









# *Candidatus Rickettsia andeanae* in *Amblyomma parvum* attached to humans in a high-altitude forest refuge within the Caatinga biome, Brazil

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Received: November 2021; Accepted: February 2022; Published: May 2022.

## ABSTRACT

**Objective.** This studied aimed to report *Amblyomma parvum* ticks parasitizing humans in a high-altitude forest within the Caatinga biome, Brazil, with notes on rickettsial infection. **Materials and methods.** The study was carried out in the Metropolitan Region of Sobral, in the Serra of Meruoca, within the Municipality of Meruoca (3° 32' 20" S; 40° 26' 56" W; altitude 650 m), in the northwest region of the State of Ceará, Brazil. All ticks were identified morphologically under stereomicroscope. Some of the collected ticks were tested molecularly for the presence of DNA of bacteria of the genus *Rickettsia*, with PCR protocols targeting two rickettsial genes: citrate synthase gene (*gltA*) and outer membrane protein gene (*ompA*). **Results.** A total of 78 ticks were collected on humans, identified as adults of *Amblyomma parvum* (62 females and 16 males). Of these, 20 females were tested, of which 15 (75%) were infected by '*Candidatus Rickettsia andeanae*'. **Conclusions.** The present study confirms one more area at human risk for tick bites in Brazil, also for the non-pathogenic rickettsia '*Ca. Rickettsia andeanae*'

**Keywords:** *Rickettsia*; spotted fever; anthropophilic; Northeast; Brazil (Source: DeCS, CAB, TGN).

## RESUMEN

**Objetivo.** Este estudio reporta garrapatas *Amblyomma parvum* que parasitan a humanos en un bosque a gran altitud dentro del bioma de Caatinga, Brasil, con nota de infección por rickettsias. **Materiales y métodos.** El presente estudio se realizó en la Región Metropolitana de Sobral, en la Serra de Meruoca, en el municipio de Meruoca (3 ° 32 '20 "S; 40° 26 '56" W; altitud 650 m), en la región noroeste del estado de Ceará, Brasil. Todas las garrapatas se identificaron morfológicamente con estereomicroscopio. Algunas de las garrapatas recolectadas se analizaron molecularmente para

### How to cite (Vancouver).

Acosta ICL, da Costa-Neto SF, Lucio C, Tavres CP, Labruna MB, Luz HR. *Candidatus Rickettsia andeanae* in *Amblyomma parvum* attached to humans in a high-altitude forest refuge within the Caatinga biome, Brazil. Rev MVZ Córdoba. 2022; 27(2):e2598. <https://doi.org/10.21897/rmvz.2598>



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detectar la presencia de ADN de bacterias del género *Rickettsia*, con protocolos de PCR dirigidos a dos genes rickettsiales: el gen de la citrato sintasa (*gltA*) y el gen de la proteína de la membrana externa (*ompA*). **Resultados.** Se recolectaron un total de 78 garrapatas en humanos, identificadas como adultos de *Amblyomma parvum* (62 hembras y 16 machos). De estas, se analizaron 20 hembras, de las cuales 15 (75%) fueron infectadas por '*Candidatus Rickettsia andeanae*'. **Conclusiones.** El presente estudio confirma una zona más de riesgo humano de picaduras de garrapatas en Brasil, también de la rickettsia no patógena '*Ca. Rickettsia andeanae*'

**Palabras clave:** *Rickettsia*; fiebre maculosa; anthropophilic; Nordeste; Brasil (*Fuentes: DeCS, CAB, TGN*).

## INTRODUCTION

Tick-borne rickettsioses are infections caused by obligate intracellular bacteria of the genus *Rickettsia*, family Rickettsiaceae (1). In Brazil, there are approximately sixteen species and/or strains of tick-associated *Rickettsia*, of which at least two species causes spotted fever group (SFG) illness in humans: *Rickettsia rickettsii*, the agent of Brazilian spotted fever, a highly lethal acute disease, and *Rickettsia parkeri*, the agent of a mild rickettsiosis with no reported lethality so far (1,2,3,4,5,6,7,8). The SFG agent '*Candidatus Rickettsia andeanae*' has been described infecting only ticks, and its role as a human pathogen has never been demonstrated (1,7). The majority of '*Ca. R. andeanae*' infections of ticks in Brazil were detected in *A. parvum* in the Pantanal biome (state of Mato Grosso do Sul) and from horses in the Cerrado biome (state of Piauí) (8).

*Amblyomma parvum* has a wide distribution in the Neotropics, found mainly in environments with moderate humidity and relatively high temperatures (9). In Brazil, this tick is endemic in the Cerrado and Caatinga biomes, and in different ecotones of the Pantanal biome (10,11). While immature stages parasitize rodents and wild birds, adults prefer large wild and domestic mammals (9). Interestingly, *A. parvum* comprises a select group of human-biting tick in South America (12). Furthermore, there are reports of its infection by *Coxiella burnetii*, and an *Ehrlichia chaffeensis*-like agent (*Ehrlichia* sp. strain San Luis) (13,14).

