Ticks and Tick-borne Diseases 7 (2016) 1057–1058

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Letter to the Editor

## First report of spotted fever group *Rickettsia* in Cuba

*To the Editor,*

The spotted fever group of *Rickettsia* consists of multiple species ranging from microorganisms with unknown pathogenicity such as "*Candidatus* Rickettsia amblyommii" (hereafter *R. amblyommii*) to potentially lethal pathogens such as *R. rickettsii*, the causative agent of Rocky Mountain spotted fever (RMSF) in humans. *Rickettsia amblyommii* is found mainly in ticks belonging to *Amblyomma* genus, and reported prevalence ranges from 0% to higher than 50% in the American continent (Mixson et al., 2006; Labruna et al., 2011). Although it has not been conclusively established that *R. amblyommii* is pathogenic to humans, there are some evidences suggesting that it is capable of causing human infection (Billeter et al., 2007; Apperson et al., 2008).

During August and September 2014, 422 ticks, individually identified following Barros-Battesti et al. (2006) and Nava et al. (2014), were collected from horses and dogs in a population settlement in Sierra del Rosario (22°42′36″N, 83°33′59″W), located in Candelaria, Artemisa province, Cuba. Specimens were grouped into 100 pools of four to five ticks each according to host, sex and life stages: 356 adults identified as *Amblyomma mixtum* (166 males and 190

