

Silent Circulation of Rickettsia Spp. In a Non-Endemic Area of Northeastern Brazil Revealed by Molecular and Serological Evidence in Animals From Probable Sites of Human Spotted Fever Infection

Clarício Alvim Bugarim-Neto

Federal University of Alagoas (UFAL)

Rodrigo Costa Maia

Federal University of Uberlândia (UFU)

Jean Phellipe Marques Nascimento

Central Public Health Laboratory (LACEN)

Camila Rose Bezerra Lima

Federal University of Alagoas (UFAL)

Gláucia Grazielle Nascimento

Federal University of Alagoas (UFAL)

Mykaella Andrade Araújo

Federal University of São Francisco Valley (UNIVASF)

Wandklebson Silva Paz

National Institute of Science and Technology on Neglected Diseases in a One Health Context in Northeastern Brazil (INCT Negli-One), Federal Rural University of Pernambuco

Waldinéa Maria Silva

State Department of Health of Alagoas (SESAU)

Márcio Bezerra-Santos

Federal University of Alagoas (UFAL)

Matias Pablo Juan Szabó

Federal University of Uberlândia (UFU)

Abelardo Silva-Júnior

Federal University of Alagoas (UFAL)

Wagner José Nascimento Porto

`wagnerporto@icbs.ufal.br`

Federal University of Alagoas

Short Report

Keywords: Rickettsia spp., Spotted fever, One Health, Sentinel species, Tick-borne diseases

Posted Date: October 22nd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11194517/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Spotted fever is a severe zoonotic disease, and Alagoas State has historically been classified as a silent area for human rickettsioses. This study aimed to investigate the occurrence of and exposure to bacteria of the genus *Rickettsia* in Alagoas to support epidemiological surveillance and assess public health risks. We collected 114 blood samples from animals, including 95 dogs and 19 horses, alongside 146 ticks, across four municipalities: Maragogi, Palmeira dos Índios, São José da Tapera, and Senador Rui Palmeira. Samples were tested for antibodies against *Rickettsia rickettsii*, *R. parkeri*, and *R. amblyommatis* by indirect immunofluorescence assay (IFA). DNA from blood clot and tick samples was extracted for molecular analyses using real-time PCR (qPCR) and conventional PCR targeting *gltA* and *ompA* genes. IFA revealed significant exposure to these agents ($p < 0.001$), with seroreactivity rates of 46.3% (44/95) in dogs and 94.7% (18/19) in horses. The highest seroreactivity frequency was observed for *R. parkeri*, followed by *R. amblyommatis* and *R. rickettsii*. Of the 146 ticks, 111 (76.0%) from dogs were identified as *Rhipicephalus sanguineus*, and 35 (24.0%) from horses were *Dermacentor nitens*. All tick samples tested negative for *Rickettsia* by qPCR. However, *Rickettsia* DNA was detected in a blood clot sample from a dog from the municipality of Maragogi. This study provides the first molecular and serological evidence of silent *Rickettsia* spp. circulation in Alagoas, a non-endemic region for spotted fever. The high seroreactivity demonstrates active exposure, highlighting dogs and horses as sentinel species within an integrated One Health framework.

Highlights

- First molecular evidence of *Rickettsia* spp. in Alagoas, Brazil.
- High *Rickettsia* spp. seroprevalence in dogs (46.3%) and horses (94.7%).
- Silent circulation of *Rickettsia* spp. detected in non-endemic areas.
- Equines and canines act as effective sentinels for rickettsial agents.
- Eco-epidemiological data support integrated One Health surveillance.

INTRODUCTION

Spotted fever is an acute infectious zoonosis caused by bacteria of the genus *Rickettsia* and transmitted by hematophagous ticks, being characterized by its high clinical severity (Donalisio et al. 2020). In Brazil, *Rickettsia rickettsii*, an obligate intracellular Gram-negative bacterium, is responsible for the most severe form of the disease, which is characterized by systemic endothelial dysfunction, multiple organ failure, and high case fatality rates when diagnosis and treatment are not promptly established. Human infection occurs incidentally within enzootic cycles in which ticks of the genus *Amblyomma* act as both vectors and reservoirs (Guedes et al., 2011; Ramírez-Hernández et al. 2020). More recently, the circulation of other species, such as *Rickettsia parkeri*, has been identified and associated with milder clinical manifestations, increasing the epidemiological and diagnostic complexity of spotted fever in Brazil (Oliveira et al., 2016; Ogrzewalska et al. 2012).

In Brazil, the first records of spotted fever date back to the early twentieth century, with the initial cases being described in the state of São Paulo, where the disease was recognized as an important public health concern (Dias and Martins 1931). Throughout the twentieth century, the occurrence of the disease was concentrated in the Southeast and South regions, particularly in areas of São Paulo characterized by intense environmental changes, high human population density, and widespread circulation of *Amblyomma* ticks (Ogrzewalska et al. 2012). More recently, epidemiological data have indicated an expansion of the disease to other regions of the country, including the Midwest, North, and Northeast, highlighting the diversity of eco-epidemiological scenarios associated with spotted fever (Oliveira et al. 2016). In São Paulo, endemic areas persist, and re-emergence has been observed since the late twentieth century, being associated with changes in land use, expansion of amplifier hosts, and increased human exposure to risk environments (Ogrzewalska et al. 2012).

In Northeastern Brazil, spotted fever has increasingly been recognized as an emerging public health concern, with a limited number of reported cases and evidence of transmission in both Atlantic Forest and semiarid environments, suggesting the existence of multiple eco-epidemiological scenarios. Human cases confirmed by molecular methods and attributed to *Rickettsia parkeri* have been described in the state of Bahia, where infections were associated with forest exposure and the presence of an inoculation eschar, reinforcing the importance of this pathogen in the region (Sevá et al. 2019). In the state of Ceará, recent investigations in an endemic area of the Baturité Massif documented the circulation of *R. parkeri* in ticks of the species *Amblyomma ovale* and evidence of exposure in dogs, highlighting the risk of domestic-sylvatic transmission (Albuquerque et al. 2024). In addition, a fatal case of spotted fever has been reported in Pernambuco, emphasizing both the severity of the disease and the importance of regional surveillance efforts (Oliveira et al. 2018).

