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SHORT NOTE

Detection of *Rickettsia aeschlimannii* and *Rickettsia sibirica mongolitimonae* in *Hyalomma marginatum* (Acari: Ixodidae) ticks from Turkey

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ABSTRACT — A total of 784 (626♀, 158♂) *Hyalomma marginatum* Koch, 1844 ticks collected from humans in Turkey were tested for the presence of spotted fever group rickettsiae using polymerase chain reaction (PCR) targeting the citrate synthase (gltA= 381 bp) and the outer membrane protein A (ompA= 532 bp) genes, followed by sequencing analysis. Of the ticks tested, 39 were infected by *Rickettsia aeschlimannii* while 2 were infected by *Rickettsia sibirica mongolitimonae*. To our knowledge, this is the first report of *R. sibirica mongolitimonae* in *H. marginatum* ticks.

KEYWORDS — *Hyalomma marginatum*, Lymphangitis-associated rickettsioses, *Rickettsia aeschlimannii*, *Rickettsia sibirica mongolitimonae*, PCR

The rickettsiae are a group of obligate intracellular gram-negative bacteria responsible for serious human diseases, such as Lymphangitis-associated rickettsioses (LAR), and Mediterranean spotted fever (Parola *et al.* 2013; Portillo *et al.* 2015). Rickettsial infections are one of the oldest known vector borne diseases, and infections are mainly spread through fleas, mites (chiggers), lice, or ticks (Portillo *et al.* 2015). To date, 10 spotted fever group (SFG) rickettsiae, namely *Candidatus Rickettsia vini*, *R. aeschlimannii*, *R. africae*, *R. conorii*, *R. felis*, *R. helvetica*, *R. hoogstraalii*, *R. monacensis*, *R. raoultii* and *R. slovaca*, have been molecularly detected in ticks mainly collected from humans in Turkey (Gargili *et*

al. 2012; Orkun *et al.* 2014; Keskin *et al.* 2014, 2016).

In the present study, a total of 784 (626♀, 158♂) *Hyalomma marginatum* Koch, 1844 ticks were collected from humans by health personnel under aseptic conditions in Tokat province, Turkey. Ticks were stored in 70 % alcohol and morphologically identified using the key and descriptions of Apanaskevich and Horak (2005). Total DNA was extracted from individual ticks by a genomic DNA Isolation Kit developed by our laboratory (Turkuaz Genomic DNA Isolation Kit, Tokat, Turkey). DNA isolation procedures were described in our previous studies (Keskin *et al.* 2014, 2016). DNA quantity and

quality was evaluated with a spectrophotometer (Eppendorf Biophotometer, Eppendorf, Hamburg, Germany) and gel electrophoresis in 1 % agarose. It should be noted that no specific test of DNA quality using a tick target was performed due to the large number of tested tick samples; prevalence may therefore be underestimated if DNA varied greatly in quality.

Primers aimed at the rickettsial citrate synthase gene (gltA) and outer membrane protein A (ompA) gene, previously described by Regnery et al. (1991), were used to detect the presence of rickettsiae in ticks and evaluate their diversity. Firstly, rickettsial DNA was screened by PCR using primers RpCS.877p (5'-GGGGACCTGCTCACGGCGG-3') and RpCS.1258n, (5'-ATTGCAAAAAGTACAGTGAACA-3') which is a 381-bp fragment of the gltA gene. Each gltA positive sample was then screened with primer pairs Rr190.70p (5'-ATGGCGAATATTTCTCCAAAA-3') and Rr190.602n (5'-AGTGCAGCATTCGCTCC-CCT-3'), targeting a 532-bp portion of the ompA gene. PCRs were performed using a Bio-Rad T100 Thermal Cycler (Hercules, CA, USA). PCR conditions for both primers were as follows: denaturation at 95 °C for 5 min, then 35 cycles of 30 s at 95 °C, 60 s at 48 °C, and 30 s at 72 °C, followed by 5 min at 72 °C. Agarose (1 %) electrophoresis was used to visualize rickettsial DNA positive samples on a gel documentation system (UVP, Upland, CA, USA). Distilled water and *Rickettsia slovaca* DNA (obtained from a previous study) were used as negative and positive controls, respectively.

