

***Rickettsia amblyommii* infecting *Amblyomma sculptum* in endemic spotted fever area from southeastern Brazil**

**Emília de Carvalho Nunes¹, Vinicius Figueiredo Vizzoni^{2,3}, Daniel Leal Navarro¹,
Felipe Campos de Melo Iani⁴, Liliane Silva Durães¹, Erik Daemon¹,
Carlos Augusto Gomes Soares³, Gilberto Salles Gazeta^{2/+}**

¹Universidade Federal de Juiz de Fora, Instituto de Ciências Biológicas, Programa de Pós-Graduação em Ciências Biológicas, Comportamento e Biologia Animal, Juiz de Fora, MG, Brasil ²Fundação Oswaldo Cruz, Instituto Oswaldo Cruz, Laboratório de Referência Nacional em Vetores das Riquetsioses, Rio de Janeiro, RJ, Brasil ³Universidade Federal do Rio de Janeiro, Instituto de Biologia, Departamento de Genética, Laboratório de Genética Molecular de Eucariontes e Simbiontes, Rio de Janeiro, RJ, Brasil

⁴Fundação Ezequiel Dias, Laboratório Central de Minas Gerais, Serviço de Virologia e Riquetsioses, Belo Horizonte, MG, Brasil

The Rickettsia bacteria include the aetiological agents for the human spotted fever (SF) disease. In the present study, a SF group Rickettsia amblyommii related bacterium was detected in a field collected Amblyomma sculptum (Amblyomma cajennense species complex) tick from a Brazilian SF endemic site in southeastern Brazil, in the municipality of Juiz de Fora, state of Minas Gerais. Genetic analysis based on genes ompA, ompB and htrA showed that the detected strain, named R. amblyommii str. JF, is related to the species R. amblyommii.

Key words: *Rickettsia amblyommii* - *Amblyomma sculptum* - southeastern Brazil - ticks

Over the past 14 years, the number of rickettsial species identified in South America increased from three to more than 10. Initially, only occurrences of *Rickettsia prowazekii*, *Rickettsia typhi*, and *Rickettsia rickettsii* were historically reported, followed by most recent detection of *Rickettsia felis*, *Rickettsia parkeri*, *Rickettsia belli*, *Rickettsia massiliae*, *Rickettsia rhipicephali*, and *Rickettsia amblyommii* from different environmental samples (Labruna 2009).

Among those cited, six species belong to the spotted fever group (SFG), including the known human pathogens *R. rickettsii*, *R. felis*, *R. parkeri*, and *R. massiliae*, each causing specific rickettsiosis, whereas *R. rhipicephali* and *R. amblyommii* are classified as with still unknown/unclear pathogenicity (Merhej et al. 2014).

R. rickettsii is the aetiological agent of the Rocky Mountain SF, the most severe of all tick-borne rickettsiosis (Parola et al. 2005). In Brazil this species causes the Brazilian SF (BSF), a disease that in the last 14 years was reported in 1,421 cases throughout Brazil, according to official data of the Information System on Notifiable Diseases (dtr2004.saude.gov.br/sinanweb/). During the period of 1995-2004, there were 334 laboratory-confirmed cases of BSF with a 31% lethality rate in the Southeast Region of Brazil. Additional 128 cases, with lethality of 29%, were confirmed from 2005-2007 only in the state of São Paulo (Labruna 2009). In the state of Minas Gerais other BSF cases were also confirmed and in the endemic area of the city of Juiz de Fora, 24 cases were actually confirmed be-

tween 2001-2014 (dtr2004.saude.gov.br/sinanweb/). According to Pacheco et al. (2011), 17 cases were notified between 1995-2008, with a lethality of 29%.

Ticks are the most important vectors for SF transmission. In South America, the tick *Amblyomma cajennense* has been considered to be the most frequent vector related with SF cases. Interestingly recent genetic and morphological/microscopic analyses showed evidence that *A. cajennense* in fact represents a complex grouping six tick species (*A. cajennense sensu lato* or *s.l.*) (Beati et al. 2013, Nava et al. 2014).

