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Identification and molecular characterization of spotted fever group rickettsiae in ticks collected from farm ruminants in Lebanon

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ABSTRACT

Tick-borne diseases have become a world health concern, emerging with increasing incidence in recent decades. Spotted fever group (SFG) rickettsiae are tick-borne pathogens recognized as important agents of human tick-borne diseases worldwide. In this study, 88 adult ticks from the species *Hyalomma anatolicum*, *Rhipicephalus annulatus*, *Rh. bursa*, *Rh. sanguineus* sensu lato, and *Rh. turanicus*, were collected from farm ruminants in Lebanon, and SFG rickettsiae were molecularly identified and characterized in these ticks. The screening showed a prevalence of 68% for *Rickettsia* spp., including the species *R. aeschlimannii*, *R. africae*, *R. massiliae* and *Candidatus* R. barbariae, the latter considered an emerging member of the SFG rickettsiae. These findings contribute to a better knowledge of the distribution of these pathogens and demonstrate that SFG rickettsiae with public health relevance are found in ticks collected in Lebanon, where the widespread distribution of tick vectors and possible livestock animal hosts in contact with humans may favor transmission to humans. Few reports exist for some of the tick species identified here as being infected with SFG *Rickettsia*. Some of these tick species are proven vectors of the hosted rickettsiae, although this information is unknown for other of these species. Therefore, these results suggested further investigation on the vector competence of the tick species with unknown role in transmission of some of the pathogens identified in this study.

1. Introduction

New tick-borne pathogens have been emerging with increasing incidence in recent decades, creating public health challenges. Among them, spotted fever group (SFG) rickettsiae are recognized as important agents of human tick-borne diseases worldwide (Raoult and Roux, 1997; Parola et al., 2005; Merhej et al., 2014). These bacteria are obligate intracellular, Gram-negative coccobacillae mainly transmitted by ticks that also act as reservoirs (Raoult and Roux, 1997; Parola et al., 2005; Merhej et al., 2014). These zoonoses are among the oldest known vector-borne diseases (Raoult and Roux, 1997; Parola et al., 2005; Merhej et al., 2014).

However, little information exists on SFG *Rickettsia* and their vectors in Lebanon, where hosts and environmental conditions can promote the existence and maintenance of these bacteria (Matossian et al., 1965). Therefore, the objective of this study was to provide evidence of the presence of SFG *Rickettsia* in ticks collected from domestic animals in Lebanon. This experimental approach is relevant to provide preliminary results of the eco-epidemiological situation of SFG *Rickettsia* in Lebanon, and results may be used to establish prevention and control measures for these tick-borne diseases.

2. Materials and methods

2.1. Study design and tick collection

The study was conducted on 30 ruminant farms located in the Lebanese provinces of Akkar, Bekaa, Nabatieh and South Lebanon

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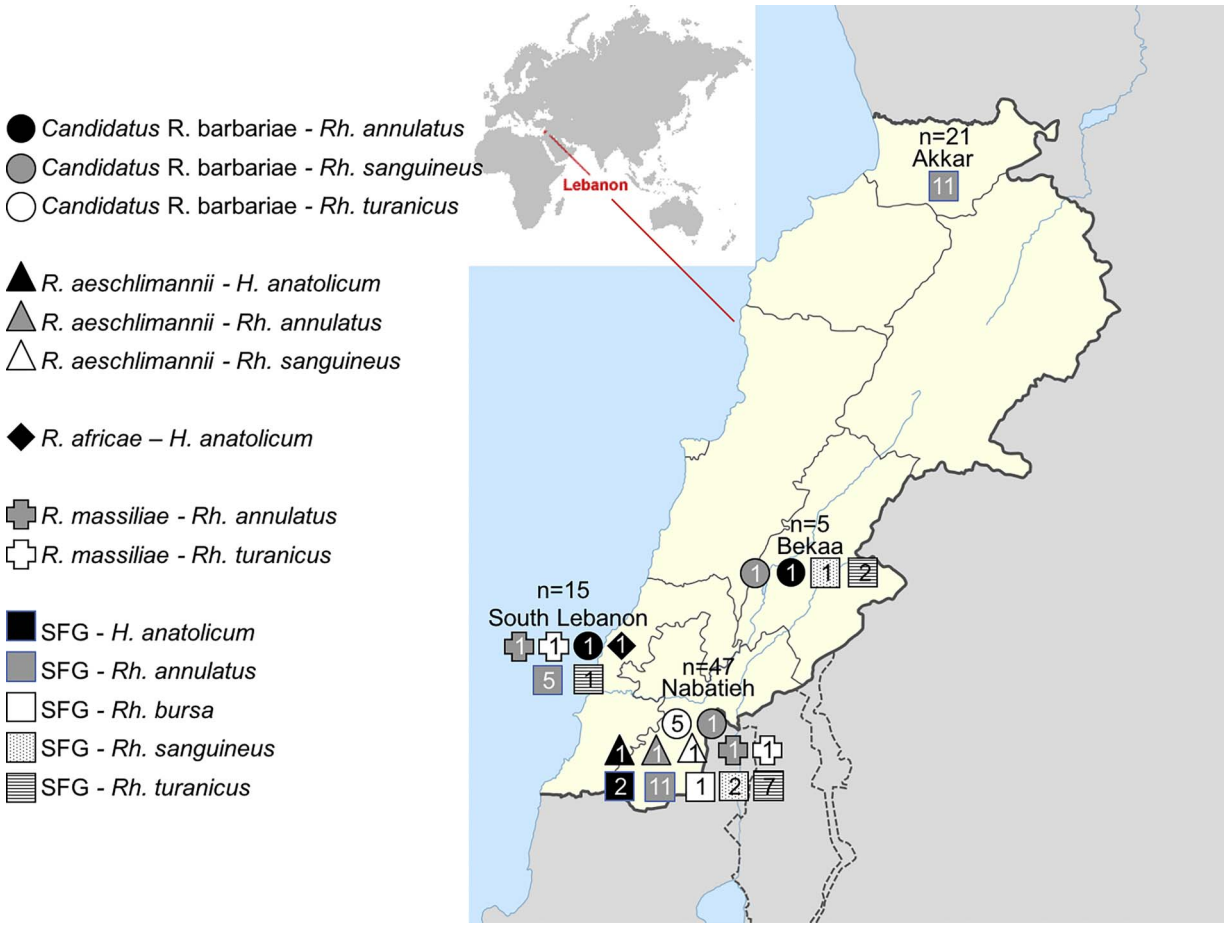