In the state of Alagoas, scientific information regarding the occurrence of spotted fever remains scarce. To date, the state has been classified as a silent area for human rickettsioses, with significant gaps in surveillance and etiological investigations (Gama et al. 2023). Recently, Gama and colleagues (2023) demonstrated the presence of tick species of public health importance parasitizing horses, including the first report of *Amblyomma sculptum* in the state, indicating ecological conditions that may favor the establishment of rickettsial transmission cycles in suitable environments. Furthermore, the first report of *Ornithodoros mimon* in opossums (*Didelphis albiventris*) from Alagoas has been published, and the authors recommended further investigations into zoonotic pathogens associated with ticks in the state (Farias et al. 2024).

Given the scarcity of information regarding the circulation of rickettsiae in the state of Alagoas and its classification as a silent area for human rickettsioses, expanding the current knowledge on the occurrence of these pathogens is essential. The recent identification of tick species of public health relevance and potential vertebrate hosts in the state reinforces the need for systematic investigations to support epidemiological surveillance and public health risk assessment. In this context, the present study aimed to investigate the occurrence and/or exposure to rickettsiae in Alagoas, thereby contributing to filling important knowledge gaps and strengthening integrated actions among public

health authorities, researchers, and the community toward the prevention and control of spotted fever and other rickettsial diseases.

MATERIALS AND METHODS

Ethical Aspects

The study was approved by the Animal Use Ethics Committee (CEUA) of the Federal University of Alagoas (UFAL) under Protocol No. 08/2023. All procedures involving animals were conducted in accordance with current ethical standards and guidelines for the use of animals in research, ensuring animal welfare throughout all stages of the study.

Study Area

The study was conducted in four municipalities in the state of Alagoas, Northeastern Brazil: Maragogi, Palmeira dos Índios, São José da Tapera, and Senador Rui Palmeira. The municipality of Maragogi is located in the state's coastal region, within the Eastern Alagoas mesoregion, and is part of the Atlantic Forest biome; the specific site is situated in a rural area (coordinates: -8.900380, - 35.192712) with a predominance of vegetation typical of coastal environments, such as restingas and mangroves. Palmeira dos Índios is located in the Agreste region of Alagoas, in a transitional area between a rural zone and a forest fragment, forming an ecotone between the Caatinga and Atlantic Forest biomes (coordinates: -9.379542, - 36.634440) that presents vegetation adapted to semiarid climatic conditions. The municipalities of São José da Tapera and Senador Rui Palmeira are located in the hinterland of Alagoas and are entirely inserted within the Caatinga biome. São José da Tapera represents a semiarid environment with a predominance of sparse vegetation (coordinates: -9.408150, - 37.552061), while Senador Rui Palmeira is characterized by a dry rural environment (coordinates: -9.424279, - 37.453043) (IBGE 2021).

Sample Collection

Biological samples were collected from 114 animals between November 2023 and April 2024. Sampling included 95 dogs (*Canis lupus familiaris*) and 19 horses (*Equus ferus caballus*) residing in Probable Sites of Infection (PSI) associated with suspected or confirmed human cases of spotted fever. The identification of PSIs was based on data obtained from the Notifiable Diseases Information System (SINAN), corresponding to notifications recorded between 2022 and 2024 and provided by the Alagoas State Health Department (SESAU).

Samples were distributed as follows: Maragogi (68 samples: 58 dogs and 10 horses), Palmeira dos Índios (30 samples: 28 dogs and 2 horses), São José da Tapera (14 samples: 7 dogs and 7 horses), and Senador Rui Palmeira (2 samples: 2 dogs). A non-probabilistic convenience sampling approach was

adopted. Blood samples were collected and stored in tubes without anticoagulant and maintained in an insulated container to allow clot retraction. The obtained serum was transferred to 1.5-mL tubes for serological analysis, whereas blood clots were also stored in 1.5-mL tubes and used for DNA extraction and molecular assays.

At the time of blood collection, animals were subjected to visual inspection for the detection and collection of ectoparasites. Specimens were removed by direct examination using anatomical forceps and preserved in isopropyl alcohol. Subsequently, ticks were morphologically identified under a stereomicroscope according to the taxonomic keys described by Barros-Battesti et al. (2006).

Indirect Immunofluorescence Assay (IFA)

All 114 serum samples were tested by indirect immunofluorescence assay (IFA) according to the protocol described by Zavala-Velazquez et al. (1996). The slides contained fixed antigens of *Rickettsia rickettsii*, *Rickettsia parkeri*, and *Rickettsia amblyommatis*. These antigens were previously prepared and provided by the Laboratory of Parasitic Diseases of the School of Veterinary Medicine and Animal Science, University of São Paulo (FMVZ/USP), allowing the detection of antibodies against spotted fever group rickettsiae.

The slides were initially washed with phosphate-buffered saline (PBS; pH 7.4) for 10 minutes and air-dried at room temperature. Twenty microliters of serum diluted at an initial screening dilution of 1:64 were added to each well. Reactive samples were further titrated by serial twofold dilutions (1:64, 1:128, 1:256, 1:512, and 1:1,024) until the absence of specific fluorescence.

The endpoint titer was defined as the highest serum dilution showing specific fluorescence under a fluorescence microscope using a 40× objective. Samples presenting antibody titers at least four times higher for one *Rickettsia* species than for the others were considered suggestive of homologous reactions to that species or to antigenically related species, according to previously established criteria (Horta et al. 2004; Labruna et al. 2007).