The rickettsia positive PCR products were sequenced with their corresponding PCR primers at a commercial facility (RefGen, Ankara, Turkey). The consensus sequences were prepared using BioEdit 7.0.4.1 (Hall 1999) and compared with other rickettsiae sequences in the NCBI GenBank database. All sequences obtained were submitted to GenBank and the corresponding accession numbers were obtained.

According to sequence analyses, 41 of the 784 *H. marginatum* ticks were found to be positive for rickettsial DNA. GltA (KU574125) and ompA (KU574126) gene sequences obtained from two *H.*

marginatum ticks were 100 % identical to sequences of *R. sibirica mongolitimonae* obtained from human patients (gltA: DQ423368, ompA: HQ728352).

This pathogenic rickettsia was first isolated in *Hyalomma asiaticum* Schulze & Schlottke, 1930 collected from Inner Mongolia (China) (Yu et al. 1993). Afterwards, *R. sibirica mongolitimonae* was detected in *H. excavatum* Koch, 1844 (Greece and Cyprus), *Hyalomma* sp. (Israel), *H. truncatum* Koch, 1844 (Niger), *Rhipicephalus pusillus* Gil Collado, 1936 (France, Portugal, and Spain) and *R. bursa* Canestrini & Fanzago, 1878 (Spain) (Parola et al. 2001, 2013; Harrus et al. 2011). To our knowledge, this is the first report of *R. sibirica mongolitimonae* in *H. marginatum*.

R. sibirica mongolitimonae, etiological agent of Lymphangitis-associated rickettsioses (LAR), is a member of *Rickettsia sibirica* species complex within the spotted fever group (SFG) of rickettsiae (Edouard et al. 2013; Parola et al. 2013). Maculopapular rash, fever, and multiple eschars are basic clinical signs and symptoms of *R. sibirica mongolitimonae* infection (Ramos et al. 2013; Portillo et al. 2015). The first human infection caused by *R. sibirica mongolitimonae* was reported in France in 1996 (Raoult et al. 1996). Since then, more than 25 *R. sibirica mongolitimonae* cases in humans have been reported in Europe (France, Greece, Portugal and Spain) and Africa (Algeria, Egypt, and South Africa) (Pretorius et al. 2004; Parola et al. 2013; Portillo et al. 2015). Although *R. sibirica mongolitimonae* was detected in Asian ticks (*H. asiaticum* in Inner Mongolia, China), there is currently no report of *R. sibirica mongolitimonae* infection in humans in Asia (Portillo et al. 2015).

In addition to infection by *R. sibirica mongolitimonae*, 39 *H. marginatum* ticks were found to be infected with *R. aeschlimannii* (Genbank similarity = 100%), which has been reported in previous studies (Gargili et al. 2012; Orkun et al. 2014; Keskin et al. 2016). *R. aeschlimannii* is broadly distributed in the Mediterranean where *H. marginatum* is common (Portillo et al. 2015). However, only a single case of *R. aeschlimannii* has been reported in Europe (Eastern Crete, Greece) to date (Germanakis et al. 2013).

H. marginatum, the main vector of Crimean-

Congo hemorrhagic fever virus, is the most frequently encountered tick species on humans in Turkey (Keskin *et al.* 2015, 2016). In addition, other tick-borne pathogens such as *R. aeschlimannii* and *Borrelia burgdorferi* sensu stricto have been detected in *H. marginatum* ticks in Turkey (Gargili *et al.* 2012; Orkun *et al.* 2014). In Turkey, the most common rickettsial infections in humans are considered to be Mediterranean spotted fever, caused by *R. conorii* (Kuloglu *et al.* 2012). However, we suggest that *H. marginatum* could also be involved in the transmission of *R. aeschlimannii* and *R. sibirica mongolitimonae* in this region. Further studies on the prevalence of rickettsiae found in ticks are now required.

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