The Caatinga biome refers to the semiarid ecosystems in northeastern Brazil, with low rainfall and humidity regimes (15). In this biome, the records of ticks and their microbial agents are more frequent on wild and domestic

animals, being scarce in association with humans (11,16,17,18,19). Interestingly, within the Caatinga biome, isolated areas of forest resembling the Atlantic rainforest exist at higher altitudes, usually >400 meters above sea level. These forest areas contrast to the typical Caatinga areas by having high humidity and mild temperature conditions (20,21).

In one of these high-altitude forests of the Caatinga biome, called "Maciço de Baturité", (22) reported a total of 10 tick species associated with environment, humans, domestic and wild animals; in addition to three rickettsial agents infecting these ticks: *R. parkeri* strain Atlantic rainforest, *Rickettsia bellii*, and '*Ca. R. andeanae*'. With the purpose to generate additional information, here we report ticks infesting humans in another high-altitude forest within the Caatinga biome. In addition, we tested the ticks for the presence of rickettsiae.

## MATERIALS AND METHODS

The present study was carried out in the Metropolitan Region of Sobral, in the Serra of Meruoca, within the Municipality of Meruoca (3° 32' 20" S; 40° 26' 56" W; altitude 650 m), in the northwest region of the State of Ceará, Northeast of Brazil. The landscape is characterized by remnants of deciduous tropical forests and subdeciduous, known as high-altitude forests of the Caatinga biome. Average annual temperature of 20 to 26°C (22,23). In addition, it has a constant presence of human beings (during the summer, consequently, due to the greater precipitation and formation of intermittent rivers), driven by agricultural and tourist activities in search of extreme sports, spas and natural waterfalls.

During a study related to the Chagas disease vectors and *Trypanosoma cruzi* in domestic and wild animals of the 4-day in the Sobral metropolitan region in June 2016, researchers observed the presence of ticks on clothes and/or biting different regions of the body after walking the local trails. Detected ticks were conveniently collected and stored individually in microtubes containing absolute isopropanol, and then sent to the laboratory for identification. All ticks were identified morphologically under stereomicroscope and according to Dantas-Torres et al (24).

Some of the collected ticks (20 females) were tested molecularly for the presence of DNA of bacteria of the genus *Rickettsia*. For this purpose, ticks were submitted individually to DNA extraction by the guanidine isothiocyanate and phenol/chloroform technique (25), and tested by a genus-specific PCR assay with primers CS-78 and CS-323, which amplify a 401-bp fragment of the rickettsial citrate synthase gene (*gltA*) (26). Samples positive in this assay were further tested by another PCR protocol using the primers Rr190.70p and Rr190.701n, which amplify a 631-bp fragment of the 190-kDa outer membrane protein gene (*ompA*) in most of the SFG rickettsiae (27). In order to increase the sensitivity of the conventional PCR, 1 µl of the product of all *ompA*-negative or weakly amplified *ompA*-positive samples in the previous *ompA* assay was used as template in a second PCR assay (hemi-nested PCR) with primers Rr190.70p and Rr190.602, targeting a 532-bp fragment of the *ompA* gene (28). Ultrapure water and DNA of *R. parkeri* strain NOD were included as negative and positive controls, respectively, in each PCR run. A 1.5% TBE agarose gel was used to visualize PCR products under UV light. The PCR products were treated with ExoSap-IT (USB, Cleveland, Ohio, USA) and DNA-sequenced in an ABI automated sequencer (Applied Biosystems/Thermo Fisher Scientific, model ABI 3500 Genetic Analyzer, Foster City, California, USA) with the same primers used for PCR. Contigs were assembled with Geneious R9 software and submitted to BLAST analyses ([www.ncbi.nlm.nih.gov/blast](http://www.ncbi.nlm.nih.gov/blast)) to infer closest identities to sequences available in GenBank.

## RESULTS

A total of 78 ticks were collected on 8 researchers, an approximate mean intensity

of infestation of 10 ticks/researcher. All ticks were morphologically identified as adults of *A. parvum* (62 females and 16 males). Of these, 40 (51.2%) ticks (9 males, 31 females) were found attached to human skin, especially the abdomen and thighs. The remaining 38 (48.8%) ticks (12 males, 26 females) were found unattached, walking on human clothing or skin, especially between the waist and knee regions.

Due to limited laboratory resources, only females biting humans (n=20 ticks) were submitted to DNA extraction and analyzed for the presence of *Rickettsia*. Of these, 15 (75%) specimens yielded amplicons using the PCR assays targeting the *gltA* and *ompA* genes. The *gltA* amplicons generated a 350-bp consensus sequence 100% identical to *gltA* sequences of '*Ca. R. andeanae*' (GenBank MG887826, KY402176). Similarly, *ompA* amplicons generated a 587-bp consensus sequence 100% identical to *ompA* sequences of '*Ca. R. andeanae*' (GenBank KY628370, KX434737).