DNA Extraction and Molecular Detection of *Rickettsia* spp.

Genomic DNA was extracted from blood clot samples and from tick specimens previously macerated using sterile pestles. DNA extraction was performed using the commercial EasyPure Blood Genomic DNA Kit (TransGen Biotech) according to the manufacturer's instructions. Following extraction, DNA was quantified and stored at – 20°C until molecular analyses were performed.

Detection of *Rickettsia* spp. was initially performed by real-time polymerase chain reaction (qPCR) as described by Labruna et al. (2004), using primers CS-5 (GAGAGAAAATTATATATCCAAATGTTGAT) and CS-6 (AGGGTCTTCGTGCATTTCTT), together with a 6-FAM/BHQ-1-labeled probe (5'-CATTGTGCCATCCAGCCTACGGT-3').

The reactions were carried out using GoTaq® Probe qPCR Master Mix (Promega) in a final volume of 25 µL using a BIO-RAD CFX96 thermocycler and included a positive control (*Rickettsia rickettsii* DNA provided by the Oswaldo Cruz Foundation—Fiocruz, Rio de Janeiro, Brazil) and a negative control (DNase-free water).

Samples testing positive by qPCR were subsequently subjected to conventional PCR for amplification of fragments of the *gltA* and *ompA* genes using primer pairs CS-78 (GCAAGTATCGGTGAGGATGTAAT)/CS-323 (GCTTCCTTAAAATTCAATAAATCAGGAT), 17kD1 (GCTCTTGCAACTTCTATGTT)/17kD2 (CATTGTTCGTCAGGTTGGCG), according to Labruna et al. (2004), and Rr190.70p (ATGGCGAATATTTCTCCAAAA)/Rr190.602n (AGTGCAGCATTCGCTCCCCCT), as described by Regnery et al. (1991).

Amplified products were subjected to electrophoresis on 2% agarose gels stained with SYBR Safe (Thermo Fisher Scientific, Waltham, MA, USA) and visualized under ultraviolet light. Subsequently, the amplified fragments were submitted for sequencing at Macrogen Inc. (Seoul, South Korea).

RESULTS

Indirect Immunofluorescence Assay (IFA)

The prevalence of seroreactivity differed significantly between dogs and horses. While 46.3% (44/95) of dogs presented antibodies against at least one *Rickettsia* species, positivity among horses was substantially higher, reaching 94.7% (18/19). This difference was statistically significant (Fisher's exact test, $p < 0.001$). Among dogs, seroreactivity varied across municipalities, ranging from 37.9% to 100%, whereas all horses sampled in Maragogi and São José da Tapera were seroreactive. These findings suggest a higher frequency of exposure of horses to spotted fever agents at the investigated Probable Sites of Infection (Table 1).

Table 1

Serological reactivity against three *Rickettsia* species in dogs and horses from four municipalities in the state of Alagoas, Northeastern Brazil, 2024.

Animal species and Locations	No. of reactive/tested sera (% positivity)	No. of reactive animals (% positivity/Titer range)			No. of probable homologous reactions
		<i>R. rickettsii</i>	<i>R. parkeri</i>	<i>R. amblyommatis</i>	
DOGS					
Maragogi (PSI)	22/58 (37.9)	9 (15.5/64-1024)	14 (24.1/64-256)	10 (17.2/64)	2 (RR); 4 (RP); 1 (RA)
Palmeira dos Índios	17/28 (60.7)	7 (25.0/64-256)	14 (50.0/64-256)	7 (25.0/64)	1 (RR); 4 (RP)
São José da Tapera	3/7 (42.9)	2 (28.6/64-256)	3 (42.9/64-256)	1 (14.3/64)	0
Senador Rui Palmeira	2/2 (100)	0	1 (50.0/256)	2 (100/64)	1 (RP)
Total	44/95 (46.3)	18 (18.9/64-1024)	32 (33.7/64-256)	19 (20.0/64)	3 (RR); 9 (RP); 1 (RA)
HORSES*					
Maragogi (PSI)	10/10 (100)	9 (90.0/64-256)	2 (20.0/1-128)	1 (10.0/64)	4 (RR); 1 (RP)
Palmeira dos Índios	1/2 (50)	1 (50.0-64)	0	0	0
São José da Tapera	7/7 (100)	6 (85.7/64-256)	7 (100/64-1024)	0	5 (RP)
Total	18/19 (94.7)	16 (84.2/64-256)	9 (47.4/64-1024)	1 (5.3/64)	4 (RR); 6 (RP)

PSI: Probable Site of Infection with confirmed cases of Brazilian spotted fever (BSF); RA = *R. amblyommatis*; RP = *R. parkeri*; RR = *R. rickettsii*.

* No horse samples were collected in Senador Rui Palmeira

Among seroreactive dogs, antibody titers ranged from 64 to 1,024, with a predominance of low to moderate titers. The highest frequency of reactivity was observed against *Rickettsia parkeri*, followed by *Rickettsia amblyommatis* and *Rickettsia rickettsii*. Higher titers (≥ 512) were observed exclusively against *R. rickettsii* in dogs from the municipality of Maragogi. Most reactions occurred with titers ranging from

64 to 256 and were distributed across the different municipalities sampled. Homologous reactions were identified mainly for *R. parkeri*, followed by *R. rickettsii* and *R. amblyommatis* (Table 1).

In horses, antibody titers ranged from 64 to 1,024, with a higher frequency of moderate to high titers compared with dogs. The most pronounced reactivity was observed against *R. rickettsii* and *R. parkeri*, particularly for *R. parkeri* in horses from São José da Tapera, where titers reached 1,024. Reactivity against *R. amblyommatis* was infrequent and was detected in only one horse. Homologous reactions in horses were more frequently attributed to *R. parkeri*, followed by *R. rickettsii*, as shown in Table 1.

