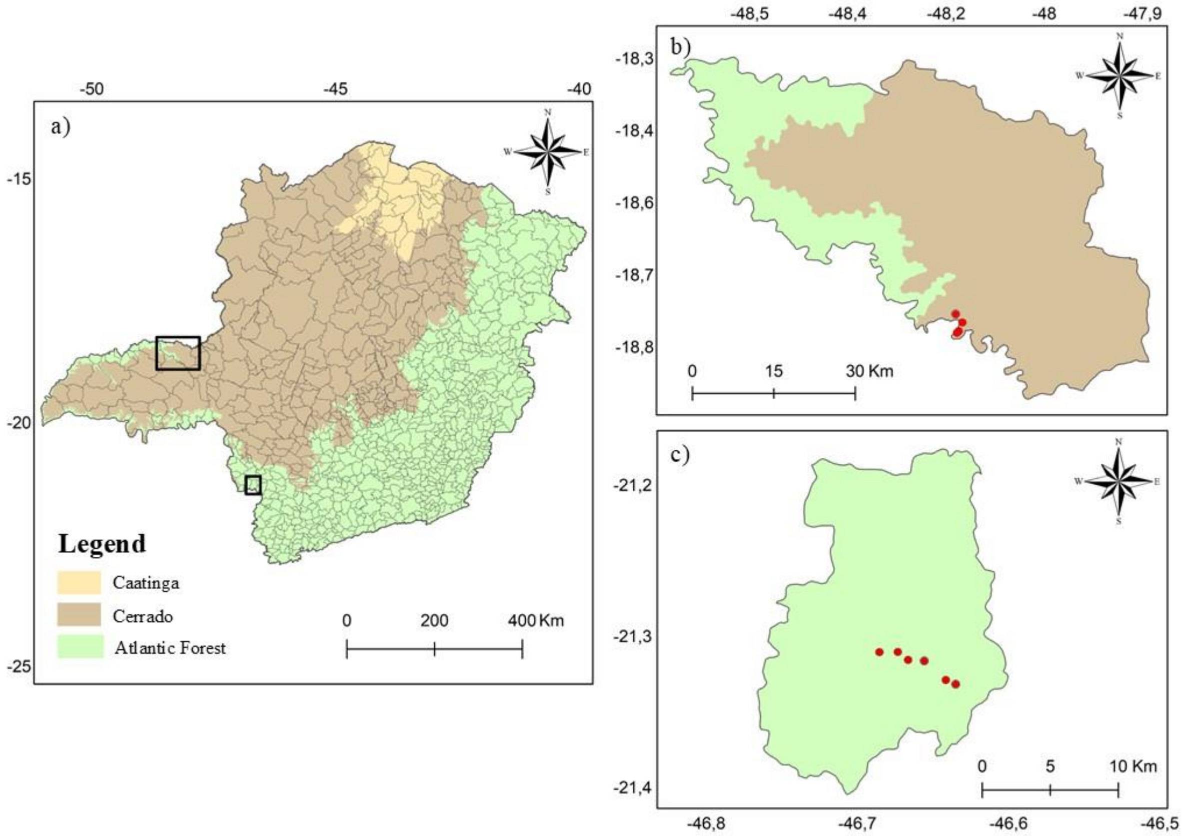

Figure 1. Map of Minas Gerais, Brazil, showing the distribution of its major biomes: Caatinga (yellow), Cerrado (brown), and Atlantic Forest (green). The black squares indicate the regions where the municipalities included in the study are located. Red dots represent the specific sampling sites where ticks were collected**.**

2.2 Ticks on dogs

Unrestrained dogs from rural properties with access to Atlantic Forest patches (Guaxupé) or Cerrado fragments (Araguari) were included in the study. Sampled dogs were classified as "rural free-ranging dogs" following Vanak and Gompper (2009), meaning that they were owned or loosely associated with human habitations but were not confined to a restricted outdoor area or kennel. Properties were selected after on-site visits to rural areas within each municipality, restricting them to those located near fragments of natural habitats. Importantly, all selected dogs originated from areas within the municipalities where the target tick species had been previously detected (Table 1). This criterion was essential because the ecological conditions required for the maintenance of these ticks, particularly *A. ovale,* are not fully understood, and their distribution in ecotonal environments (i.e., interfaces between natural and anthropized areas) may be highly focal (Szabó et al., 2018).

Table 1. Coordinates and altitude of tick collection sites on dogs in the municipalities of Araguari and Guaxupé, Minas Gerais, Brazil.

Property	Coordinates (S;W)[1]	Elevation (m)
Guaxupé-1	21° 18′ 39.26″; 46° 41′ 07.48″	862
Guaxupé-2	21° 18′ 37.58″; 46° 40′ 24.44″	867
Guaxupé-3	21° 18′ 56.33″; 46° 40′ 00.38″	889
Guaxupé-4	21° 19′ 46.33″; 46° 38′ 28.93″	931
Guaxupé-5	21°18′48.00″ ; 46°40′04.65″	881
Guaxupé-6	21°19′46.36″; 46°38′27.41″	932
Araguari-1	18° 45′ 50.34″; 48° 10′ 57.16″	609
Araguari-2	18° 46′ 42.54″; 48° 11′ 18.99″	578
Araguari-3	18° 46′ 52.19″; 48° 11′ 29.37″	573
Araguari-4	18°45′22.39″; 48°11′34.60″	586

[1]Coordinates refer to the reference properties; dogs from neighboring properties were sampled when those from the reference properties were unavailable.

Dog selection followed a targeted approach based on three main criteria: (i) location in areas prone to tick occurrence, (ii) owner-reported roaming behavior and access to near fragments of natural habitats, and (iii) prior evidence of infestation by the target tick species in each municipality. In several cases, access of dogs to fragments of natural habitats was supported by the presence of injuries and scars consistent with encounters with wildlife, such as porcupines and anteaters.

Dogs were examined monthly from November 2022 to November 2024 in Guaxupé, and from June 2022 to November 2024 in Araguari, allowing the assessment of tick infestation patterns twice across all seasons. Each month, at least 10 dogs from a minimum of six properties in Guaxupé and four properties in Araguari were thoroughly examined. The sample size was not estimated to represent the overall dog population of the municipalities, as the study was not designed for population-level inference of tick infestation. Instead, the sampling strategy aimed to capture seasonal variation in infestation and was considered representative of the population of dogs exposed to the target tick species within each municipality over the study period. Further, dogs were not followed as a fixed cohort; rather, they were sampled at each time point, depending on their availability on the area of the study. This approach is particularly relevant in rural areas of Brazil, where high turnover rates of dogs are commonly observed, with frequent replacement of animals over time; annual replacement rates of up to 59% have been reported (Brandão et al., 2020). Although several animals were repeatedly examined throughout the two-year study period, many others were evaluated only once across sampling events.

All animals underwent thorough physical examination, and all visible ticks were collected. In cases of heavy infestations (>100 ticks per animal), representative samples were collected. Collected ticks were preserved in 70% ethanol. Dogs that had been recently treated with antiparasitic products were not included in the sampling. Information regarding previous treatments was obtained from owners when available.

2.3 Seasonality of host-questing *Amblyomma ovale* ticks

Since the seasonality of *A. ovale* ticks has not been specifically addressed before, monthly environmental sampling of host-questing ticks was performed in Guaxupé from November 2022 to November 2024, coinciding with dog examinations. A 100-m stretch of dense bamboo vegetation near the sampled properties was selected after preliminary surveys; the site remained shaded, cool, and maintained a persistently humid litter layer. Each month, ticks were collected by dragging a white flannel cloth (1 m × 1.8 m) for a total of 60 minutes (20 minutes per collector × three collectors) along the perimeter and through the bamboo grove. Specimens were preserved in 70% ethanol.

2.4 Tick identification

Ticks were identified based on external morphology following dichotomous keys (Martins et al., 2010; Barros-Battesti et al., 2025; Martins et al., 2026). Almost fully engorged larvae of *Amblyomma* spp. were transferred to an incubator maintained at 27 °C and 85% relative humidity to allow ecdysis and subsequent identification at the nymphal stage. In many cases, *A. sculptum* larvae were found in large, tightly aggregated clusters, particularly along the margins of the dogs' ears. The removal of these densely packed larvae using forceps, especially in restless dogs not accustomed to handling, resulted in damage to some specimens. In such cases, after the identification of representative larvae within a given cluster, the remaining specimens in the same aggregate on the host were conservatively assigned to the same species.

Representative specimens of the *Rhipicephalus sanguineus* complex were subjected to molecular analysis due to the limitations of morphological identification within this group. Molecular identification of two *Rhipicephalus sanguineus* sensu lato specimen for each municipality was carried out. DNA extraction was carried out using the guanidine isothiocyanate-phenol method (Sangioni et al., 2005) and subjected to polymerase chain reaction (PCR) for amplification of a fragment of the 16S rRNA gene (Mangold et al., 1998). Briefly, PCR was performed using primers 16S-F (CCG GTC TGA ACT CAG ATC AAG T) and 16S-R (GCT CAA TGA TTT TTT AAA TTG CTG T). PCR reactions consisted of an initial denaturation at 95 °C for 10 min, followed by 35 cycles of 95 °C for 30s, 48 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 7 min, producing a 460 bp amplicon. The amplicons were visualized by electrophoresis on a 1.2% agarose gel. The fragment was purified using the PureLink® kit (Invitrogen Life Technologies). The purified material was then sent for sequencing by the Sanger method at a certified laboratory (Genoma USP®). The partial sequence obtained was manually edited using SeqMan Pro from the Lasergene package, version 15 (DNASTAR Inc., Madison, WI, USA), and compared through the BLASTn tool (Altschul et al., 1990). The generated sequence was submitted to BLAST analysis (www.ncbi.nlm.nih.gov/blast) to infer the closest similarities available in the GenBank.

2.5 Seasonality of human rickettsiosis

Following the observation of a well-defined seasonal pattern of adult *A. ovale* infesting dogs in the study area, we sought to investigate whether a corresponding, and until now unknown, seasonal pattern could be observed in human rickettsiosis caused by *R. parkeri* (ARF), based on data from the Notifiable Diseases Information System (SINAN) of the Brazilian Ministry of Health. This comparison was not part of the original study design but emerged as a relevant question after the identification of consistent seasonality in the vector.

Human case data from the state of Santa Catarina, southern Brazil, were used as a proxy to assess seasonality. Although geographically distinct from the study area, this dataset represents the only currently available epidemiological scenario in Brazil in which human rickettsiosis is predominantly associated with *R. parkeri* ARF, minimizing confounding due to co-circulation of other rickettsial species.

Spotted fever has been considered a notifiable disease in Brazil since 2001 due to its public health relevance, and its reporting to SINAN is mandatory and immediate (Brazil, 2001). However, the laboratory diagnosis recommended by the Brazilian Ministry of Health relies primarily on serological evidence obtained by indirect immunofluorescence assay (IFA) using *R. rickettsii* antigens (Oliveira et al., 2016). Due to serological cross-reactivity among spotted fever group rickettsiae, this approach does not allow reliable differentiation among distinct rickettsial species, representing a major limitation for species-specific epidemiological analyses in Brazil (Faccini-Martínez et al., 2021). Unlike other Brazilian states with a high incidence of spotted fever, where serological results may include infections caused by both *R. rickettsii* and *R. parkeri*, leading to temporal overlap of distinct rickettsioses, human cases in Santa Catarina appear to be exclusively associated with *R. parkeri* ARF (Angerami et al., 2009; Barbieri et al., 2014; Oliveira et al., 2016; Faccini-Martínez et al., 2021). This characteristic makes Santa Catarina data the only suitable in Brazil for assessing the seasonality of human rickettsiosis likely attributable to *R. parkeri* ARF.