The GenBank nucleotide sequence accession numbers for the *gltA* and *ompA* partial sequences generated in this study are OK050181 and OK050182, respectively.

## DISCUSSION

In the present study, we provide new records of *A. parvum* infesting humans in a high-altitude forest within the Caatinga biome, Ceará State, Northeast Brazil. Notably, 15 (75%) out of 20 female ticks biting humans were infected with '*Ca. R. andeanae*'. These records show an increase risk of tick parasitism on humans and possible transmission of rickettsial agents in the study area.

Although widely distributed in the Neotropics (Mexico to Argentina), *A. parvum* is most often found in ecoregions with high temperatures and low to moderate humidity, including the Cerrado and Caatinga biomes, and in Cerrado-Pantanal ecotones in Brazil (9,16,19). Interesting, there are records of *A. parvum* parasitizing wildlife (Dasypodidae and Cervidae) on another high-altitude forest area of Ceará state, the "Maciço de Baturité" (22), which is located ≈200 Km distant from the present study area.

Most of the reports of *A. parvum* in the Caatinga biome have been from the more typical semiarid

phytophysiognomies, where immature (larvae and nymphs) of this tick species parasitizes mainly rodents, marsupials and wild birds. In contrast, adults of *A. parvum* prefer medium- to large-sized domestic and wild mammals (e.g., goat, sheep, dog, horse, fox) (11,18,19,29,30). Interestingly, adults of *A. parvum* are quite anthropophilic and records on human in Brazil are concentrated mainly in the Cerrado and Pantanal biomes, with rare records in the Caatinga biome (12,18,31,32). This reinforces the importance of the encounter of *A. parvum* biting humans in the present study.

The bacterium '*Ca. R. andeanae*' has been reported infecting different tick species of the New World, including *Amblyomma maculatum*, *Ixodes boliviensis* and *Rhipicephalus sanguineus* sensu lato in Peru (33,34), *A. maculatum* in the United States (35,36), *A. parvum* and *Amblyomma pseudoconcolor* in Argentina (37,38), and *Amblyomma triste* in Chile (39). In Brazil, '*Ca. R. andeanae*' was reported infecting *A. parvum* ticks from the Pantanal and Cerrado biomes (8,40). In addition, in the Caatinga biome, '*Ca. R. andeanae*' was detected in specimens of *A. parvum* parasitizing *Monodelphis domestica*, *Canis familiaris* (18,19) and numerous species of wild birds (11). Furthermore, an uncharacterized *Rickettsia* agent related to SFG was detected in *A. parvum* adults in the Caatinga biome, identified as *Rickettsia* sp. haplotype ApBA1 and ApBA2 (19). Although there are reports of *A. parvum* on humans in the Caatinga biome (18), the present study is the first to report *A. parvum* specimens infected with '*Ca. R. andeanae*' parasitizing humans in this biome.

The present 75% infection rate of '*Ca. R. andeanae*' in *A. parvum* is in accordance with previous studies that reported that most of the *A. parvum* ticks from Argentina (69.2%) and from Brazil (63.5% in Pantanal biome;

66.7% in Cerrado biome) were infected with '*Ca. R. andeanae*' (8). Regardless, the role of '*Ca. R. andeanae*' as a human pathogen is unknown. Because *A. parvum* is an important human-biting tick in South America (12), further studies are needed to evaluate the vector competence of *A. parvum* for '*Ca. R. andeanae*' and its pathogenicity to humans or other vertebrate hosts. In our study, none of the infested humans reported symptoms compatible with rickettsial infection (e.g., fever, myalgia, vomiting or petechiae) during the subsequent 14 days after infestations with '*Ca. R. andeanae*'-infected ticks. It is possible to speculate on three possible scenarios: (i) tick did not remain attached for a minimal period required for rickettsial transmission; (ii) human infection occurred, but symptoms were unnoticeable due to little or no pathogenicity; or (iii) '*Ca. R. andeanae*' is not tick-transmitted; it is just a tick endosymbiont.

Finally, several ecological and behavioral traits of ticks or hosts may either favor or restrict human encounters and, therefore, tick bite and disease agent transmission. In this regard, preferences of tick species for specific hosts as well as space and time overlapping of parasite and host, are key features for tick-borne disease epidemiology (32). The Serra Meruoca has a rich biodiversity and innumerable natural landscapes, which attracts thousands of tourists throughout the year. In this scenario, added to the records of the present study, the Serra of Meruoca enters the select group of priority areas for future investigations of the transmission of pathogenic agents by ticks to animals and humans.

### Conflicts of interest

The authors declare having no conflicts of interest.

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