The distribution of antibody titers varied according to both *Rickettsia* species and municipality. In Maragogi, seroreactivity to all three evaluated species was observed in both dogs and horses, including high titers against *R. rickettsii*. In Palmeira dos Índios, reactivity against *R. parkeri* predominated in dogs, with titers reaching 256, whereas horse samples did not show significant seroreactivity. In São José da Tapera, both the frequency and magnitude of titers against *R. parkeri* were particularly high in horses. In Senador Rui Palmeira, seroreactivity was restricted to dogs and was characterized by low to moderate titers (Table 1).

Tick Identification and Molecular Detection of *Rickettsia* spp.

A total of 146 ticks were collected, of which 111 (76.0%) were recovered from dogs and identified as *Rhipicephalus sanguineus*, whereas 35 (24.0%) were collected from horses and identified as *Dermacentor nitens*. All specimens were subjected to real-time PCR (qPCR) for the detection of *Rickettsia* spp.; however, no amplification was observed for any of the investigated targets in any of the samples analyzed.

Molecular Detection of *Rickettsia* spp. in Animal Samples

Among the blood clot samples analyzed, specific amplification was detected in only one sample obtained from a seroreactive dog, indicating the presence of *Rickettsia* DNA. This positive sample originated from the municipality of Maragogi, in an area located near the region where the first fatal case of spotted fever in Alagoas was recorded (Fig. 5).

The positive animal presented antibody titers of 64 against *R. rickettsii* and *R. amblyommatis* and was seronegative for *R. parkeri*. The amplification curves obtained by real-time PCR highlighted the pattern observed for the positive sample and the positive control. Following qPCR, a conventional PCR assay successfully amplified a 208-base pair fragment of the *gltA* gene. This study provides the first molecular evidence of *Rickettsia* in Alagoas, a state historically classified as a silent area for human rickettsioses. The positive sample was subsequently subjected to sequencing of fragments of the *gltA* and *ompA* genes; however, sequence quality was insufficient to allow species-level identification of *Rickettsia*.

DISCUSSION

In the present study, we provide evidence of the circulation of rickettsiae in Alagoas, filling important knowledge gaps in a region historically classified as a silent area for human rickettsioses. The observed seropositivity, together with the detection of antibodies against different *Rickettsia* species and the spatial heterogeneity of antibody titers, suggests previous exposure to these pathogens in distinct eco-epidemiological settings. Our findings indicate a potential zoonotic risk in the state and highlight the importance of strengthening epidemiological surveillance and integrated investigations involving vertebrate hosts, vectors, and the human population.

Our results demonstrated substantially higher seroreactivity in horses than in dogs, suggesting important differences in the exposure of domestic animals to *Rickettsia* spp. at the investigated sites. These findings corroborate previous studies describing dogs and horses as useful sentinels for the circulation of spotted fever group rickettsiae in both endemic and non-endemic areas of Brazil (Batista et al. 2010; Neves et al. 2023). In endemic regions, studies conducted by Souza et al. (2016) and Toledo et al. (2011) reported high seroprevalence in horses, frequently exceeding that observed in dogs, reflecting a more intense and continuous exposure to tick vectors. The high seroreactivity observed in horses in our study suggests a greater frequency of contact with vectors or infected environments, which may influence the local epidemiological risk associated with pathogens such as *R. rickettsii* and *R. parkeri* (Albuquerque et al. 2024).

It is noteworthy, however, that *Amblyomma sculptum*, considered the principal vector of Brazilian spotted fever, was not detected among the evaluated horses. Although this species has previously been reported in Alagoas, its prevalence appears to be low (Gama et al. 2023). Therefore, further investigations on tick fauna in the municipalities included in this study are necessary to clarify the role of these ectoparasites as vectors of rickettsiae. Collectively, these findings reinforce the need for integrated surveillance strategies that consider multiple host species and distinct geographic contexts.

The predominance of low to moderate antibody titers (64–256) among seroreactive dogs identified in this study is consistent with previous reports indicating frequent, although not necessarily recent, exposure to *Rickettsia* spp. in regions with enzootic circulation (Gonçalves et al. 2022). The higher reactivity against *R. parkeri* and *R. amblyommatis* observed in our study has also been reported in canine serological surveys conducted in Brazil, suggesting widespread exposure to less pathogenic species frequently associated with ticks of the genus *Amblyomma* (Weck et al. 2020; Kmetiuk et al. 2025).

However, among the dogs evaluated in our study, only ticks belonging to the *Rhipicephalus sanguineus* complex were identified, and these are not recognized vectors of *R. parkeri* or *R. amblyommatis*. Furthermore, there is currently no definitive evidence demonstrating the transmission of *R. rickettsii* by this tick species in our study area. These findings reinforce the need for complementary investigations to improve our understanding of local transmission chains. On the other hand, high titers (≥ 512) directed exclusively against *R. rickettsii* may indicate a more recent infection or a stronger immune response, as previously observed in endemic areas (Cunha et al. 2014). Therefore, the occurrence of homologous

reactions supports the hypothesis of simultaneous circulation of different *Rickettsia* species in the municipalities investigated.

Dogs play a strategic role as sentinel hosts for tick-borne pathogens, particularly spotted fever group rickettsiae, because of their close ecological and domestic association with humans (Qurollo et al. 2025). Canine seropositivity has consistently been associated with the local circulation of *Rickettsia* spp., including the possibility of human cases, reinforcing their utility in epidemiological surveillance (Bowser and Anderson 2018). More recent studies have also highlighted that serological surveys in dogs allow the detection of emerging species or species with uncertain pathogenicity, thereby increasing the sensitivity of surveillance programs in silent areas or regions with low clinical incidence (Neves et al. 2023; Foley et al. 2025).