Despite the geographic mismatch between vector seasonality in southeastern Brazil and human cases in southern Brazil, it is noteworthy that previous observations suggesting a seasonal pattern of *A. ovale* were first reported in southern Brazil (e.g., Suzin et al., 2020), which is consistent with the seasonal pattern identified in the present study. Therefore, this analysis was conducted as an exploratory, hypothesis-generating approach rather than to establish a direct epidemiological relationship.

2.6 Data analysis

Descriptive statistics were used to analyze tick infestation parameters, including prevalence (number of tick-infested dogs / total number of examined dogs), mean intensity of infestation (total number of ticks / number of infested dogs), and mean infestation abundance (total number of ticks / total number of examined dogs).

To assess the seasonality of tick infestation, mean infestation abundance was used as a proxy for the seasonal distribution of each tick species. For *A, sculptum* and *A. ovale*, circular statistics were applied using the circular package (Lund et al., 2017) in R software (version 4.5.2; R Core Team, 2025). The Rayleigh test was selected due to its robustness in detecting non-uniformity in circular distributions, allowing the identification of significant seasonal patterns in monthly infestation data. The analysis focused on identifying periods of significant temporal aggregation.

Differences between the abundance of *R. linnaei* adults and *A. sculptum* nymphs, most abundant in, respectively, Guaxupé and *A. sculptum*, were evaluated using generalized linear models (GLMs). A Poisson error distribution with a log link function was used for *R. linnaei* adults, as no overdispersion was detected, whereas a negative binomial GLM was applied for *A. sculptum* nymphs due to significant overdispersion. Tick counts were used as the response variable, and study location was included as the explanatory variable of interest. Sampling effort, which varied according to the number of examined dogs, was accounted for by including the number of examined dogs as a log-transformed offset in all models. Model assumptions were assessed using simulation-based residual diagnostics implemented in the DHARMa package (Hartig, 2022). Statistical analyses were performed in R software (version 4.5.2; R Core Team, 2025).

Data on reported cases of spotted fever (SF) in the state of Santa Catarina were extracted for the period 2001–2024 (Brazil, 2025a; 2025b) using the TabNet platform of DataSUS (Department of Informatics of the Unified Health System). The retrieved data were exported and analyzed using Microsoft Excel and GraphPad Prism version 8.0.1 (GraphPad Software, San Diego, CA, USA). Statistical comparisons among months were performed using the Kruskal–Wallis test followed by Dunn's multiple comparisons test. To allow direct comparison with tick seasonality, monthly human case data were aggregated across years and analyzed using the same circular statistical framework described for tick data. To assess the seasonality of human rickettsiosis cases, monthly case frequencies were treated as circular data, with months converted into angular measurements (radians) over a 12-month cycle. Circular statistical analyses were performed using the *circular* package in R software as described above for tick seasonality. The Rayleigh test was applied to evaluate deviations from a uniform distribution and to detect significant seasonal clustering of cases. Descriptive circular parameters, including the mean angle and angular standard deviation, were calculated to estimate the timing and dispersion of peak occurrence.

2.7 Ethics

All procedures involving dog sampling protocols were approved by the Ethics Committee on Animal Experimentation of the Federal University of Uberlândia (CEUA-UFU) under protocol number 23117.016236/2022-70. Environmental tick collection was authorized by the Brazilian Ministry of the Environment (SISBIO permit nº 48141−5).

3 Results

3.1 Ticks on dogs

In Araguari, 342 dog evaluations (n = 52 dogs; monthly mean = 11.4) yielded 2097 ticks belonging to four species: *Amblyomma sculptum* (n = 2,064), *Rhipicephalus linnaei* (n = 14) and *Rhipicephalus microplus* (n = 18) (Table 2). A single adult *Amblyomma ovale* was also collected in August 2024. In June and July 2022 and June and July 2024, >100 *Amblyomma* spp. larvae were recorded on one animal each month. In September and October 2022, August 2023 (two animals), and September 2023, >100 *A. sculptum* nymphs were recorded on one animal each month. Nine *Amblyomma* spp. larvae collected from three dogs successfully moulted, yielding *A. sculptum* nymphs.

In Guaxupé, 293 dog evaluations (n = 58 dogs; monthly mean = 11.7) yielded 1948 ticks, predominantly *R. linnaei* (n = 1,422), followed by *A. sculptum* (n = 300), adult *A. ovale* (n = 201) and *R. microplus* (n = 25) (Table 2). In March, April, June, and August 2024, >100 adult *R. linnaei* ticks were recorded on one animal each month; in July and November 2024, on two animals per month; and in May 2024, >100 *R. linnaei* nymphs were recorded on one animal. Overall, these nine occurrences were recorded in four animals, with two dogs each presenting three episodes of intense infestation.

Table 2. Tick numbers, prevalence and mean intensity of infestation according to species and stage, collected in dogs from Araguari (52 dogs, 342 evaluations) and Guaxupé (58 dogs, 293 evaluations), 2022-2024.

	Araguari				**Guaxupé**			
	n	Preva-lence	Abun-dance	Inten-sity	n	Preva-lence	Abun-dance	Inten-sity
A. sculptum larvae	657	0.13	1.91	15.33	60	0.02	0.21	10
A. sculptum nymphs	1395	0.41	4.07	9.9	234	0.19	0.79	4.03
A. sculptum adults	12	0.03	0.003	1.33	6	0.02	0.02	1.17
A. ovale adults	1	0.001	0.05	1	201	0.23	0.68	3
R. linnaei larvae	0	0	0	0	3	0.01	0.01	1.5
R. linnaei nymphs	2	0.002	0.005	2	153	0.03	0.52	5.88
R. linnaei[1] adults	12	0.02	0.03	1.5	1266	0.29	4.32	14.89
R . microplus larvae	5	0.01	0.01	1.6	0	0	0	0
R . microplus nymphs	2	0.01	0.005	1	10	0.02	0.03	2
R . microplus adults	11	0.02	0.03	1.37	15	0.02	0.05	2.14

Total	2097	0.44	6.11	13.11	1948	0.56	6.65	11.33

[1]Confirmed by molecular sequencing

Notably, dogs (n = 3–6 dogs) from a property in Guaxupé underwent animal replacement due to deaths. The newly introduced dogs, although not physically confined, exhibited a more home-based lifestyle, which was associated with an increase in *R. linnaei* infestations and a decrease in *A. ovale* infestations. Table 3 compares the evaluated parameters between dogs from this home-based property and those from the remaining more free-ranging dog population in Guaxupé.

Table 3. *Rhipicephalus linnaei* and *Amblyomma ovale* counts, prevalence, and mean intensity of infestation by species and life stage in dogs from the home-based property and more free-ranging dogs, Guaxupé, Minas Gerais.

	Home-based dogs				Free-ranging			
	n	Prevalence	Abundance	Intensity	n	Prevalence	Abundance	Intensity
A. ovale adults	18	0.10	0.17	1.29	183	0.65	0.96	3.27
R. linnaei nymphs	136	0.19	1.31	6.48	17	0.09	0.09	2.13
R. linnaei adults	1175	0.61	11.29	18.36	91	0.22	0.48	5.06

In Araguari, the most abundant ticks were *A. sculptum* nymphs, followed by larvae of the same species. In contrast, in Guaxupé, adult *R. linnaei* were the most abundant, followed by *A. sculptum* nymphs and adult *A. ovale*. In Guaxupé, adult *R. linnaei* were markedly more abundant than in Araguari (Poisson GLM with log link: $p < 0.001$). In contrast, *A. sculptum* nymphs were significantly less abundant in Guaxupé (negative binomial GLM accounting for overdispersion: $p < 0.001$).

In Araguari, the highest mean intensity of infestation was recorded for *A. sculptum* larvae in June 2024 (n = 55.5), followed by *A. sculptum* nymphs in August 2023 (n = 24.8). The highest prevalence (100%) was observed during the same period for this *A. sculptum* stage. Only one adult *A. ovale* was collected, in August 2024.

In Guaxupé, the highest mean intensity of infestation was recorded for adult *R. linnaei* in November 2024 (n = 67.7). The highest prevalences was observed for *A. sculptum* nymphs (71%) in September 2023, *A. ovale* adults (62%) in October 2024 and for adult *R. linnaei* in March 2024 (62%). The mean abundance of adult *R. linnaei* was also highest in March 2024 ($\bar{x}$ = 21.11).

3.2 Seasonality of ticks

3.2.1 *Amblyomma sculptum*

Only a few adult *A. sculptum* were recovered from dogs in both municipalities, mostly during the summer months, whereas larval abundance peaked during the winter months in both municipalities. *Amblyomma sculptum* nymphs showed infestation peaks in winter extending into spring.

Analyses of *A. sculptum* nymph was performed for both municipalities. In Guaxupé the Rayleigh test ($R = 0.759$, $p = 0.0029$), indicate a non-uniform distribution throughout the annual cycle. The mean angle of occurrence was 3.65 radians (SD = 0.74), corresponding to a peak in August (Figure 2a). In Araguari the analysis revealed a highly concentrated seasonal pattern in tick occurrences, with events significantly clustered around a mean angle of 4.09 radians (SD = 0.56; rayleigh test: $R = 0.855$, $p < 0.001$), indicating a strong distribution peaking in September (Figure 2b).

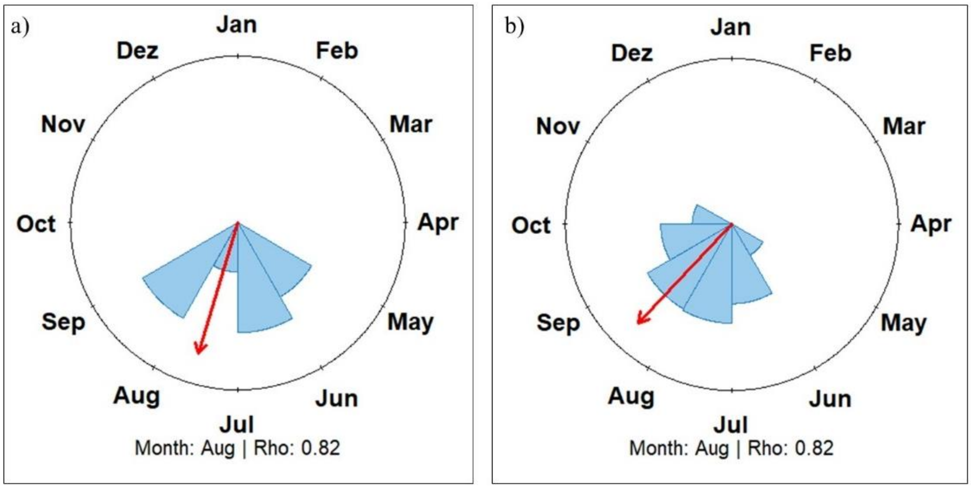

Figure 2. Circular representation of the average distribution of Amblyomma sculptum nymphs on dogs from Guaxupé, Minas Gerais (a), between November 2022 and November 2024, and from Araguari, Minas Gerais (b), between June 2022 and November 2024. Blue areas represent overlapping distribution patterns. Arrows indicate the mean angle (mean month), and the rho value represents the degree of concentration around the main direction.

3.2.2 *Amblyomma ovale*

Only *A. ovale* ticks were collected in the bamboo vegetation. In both the environment and on dogs, only adult stages of this species were found. The mean infestation abundance of adult *A. ovale* on dogs, as well as the absolute numbers collected from the environment, are presented for Guaxupé in Figure 3 and Table 4.

