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 (AKZ00801, AB198746), R. rickettsii (AAC15675, AE08252), R. conorii subsp. indica (AFK77691, WP_010977866), R. africae (AEJ87980, AEK27861), R. parkeri (ALJ96913, AJK25438), R. sibirica (ALB00644, AGA84453), R. slovaca (AKF77692, A1T11544), R. honei (AAB49564, AAB20974), R. japonica (ABI84239, AMK48118), R. heilongiangensis (BAJ09685, AAG43416), Candidatus R. barbaraiae (AEJ87979, ALE33680), R. massiliae (ADJ2092, ADH15757), R. rhopiceps (ABW90982, ABJ96975), R. aeschlimannii (ALS39863, ADJ27462), R. montanensis (AAC15678, AAB12722), R. raoultii (AGA12765, AKN34277), R. tamaure (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.

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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. rhopiceps was used as PCR positive control. Sequencing of the gel-purified amplicons was performed using the GeneReadTM 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 95% 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 (Mixon 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 is continuously recordedbiting 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.

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References


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