In this context, Durães et al. (2024), in Minas Gerais, demonstrated serological evidence of the circulation of tick-borne pathogens in a preserved area, reinforcing the value of dogs as early indicators of zoonotic risk and supporting integrated public health strategies under the One Health framework. Although horses do not play a direct role in the epidemiology of Brazilian spotted fever, since they do not develop rickettsemia and do not serve as a source of infection for ticks, we detected antibody titers ranging from 64 to 1,024, predominantly against *R. parkeri* and *R. rickettsii*. These findings are consistent with the understanding that, similarly to dogs, horses can act as important sentinels of exposure to spotted fever group rickettsiae in Brazil.

For example, Oliveira et al. (2019) demonstrated that horses from an Atlantic Forest area in Bahia exhibited measurable seroreactivity to *R. rickettsii* and *R. parkeri* and generally showed a higher proportion of positivity than dogs, compatible with greater exposure to ticks associated with equids. Similar findings have also been reported in horses from São Paulo, with antibody titers reaching 1,024 against multiple rickettsial species, including *R. parkeri*. Furthermore, the predominance of reactivity against *R. parkeri* is consistent with its ecological maintenance in cycles involving *Amblyomma* ticks (Suzin et al. 2022), further supporting the role of horses as sensitive indicators of the circulation of epidemiologically relevant rickettsiae in our study area.

The spatial variation in antibody titers among the investigated municipalities, each characterized by distinct environmental conditions, suggests a possible influence of the eco-epidemiological cycles of spotted fever group rickettsiae. In Atlantic Forest areas, such as Maragogi, the detection of reactivity against multiple *Rickettsia* species is compatible with scenarios in which *Amblyomma* ticks maintain transmission cycles involving dogs and wild hosts, thereby favoring greater pathogen diversity (Barbieri et al. 2014; Faccini-Martínez et al. 2020). In the semiarid Caatinga environments of São José da Tapera and Senador Rui Palmeira, we detected only *R. parkeri*, in contrast to the diversity of *Rickettsia* species reported in the literature for this biome, including several members of the spotted fever group (Gazeta et al. 2025). This difference may reflect a more restricted local circulation, differences in vector and host composition, or sampling limitations. Therefore, our findings reinforce the concept that the diversity of

rickettsiae in the Caatinga biome is heterogeneous and strongly dependent on local eco-epidemiological contexts.

We also collected ectoparasites to investigate the presence of *Rickettsia* spp.; however, no molecular detection was achieved in any of the tick species identified in this study. The absence of molecular detection does not exclude the epidemiological relevance of these species, since natural infection rates in tick populations are generally low, even in endemic areas (Backus et al. 2025).

Rhipicephalus sanguineus was the predominant species in our study and is widely recognized as an important vector of spotted fever group rickettsiae in dogs (Fernandes et al. 2024), including *R. rickettsii*. Experimental and field evidence has confirmed its vector competence in urban transmission cycles in Brazil (Zulzke et al. 2023). Additionally, *Dermacentor nitens*, although classically associated with horses, has been identified in endemic areas of spotted fever and may act as a host for rickettsiae, contributing to the eco-epidemiological complexity of the disease (Mota et al. 2026). Therefore, the identification of these species, even in the absence of molecular detection, reinforces the need for continuous surveillance considering their potential role in the transmission of rickettsiae of public health importance.

The molecular detection of *Rickettsia* DNA in a canine sample, although limited to a single animal, constitutes the first direct evidence of the circulation of spotted fever agents in the state of Alagoas, historically classified as a silent area for human rickettsioses. This finding reinforces recent evidence indicating that areas previously considered non-endemic may actually harbor active transmission cycles that remain undetected because of limitations in surveillance and laboratory diagnosis (Silva et al. 2026).

Recent studies have demonstrated that eco-epidemiological investigations conducted in Alagoas identified laboratory-confirmed human cases, seropositive dogs, and ticks infected with spotted fever group rickettsiae, suggesting the circulation of these pathogens even in the absence of previous epidemiological recognition (Silva et al. 2026). In this context, the molecular confirmation presented in our study strengthens the hypothesis of local transmission and highlights the need to intensify integrated surveillance under the One Health approach, including the monitoring of sentinel animals and vectors. These findings also raise concerns regarding the potential occurrence of human cases in areas located at the interface between natural environments and human activities.

CONCLUSION

In this study, we demonstrated evidence of the circulation of *Rickettsia* spp. in the state of Alagoas through the detection of high seropositivity rates in dogs and horses and the molecular detection of *Rickettsia* DNA in a canine sample, representing the first molecular evidence of this pathogen in the state. These findings fill an important knowledge gap, as the region has historically been considered a silent area for human rickettsioses, while simultaneously revealing eco-epidemiological scenarios favorable to the transmission of these agents.

The higher seroreactivity observed in horses reinforces the role of these animals as valuable sentinels for the early detection of rickettsial circulation. Furthermore, our results highlight the importance of integrated surveillance strategies encompassing animal, environmental, and human health components to identify risk areas and prevent human cases of spotted fever in the state.

In light of our findings, further studies integrating serological, molecular, and entomological approaches are of paramount importance to elucidate the *Rickettsia* species circulating in Alagoas and to better understand their transmission cycles across the state's different biomes. In addition, investigations involving vectors, wildlife hosts, and human populations are essential to characterize the local eco-epidemiological dynamics and to assess the zoonotic risk associated with these pathogens.

The expansion of diagnostic capacity and active surveillance, particularly in areas where suspected cases have been reported, may substantially improve our understanding of spotted fever in Alagoas. Our study demonstrates that regions with few or no recognized cases may still harbor undetected transmission cycles, emphasizing the need for integrated and sensitive surveillance strategies aimed at the early identification of rickettsial diseases.