The endemicity of BSF leads Juiz de Fora Health Office to promote a constant environmental monitoring of ticks. Forty-eight horses fed *A. sculptum* ticks were obtained during this vigilance and the specimens were identified according to the new description of species that belong to the *A. cajennense* complex (Nava et al. 2014). The neighbourhoods known as Previdenciários and Monte Castelo were visited and both areas had confirmed human BSF cases. Sampled ticks were processed for molecular analysis and initially submitted to DNA extraction as described elsewhere [method with NaCl (Aljanabi & Martinez 1997)].

Rickettsia infected ticks were identified by polymerase chain reaction (PCR) screening for the rickettsial *ompA*, *ompB* and *htrA* genes in 25 µL conventional PCR reactions under the temperature/time cycle: 94°C 3 min and 30 s (94°C 30 s, 55°C 30 s, 72°C 1 min/Kb) 40X, 72°C 7 min, 20°C ∞. *R. parkeri* str. AT#24 DNA was used as a positive control. The primers used were Rr190.70F and Rr190.602R (Regnery et al. 1991) for *ompA* gene, Rr1175F and Rr2608R (Blair et al. 2004) for *htrA* gene and ompB3064-F (5'gggtatagccggaatagtttgacg, present study) and ompB4271-R (5'tcagtttagtgat-accgatagcagc, present study) for *ompB* gene. PCR products were purified using HiYield™ Gel/PCR DNA Mini Kit according to manufacturer (Real Genomics™, New

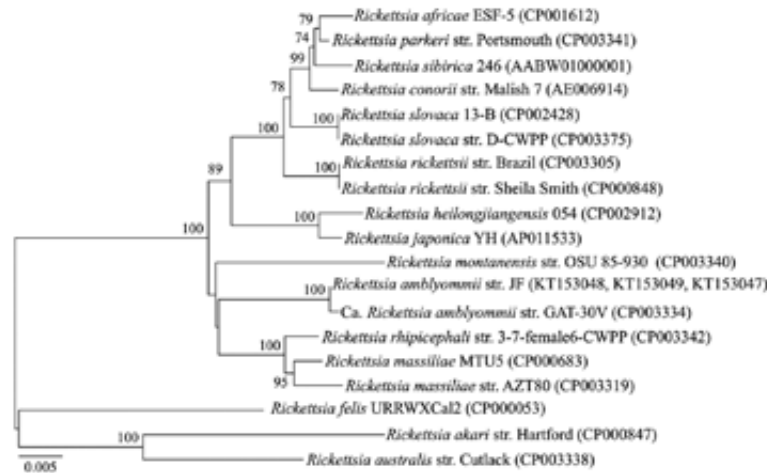
doi: 10.1590/0074-02760150266

ECN and VFV contributed equally to this work.

+ Corresponding author: gsgazeta@ioc.fiocruz.br

Received 15 July 2015

Accepted 6 November 2015



Phylogenetic tree of concatenated spotted fever group rickettsiae *ompA*, *ompB* and *htrA* genes constructed by neighbour-joining method with Kimura two-parameter as evolution model and based on the nucleotide sequences. The GenBank accession codes are presented in parenthesis. The numbers at nodes are the bootstrap values obtained from 1,000 re-samplings. Bootstrap values below 70% are not present.

Zealand), sequenced in both directions on an automated ABI 3130xl Genetic Analyser (Applied Biosystems®, USA) and using the same primers applied for the initial PCR amplifications. Sequence edition was performed with Lasergene software packages (DNASTAR, USA).

One *A. sculptum* female was positive for *Rickettsia* infection as determined by the PCR tests. PCR amplicons of 512 bp, 1,208 bp, and 434 bp were obtained using the primer sets for *ompA*, *ompB* and *htrA*, respectively.

A phylogenetic tree was constructed with concatenated *ompA*, *ompB* and *htrA* sequences, neighbour-joining methods [MEGA 5.2 (Tamura et al. 2011)], and Kimura two-parameter model to estimate genetic divergence (Kimura 1980) and bootstrap values were obtained from 1,000 randomly generated trees. The resulting tree showed that the presently identified strain, here named *R. amblyommii* str. JF, is most closely related to *R. amblyommii* (Figure).