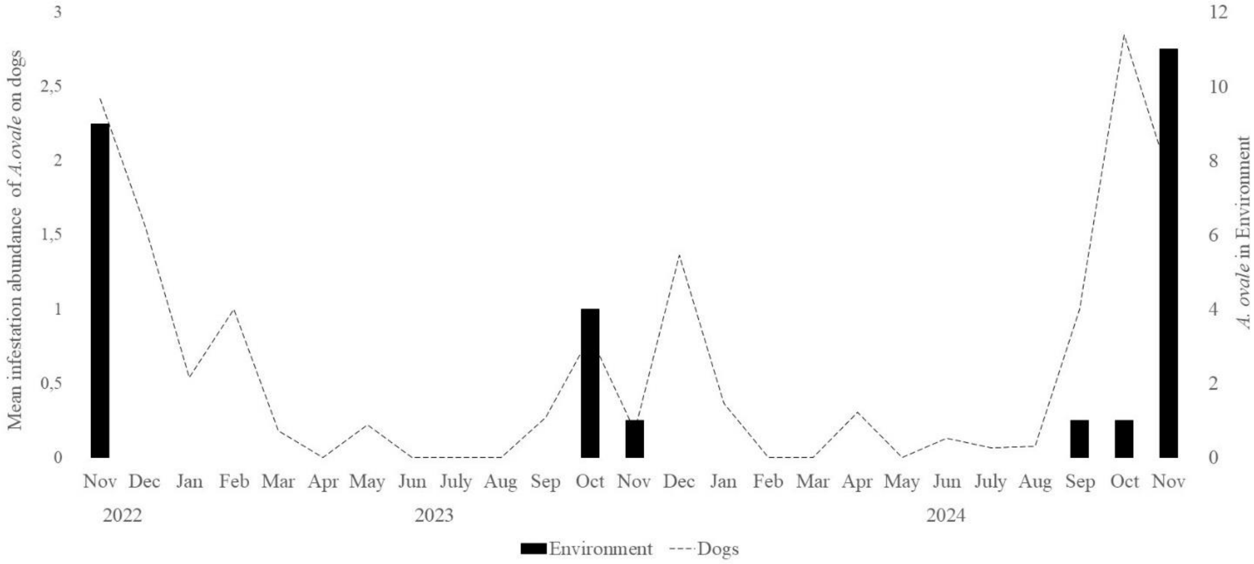

Figure 3. Mean monthly infestation abundance of Amblyomma ovale collected on dogs and the absolute monthly number of A. ovale collected in the environment in Guaxupé, Minas Gerais, Brazil, from 2022 to 2024.

Table 4. Mean monthly infestation abundance of *Amblyomma ovale* collected on dogs and the absolute monthly number of *A. ovale* collected in the environment in Guaxupé, Minas Gerais, Brazil, from 2022 to 2024.

Year	Month	Mean Abundance Dogs	Environment
2022	Nov	2.42	9
	Dec	1.57	0.00
2023	Jan	0.54	0.00
	Feb	1.00	0.00
	Mar	0.18	0.00
	Apr	0.00	0.00
	May	0.22	0.00
	Jun	0.00	0.00
	Jul	0.00	0.00
	Aug	0.00	0.00
	Sep	0.27	0.00
	Oct	0.82	4.00
	Nov	0.18	1.00
	Dec	1.36	0.00
2024	Jan	0.36	0.00
	Feb	0.00	0.00

Mar	0.00	0.00
Apr	0.31	0.00
May	0.00	0.00
Jun	0.13	0.00
Jul	0.07	0.00
Aug	0.08	0.00
Sep	1.00	1.00
Oct	2.85	1.00
Nov	1.90	11.00

On dogs, circular analysis of *A. ovale* data in Guaxupé revealed a non-uniform distribution of occurrences across the annual cycle (Rayleigh test: $R = 0.774$, $p = 0.002$), indicating significant temporal clustering. The mean angle of occurrence was 5.26 radians (SD = 0.72), corresponding to a peak in November (Figure 4a). In the environment, a highly concentrated seasonal pattern was observed, with occurrences significantly clustered around a mean angle of 5.11 radians (SD = 0.27; Rayleigh test: $R = 0.965$, $p < 0.001$), indicating that events were tightly grouped around November (Figure 4b).

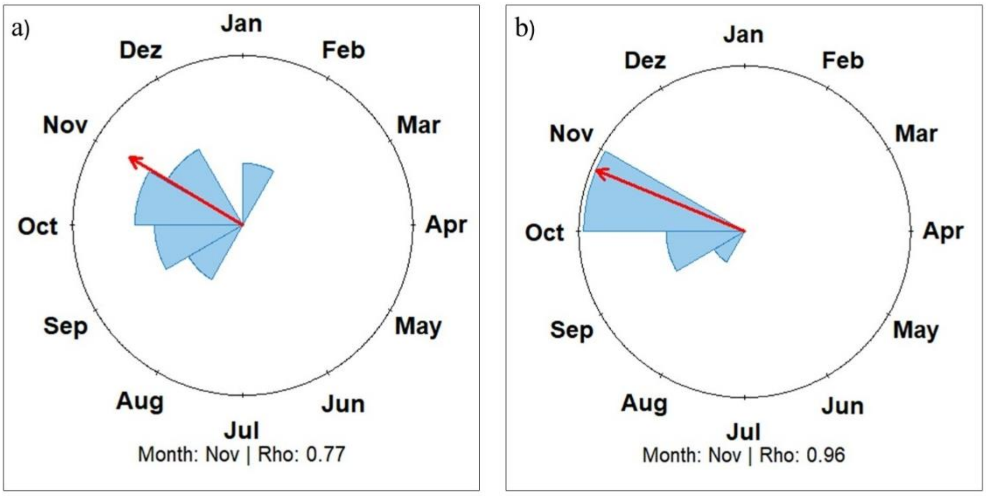

Figure 4. Circular representation of the average distribution of Amblyomma ovale in both dog cohorts (a) and in the environment (b) in Guaxupé, Minas Gerais, Brazil, between November 2022 and November 2024. Blue areas represent overlapping distribution patterns. Arrows indicate the mean angle (mean month), and the rho value represents the degree of concentration around the main direction.

3.2.3 *Rhipicephalus linnaei*

Molecular analysis of the *R. sanguineus* lato sensu of all samples revealed 100% identity with *R. linnaei* (GenBank accession no. OM984677.1). The obtained sequences were deposited in GenBank under accession numbers PZ213154 and PZ213155. The mean abundance on dogs in Araguari was lower and largely concentrated within the first year and a half of the study. In contrast, in Guaxupé, higher levels were observed from late 2023 onward, with markedly elevated mean abundance. Generally, infestation peaks were driven primarily by adult ticks and did not exhibit a clear temporal pattern. The high infestations with this tick species observed during the second half of the study in Guaxupé were attributable to dogs from the property that exhibited marked behavioural changes.

3.3 Human infection seasonal occurrence

A total of 806 human rickettsiosis cases were compiled in the 20 microregions of Santa Catarina state between 2001 and 2024, originating from 77 municipalities. The highest number of cases occurred during spring and summer, with a peak in November (Table 5). The number of cases recorded in November was compared with those of the other months and differed significantly from those observed between March and August (Table 5). Circular analysis revealed a highly concentrated seasonal pattern in human rickettsiosis cases, with events significantly clustered around a mean angle of 5.42 radians (SD = 1.08; rayleigh test: R = 0.513, p < 0.001), indicating a strong distribution peaking in November (Figure 5).

Table 5. Monthly distribution (mean, median, and range) of human rickettsiosis cases in municipalities (n = 77) of Santa Catarina State, Brazil, 2001–2024 (n = 806).

	Median	Mean	SD	Range	Vs November (Dunn)[1]
January	5	10,8	24,9	0-124	ns
February	4	6,6	15,2	0-76	ns
March	2	3,2	7,3	0-36	*
April	1	1,7	3,9	0-19	**
May	1	2	4,7	0-23	**
June	0	1	2,5	0-12	**
July	1	1,5	3,5	0-17	**
August	1	1,7	4,1	0-20	**
September	4	7,7	17,8	0-88	ns
October	7	11,7	27,1	0-135	ns
November	9	13,5	31,2	0-155	--
December	4	8,7	20,1	0- 100	ns

[1]Differences among months were assessed using the Kruskal–Wallis test followed by Dunn's multiple comparisons test. Significance refers to comparisons with November. ns: not significant; *p = 0.0017; **p < 0.0001.

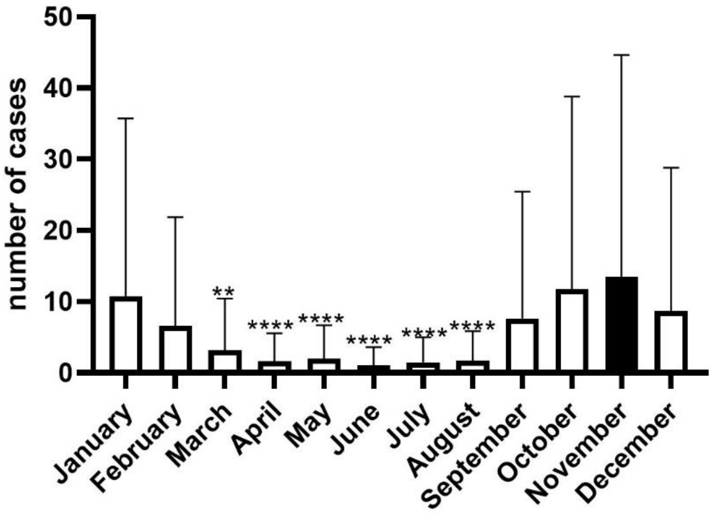

Figure 5. Mean monthly number of human rickettsiosis cases in the state of Santa Catarina, Brazil, from 2001 to 2024. Significant differences between November and the other months are indicated as **P = 0.0017 or ****P< 0.0001.

4 Discussion

In Araguari, located within the Cerrado biome, we observed a clear predominance of *A. sculptum* ticks on dogs, overwhelmingly represented by immatures, mainly nymphs, whereas only a few adult ticks and specimens of other species (*R. linnaei*, *R. microplus*, and *A. ovale*) were detected.

Amblyomma sculptum, considered the main vector of Brazilian spotted fever caused by *R. rickettsii* in Brazil, is primarily associated with the Brazilian savanna biome (Cerrado) but has expanded into large areas of the Atlantic Forest that have been heavily modified or degraded (Szabó et al., 2009; Martins et al., 2016). The low occurrence of *A. sculptum* adults on dogs reinforces that infestation by immature stages depends on the presence of primary hosts, such as capybaras and horses, which sustain adult tick populations (De Paula et al., 2022).

The seasonal distribution of *A. sculptum* on dogs observed in this study followed the well-established pattern for the species, with nymphs predominating in late winter and spring, corresponding to the driest period of the year (Labruna et al., 2002; Barbieri et al., 2019; De Paula et al., 2022). Likewise, the seasonality of *Amblyomma* larvae, with peak host-seeking activity between April and July coinciding with the onset of autumn and the cooler temperatures, is also characteristic of *A. sculptum* populations (Labruna et al., 2002; Barbieri et al., 2019; De Paula et al., 2022). During the peak period of larval occurrence, dense feeding clusters of *A. sculptum* larvae were observed on the ear margins of some dogs, a pattern appears to reflect a common behaviour of this species at least on this host.

In endemic areas of *R. rickettsii* rickettsiosis, most cases of spotted fever occur between June and September, coinciding with the peak abundance of the nymphal stages of *A. sculptum* (Angerami et al., 2012; Szabó et al., 2013a). The predominance of *A. sculptum* nymphs parasitizing dogs with unrestricted access to fragments of natural environments in our study highlights the high level of exposure of this host to tick-borne pathogens transmitted by the immature stages of this species. It is important to emphasize that endemic areas for this rickettsiosis are much more restricted than the overall distribution range of its vector species. In particular, endemic human rickettsiosis occurs mainly in areas where tick populations are maintained by capybaras in degraded Atlantic Forest environments and ecotones, rather than in the Cerrado biome (Nunes et al., 2022). Moreover, when transmitted by adults of *A. aureolatum*, this pathogen is even more geographically restricted, being associated with more humid Atlantic Forest environments at higher altitudes, particularly along the margins of the metropolitan region of São Paulo (Pinter et al., 2004; Sabatini et al., 2010; Szabó et al., 2013a). In this latter scenario, no well-defined seasonal pattern of rickettsiosis has been identified, as both dog infestation and human cases are distributed throughout the year (Pinter et al., 2004; Angerami et al., 2012). Regardless of the transmission context, the close association of dogs with humans and their frequent infestation by vector ticks make them important sentinels for the circulation of *R. rickettsii*, serving as early indicators of human risk for Brazilian spotted fever in endemic areas (Horta et al., 2004; 2007; Silva et al., 2010; Poubel et al., 2018; Neves et al., 2023).