Declarations

Acknowledgments

The authors thank the Alagoas State Health Department (SESAU) for providing epidemiological data and logistical support. The authors would like to thank the Central Public Health Laboratory of Alagoas (LACEN-AL) for its technical support, collaboration in sample processing, and contributions to the epidemiological surveillance activities that made this study possible. The Laboratory of Parasitic Diseases of the School of Veterinary Medicine and Animal Science of the University of São Paulo (FMVZ/USP) for providing the antigens used in the serological assays, and the Oswaldo Cruz Foundation (Fiocruz) for providing the positive control DNA used in the molecular analyses.

Declaration of Competing Interest

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

Funding

This work was supported by the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq) and the National Institute of Science and Technology on Neglected Diseases in a One Health Context in Northeastern Brazil (INCT Negli-One) under Grant number 408531/2024-0.

Author contributions: CRediT

Clarício Alvim Bugarim-Neto: Conceptualization, Methodology, Investigation, Writing – original draft. **Rodrigo da Costa Maia:** Investigation, Data curation, Validation. **Jean Phellipe Marques do Nascimento:** Resources, Investigation. **Camila Rose Bezerra de Lima:** Methodology, Investigation. **Gláucia Grazielle Nascimento:** Investigation, Data curation. **Mykaella Andrade de Araújo:** Investigation, Validation, Writing – review and editing. **Wandklebson Silva da Paz:** Visualization, Writing – review and editing. **Waldinéa Maria da Silva:** Resources, Data curation. **Márcio Bezerra-Santos:** Methodology, Validation, Writing – review and editing. **Matias Pablo Juan Szabó:** Validation, Writing – review and editing. **Abelardo Silva-Júnior:** Supervision, Resources, Project administration. **Wagner José Nascimento Porto:** Conceptualization, Project administration, Supervision, Writing – review and editing.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Albuquerque MP, Horta MC, Melo DRX, Takeda GACG, Arraes-Santos AI, Martins TF, Pinter A (2024) Eco-epidemiological analysis of *Rickettsia parkeri* in domestic dogs and *Amblyomma ovale* ticks in the Atlantic rainforest of Northeast Brazil. *Rev Bras Parasitol Vet* 33:e012524. <https://doi.org/10.1590/S1984-29612024077>
2. Backus L, Rubino F, López-Pérez AM, Zazueta OE, Borboa J, Quintana AC, Probert W, Hacker JK, Gómez-Castellanos P, Inustroza-Sánchez LC, Herrera Olivas C, Paddock CD, Foley J (2025) *Rickettsial* pathogens in dogs and ticks during an epidemic of Rocky Mountain spotted fever in Ensenada, Baja California, México. *Am J Trop Med Hyg* 112:1096–1112. <https://doi.org/10.4269/ajtmh.24-0497>
3. Barbieri AR, Filho JM, Nieri-Bastos FA, Souza JC Jr, Szabó MP, Labruna MB (2014) Epidemiology of *Rickettsia* sp. strain Atlantic rainforest in a spotted fever-endemic area of southern Brazil. *Ticks Tick Borne Dis* 5:848–853. <https://doi.org/10.1016/j.ttbdis.2014.07.010>
4. Barros-Battesti DM, Arzua M, Bechara GH (2006) Carrapatos de importância médico-veterinária da Região Neotropical: um guia ilustrado para identificação de espécies. Vox/International Consortium on Ticks and Tick-borne Diseases/Instituto Butantan, São Paulo
5. Batista FG, Silva DM, Green KT, Tezza LB, Vasconcelos SP, Carvalho SG, Silveira I, Moraes-Filho J, Labruna MB, Fortes FS, Molento MB (2010) Serological survey of *Rickettsia* sp. in horses and dogs in a non-endemic area in Brazil. *Rev Bras Parasitol Vet* 19:205–209. <https://doi.org/10.1590/s1984-29612010000400003>
6. Bowser NH, Anderson NE (2018) Dogs (*Canis familiaris*) as sentinels for human infectious disease and application to Canadian populations: a systematic review. *Vet Sci* 5:83. <https://doi.org/10.3390/vetsci5040083>