The first detection of *R. amblyommii* occurred after analysis of *Amblyomma americanum* ticks in the United States of America (Burgdorfer et al. 1981). After that, *R. amblyommii* and other genetically related species were found in a wide variety of tick species in different countries in the New World (Labruna et al. 2007, Zanetti et al. 2008, Hun et al. 2011, Saraiva et al. 2013). In the present study, a *R. amblyommii* related infection was characterised in an *A. sculptum* sample, an *A. cajennense*-complex species abundantly observed in Juiz de Fora. Other bacteria genetically related to *R. amblyommii* was previously found associated with *A. cajennense* s.l. from Brazil (Labruna et al. 2004, Soares et al. 2015) and Alves et al. (2014) provided the first description of *R. amblyommii*-like infection in *A. sculptum* tick.

Despite the amount of studies on *Rickettsia* performed in thousands of ticks sampled in southeastern Brazil (Guedes et al. 2005, Gehrke et al. 2009, Pacheco et al. 2009), the present paper is the first report of a *R. amblyommii* related SFG infecting *A. sculptum* in this region.

Several reports indicate that *R. amblyommii* is commonly found in ticks parasitising human (Jiang et al. 2010, Lee et al. 2014) and the observed *R. amblyommii* in Juiz de Fora could distinctly represent an unusual rickettsiosis agent. Primarily nonpathogenic *Rickettsia* is able to cause disease under some circumstances, as reported for *R. parkeri* (Paddock et al. 2004). The pathogenic potential of *R. amblyommii* and genotypically similar strains is still speculative, but increasingly studies have associate these bacteria to rickettsiosis cases (Taylor et al. 1985, Yevich et al. 1995, Billeter et al. 2007, Apperson et al. 2008).

The potential pathogenic capacity of *R. amblyommii* would bring major concerns in terms of public health since this bacterium can infect a variety of vertebrate hosts with wide distribution, including species with dense populations and commonly found in parks or recreational areas (Blanton et al. 2014).

Despite that not all ticks can actively feed and parasitise humans, serological data obtained from dogs and horses showed the circulation of *R. amblyommii* in domestic animals (Barrett et al. 2014). Frequent infestation of these animals with specific tick species could facilitate the transmission of this bacterium to humans. Indeed, ticks frequently found on dogs and horses have been reported to be able to support *R. amblyommii* infection (Bermúdez et al. 2009, Eremeeva et al. 2009) and this bacterium seems to be well adapted to its hosts, presenting highly successful transmission rates (Burgdorfer et al. 1981, Saraiva et al. 2013).

Taking together, these observations suggest that this bacterium may be involved in cases of atypical SF cases and show the need for further studies to elucidate its real pathogenic potential. Although the *R. amblyommii* pathogenic status remains unclear, the present study brings new contribution to the understanding of its complex vector/host network.

ACKNOWLEDGEMENTS

To the vectors collection maintained at Oswaldo Cruz Institute, Collection of Apteroous Arthropod Vectors of Community Health Importance, for tick specimens.