Rhipicephalus microplus a lesser tick on dogs is a synanthropic and exotic one-host tick species in Brazil, believed to have originated in Southeast Asia (Estrada-Peña et al., 2006). It is widely distributed throughout the country in close association with cattle and infested pastures (Pereira et al., 2008). Although bovines are its main hosts, *R. microplus* may occasionally infest carnivores, including dogs (Labruna et al., 2005; Szabó et al., 2001; 2010). The detection of this species on dogs in the present study indicates that these animals had access to cattle pastures and it is not an unexpected finding for rural dogs.

Tick infestation in dogs from Guaxupé showed a distinct pattern, with *A. sculptum* being much less prevalent, whereas *R. linnaei* was the dominant species. Although *A. sculptum* is not typical of the Atlantic Forest biome, it has been reported in degraded environments within this biome (Szabó et al., 2009) which accounted for the observed dog infestations.

Rhipicephalus linnaei, a member of the *Rhipicephalus sanguineus* species complex, is considered an exotic tick species in Brazil. Of the two species currently recognized in the country (*R. linnaei* and *R. sanguineus* sensu stricto), the latter is restricted to the extreme southern region (Moraes-Filho et al., 2011; Dantas-Torres et al., 2024). Molecular identification of *R. sanguineus* s.l. specimens from Araguari and Guaxupé as *R. linnaei*, together with its known geographic distribution, indicates that this species represents the complex in these municipalities. *Rhipicephalus linnaei* tick is synanthropic and endophilic, being strongly associated with human dwellings (Serpa et al., 2026) and is usually not recorded in natural or anthropized green areas (Barbieri et al., 2019; Szabó et al., 2007, 2009, 2013b, 2018, 2024) or on wild hosts unless these have access to human dwellings (Labruna et al., 2005; Szabó et al., 2019). In the present study, infestations by *R. linnaei* increased markedly in dogs from a property that became more confined to the household environment and accounted for the highest abundance of this tick species in Guaxupé. This pattern contrasts with that observed in dogs from Araguari, none of which were confined to the household environment, and reinforces the strong association of *R. linnaei* with domiciliary settings and confined dogs.

Despite the high number of ticks recorded in Guaxupé, no clear seasonal pattern was observed for *R. linnaei* infestations. The rapid development of infestations by this species in the second year of the study in the more confined dogs supports a continuous life cycle without diapause, with development being accelerated or slowed by higher or lower temperatures, respectively and as previously described under experimental settings (Labruna et al., 2017).

In contrast to Araguari, *A. ovale* exhibited higher prevalence and abundance on dogs in Guaxupé. This higher level of canine infestation is likely associated with the species' dependence on more humid forested environments (Szabó et al., 2009, 2013b, 2018), which were provided in Guaxupé by Atlantic Forest fragments and humid vegetation such as the bamboo grove.

Notably, in Guaxupé, infestation of both dogs and the environment by adult *A ovale* revealed a distinct seasonal pattern, with significant aggregation of adult ticks in spring, particularly in November, and infestations extending into summer. This observation reinforces the notion that many *Amblyomma* ticks in Brazil exhibit a one-year life cycle with distinct peak periods for each developmental stage (Labruna et al., 2009), but which remains to be determined for most species across the country. Although the seasonality of *A. ovale* has not been specifically characterized, previous studies conducted in the states of São Paulo and Paraná have suggested a similar seasonal pattern of adult ticks across a broad geographic range in southeastern and southern Brazil (Szabó et al., 2009; Suzin et al., 2020; Szabó et al., 2026).

Human rickettsiosis caused by *R. parkeri* ARF and transmitted by *A. ovale* remains poorly characterized in Brazil. Only six cases have been confirmed by molecular methods (Rodrigues et al., 2023; Albuquerque et al., 2025), distributed across different periods of the year, which has hindered the recognition of a clear seasonal pattern. This low number of confirmed cases is likely related to the typically milder clinical presentation of the disease (Faccini et al., 2021; Rodrigues et al., 2023), which reduces the likelihood of specific diagnostic investigation. Nevertheless, surveillance data from the Brazilian Ministry of Health, particularly from Santa Catarina and the municipality of Blumenau (Brasil, 2025), suggest that the true number of infections is substantially higher.

The seasonal distribution of human rickettsiosis cases in Santa Catarina followed a pattern similar to that observed for adult *A. ovale*, with cases occurring throughout the year but peaking during spring and early summer, particularly in November. While this temporal correspondence is noteworthy, it should be interpreted with caution. Ideally, the seasonality of adult *A. ovale* would be compared with human rickettsiosis cases from Minas Gerais, where the present study was conducted. However, such a comparison is currently limited by the epidemiological context in Brazil. In several Brazilian states with a high incidence of rickettsioses, including São Paulo, Rio de Janeiro, and Minas Gerais (Faccini et al., 2021; Oliveira et al., 2016), infections caused by *R. rickettsii* co-occur with those caused by *R. parkeri* ARF, and surveillance data, largely based on serological methods, may reflect a mixture of distinct rickettsioses, limiting species-specific interpretations. In contrast, no human cases caused by *R. rickettsii* have been reported in Santa Catarina; instead, reported cases are attributed to *R. parkeri* ARF, with many specific clinical and epidemiological features (Angerami et al., 2009; Barbieri et al., 2014). This makes Santa Catarina the only suitable epidemiological reference currently available for this purpose.

Although the comparison involves different geographic regions (southeastern vs. southern Brazil), previous studies have reported a similar seasonal pattern of adult *A. ovale* across a broad geographic range, including southeastern and southern Brazil (Szabó et al., 2009; Suzin et al., 2020; Szabó et al., 2026). Notably, Suzin et al. (2020) reported a peak of adult ticks in November in Paraná, at a latitude comparable to that of Santa Catarina, supporting the plausibility of a broader and consistent seasonal pattern for this species. In this context, the seasonal pattern observed here may represent the first indication of a defined seasonality for human rickettsiosis caused by *R. parkeri* ARF in Brazil. If confirmed, this finding may have important implications for public health, particularly for improving clinical suspicion, diagnosis, and preventive measures coordinated by the Brazilian Ministry of Health. However, this interpretation should be regarded as preliminary. Further studies conducted in different regions across the distribution range of *A. ovale* are necessary to confirm the consistency, geographic extent, and epidemiological significance of this seasonal pattern.

In conclusion, our findings provide the first clear demonstration of a well-defined seasonal distribution of adult *A. ovale*, a vector of *R. parkeri* ARF in the Atlantic Forest biome. These observations should be complemented by seasonal investigations of *A. ovale* populations in other regions where this tick species occurs in South and Central America, given that ticks infected with *R. parkeri* ARF have been reported across this broad geographic range. The potential relationship between tick seasonality and the seasonality of human rickettsiosis warrants further investigation, as it may represent a key step toward improving surveillance strategies, and the timing of preventive measures for this emerging disease.

CRediT authorship contribution statement

Rodrigo da Costa Maia: Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Vinicius da Silva Rodrigues**: Methodology, Investigation, Formal analysis, Resources. **Maria Marlene Martins**: Resources, Methodology. **Adriane Suzin**: Writing – review & editing, Methodology, Formal analysis, Data curation. **Cecília de Aquino Pinto Palis**: Formal analysis, Writing – review & editing, Methodology. **Matias P. J. Szabó**: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflict of interest

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Data availability

Data will be made available on request.

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3.2 Tick vectors and exposure of dogs to spotted fever group rickettsiae in Minas Gerais, Brazil

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Abstract

Tick-borne rickettsioses remain a major public health concern in Brazil, where their distribution is determined by the ecology of vector tick species. In Minas Gerais, human cases of *Rickettsia rickettsii* infection are recurrent, whereas the occurrence of Atlantic Forest spotted fever caused by *Rickettsia parkeri* strain Atlantic Forest (ARF) has not yet been documented, despite favorable environmental conditions and records of its vector, *Amblyomma ovale*, in the state. This study aimed to investigate the presence of *R. parkeri* ARF in *A. ovale* ticks infesting dogs and to assess canine exposure to spotted fever group (SFG) *Rickettsia* across ten municipalities representing nine mesoregions of Minas Gerais. A total of 300 dogs were examined, yielding 1,283 ticks from eight species. *Rhipicephalus sanguineus* sensu lato, *Amblyomma sculptum*, and *A. ovale* were the most prevalent species, with marked geographic variation in species composition and infestation indices. Serological analysis revealed SFG *Rickettsia* exposure in dogs from multiple municipalities, with varying geometric mean titers. PCR screening of *A. ovale* ticks detected *Rickettsia* DNA sequences consistent with *R. parkeri* ARF. These findings demonstrate widespread exposure of dogs to SFG *Rickettsia* and heterogeneous tick assemblages across Minas Gerais, and confirm *R. parkeri* ARF circulation in the State. This study represents the first statewide systematic survey of canine ticks in Minas Gerais and provides essential data to improve understanding of the eco-epidemiology of tick-borne diseases and to support targeted surveillance strategies for rickettsioses in the region.

Key words: Minas Gerais, *Rickettsia*, dogs, vector ticks

1. Introduction

Rickettsioses are the most important tick-borne zoonotical diseases in Brazil (de la Fuente et al., 2023). Two *Rickettsia* species are known to be pathogenic to humans in the country: *Rickettsia rickettsii*, the etiological agent of Brazilian spotted fever, which is associated with high lethality, and *Rickettsia parkeri* sensu lato, which causes a milder, non-lethal disease (Labruna et al., 2011; Faccini-Martínez et al., 2021). However, phylogenetic evidence indicates the existence of multiple strains of *R. parkeri* in Brazil, two of which are known to be pathogenic to humans: *Rickettsia parkeri* sensu stricto and *Rickettsia parkeri* strain Atlantic rainforest (ARF) (Nieri et al., 2018). The latter is the one that has been associated with several confirmed human cases of rickettsiosis in the country (Faccini-Martínez et al., 2021; Rodrigues et al., 2023).

Rickettsiae are obligate intracellular Gram-negative bacteria with high diversity in nature, most of which are non-pathogenic or have unknown pathogenicity (Merhej et al., 2014). Although some rickettsial species may be transmitted by multiple tick species, pathogenic rickettsiae are typically associated with a limited number of vectors (Labruna et al., 2011; Szabó et al., 2013a; Blanton, 2019). Consequently, the geographic distribution of rickettsioses is largely constrained by the distribution of these tick species and may be even more restricted in areas where vector populations are not infected (Barbieri et al., 2023; Szabó et al., 2026), a pattern that remains poorly understood and warrants further investigation.

Brazil is a country of continental dimensions, and the distribution of tick species, and therefore of rickettsioses, is heterogeneous, being restricted to specific eco-epidemiological contexts determined primarily by the distribution of the vector tick species (Labruna et al., 2011; Szabó et al., 2013a; Blanton, 2019; Faccini -Martínez et al., 2020;). Although the precise distribution of rickettsioses in Brazil is not fully established, Brazilian spotted fever caused by *R, rickettsii* occurs predominantly in southeastern Brazil, often in focal areas (Ogrzewalska et al., 2012; Szabó et al., 2013a; Luz et al., 2019; Foley et al., 2025). In most of these settings, transmission is primarily associated with *Amblyomma sculptum* ticks maintained by capybaras (*Hydrochoerus hydrochaeris*), whereas in the metropolitan region of São Paulo transmission occurs more restrictively and is mainly linked to the tick *Amblyomma aureolatum* (Luz et al., 2019; Ogrzewalska et al., 2012; Foley et al., 2025).