Fig. 1. Study area with Lebanon provinces in which ticks were collected. For each location, the number of identified *Rickettsia* spp. and the tick species in which were identified are shown. *R.* – *Rickettsia*, *Rh.* – *Rhipicephalus*, SFG – Spotted fever group, *H.* – *Hyalomma*.

(Fig. 1). In these farms, 88 adult ticks were randomly collected for this study to characterize two or three ticks from each farm. Consent was obtained from farm owners to access their animals and collect ticks after the approval of the Animal Resource Directorate of Lebanon from the Lebanese Ministry of Agriculture. Late spring and early summer represent the period of tick activity in these provinces, and therefore ticks were collected in June 2014. Farms contained different types of livestock including imported and local races and mixed or mono species of ovine, bovine and caprine. The farms rear cattle originally imported from Holland and Germany (Holstein breed) and from France (Normandie breed), and goats originally introduced from Syria (Shami breed).

Partially or fully engorged adult ticks were collected in the morning or evening on animals using fine forceps, stored at room temperature in 70% ethanol and morphologically identified using the guidelines for tick identification (Hoogstraal and Kaiser, 1959; Kaiser et al., 1974; Walker et al., 2014). Additionally, molecular identification of tick species was performed on the sampled ticks using the mitochondrial small subunit *12S rRNA* gene amplification and sequencing (Shemshad et al., 2012; Beati and Keirans, 2001).

2.1.1. Identification and characterization of Rickettsia spp. in ticks

Ticks were sectioned longitudinally and one half per each tick was used for DNA extraction. Total DNA was extracted using the PureLink Genomic DNA kit (Invitrogen, Life Technologies Corporation, Carlsbad, CA, USA) and used for tick molecular identification and characterization of *Rickettsia* spp. DNA by PCR, cloning, and sequence analysis of the amplicons. Genes targeted for *Rickettsia* spp. identification included fragments of 17 kDa protein gene (*htrA*), *ompA*, *ompB*, *gltA*, *atpA*, *dnaK*, *dnaA*, *recA* and *16S rRNA* (Giudice et al., 2014; Oteo et al., 2006; Choi et al., 2005; Fernández de Mera et al., 2013). PCR conditions and primer sequences are shown in Table 1. Each PCR reaction included a positive control, consisting of DNA from *Rickettsia conorii* Malish 7 strain cultured in VERO cells, and a negative control, in which DNA was replaced by nuclease-free water (Promega, Madison, WI, United States). PCR products were visualized after electrophoretic migration on 1.5% agarose gels containing SYBR Safe 10X (Invitrogen, Life Technologies Corporation, Carlsbad, CA, USA). PCR products were purified using the Wizard SV Gel and PCR Clean-up System (Promega), quantified, cloned into the pGEM-T vector (Promega) and sequenced (Macrogen Inc., Amsterdam, The Netherland). At least three clones were sequenced for each amplicon. Obtained sequences were analyzed using Bioedit (Ibis Biosciences, Carlsbad, CA, USA) and ClustalW version 2.0.10 (www.ebi.ac.uk/clustalw), and aligned to the sequences of the reference strains reported in the GenBank using the Basic Local Alignment Search Tool (BLAST). Furthermore, a multilocus sequence analysis was carried out using *ompA-ompB* sequences and *in silico Pst* I and *Rsa* I restriction analysis of *ompA* sequences (Fernández de Mera et al., 2013).

2.1.2. Phylogenetic analysis of ompA sequences

For phylogenetic analysis, a maximum likelihood phylogenetic tree was constructed based on *Rickettsia ompA* DNA sequences. The *ompA* sequences were collected from reference SFG rickettsiae, *R. massiliae* (GenBank accession number KR401146), *R. sibirica* (KT345980), *R. slovaca* (KX506733), *R. raoultii* (KX506737), *R. conorii* (KR401144), *R. parkeri* (KJ158741), *R. australis* (AF149108), *R. montanensis* (U43801), *R. rickettsii* (KX544816), *R. rhipicephali* (U43803), *R. mongolotimonae* (DQ097082), *Candidatus* R. barbariae (EU272186) and *R. africae*