REFERENCES

- Aljanabi SM, Martinez I 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Res* 25: 4692-4693.
- Alves AS, Melo ALT, Amorim MV, Borges AMCM, Gaiva e Silva L, Martins TF, Labruna MB, Aguiar DM, Pacheco RC 2014. Sero-prevalence of *Rickettsia* spp in equids and molecular detection of *Candidatus Rickettsia amblyommii* in *Amblyomma cajennense* sensu lato ticks from the Pantanal region of Mato Grosso, Brazil. *J Med Entomol* 51: 1242-1247.
- Apperson CS, Engber B, Nicholson WL, Mead DG, Engel J, Yabsley MJ, Dail K, Johnson J, Watson DW 2008. Tick-borne diseases in North Carolina: is *Rickettsia amblyommii* a possible cause of rickettsiosis reported as Rocky Mountain spotted fever? *Vector Borne Zoonotic Dis* 8: 597-606.
- Barrett A, Little SE, Shaw E 2014. *Rickettsia amblyommii* and *R. montanensis* infection in dogs following natural exposure to ticks. *Vector Borne Zoonotic Dis* 14: 20-25.
- Beati L, Nava S, Burkman EJ, Barros-Battesti DM, Labruna MB, Guglielmone AA, Cáceres AG, Guzmán-Cornejo CM, León R, Durden LA, Faccini JL 2013. *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae), the Cayenne tick: phylogeography and evidence for allopatric speciation, Panama. *BMC Evol Biol* 13: 267.
- Bermúdez SE, Eremeeva ME, Karpathy SE, Samudio F, Zambrano ML, Zaldivar Y, Motta JA, Dasch GA 2009. Detection and identification of rickettsial agents in ticks from domestic mammals in eastern Panama. *J Med Entomol* 46: 856-861.
- Billeter SA, Blanton HL, Little SE, Levy MG, Breitschwerdt EB 2007. Detection of *Rickettsia amblyommii* in association with a tick bite rash. *Vector Borne Zoonotic Dis* 7: 607-610.
- Blair PJ, Jiang J, Schoeler GB, Moron C, Anaya E, Cespedes M, Cruz C, Felices V, Guevara C, Mendoza L, Villaseca P, Sumner JW, Richards AL, Olson JG 2004. Characterization of spotted fever group rickettsiae in flea and tick specimens from northern Peru. *J Clin Microbiol* 42: 4961-4967.
- Blanton LS, Walker DH, Bouyer DH 2014. Rickettsiae and ehrlichiae within a city park: is the urban dweller at risk? *Vector Borne Zoonotic Dis* 14: 168-170.
- Burgdorfer W, Hayes SF, Thomas LA, Lancaster JL 1981. A new spotted fever group *Rickettsia* from the lone star tick, *Amblyomma americanum*. In: W Burgdorfer, RL Anacker, *Rickettsiae and rickettsial diseases*, Academic Press, New York, p. 595-602.
- Eremeeva ME, Karpathy SE, Levin ML, Caballero CM, Bermudez S, Dasch GA, Motta JA 2009. Spotted fever rickettsiae, *Ehrlichia* and *Anaplasma* in ticks from peridomestic environments in Panama. *Clin Microbiol Infect* 2: 12-14.
- Gehrke FS, Gazeta GS, Souza ER, Ribeiro A, Marrelli MT, Schumaker TT 2009. *Rickettsia rickettsii*, *Rickettsia felis* and *Rickettsia* sp. TwKM03 infecting *Rhipicephalus sanguineus* and *Ctenocephalides felis* collected from dogs in a Brazilian spotted fever focus in the state of Rio de Janeiro/Brazil. *Clin Microbiol Infect* 2: 267-268.
- Guedes E, Leite RC, Prata MCA, Pacheco RC, Walker DH, Labruna MB 2005. Detection of *Rickettsia rickettsii* in the tick *Amblyomma cajennense* in a new Brazilian spotted fever-endemic area in the state of Minas Gerais. *Mem Inst Oswaldo Cruz* 100: 841-845.
- Hun L, Troyo A, Taylor L, Barbieri AM, Labruna MB 2011. First report of the isolation and molecular characterization of *Rickettsia amblyommii* and *Rickettsia felis* in Central America. *Vector Borne Zoonotic Dis* 11: 1395-1397.
- Jiang J, Yarina T, Miller MK, Stromdahl EY, Richards AL 2010. Molecular detection of *Rickettsia amblyommii* in *Amblyomma americanum* parasitizing humans. *Vector Borne Zoonotic Dis* 10: 329-340.
- Kimura M 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16: 111-120.