Rickettsia parkeri ARF was only recently recognized as a human pathogen (Spolidorio et al., 2010). This agent is transmitted to humans by adult *A. ovale* ticks (Faccini -Martínez et al., 2021; Rodrigues et al., 2023), which parasitize wild carnivores and domestic dogs

(Guglielmone et al., 2021). Rickettsiosis caused by *R. parkeri* ARF is mainly associated with remnants of the Atlantic Forest biome along the Brazilian coast, from southern to northeastern Brazil (Krawczak et al., 2016; Rodrigues et al., 2023) and infected *A. ovale* ticks have been detected in the Amazon and Cerrado biomes as well (Aguirre et al., 2022; Garcia et al., 2022).

Minas Gerais, located in southeastern Brazil, is endemic for *R. rickettsii* rickettsioses in specific areas, with recurrent human cases reported annually, particularly in the metropolitan region of Belo Horizonte (de Carvalho Nunes et al., 2022). In contrast, although *A. ovale* ticks infected with *Rickettsia parkeri* ARF have been detected in all Brazilian states bordering Minas Gerais, their occurrence has not yet been documented within the state. In Minas Gerais, remnants of Atlantic Forest occur in a mosaic with Cerrado and anthropized landscapes. This ecological transition zone, coupled with the documented presence of the vector *A. ovale* (Nogueira et al., 2023; Szabó et al., 2010; 2018), creates a favorable environment to support sylvatic transmission cycles that may extend into peri-urban areas. Thus, the apparent absence of human rickettsiosis caused by *R. parkeri* ARF in Minas Gerais is more likely related to the lack of systematic investigations targeting specific eco-epidemiological scenarios than to the true absence of the pathogen.

Identifying the pathogen and/or serological evidence of exposure in sentinel hosts may improve the recognition of *R. parkeri* ARF infection among human febrile illnesses of unknown etiology. In such areas, preventive measures may be implemented, including restricting dog access to natural areas and improving tick control in dogs. Importantly, such data is essential to discriminate between tick-borne rickettsioses caused by *R. rickettsii* and *R. parkeri* ARF in Minas Gerais, thereby strengthening public health surveillance and control strategies.

Therefore, this study aimed to investigate the presence of *R. parkeri* ARF DNA in *A. ovale* ticks collected from dogs with access to remnants of natural areas, mainly forest fragments, across different regions of Minas Gerais and to assess canine exposure through serological analysis. In addition, this study represents the first statewide, systematic survey of ticks on dogs in Minas Gerais, providing data to improve epidemiological understanding of canine tick-borne diseases in the state.

2. Material and Methods

2.1 Study site

The study was conducted in rural areas of ten municipalities in Minas Gerais, Brazil, representing nine mesoregions. The geographic location of the municipalities is shown in Figure 1, and detailed information is provided in Table 1.

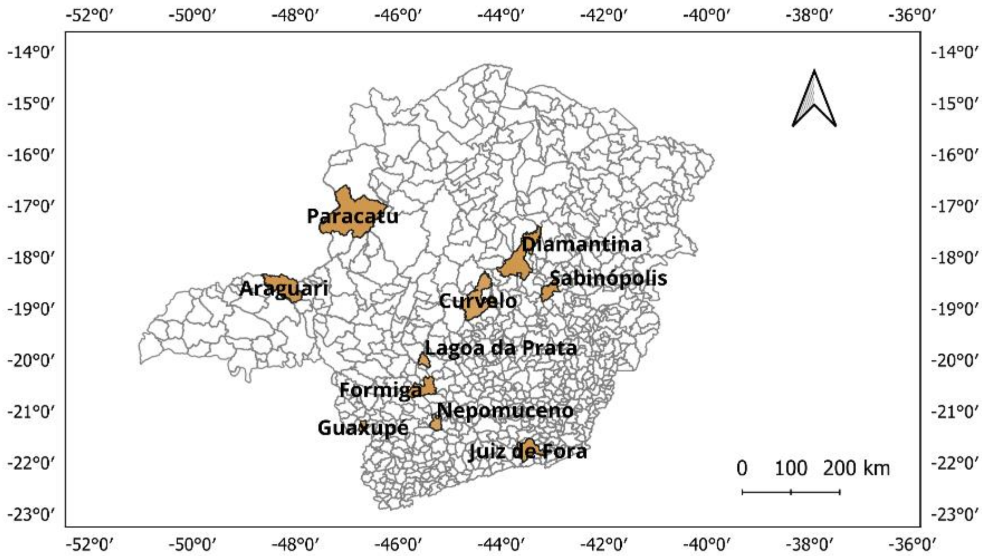

Figure 1. Municipalities of dog sampling in Minas Gerais, Brazil

Table 1. Geographic, climatic, and environmental characteristics of the municipalities of study, Minas Gerais, Brazil (Alvares et al., 2013)

Municipality	Dominant Biome	Mesoregion	Köppen-Geiger classification	Average Temperature (°C)	Annual Precipitation (mm)	Average Elevation (m)
Araguari	Cerrado	Triângulo Mineiro/Alto Paranaiba	Aw	22.5	1250	755
Curvelo	Atlantic Forest	Central Mineira	Aw	23.1	1110	632
Diamantina	Cerrado	Jequitinhonha	Cwb	19.1	1404	1280
Formiga	Cerrado	Oeste de Minas	Cwa	20.8	1266	840
Guaxupé	Atlantic Forest	Sul e Sudoeste de Minas	Cwa	20.5	1856	920
Lagoa da Prata	Cerrado	Central Mineira	Aw	23.0	1180	630
Juiz de Fora	Atlantic Forest	Zona da Mata	Cwa	19.8	1500	720

Nepomu-ceno	Atlantic Forest	Campo das Verten-tes	Cwa	20.6	1450	848
Paracatu	Cerrado	Noroeste de Minas	Aw	22.9	1400	688
Sabinó-polis	Atlantic Forest	Vale do Rio Doce	Cwa	20.0	1350	910

2.2 Dog sampling

Tick sampling was conducted from November to January in 2023, 2024 and 2025, coinciding with the peak occurrence of adult *Amblyomma* ticks, particularly *A. ovale*, the vector of *R. parkeri* ARF (Labruna et al., 2009; Maia et al., 2026; Szabó et al., 2026). In each municipality, 30 dogs classified as "rural free-ranging dogs," following Vanak and Gompper (2009), were examined. These dogs were owned or loosely associated with human habitations but were not confined to restricted outdoor areas or kennels and had access to remnants of natural habitats in the region. To ensure broad geographic coverage, animals from various rural properties surrounding each municipality were included. Additional inclusion criteria comprised dogs that had not been recently treated with antiparasitic drugs. Each dog was thoroughly examined, and all ticks were collected; when infestations exceeded 100 ticks, representative samples were obtained. Collected ticks were preserved in 70% ethanol. Blood samples (3 mL) were collected from each dog under manual restraint by cephalic vein puncture using sterile needles and syringe.

2.3 Tick identification

Ticks were identified based on external morphology following dichotomous keys (Martins et al., 2010; Barros-Battesti et al., 2025; Martins et al., 2026).

2.4 Serology

Blood samples were refrigerated after collection; sera were separated and stored frozen until analysis. Dog sera were tested by indirect immunofluorescence assay (IFA) using crude antigens from three Brazilian *Rickettsia* isolates (*Rickettsia bellii* strain Mogi, *R. rickettsii* strain Taiaçu, and *R. parkeri* strain At24), as described by Labruna et al. (2007). Sera were subjected to two-fold serial dilutions in phosphate-buffered saline (PBS), starting at 1:64. Slides were incubated with fluorescein isothiocyanate (FITC)-labelled rabbit anti-dog IgG (Sigma, St.

Louis, MO, USA). Endpoint IgG titers were determined for each antigen. A titer at least four-fold higher for one *Rickettsia* species than for the others was considered probably homologous to that species or a closely related one (Labruna et al., 2007). Each slide included known negative and positive control sera.

2.5 *Rickettsia* DNA in ticks

Tick samples from municipalities where *A. ovale* where found was submitted to DNA extraction using the guanidine isothiocyanate–phenol method (Sangioni et al., 2005) for *Rickettsia* detection. Ticks were processed either individually or in pools. For the adult pools, a 1/4 of four individual ticks was combined, whereas nymphs were grouped in pools of 2–10 individuals from the same host. If a pool tested positive for *Rickettsia* spp., the remaining tissues from the respective individuals were subsequently processed and tested separately to identify the positive specimen. DNA quality was verified by PCR targeting tick mitochondrial 16S rDNA (Mangold et al., 1998). PCR amplification was carried out using DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol and using the cycling conditions as previously described. The extracted DNA was initially subjected to screening for *Rickettsia spp*. by PCR targeting a 401 bp fragment of the rickettsial *gltA* gene (primers CS-78 and CS-323) (Labruna et al., 2004). Samples that tested positive were subsequently subjected to PCR targeting a 532 bp fragment of the rickettsial *ompA* gene, with primers Rr190.70F and Rr190.602R (Regnery et al., 1991). PCR runs included positive (*Rickettsia vini*–infected Vero cells) and negative controls. Amplicons of the *ompA*-PCR assay were purified (PureLink®, Invitrogen) and Sanger-sequenced; sequences were edited in SeqMan Pro (Lasergene v15) to generate consensus sequences and compared with GenBank entries using BLASTn (Altschul et al., 1990).

2.6 Data analysis

Descriptive statistics were used to analyze tick infestation parameters, including prevalence (number of tick-infested dogs/total number of examined dogs), mean intensity of infestation (total number of ticks/number of infested dogs), and mean infestation abundance (total number of ticks/total number of examined dogs). Antibody titers were expressed as geometric mean titers (GMT). Individual titers from dogs were grouped by municipality (n = 30 per group) and analyzed using GraphPad Prism version 8.0.1 (GraphPad Software, San Diego, CA, USA). GMTs and their respective 95% confidence intervals were calculated after log10 transformation of the data. Samples with antibody titers below the cutoff dilution (1:64) were considered non-reactive and assigned a value corresponding to half of the cutoff (1:32) for GMT calculation. Comparisons of antibody titers among municipalities were performed using the non-parametric Kruskal–Wallis test and Dunn's post hoc test with significance set at $p < 0.05$. Simple linear regression analyses were performed to evaluate the association between the geometric mean titer (GMT) of antibodies against *R. parkeri* and the mean intensity and mean abundance of *A. ovale* across municipalities. For each municipality (n = 10), GMT values were used as the dependent variable and parasitological indices as independent variables. The significance of the regression slope was assessed using the F test to determine whether it differed from zero. Statistical significance was set at $p < 0.05$.

2.7 Ethics

All procedures involving dog sampling protocols were approved by the Ethics Committee on Animal Experimentation of the Federal University of Uberlândia (CEUA-UFU) under protocol number 23117.016236/2022-70. Environmental tick collection was authorized by the Brazilian Ministry of the Environment (SISBIO permit nº 48141−5).

3. Results

3.1 Tick species and numbers

Across the ten municipalities, a total of 1,284 ticks belonging to eight species and three genera were collected from 300 dogs (Table 2). Overall prevalence, mean intensity of infestation, and mean abundance were 0.45, 9.5, and 4.3, respectively. However, both tick species composition and parasitological indices varied markedly among municipalities (Tables 3-4). Dogs with tick counts over 100 *R. sanguineus* s.l. ticks were recorded in Guaxupé (n = 1), Curvelo (n = 3), and Paracatu (n = 1). Partial results of ticks from Araguari and Guaxupé were presented elsewhere (Maia et al., 2026).