7. Cunha NCD, Lemos ED, Rozental T, Teixeira RC, Cordeiro MD, Lisboa RS et al (2014) Rickettsiae of the Spotted Fever group in dogs, horses and ticks: an epidemiological study in an endemic region of the State of Rio de Janeiro, Brazil. *Braz J Vet Med* 36:294–300
8. Dias E, Martins AV (1931) Spotted fever in Brazil: a summary. *J Trop Med Hyg* 34:103–108
9. Donalisio MR, Souza CE, Angerami RN, Samy AM (2020) Mapping Brazilian spotted fever: linking etiological agent, vectors, and hosts. *Acta Trop* 207:105496. <https://doi.org/10.1016/j.actatropica.2020.105496>
10. Durães LS, Duré ÁI, Fournier G, Reis VM, Tolescano-Pascoli G, Ramirez D et al (2024) Dogs as sentinels for emergent pathogens: serological evidence in a preserved area in Brazil. <https://doi.org/10.20944/preprints202406.2004.v1>. Preprints
11. Faccini-Martínez ÁA, Muñoz-Leal S, Krawczak FS, Acosta ICL, Martins TF, Serpa MCA, Barbieri ARM, Tovar JR, Cerutti Junior C, Labruna MB (2020) Epidemiological aspects of *Rickettsia parkeri* in the Atlantic forest biome of Espírito Santo state, Brazil. *Ticks Tick Borne Dis* 11:101319. <https://doi.org/10.1016/j.ttbdis.2019.101319>
12. Farias Júnior EC, Lopes ACPA, Oliveira GMB, Vieira RFDC, Labruna MB, Almeida JC (2024) First report of the soft tick *Ornithodoros mimon* (Ixodida: Argasidae) in Alagoas State, Northeastern Brazil. *Rev Bras Parasitol Vet* 33:e013424. <https://doi.org/10.1590/S1984-29612024056>
13. Fernandes NNU, Jorge FR, Costa VMD, Rodrigues AKPP, Magalhães MML, Junior RSL, Braz GF, Cavalcante FRA, de Azevedo Serpa MC, Martins TF, Muñoz-Leal S, Labruna MB, Teixeira BM (2024) Evaluating the circulation of *Ehrlichia canis* and *Rickettsia* spp. in domestic dogs from a semiarid region in Brazil. *Vet Parasitol Reg Stud Rep* 52:101041. <https://doi.org/10.1016/j.vprsr.2024.101041>
14. Foley J, Backus L, López-Pérez AM (2025) Focus on brown dog tick–transmitted Rocky Mountain spotted fever in dogs and people: shared threats and solutions. *J Am Vet Med Assoc* 263:293–300. <https://doi.org/10.2460/javma.24.11.0756>
15. Gama BC, Martins TF, Labruna MB, Vieira RFDC, de Almeida JC (2023) First report of *Amblyomma sculptum* (*Amblyomma cajennense* complex) in a Brazilian state classified as a silent area for human rickettsiosis. *Vet World* 16:2200–2204. <https://doi.org/10.14202/vetworld.2023.2200-2204>
16. Gazeta JFA, Pais Borsoi AB, Bitencourth K, Côrtes MG, Machado IB, Melo da Silva BP, Cavalcante R, Gazeta GS, Costa da Cunha N (2025) The mosaic of Rickettsiae from the Caatinga Biome, Brazil: new records and first interactions. *PLoS Negl Trop Dis* 19:e0013670. <https://doi.org/10.1371/journal.pntd.0013670>
17. Gonçalves ES, Cordeiro MD, Dos Santos LMR, Araújo IM, da Fonseca AH, Labruna MB, Guedes E (2022) Research of *Rickettsia* spp. and *Borrelia* spp. in dogs in Southeast Brazil. *Vet Parasitol Reg Stud Rep* 30:100706. <https://doi.org/10.1016/j.vprsr.2022.100706>
18. Gual-Gonzalez L, Torres ME, Self SCW, Cantillo-Barraza O, Nolan MS (2024) Spotted Fever Group *Rickettsia* spp. molecular and serological evidence among Colombian vectors and animal hosts: a historical review. *Insects* 15:170. <https://doi.org/10.3390/insects15030170>

19. Guedes E, Leite RC, Pacheco RC, Silveira I, Labruna MB (2011) *Rickettsia* species infecting *Amblyomma* ticks from an area endemic for Brazilian spotted fever in Brazil. *Rev Bras Parasitol Vet* 20:308–311. <https://doi.org/10.1590/s1984-29612011000400009>
20. Horta MC, Labruna MB, Sangioni LA, Vianna MCB, Gennari SM, Galvão MAM, Mafra CL, Vidotto O, Schumaker TTS, Walker DH (2004) Prevalence of antibodies to Spotted Fever Group *Rickettsiae* in humans and domestic animals in a Brazilian Spotted Fever-endemic area in the state of São Paulo, Brazil: serologic evidence for infection by *Rickettsia rickettsii* and another Spotted Fever Group *Rickettsia*. *Am J Trop Med Hyg* 71:93–97. <https://doi.org/10.4269/ajtmh.2004.71.93>
21. Instituto Brasileiro de Geografia e Estatística (2021) Censo Demográfico de 2021: população e domicílios – resultados preliminares. IBGE. <https://www.ibge.gov.br>. Acessado em 20 de março de 2026
22. Kmetiuk LB, Santarém VA, Rodrigues D, Resende STF, Ferreira IB, Giuffrida R, da Silva BBF, Neves LC, Bittencourt RBM, Biondo LM, Figueiredo FB, Krawczak FS, Biondo AW (2025) One health approach to *Rickettsia* spp.: Brazilian indigenous individuals, their dogs and ticks, and healthcare professionals. *One Health* 20:101025. <https://doi.org/10.1016/j.onehlt.2025.101025>
23. Labruna MB, Whitworth T, Horta MC, Bouyer DH, McBride JW, Pinter A, Popov V, Gennari SM, Walker DH (2004) *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the state of São Paulo, Brazil, where Brazilian spotted fever is endemic. *J Clin Microbiol* 42:90–98. <https://doi.org/10.1128/JCM.42.1.90-98.2004>
24. Labruna MB, Horta MC, Aguiar DM, Cavalcante GT, Pinter A, Gennari SM, Camargo LMA (2007) Prevalence of *Rickettsia* infection in dogs from the urban and rural areas of Monte Negro Municipality, Western Amazon, Brazil. *Vector Borne Zoonotic Dis* 7:249–255. <https://doi.org/10.1089/vbz.2006.0621>
25. Mota RA, Silito IS, Martins MP, Oliveira PRF, Melo RPB, Vieira da Silva V, Jorge FR, Martins TF, Labruna MB (2026) Serological evidence of *Rickettsia* in horses and survey of tick-borne agents in ticks from horses and wildlife in the state of Pernambuco, Brazil. *J Med Entomol* 63:tjaf155. <https://doi.org/10.1093/jme/tjaf155>
26. Neves LC, Paula WV, de d F LGF, da Silva BBF, Dias SA, Pereira BG, Silva BSA, Sevá A, d P, Dantas-Torres F, Labruna MB, Krawczak F (2023) d S Detection of *Rickettsia* spp. in animals and ticks in Midwestern Brazil, where human cases of rickettsiosis were reported. *Animals* 13:1288. <https://doi.org/10.3390/ani13081288>
27. Oliveira PB, Harvey TV, Fehlberg HF, Rocha JM, Martins TF, Acosta ICL, Labruna MB, Faccini JLH, Albuquerque GR (2019) Serologic and molecular survey of *Rickettsia* spp. in dogs, horses and ticks from the Atlantic rainforest of the state of Bahia, Brazil. *Exp Appl Acarol* 78:431–442. <https://doi.org/10.1007/s10493-019-00397-x>
28. Ogrzewalska M, Saraiva DG, Moraes-Filho J, Martins TF, Costa FB, Pinter A, Labruna MB (2012) Epidemiology of Brazilian spotted fever in the Atlantic Forest, state of São Paulo, Brazil. *Parasitology* 139:1283–1300. <https://doi.org/10.1017/S0031182012000546>