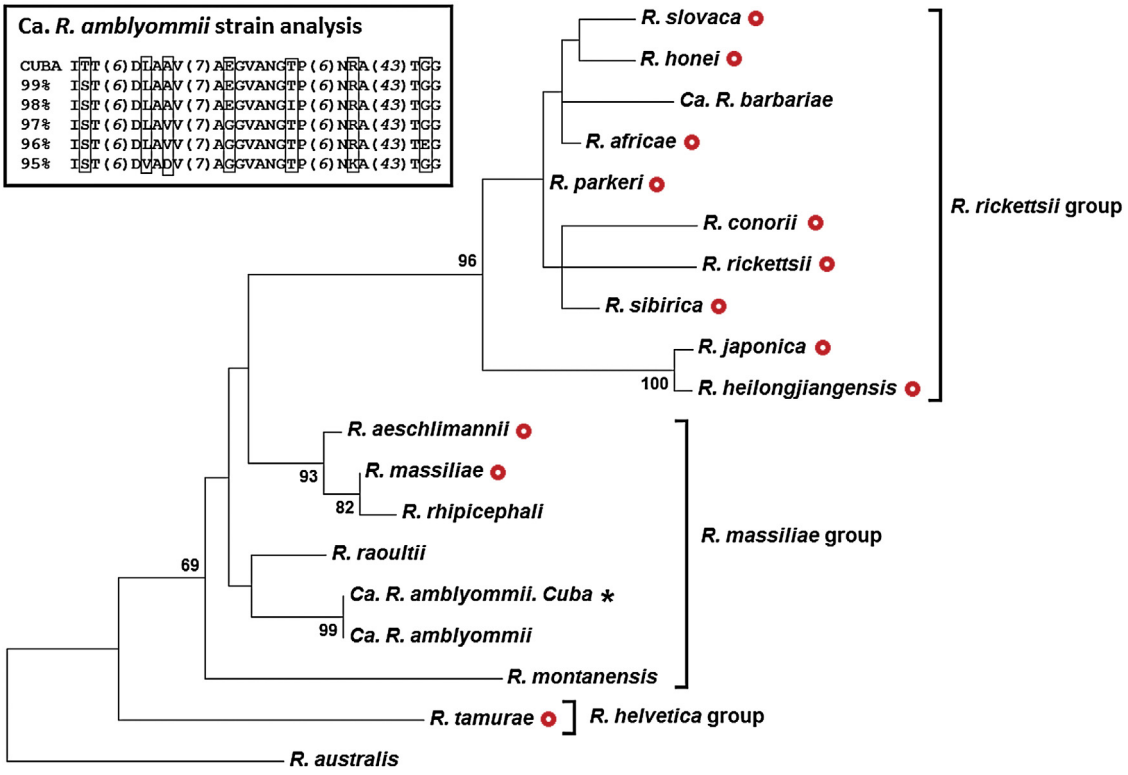


**Fig. 1.** Phylogenetic tree of *Rickettsia* spp. built with MEGA 6. The classification of *Rickettsia* spp. groups was based on Merhej and Raoult (2011). The strain of *R. amblyommii* identified in ticks from Cuba is indicated with an asterisk. Numbers on branches are bootstrap support values. *Rickettsia* spp. with known vertebrate pathogenic effect as in Merhej and Raoult (2011) are indicated with red circles. The *Rickettsia* species and GenBank accession numbers of *ompA* and *gltA* used in the analysis were as follow: *Candidatus R. amblyommii* (AKZ20801, AIU98746), *R. rickettsii* (AAC15675, AED88252), *R. conorii* subsp. Indica (AFK77691, WP_010977866), *R. africae* (AEJ87980, AER27861), *R. parkeri* (ALI96913, AIK25438), *R. sibirica* (ALB00644, AGA84543), *R. slovaca* (AFK77692, AIT11544), *R. honei* (AAB49564, AAB02974), *R. japonica* (ABI84239, AMK48118), *R. heilongjiangensis* (BAJ09685, AAG43416), *Candidatus R. barbariae* (AEJ87979, ALE33680), *R. massiliae* (ADC32092, ADH15757), *R. rhipicephali* (ABW90982, ABI96975), *R. aeschlimannii* (ALS39863, ADU77462), *R. montanensis* (AAC15678, AAB18272), *R. raoultii* (AGA12765, AKN34277), *R. tamurae* (BAC79495, AMK48116) and *R. australis* (AAD39531, WP_014412205). The best-fit model of the sequence evolution was selected based on Corrected Akaike Information Criterion and Bayesian Information Criterion implemented in Molecular Evolutionary Genetics Analysis (MEGA 6) (Tamura et al., 2013). Maximum likelihood method, implemented in MEGA, was used to obtain the best tree topology. *Rickettsia australis* was used as outgroup. It was also analyzed 19, 4, 28, 8 and 2 sequences with 99, 98, 97, 96 and 95% identity, respectively. Amino acid polymorphisms were addressed in back boxes after the sequences alignment and the identical regions were removed from the figure. The number of identical amino acids has been placed into brackets.

http://dx.doi.org/10.1016/j.ttbdis.2016.06.004

females) and 66 *Amblyomma* sp. nymphs. DNA was isolated by using a QIAamp DNA Mini-Kit (Qiagen, Chatsworth, California, USA).

The DNA aliquots were screened with the *Rickettsia*-specific primers for the outer membrane protein A (*ompA*) and citrate synthase (*gltA*) genes (Labruna et al., 2004). Genomic DNA from *R. rhipicephali* was used as PCR positive control. Sequencing of the gel-purified amplicons was performed using the GenomeLabTM Dye Terminator Cycle Sequencing with Quick Start Kit (Beckman Coulter®; Washington, D.C., USA). Generated sequences were submitted for basic local alignment search tool (BLAST) analysis to determine closest similarities to available corresponding DNA sequences in GenBank. A maximum likelihood (ML) phylogenetic tree was built using concatenated *Rickettsia* OmpA and GltA protein sequences.

Rickettsial DNA was amplified in 73% of tested tick pools and sequence analysis demonstrated that the *Rickettsia*-positive samples aligned with the *ompA* and *gltA* genes of *R. amblyommii*. All the *ompA* and *gltA* sequences from positive samples were identical (GenBank accession numbers of Cuban strains: KU234520 and KU214431). The ML phylogenetic tree (Fig. 1) indicates that the *R. amblyommii* identified in Cuban cluster together with *Rickettsia* spp. of the *R. massiliae* group. Further strain analysis using OmpA amino acid fragment suggests that the Cuban isolates are a new strain of *R. amblyommii* very similar to strains from USA and South America. Seven amino acid polymorphisms were found in the OmpA fragment analyzed (Fig. 1). When the OmpA sequence from the Cuban isolates was compared with 19 OmpA sequences available in GenBank that share 99% identity, only one amino acid substitution was found (Threonine (T) → Serine (S)). These 19 OmpA sequences belong to isolates from USA (11), Brazil (4), Panama (2), Costa Rica (1) and Honduras (1).

Our results show high prevalence of the infection by *R. amblyommii* in *A. mixtum* ticks, which is in agreement with results from areas in the USA, where such high infection rates have been reported for *Amblyomma* ticks (Mixson et al., 2006). In that same country, a localized rash in a woman was attributed to *R. amblyommii* after a tick bite by *A. americanum* (Billeter et al., 2007) and seroconversion with a fourfold or greater rise in IgG titers to *R. amblyommii*, but not to *R. rickettsii*, was demonstrated in patients with a presumptive clinical diagnosis of RMSF in North Carolina, USA (Apperson et al., 2008). In Tennessee, where cases of RMSF are frequently observed, a study failed to detect *R. rickettsii* in ticks, but found a high prevalence of *R. amblyommii* in *A. americanum* (Moncayo et al., 2010). These findings alert to the potential role of *R. amblyommii* to humans.

We report the identification of *R. amblyommii* in *A. mixtum*, a tick that rests essentially important from a medical point of view given its pronounced anthropophilic character. The finding of this *Rickettsia* member may be a public health concern in the studied area, where a raised infestation by ticks are continuously recorded biting humans; ticks that were also found infected with *Coxiella burnetii*, the agent of Q fever (Noda et al., 2016). Additional surveys of *A. mixtum* ticks spreading and the prevalence of *R. amblyommii* infection in ticks and humans should be conducted in Artemisa and in other Cuban regions.

## Acknowledgments

We kindly thank Córdoba University, Colombia for the financial support intended for the present research. Also thanks to Jorge Polo (National Zoological Park) and Maritza Miranda for their cooperation in collecting tick samples. Finally, we are very thankful to Dr. Marcelo B. Labruna, form University of Sao Paulo, who gently donated the genomic DNA from R. rhipicephali used as positive control for PCR.

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28 April 2016
Available online 21 June 2016