Table 1. Number of ticks according to species and stage collected on dogs in 10 municipalities in Minas Gerais, Brazil

	R. sanguineus s.l.[1]			*R. microplus*		*D. nitens*	*A. sculptum*			*A. ovale*	*A. aureolatum*	*A. parvum*	*A. tigrinum*
Tick stage[2]	L	N	A	N	A	L	L	N	A	A	A	A	A
Araguari			5				2	19	1				
Guaxupé		0	113[3]	0	3			4	1	67			
Nepomuceno		71	62	3	3					8			
Formiga		13	105					57	2	52			
Lagoa da Prata		5	6		1				2	1			
Juiz de Fora			29					32		3	4		
Curvelo	1	6	336[3]					6	1	6			
Diamantina		8	33			1		2	1	5		4	13
Sabinópolis								37		4	1		
Paracatu		8	134[3]					3					
Total	1	111	823	3	7	1	2	160	8	146	5	4	13

[1]s.l. sensu lato
[2]L-Larva; N-Nymph; A-Adult
[3]Dogs with tick counts over 100 ticks - Guaxupé (n = 1), Curvelo (n = 3), Paracatu (n = 1).

Considering tick species, prevalence, mean intensity of infestation, and mean abundance followed a decreasing order of *R. sanguineus* sensu lato, *A. sculptum*, and *A. ovale*. *Rhipicephalus sanguineus* s.l. was detected in all municipalities except Sabinópolis, *A. sculptum* was absent only in Nepomuceno, and *A. ovale* was not recorded in Paracatu. The remaining species were collected in much lower numbers and were restricted to few municipalities. Notably, *Amblyomma parvum*, *A. tigrinum*, and *Dermacentor nitens* were recorded exclusively in Diamantina, where *Amblyomma tigrinum* was the second most abundant species, whereas *A. aureolatum* was collected only in Juiz de Fora and Sabinópolis. Most ticks were collected at the adult stage, with fewer nymphs and larvae recorded.

Table 2. Prevalence of tick infestation on dogs by municipality and tick species in Minas Gerais, Brazil

	Total	*R. sanguineus* s.l.[1]	*R. microplus*	*D. nitens*	*A. sculptum*	*A. ovale*	*A. aureolatum*	*A. parvum*	*A. tigrinum*
Araguari	0.33	0.07	–	–	0.30	–	–	–	–
Guaxupé	0.60	0.17	0.03	–	0.10	0.50	–	–	–
Nepomuceno	0.63	0.50	0.03	–	–	0.23	–	–	–
Formiga	0.50	0.37	–	–	0.10	0.20	–	–	–
Lagoa da Prata	0.27	0.17	0.03	–	0.07	0.03	–	–	–
Juiz de Fora	0.50	0.17	–	–	0.23	0.07	0.10	–	–
Curvelo	0.47	0.43	–	–	0.13	0.10	–	–	–
Diamantina	0.50	0.30	–	0.03	0.07	0.07	–	0.13	0.23
Sabinópolis	0.37	–	–	–	0.30	0.13	0.03	–	–
Paracatu	0.33	0.30	–	–	0.03	–	–	–	–

[1]s.l. sensu lato

Table 3. Mean intensity of tick infestation on dogs by municipality and tick species in Minas Gerais, Brazil

	Total	*R. sanguineus* s.l.[1]	*R. microplus*	*D. nitens*	*A. sculptum*	*A. ovale*	*A. aureolatum*	*A. parvum*	*A. tigrinum*
Araguari	2.7	2.5	–	–	2.4	–	–	–	–
Guaxupé	10.4	22.6	3.0	–	1.7	4.5	–	–	–
Nepomuceno	7.7	8.9	6.0	–	–	1.1	–	–	–
Formiga	15.3	10.7	–	–	19.7	8.7	–	–	–
Lagoa da Prata	1.9	2.2	1.0	–	1.0	1.0	–	–	–
Juiz de Fora	4.5	5.8	–	–	4.6	1.5	1.3	–	–
Curvelo	25.4	26.4	–	–	1.8	2.0	–	–	–
Diamantina	4.4	4.6	–	1.0	1.5	2.5	–	2.0	1.9
Sabinópolis	3.8	–	–	–	4.1	1.0	1.0	–	–
Paracatu	14.5	15.8	–	–	3.0	–	–	–	–

[1]s.l. sensu lato

Table 4. Mean abundance of tick infestation on dogs by municipality and tick species in Minas Gerais, Brazil

	Total	*R. sanguineus* s.l.[1]	*R. microplus*	*D. nitens*	*A. sculptum*	*A. ovale*	*A. aureolatum*	*A. parvum*	*A. tigrinum*
Araguari	0.90	0.17	–	–	0.73	–	–	–	–
Guaxupé	6.27	3.77	0.10	–	0.17	2.23	–	–	–
Nepomuceno	4.90	4.43	0.20	–	0.00	0.27	–	–	–
Formiga	7.63	3.93	–	–	1.97	1.73	–	–	–
Lagoa da Prata	0.50	0.37	0.03	–	0.07	0.03	–	–	–
Juiz de Fora	2.27	0.97	–	–	1.07	0.10	0.13	–	–

Curvelo	11.87	11.43	–	–	0.23	0.20	–	–	–
Diamantina	2.20	1.37	–	0.03	0.10	0.17	–	0.10	0.43
Sabinópolis	1.40	0.00	–	–	1.23	0.13	0.03	–	–
Paracatu	4.83	4.73	–	–	0.10	–	–	–	–

[1]s.l. sensu lato

3.2 Serology

One hundred and sixteen animals (60.5%) were seroreactive to at least one *Rickettsia* spp. antigen. Seroprevalence against *Rickettsia* spp. (Table 5) was lowest in Nepomuceno (23%), followed by Sabinópolis (47%). In all other municipalities, seroprevalence exceeded 50%, reaching 76.7% in Formiga and Lagoa da Prata. Except for *R. bellii* in Nepomuceno, serological reactivity to all three *Rickettsia* species was detected in dogs from all municipalities (Table 5). Homologous reactions were detected in 58 dogs for *R. rickettsii*, 59 for *R. parkeri*, and 14 for *R. bellii*. The number of homologous reactions varied among municipalities, particularly for *R. parkeri*, for which more than half of the dogs from Lagoa da Prata showed homologous reactivity, followed by Formiga (n = 10). In contrast, homologous reactions to *R. rickettsii* were more frequent in Curvelo (n = 14) and Paracatu (n = 13).

Table 5. Seroreactivity to three *Rickettsia* species of 30 dogs from each of nine municipalities of Minas Gerais State, Brazil, 2024-2025.

Municipality	Nº seroreactive dogs to each of the *Rickettsia* species (% seroreactivity)				Nº of dogs with homologous reaction[2]
	R. parkeri	*R. rickettsii*	*R. bellii*	*Rickettsia* spp.	
Araguari	11 (36.7)	9 (30.0)	6 (20.0)	19 (63.3)	8 (*R. parkeri*) 4 (*R. rickettsii*) 1 (*R. bellii*)
Guaxupé[1]	15 (46.9)	16 (50.0)	7 (21.9)	23 (71.9)	8 (*R. parkeri*) 5 (*R. rickettsii*) 3 (*R. bellii*)
Nepomuceno	4 (13.3)	7 (23.3)	0 (0.0)	7 (23.3)	5 (*R. rickettsii*)
Formiga	20 (66.7)	15 (50.0)	1 (3.3)	23 (76.7)	10 (*R. parkeri*) 4 (*R. rickettsii*) 1 (*R. bellii*)
Lagoa da Prata	20 (66.7)	5 (16.7)	1 (3.3)	23 (76.7)	17 (*R. parkeri*) 3 (*R. rickettsii*) 1 (*R. bellii*)
Juiz de Fora	12 (40.0)	9 (30.0)	2 (6.7)	16 (53.3)	5 (*R. parkeri*) 4 (*R. rickettsii*) 2 (*R. bellii*)
Curvelo	7 (23.3)	15 (50.0)	1 (3.3)	20 (66.7)	4 (*R. parkeri*) 14 (*R. rickettsii*)

					1 (*R. bellii*)
Diamantina	11 (36.7)	17 (56.7)	5 (16.7)	21 (70.0)	4 (*R. parkeri*) 6 (*R. rickettsii*) 1 (*R. bellii*)
Sabinópolis	8 (26.7)	8 (26.7)	3 (10.0)	14 (46.7)	3 (*R. parkeri*) 3 (*R. rickettsii*) 3 (*R. bellii*)
Paracatu	8 (26.7)	13 (43.3)	4 (13.3)	17 (56.7)	3 (*R. parkeri*) 13 (*R. rickettsii*) 1 (*R. bellii*)

[1]Refer to 32 dogs

[2]A homologous reaction was determined when an end point titer to a *Rickettsia* species was at least 4-fold higher than those observed for the other *Rickettsia* species. In this case, the *Rickettsia* species or a closely related species was considered the possible antigen involved in a homologous reaction

Along with these differences in frequency, the magnitude of the serological responses also varied, with *R. parkeri* and *R. rickettsii* eliciting antibody titers as high as 4096 and *R. bellii* reaching once 1024 (in Diamantina). Considering, empirically, an antibody titer of 1024 as the cutoff for high titers against *R. parkeri* and *R. rickettsii* (Table 6), Guaxupé showed the highest titers, specifically against *R. parkeri*. In Formiga and Sabinópolis, high titers were observed against both pathogenic *Rickettsia* species, in Nepomuceno, Lagoa da Prata and Paracatu, solely against *R. rickettsii* and in Curvelo and Diamantina solely against *R. parkeri*.

Table 6. Number of dogs with high antibody titers (≥ 1:1024) against *Rickettsia parkeri* and *Rickettsia rickettsii*, according municipality in Minas Gerais, Brazil.

Municipality	*Rickettsia* species	Endpoint titer		
		1024	2048	4096
Guaxupé	*R. parkeri*	2	1	2
Nepomuceno	*R rickettsii*	0	1	0
Formiga	*R rickettsii*	0	2	1
	R. parkeri	1	3	1
Lagoa da Prata	*R rickettsii*	1	0	0
Curvelo	*R. parkeri*	1	0	0
Diamantina	*R. parkeri*	0	1	0
Sabinópolis	*R rickettsii*	0	0	2
	R. parkeri	1	2	0

Paracatu	*R rickettsii*	2	4	0

Nevertheless, serological reactivity of dogs against *R. bellii* and *R. rickettsii* did not differ significantly among municipalities. In contrast, serological reactivity against *R. parkeri* varied significantly among municipalities (Figure 2). Dogs from Formiga showed significantly higher serological reactivity than those from Nepomuceno (p = 0.0002), Curvelo (p = 0.0084), and Paracatu (p = 0.0305). Similarly, Lagoa da Prata exhibited higher reactivity compared with Nepomuceno (p = 0.0015) and Curvelo (p = 0.0431). In addition, dogs from Guaxupé presented higher serological reactivity than those from Nepomuceno (p = 0.0484). No other pairwise comparisons were statistically significant.