- Labruna MB 2009. Ecology of *Rickettsia* in South America. *Ann NY Acad Sci* 1166: 156-166.
- Labruna MB, Pacheco RC, Nava S, Brandão PE, Richtzenhain LJ, Guglielmone AA 2007. Infection by *Rickettsia bellii* and *Candidatus Rickettsia amblyommii* in *Amblyomma neumanni* ticks from Argentina. *Microb Ecol* 54: 126-133.
- Labruna MB, Whitworth T, Bouyer DH, McBride JW 2004. *Rickettsia bellii* and *Rickettsia amblyommii* in *Amblyomma* ticks from the state of Rondônia, western Amazon, Brazil. *J Med Entomol* 41: 1073-1081.
- Lee S, Kakumanu M, Ponnusamy L, Vaughn M, Funkhouser S, Thornton H, Meshnick SR, Apperson CS 2014. Prevalence of rickettsiales in ticks removed from the skin of outdoor workers in North Carolina. *Parasit Vectors* 7: 607.
- Merhej V, Angelakis E, Socolovschi C, Raoult D 2014. Genotyping, evolution, and epidemiological findings of *Rickettsia* species. *Infect Genet Evol* 25: 122-137.
- Nava S, Beati L, Labruna MB, Cáceres AG, Mangold AJ, Guglielmone AA 2014. Reassessment of the taxonomic status of *Amblyomma cajennense* (Fabricius, 1787) with the description of three new species, *Amblyomma tonelliae* n. sp., *Amblyomma interandinum* n. sp. and *Amblyomma patinoi* n. sp., and reinstatement of *Amblyomma mixtum* Koch, 1844, and *Amblyomma sculptum* Berlese, 1888 (Ixodida: Ixodidae). *Ticks Tick Borne Dis* 5: 252-276.
- Pacheco RC, Horta MC, Pinter A, Moraes-Filho J, Martins TF, Nardi MS, Souza SS, Souza CE, Szabó MP, Richtzenhain LJ, Labruna MB 2009. Survey of *Rickettsia* spp in the ticks *Amblyomma cajennense* and *Amblyomma dubitatum* in the state of São Paulo. *Rev Soc Bras Med Trop* 42: 351-353.
- Pacheco RC, Moraes-Filho J, Guedes E, Silveira I, Richtzenhain LJ, Leite RC, Labruna MB 2011. Rickettsial infections of dogs, horses, and ticks in Juiz de Fora, southeastern Brazil, and isolation of *Rickettsia rickettsii* from *Rhipicephalus sanguineus* ticks. *Med Vet Entomol* 25: 148-155.
- Paddock CD, Sumner JW, Comer JA, Zaki SR, Goldsmith CS, Goddard J, McLellan SL, Tamminga CL, Ohl CA 2004. *Rickettsia parkeri*: a newly recognized cause of spotted fever rickettsiosis in the United States. *Clin Infect Dis* 38: 805-811.
- Parola P, Paddock CD, Raoult D 2005. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. *Clin Microbiol Rev* 18: 719-756.
- 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.
- Saraiva DG, Nieri-Bastos FA, Horta MC, Soares HS, Nicola PA, Pereira LC, Labruna MB 2013. *Rickettsia amblyommii* infecting *Amblyomma auricularium* ticks in Pernambuco, northeastern Brazil: isolation, transovarial transmission, and transstadial perpetuation. *Vector Borne Zoonotic Dis* 13: 615-618.

- Soares HS, Barbieri AR, Martins TF, Minervino AH, de Lima JT, Marcili A, Gennari SM, Labruna MB 2015. Ticks and rickettsial infection in the wildlife of two regions of the Brazilian Amazon. *Exp Appl Acarol* 65: 125-140.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28: 2731-2739.
- Taylor JP, Tanner WB, Rawlings JA, Buck J, Elliott LB, Dewlett HJ, Taylor B, Betz TG 1985. Serological evidence of subclinical Rocky Mountain spotted fever infections in Texas. *J Infect Dis* 151: 367-369.
- Yevich SJ, Sánchez JL, de Fraites RF, Rives CC, Dawson JE, Uhaa IJ, Johnson BJ, Fishbein DB 1995. Seroepidemiology of infections due to spotted fever group rickettsiae and *Ehrlichia* species in military personnel exposed in areas of the United States where such infections are endemic. *J Infect Dis* 171: 1266-1273.
- Zanetti AS, Pornwiroon W, Kearney MT, Macaluso KR 2008. Characterization of rickettsial infection in *Amblyomma americanum* (Acari: Ixodidae) by quantitative real-time polymerase chain reaction. *J Med Entomol* 45: 267-275.