29. Oliveira SV, Caldas EP, Colombo S, Gazeta GS, Labruna MB (2016) An update on the epidemiological situation of spotted fever in Brazil. *J Venom Anim Toxins Incl Trop Dis* 22:1–8.
<https://doi.org/10.1186/s40409-016-0077-4>
30. Oliveira SV, Costa RMF, Ferreira G, Pereira SVC, Amorim M, Monteiro MFM, Alves LC, Gazeta GS (2018) Fatal case of spotted fever in a patient from Northeastern Brazil. *Rev Inst Med Trop Sao Paulo* 60:e21. <https://doi.org/10.1590/s1678-9946201860021>
31. Qurollo BA, Korla P, Karounos M, Clarke S, Robveille C, Wilson J, Breitschwerdt E, Birkenheuer A (2025) Isolation and characterization of *Rickettsia finnyi*, novel pathogenic spotted fever group *Rickettsia* in dogs, United States. *Emerg Infect Dis* 31:2118–2127.
<https://doi.org/10.3201/eid3111.250681>
32. Ramírez-Hernández A, Uchoa F, de Azevedo Serpa MC et al (2020) Clinical and serological evaluation of capybaras (*Hydrochoerus hydrochaeris*) successively exposed to an *Amblyomma sculptum*-derived strain of *Rickettsia rickettsii*. *Sci Rep* 10:924. <https://doi.org/10.1038/s41598-020-57607-5>
33. Regnery RL, Spruill CL, Plikaytis BD (1991) Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. *J Bacteriol* 173:1576–1589.
<https://doi.org/10.1128/jb.173.5.1576-1589.1991>
34. Sevá AP, Martins TF, Muñoz-Leal S, Rodrigues AC, Pinter A, Luz HR, Angerami RN, Labruna MB (2019) A human case of spotted fever caused by *Rickettsia parkeri* strain Atlantic rainforest and its association to the tick *Amblyomma ovale*. *Parasit Vectors* 12:471. <https://doi.org/10.1186/s13071-019-3730-2>
35. Silva APB, De Castro Araújo Pacheco C, De Faria ACM, Borsoi ABP, Bitencourth K, Neto C, a B, Da Silva WM, Hora DPG, Da Silva CE, Leite AB, De Lima Duré AÍ, Da Silva FCL, Soares EISC, Da Silva Oliveira MJ, Angerami RN, Da Silva DCC Almeida Guimarães LN, Cabrera L a A, Gazêta GS (2026) Environmental and laboratorial investigation of two potential spotted-fever group rickettsioses cases in a non-endemic area of Brazil. *WebLog J Public Health Epidemiol* a0301.
<https://doi.org/10.5281/zenodo.18212574>
36. Souza CE, Camargo LB, Pinter A, Donalisio MR (2016) High seroprevalence for *Rickettsia rickettsii* in equines suggests risk of human infection in silent areas for the Brazilian spotted fever. *PLoS ONE* 11:e0153303. <https://doi.org/10.1371/journal.pone.0153303>
37. Suzin A, da Silva MX, Tognolli MH, Vogliotti A, Adami SF, Moraes MFD et al (2022) Ticks on humans in an Atlantic rainforest preserved ecosystem in Brazil: species, life stages, attachment sites, and temporal pattern of infestation. *Ticks Tick Borne Dis* 13:101862.
<https://doi.org/10.1016/j.ttbdis.2021.101862>
38. Toledo RS, Tamekuni K, Silva Filho MF, Haydu VB, Barbieri ARM, Hiltel AC, Pacheco RC, Labruna MB, Dumler JS, Vidotto O (2011) Infection by spotted fever rickettsiae in people, dogs, horses and ticks in Londrina, Paraná State, Brazil. *Zoonoses Public Health* 58:416–423.
<https://doi.org/10.1111/j.1863-2378.2010.01382.x>

39. Weck B, Krawczak FS, Costa FB, Dall'Agnol B, Marcili A, Reck J, Labruna MB (2020) *Rickettsia parkeri* in the Pampa biome of southern Brazil: isolation, molecular characterization, and serological evidence of canine infection. *Vet Parasitol Reg Stud Rep* 22:100448. <https://doi.org/10.1016/j.vprsr.2020.100448>
40. Zavala-Velazquez JE, Yu XJ, Walker DH (1996) Unrecognized spotted fever group rickettsiosis masquerading as dengue fever in Mexico. *Am J Trop Med Hyg* 55:157–159. <https://doi.org/10.4269/ajtmh.1996.55.157>
41. Zulzke L, Labruna MB, Silva BRFD, Marcili A, Martins TF, Moraes-Filho J (2023) Molecular detection of *Rickettsia rickettsii*, *Ehrlichia canis* and *Rangelia vitalli* in *Rhipicephalus sanguineus* sensu lato collected from dogs in Brazil. *Arq Bras Med Vet Zootec* 75:843–848. <https://doi.org/10.1590/1678-4162-12864>

Figures



Figure 1

Figure 5. Spatial distribution of the location where the PCR-positive dog resided and the site of the first human fatality recorded in Alagoas. The geographical proximity between the seroreactive animal and the

probable site of infection of the fatal case suggests a potential epidemiological relationship.

Source: Google Earth/Authors' archive, 2024.