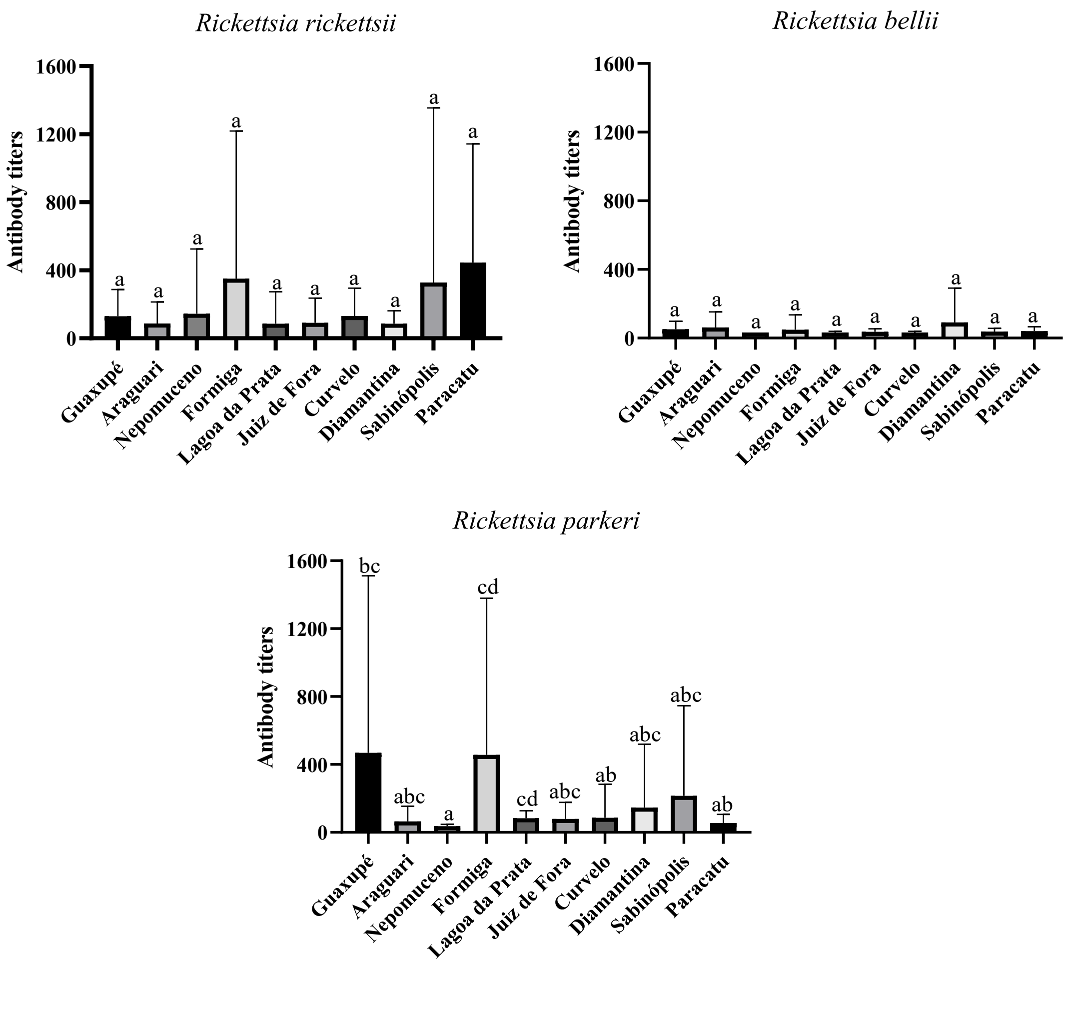

Figure 2. Mean antibody titers to three *Rickettsia* species (*R. rickettsii*, *R. bellii*, and *R. parkeri*) in dogs (n = 30 per municipality) from ten municipalities in Minas Gerais State, Brazil

(2024–2025). Comparisons among municipalities were performed using the Kruskal–Wallis test followed by Dunn's post hoc test. Different lowercase letters above bars indicate statistically significant differences among municipalities ($p < 0.05$).

3.3 Association between antibody titers against *R. parkeri* and *A. ovale* dog infestation

Linear regression analysis revealed a significant positive association between the geometric mean titer (GMT) against *R. parkeri* and both the mean intensity ($F_{1,8} = 32.16$, $p = 0.0005$) and mean abundance ($F_{1,8} = 21.42$, $p = 0.0017$) of *A. ovale* across municipalities (Figure 3).

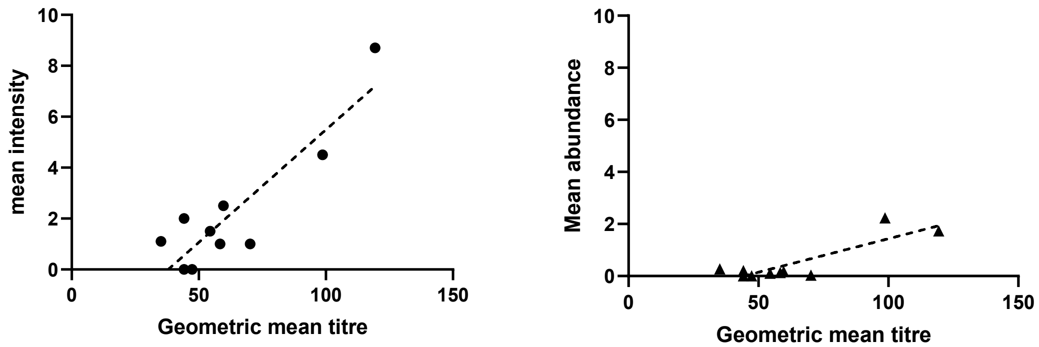

Figure 3. Linear regression between the geometric mean antibody titer against *Rickettsia parkeri* and the mean intensity and mean abundance of *A. ovale* ticks on dogs across ten municipalities in Minas Gerais, Brazil. Each point represents one municipality.

3.4 *Rickettsia* DNA in ticks

A total of 276 ticks were screened for *Rickettsia* DNA in municipalities where *A. ovale* was found on dogs (Table 7). The sample comprised 126 adult *A. ovale*, 119 specimens of *R. sanguineus* s.l. (81 adults and 38 nymphs), 13 adult *A. tigrinum*, nine adult *A. aureolatum*, four specimens of *A. sculptum* (three adults and one nymph), three adult *Rhipicephalus microplus*, and two adult *A. parvum*. Only two adult *A. ovale* ticks collected in Formiga, both from the same dog, tested positive for both target genes by PCR. The *ompA* sequences obtained from these two ticks were 100% identical (463/463 bp) to several sequences of *R. parkeri* strain

Atlantic rainforest from GenBank (CP040325, MF536975, KM116015, MN027565). The sequences generated in the present study have been submitted to GenBank under accession numbers PZ282102 and PZ282103.

Table 7. Number of ticks screened for *Rickettsia* DNA according to species, developmental stage, and municipality of collection.

Municipality	*R. sanguineus* s.l.			*R. microplus*			*A. sculptum*			*A. ovale*		*A. aureolatum*		*A. parvum*		*A. tigrinum*	
	N	M	F	N	M	F	N	M	F	M	F	M	F	M	F	M	F
Curvelo	0	0	0	0	0	0	0	0	0	2	4	0	0	0	0	0	0
Diamantina	0	0	0	0	0	0	0	0	0	2	3	0	0	1	1	9	4
Formiga	3	21	8	0	0	0	0	1	1	28	28	0	0	0	0	0	0
Guaxupé	0	5	5	0	1	1	1	0	1	28	23	0	0	0	0	0	0
Lagoa da Prata	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Juiz de |Fora	0	0	0	0	0	0	0	0	0	0	1	2	6	0	0	0	0
Nepomuceno	35	25	17	0	1	0	0	0	0	2	0	0	0	0	0	0	0
Sabinópolis	0	0	0	0	0	0	0	0	0	0	4	0	1	0	0	0	0
Total	38	51	30	0	2	1	1	1	2	62	64	2	7	1	1	9	4

4. Discussion

All eight tick species found parasitizing dogs in this study had been previously reported for this host species (Guglielmone et al., 2021). Of these, *R. sanguineus* s.l. and *R. microplus* are exotic to the country. Based on the currently recognized geographic distribution of the *R. sanguineus* complex in Brazil, the specimens identified as *R. sanguineus* s.l. in the present study were most likely *Rhipicephalus linnaei*. Of the two species recognized in the country (*R. linnaei* and *R. sanguineus* sensu stricto), the latter is restricted to the extreme southern region (Moraes-Filho et al., 2011; Dantas-Torres et al., 2024). Both species are synanthropic and endophilic (Fachet-Lehmann et al., 2025; Serpa et al., 2026), with *R. linnaei* in Brazil being strongly associated with human dwellings (Serpa et al., 2026) regardless of the local biome or ecosystem.

Interestingly, although *R. linnaei* is recognized as the primary vector of *R. rickettsii* in the southern United States and northern Mexico (Foley et al., 2025), there are no confirmed reports of transmission of *R. rickettsii* to humans by this species in Brazil, and its role in the epidemiology of Brazilian spotted fever appears to be limited (Foley et al., 2025). Finally, if left uncontrolled, this tick can rapidly reach extremely high infestation levels (Maia et al., 2026), which likely explains the overall heaviest tick burdens (both mean infestation intensity and abundance) observed in dogs in the present study.

Rhipicephalus microplus a lesser tick on dogs, is widely distributed throughout the country in close association with cattle and infested pastures (Pereira et al., 2008; Grisi et al., 2014). Although bovines are its main hosts, *R. microplus* may occasionally parasite carnivores, including dogs (Labruna et al., 2005; Szabó et al., 2001; 2010). The detection of this species on dogs in the present study indicates that these animals had access to cattle pastures and should not be considered an unexpected finding for rural dogs anywhere in the country.

The remaining six species are of Neotropical origin; however, their current distributions have been modified by anthropogenic activities. Although *D. nitens* is considered to have evolved in the New World (Lado and Klompen, 2019), it has expanded across all major Brazilian biomes in association with equines and pasturelands (Labruna and Faccini, 2020). Notably, Aragão (1936) reported no records of the genus *Dermacentor* in Brazil at that time. Later, Aragão and Fonseca (1953) documented the rapid spread of *D. nitens* in South America, then described as *Anocentor nitens*, suggesting its introduction from the Caribbean. This tick has occasionally been recorded on dogs (Guglielmone et al., 2021), likely associated with horses and pastures, as was the case in the present study.

Except for *A. sculptum*, all *Amblyomma* species found on dogs in the present study are commonly reported parasitizing dogs in the adult stage (Guglielmone et al., 2021). These tick species are primarily maintained in natural environments through live cycles involving wild vertebrate hosts. Consequently, infestation of dogs occurs mainly when they access natural or semi-natural habitats.

Amblyomma sculptum more frequently parasitizes dogs as larvae and nymphs (Maia et al., 2016), while adults are more commonly associated with medium to large-sized wild and domestic ungulates, capybaras and giant anteaters (Nava et al., 2017; Luz et al., 2019; Szabó et al., 2019; Maia et al., 2026). This species is a Brazilian Savanna Biome (Cerrado) tick and has expanded into large areas of the Atlantic Forest that have been heavily modified or degraded (Szabó et al., 2009; Martins et al., 2016).

The higher prevalence, mean intensity, and mean abundance of infestation by *A. sculptum* among *Amblyomma* species can be explained by its strong ecological association with the Cerrado biome and degraded areas of the Atlantic Forest, that predominated in the study area, particularly the former. Although sampling did not coincide with the peak seasonal activity of nymphs, most infestations involved this stage, as adults are less prone to parasitize dogs. These findings highlight the widespread exposure of dogs in Minas Gerais to the primary vector of *R. rickettsii* in Brazil (Foley et al., 2025).

In contrast, the reduction of the Atlantic Forest has likely led to a contraction in the distribution of tick species associated with this habitat, *A. ovale* and *A. aureolatum* (Szabó et al., 2009; Pinter et al.,2004). However, *A. aureolatum* appears to require substantially more humid environmental conditions for development, typically found in moist forested habitats and favored by cooler climates at southern latitudes and/or at higher elevations (Barbieri et al., 2015). Indeed, *A. ovale* may occur in the Cerrado biome, provided that more humid phytophysiognomies are present, such as gallery forests and riparian corridors (Szabó et al., 2018). This ecological association likely explains the second highest prevalence, mean intensity, and mean abundance of infestation by this species among *Amblyomma* ticks in most evaluated municipalities. In addition, adult ticks of this species primarily parasitize carnivores (Labruna et al., 2005), which contributes to their frequent occurrence on dogs that access natural or semi-natural environments.

