

Simultaneous Detection of “*Rickettsia mongolotimonae*” in a Patient and in a Tick in Greece

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***Rickettsia conorii*, a spotted fever group rickettsia which is transmitted by *Rhipicephalus* sp. complex ticks, was considered until now the only pathogenic rickettsia prevalent in Greece. Here, we report the presence of “*Rickettsia mongolotimonae*” (proposed name) detected simultaneously in a patient and in a *Hyalomma anatolicum excavatum* tick, sampled on the patient.**

CASE REPORT

A 76-year-old man, was admitted on 9 December 2002 to the hospital of Sitia (Crete, Greece) with fever (38.2°C), malaise, marked fatigue, lumbar and shoulder myalgias, and knee and hand arthralgias for 7 days prior to admission. He reported no direct contact with domestic animals except that he had been collecting olives (for 6 days) in fields that were visited by goats and sheep. On the day of admission the physician removed an engorged tick from his scrotum, which was morphologically identified as a female belonging to the *Hyalomma anatolicum excavatum* species. The patient reported that he removed another engorged tick 3 days before the onset of symptoms.

On physical examination, a light discrete hyperemic maculopapular skin rash was observed, with elements 3 to 5 mm in diameter, mostly on the trunk and proximal extremities. There was no inoculation eschar at the tick bite sites. We observed, however, a single, large (17 mm in diameter) papule at the epigastrium with a small (1 mm in diameter) central crust that persisted for about 4 weeks and a painless enlarged lymph node of 1 cm in diameter in the right inguinal region. During the next two days a few elements of the rash presented petechias in their centers.

Initial laboratory examination demonstrated moderate anemia (hemoglobin, 11.5 g/dl), marked increase in serum urea (178 mg/dl) and creatinine (6 mg/dl), increased levels of lactate dehydrogenase (1,486 IU/liter) triglycerides and uric acid, elevated C-reactive protein (229 mg/liter), and mildly elevated serum concentrations of aspartate aminotransferase (67 IU/liter) and alanine aminotransferase (72 IU/liter). The electrocardiogram revealed T-wave inversions in leads I and aVL while, at the second day of hospitalization, paroxysmal atrial fibrillation emerged. The patient was treated with doxycycline (200 mg/day for 10 days) and rapidly recovered.

Serum samples were taken from the patient on days 7 and 15 after the onset. Antibodies to *Rickettsia typhi*, *Coxiella burnetii*,

Rickettsia conorii, “*Rickettsia mongolotimonae*” (proposed name), and *Rickettsia sibirica* were determined by microimmunofluorescence (antigens were kindly provided by D. Raoult). The first serum was negative for all tested antigens. The second sample yielded immunoglobulin G (IgG) titers of 1/256, 1/1,024, and 1/256 and IgM titers of 1/64, 1/256, 1/128 against *R. conorii*, *R. mongolotimonae*, and *R. sibirica*, respectively, but was negative for *C. burnetii* and *R. typhi*.

DNA was extracted from the removed tick and the whole-blood sample by using the QIAmp tissue kit and QIAmp blood minikit, respectively (QIAGEN GmbH, Hilden, Germany), according to the manufacturer’s instructions. For rickettsia detection we amplified by PCR a fragment of the *gltA* and the *rOmpA* genes, as described previously (10, 11). The PCR products were cloned in the TOPO vector (Invitrogen) and sequenced using the Thermo Sequenase primer cycle sequencing kit (Amersham Biosciences). Nucleotide composition was obtained in a LI-COR 4200 double-beam automated sequencer, and derived sequences were aligned with the GenBank/EMBL database. Sequence alignment based on the *gltA* and *rOmpA* amplicons obtained both from the patient and the tick demonstrated 99% homology with *R. mongolotimonae*.

R. conorii has long been considered to be the only pathogenic spotted fever group (SFG) rickettsia in Greece (1). In this study, we report the presence and the partial identification of a second tick-transmitted pathogenic rickettsia, in an area in which Mediterranean spotted fever (MSF) is endemic (Sitia, eastern Crete, Greece). *R. mongolotimonae* was detected simultaneously from a patient and from a *Hyalomma anatolicum excavatum* tick, which parasitized on the patient during the acute phase of the infection. The clinical manifestation of this infection and the epidemiological characteristics (occurrence of this case during the winter and the unusual tick vector) differ from the common description of MSF in Greece.

In our case the diagnosis of infection by *R. mongolotimonae* is confirmed by higher antibody levels to this rickettsia than to others (including *R. sibirica*) and by the detection of an *R. mongolotimonae* *rOmpA* sequence in the blood sample. This is the first laboratory-confirmed case of *Rickettsia mongolotimo-*

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nae infection reported in Greece. Two further cases have been reported in France (4, 9) and one case in South Africa (7).

Up to now in Greece, *R. conorii*, along with two nonpathogenic rickettsial species (*Rickettsia rhipicephali* and a variant of *Rickettsia massiliae*) has been found only in the *Rhipicephalus* species complex ticks (2, 8). This study represents the first description of a spotted fever group rickettsia in *Hyalomma* sp. ticks.

Due to the fact that MSF is the only known tick-borne rickettsiosis in Greece, serological diagnosis is based on the use of *R. conorii* antigen. As *H. anatolicum excavatum* is widely spread in Greece and frequently bites humans, it is reasonable to assume that *R. mongolotimonae* may be the causative agent of rickettsiosis in other areas of Greece, but reduced serological specificity and lack of molecular data have not allowed us to identify it successfully. We suggest that *R. mongolotimonae* should be considered in the differential diagnosis of rickettsioses in Greece.

Species of *Hyalomma* ticks are widespread (Africa, southeastern Europe, Asia), parasitizing on livestock hosts and infesting domestic animals and occasionally humans. Two SFG rickettsiae have been detected in three species of *Hyalomma* ticks: *R. aeschlimanni*, from *Hyalomma marginatum* ticks (3, 5) and *R. mongolotimonae*, from *Hyalomma asiaticum* and from *Hyalomma truncatum* ticks (6). To our knowledge this is the first detection of an SFG rickettsia in *Hyalomma anatolicum excavatum* ticks. *H. anatolicum excavatum* is a perennial species distributed in areas of the Near East, Asia Minor, southern Europe, southern Russia, and India. Our findings add to the accumulating data on *Hyalomma*-transmitted SFG rickettsiae.

The distribution of *R. mongolotimonae* may be potentially as

wide as that of its arthropod hosts. An active search for its presence in *Hyalomma* ticks will be conducted in order to determine the distribution and prevalence of *R. mongolotimonae*. The epidemiology and the pathogenicity of this rickettsia have yet to be investigated.

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