On the other hand, *A. aureolatum* is more strictly associated with the Atlantic Forest biome in southeastern and southern Brazil. This ecological pattern was confirmed in the present study and whereas *A. ovale* was recorded in most surveyed municipalities, *A. aureolatum* was restricted to two municipalities originally covered by Atlantic Forest and located at elevations above 700 meters above sea level as previously observed (Barbieri et al., 2015).

Information on the original environments of two common ticks on dogs, *A. parvum* and *A. tigrinum*, of our study is more limited. Although *A. tigrinum* has been described as exhibiting considerable ecological plasticity (Nava et al., 2017), reports from Minas Gerais are relatively rare and mainly involve dogs or wild canids inhabiting slightly higher-altitude areas predominantly within the Cerrado biome (Abel et al., 2006; Martins et al., 2015; Szabó et al., 2018; Arrais et al., 2021) as observed in the present study. This species appears to be epidemiologically relevant, as it has been associated with the transmission to humans of the pathogen *R. parkeri* sensu stricto in southern Brazil (Weck et al., 2016).

Amblyomma parvum is widely distributed throughout the Neotropical region, from Mexico to Argentina. Across this broad geographic range, it is better adapted to hot and dry environments (Nava et al., 2017) and there are no records from humid forested areas. Adults of this tick species exhibit exceptionally low host specificity (Olegario et al 2011) and are considered highly aggressive toward humans (Szabó et al., 2020). *Amblyomma parvum* has been found naturally infected with '*Candidatus* Rickettsia andeanae´, a rickettsial agent of unknown pathogenicity (Nieri-Bastos et al., 2014). The species completes one generation per year, with a predominance of adults during the spring–summer period (Nava et al., 2017; Barbieri et al., 2019). In the present study, four adults were recorded in only one municipality, characterized by a drier rocky Cerrado ("cerrado rupestre"), which is consistent with the known ecological preferences of this tick species.

It must be highlighted, however, that the total number of ticks and species recorded in this study was likely underestimated. Immature stages, particularly larvae, may not have been fully collected due to their small size and limited visibility during field examinations. In addition, five dogs infested with *R. sanguineus* s.l. harbored more than 100 ticks each, which precluded complete quantification of all specimens. Further, tick infestations were assessed exclusively during late spring and summer, a period chosen to favor the detection of adult *A. ovale*. Consistent with this seasonal bias, Maia et al. (2026) reported substantially higher infestations of *A. sculptum* nymphs and larvae on dogs in Araguari during autumn, winter, and early spring, displaying the strong influence of seasonal dynamics on tick stage composition and infestation intensity.

Serological reactivity of dogs to the three tested *Rickettsia* species should be interpreted with caution, as it likely reflects prior exposure to rickettsiae associated with the tick species identified in this study, but may also result from exposure to other tick species and stages, ectoparasites such as fleas (Horta et al., 2010), or multiple *Rickettsia* species co-circulating in the same area. Likewise, homologous reactions should be interpreted cautiously, since only three *Rickettsia* antigens were tested, and these reactions may reflect exposure to untested *Rickettsia* species as well.

Seroreactivity to *R. bellii* was the lowest and was likely related to cross-reactivity with other *Rickettsia* species or transmitted by other vectors. *Rickettsia bellii* maintains a symbiotic relationship with ticks and can persist through vertical transmission without requiring vertebrate hosts (Krawczak et al., 2018). Although serological evidence of *R. bellii* exposure has been reported in rodents (Pacheco et al., 2007; Coelho et al., 2016), Sakai et al. (2014) observed no seroconversion in guinea pigs infested with *Amblyomma dubitatum* ticks that were 100% infected with *R. bellii*. Therefore, it is possible that infected ticks do not efficiently inoculate *R. bellii* into dogs during feeding or alternatively, the inoculated *R. bellii* may not elicit a detectable humoral response, as suggested by Binder et al. (2026). Either case may explain the low frequency and magnitude of antibody responses observed.

On the other hand, higher canine seroprevalence and antibody titers are more likely elicited by the *Rickettsia* species that is most prevalent in the area and to which dogs have been more frequently exposed. Furthermore, although not absolute, tick-borne rickettsiae exhibit strong ecological associations with specific tick species (Parola et al., 2013). Therefore, higher seroprevalence rates and elevated antibody titers may indicate exposure to a locally dominant tick–*Rickettsia* system. Conversely, lower seroprevalence rates and lower antibody titers may reflect less frequent exposure but may also result from cross-reactivity with other antigenically related *Rickettsia* species.

Considering the known epidemiology of spotted fever in Brazil and the tick species involved, the wider distribution of the two main tick vectors found on dogs in the present study, *A. sculptum* and *A. ovale*, suggests that these species are the most likely responsible for the transmission of *R. rickettsii* and *R. parkeri*, respectively. It should also be considered that both pathogens belong to the spotted fever group (SFG), and cross-reactivity among species within this group is expected (Rivas et al., 2015).

In non-endemic areas of Brazil, both seroreactivity and antibody titers against these two *Rickettsia* species in dogs are generally low, with seroprevalence around or below 20% and titers not exceeding 1,024 (Batista et al., 2010; Szabó et al., 2026). In contrast, studies conducted in areas with confirmed recent transmission of *R. rickettsii* and documented human cases of Brazilian spotted fever have reported substantially higher seroprevalence and antibody titers. For example, Pacheco et al. (2011), in Juiz de Fora, reported titers up to 131,072, with 51.3% of dogs exhibiting endpoint titers ≥1,024. Similarly, during a spotted fever outbreak in Rio de Janeiro, Poubel et al. (2018) found that 67.6% of 253 dogs were seroreactive to *R. rickettsii* and 11.2% to *R. parkeri*, with maximum titers reaching 131,072 and 4,096, respectively; notably, 88% of *R. rickettsii* seroreactive dogs had titers 1,024.

Likewise, in areas with confirmed circulation of *R. parkeri* strain ARF and human cases, canine serological responses are also elevated. Barbieri et al. (2014), in Blumenau, southern Brazil, reported titers up to 32,768, with 36.5% of dogs showing titers ≥1,024 against *R. parkeri*. Similarly, Szabó et al. (2013b) observed canine seroprevalence exceeding 80%, with titers up to 32,768 and higher titers against *R. parkeri*.

In our study, seroprevalence against *Rickettsia* spp. was as low as 20% in one municipality; however, in eight of the ten municipalities, it exceeded 50%, with four of these showing values above 70%, indicating widespread exposure of dogs to *Rickettsia* spp. It should be considered that the sampling was targeted toward free-ranging dogs with access to green areas in rural settings, whereas animals without this behavior were not included. This sampling approach likely contributed to the higher seroprevalence observed compared to other studies. In contrast, although homologous reactions to *R. rickettsii* were detected in 58 dogs, titers were generally low and did not differ among municipalities. This pattern suggests limited exposure and/or serological cross-reactivity with related species, rather than a recent ongoing transmission of *R. rickettsii*.

In the case of *R. parkeri*, although the maximum antibody titer observed in this study (4,096) was not typically indicative of areas with intense canine exposure, there was a significantly greater concentration of the higher titers in two municipalities (Guaxupé and Formiga), which were also associated with higher specific seroprevalence (around 50%). These municipalities likewise showed the highest mean intensity and mean abundance of infestation by *A. ovale*, resulting in a strong positive association between antibody titers and infestation by this tick species. Additionally, *R. parkeri* DNA was detected in ticks collected in Formiga, further supporting the local presence and circulation of this pathogen.

The infection rate of *A. ovale* ticks with *R. parkeri* however was only 1.6%, which can be considered low, as in endemic areas infection rates approach 10% (Szabó et al., 2013b; Barbieri et al., 2014). Based on these findings, it can be inferred that *R. parkeri* is circulating in these two municipalities, albeit at low levels. Consequently, human exposure is likely limited but still possible and should not be excluded from the differential diagnosis of febrile illnesses.

The significant positive association between the geometric mean antibody titers against *R. parkeri* was stronger for mean intensity of infestation than for mean abundance, as indicated by the higher regression coefficient and F value. This finding suggests that the magnitude of individual tick burden, rather than population-level exposure alone, plays a more important role in shaping the humoral immune response of dogs. In practical terms, dogs harboring higher numbers of *A. ovale* ticks per infested animal are more likely to develop elevated antibody titers. These results reinforce the epidemiological relevance of *A. ovale* infestation intensity as a principal driver of local transmission dynamics of *R. parkeri*. Moreover, this pattern highlights the marked focality of *A. ovale* infestations and *R. parkeri* transmission within municipalities.

A similar pattern may also occur in the transmission of *R. rickettsii* to dogs by *A. sculptum* or *A. aureolatum*. In the present study, dogs from Juiz de Fora showed substantially lower seroreactivity and prevalence than those reported by Pacheco et al. (2011) in the same municipality. We speculate that we did not capture an active focus of *R. rickettsii* transmission, as observed by Pacheco et al. (2011). Consequently, targeted sampling strategies and timing are essential to accurately identify these eco-epidemiological scenarios, as the inclusion of non-exposed animals may dilute seropositivity prevalence and preclude sampling of dogs with high antibody titers, thereby reducing the sensitivity of municipal-level epidemiological assessments.

In conclusion, we report for the first time the detection of the agent of mild spotted fever, *R. parkeri* ARF, in Minas Gerais State, and demonstrate canine exposure to this agent in multiple municipalities. We identified a significant association between infestation by *A. ovale* ticks and canine exposure to the pathogen in dogs with access to natural or semi-natural environments. Although the circulation of *R. parkeri* appears to occur at low levels, the combined serological and molecular findings support its focal presence in municipalities. Taken together, these results, along with existing evidence, contribute to a better understanding of the eco-epidemiology of tick-borne rickettsioses not only in Minas Gerais but Brazil as well, and may support improvements in public health strategies for the surveillance, diagnosis, and prevention of tick-borne febrile diseases.

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5. CONCLUSÕES

Neste trabalho mostramos a avaliação abrangente de carrapatos que infestam cães rurais em diferentes mesorregiões de Minas Gerais, revelando ampla diversidade de espécies e forte variação geográfica na composição da fauna de ixodídeos. *R.s sanguineus* s.l., *A. sculptum* e *A. ovale* foram as espécies predominantes e definiram os principais cenários de risco para transmissão de riquétsias. A sorologia demonstrou exposição disseminada de cães a riquétsias do grupo da febre maculosa, com maior reatividade para *R. parkeri* em municípios específicos, associada à intensidade e abundância de *A. ovale*, evidenciando sua relevância epidemiológica como potencial vetor no estado. Embora pequeno número de carrapatos tenha apresentado DNA compatível com *R. parkeri* ARF, os resultados sugerem circulação focal e de baixa intensidade da bactéria, reforçando a importância de vigilância direcionada e de estudos ecoepidemiológicos contínuos para aprimorar estratégias de prevenção e diagnóstico da febre maculosa no estado.

Além disso a análise da sazonalidade dos carrapatos vetores em Minas Gerais revelou padrões ecológicos contrastantes entre os biomas Cerrado e Mata Atlântica. Em Araguari, *A. sculptum* predominou, especialmente em estágio ninfal, confirmando a forte exposição de cães ao vetor primário de *R. rickettsii*. Em Guaxupé, a dominância de *R. linnaei* refletiu seu comportamento sinantrópico e ciclo contínuo, enquanto *A.ovale* apresentou elevada abundância e forte sazonalidade, com pico marcado na primavera. A coincidência entre o aumento de adultos de *A. ovale* e o pico de casos humanos de rickettsiose por *R. parkeri* cepa Mata Atlântica sugere estreita relação entre a atividade sazonal do vetor e o risco humano de infecção. Assim, quadros febris no período primaveril em áreas com ocorrência de *A. ovale* devem considerar essa rickettsiose no diagnóstico diferencial. Esses achados destacam a necessidade de vigilância contínua e de estudos sazonais adicionais em outras regiões onde a espécie ocorre.