Short Communication

Possible *Rickettsia massiliae* Infection in Greece: an Imported Case

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SUMMARY: Tick-borne rickettsioses are endemic in Greece; however, until recently, only *Rickettsia typhi* and *R. conorii* were tested routinely in human samples arriving at the National Reference Center. During the last few years, the identification of different rickettsia species in ticks led to the introduction of other spotted fever group rickettsiae in routine analysis. Under the new scheme, *R. massiliae* is now tested routinely in human samples; herein, we describe a human case of this infection.

A 62-year-old English man permanently residing in Crete since the last several years, presented to the health center of a small village in Chania, at the end of July 2013, with headache, severe myalgia, and weakness. No visual disturbances were found. He reported that the symptoms began at the beginning of July and gradually worsened until they were affecting his daily activities. Since then, he had fever up to 38°C daily, for which he was taking ibuprofen, with temporary improvement of the condition.

The patient reported being bitten by a tick on June 15, while camping at the Exmoor National Park in southwest England; the removal of the tick from the arm proved to be difficult, according to his report. He attributed the tick bite to the presence of numerous deer in that area, with which he stated having had close contact.

A clinical examination revealed an approximate 5-mm-diameter eschar on the arm, and a macular rash on the upper extremities. Regarding the patient's clinical signs, low-grade fever was recorded along with normal pulse (65 beats/minute) and respiratory rate (14 breaths/minute). No hepatomegaly, splenomegaly, or lymph enlargement was recorded. Laboratory evaluation showed a slight lymphopenia (white blood cell count: 7.37/μl, lymphocytes: 1.39/μl), while the c-reactive protein level (0.28 mg/dl) and erythrocyte sedimentation rate (11 mm/h) were within normal range and all other test results were unremarkable.

Treatment with doxycycline, 100 mg twice per day, was initiated on August 9, and the patient became afebrile within 48 hours. However, 7 days later the patient was feverish and myalgia persisted. He was, therefore, hospitalized at the General Hospital of Chania, where ofloxacin was added to the antibiotic regimen and the therapy was continued for another week. Fifteen days after the end of treatment, the patient

showed remarkable improvement with the exception of myalgia, which persisted and for which he was prescribed painkillers.

Two serum samples and whole blood samples were drawn, the first on presentation at the Health Center and the second one month later. No eschar was observed at the time of clinical inspection. All samples were sent to the Regional Laboratory of Public Health of Crete-Laboratory of Clinical Bacteriology, Parasitology, Zoonoses, and Geographical Medicine, University of Crete, for further testing. DNA was extracted from the samples by using the QIAamp DNA blood mini Kit (QIAGEN, Hilden, Germany) according to manufacturer instructions. Real-time polymerase chain reaction (PCR) targeting the *gltA* gene of *Rickettsia* spp was performed as previously described (1). Immunoglobulin (Ig)G and IgM antibody titers were determined to detect the presence of antibodies against *Rickettsia* spp by using a slide that could test against *R. conorii*, *R. siberica mongolotimonae*, *R. slovaca*, *R. felis*, *R. massiliae*, and *R. typhi* as individual antigens (Fuller Laboratories, Fullerton, CA, USA) by an immunofluorescence antibody assay (IFA) that is based on the targeting of the rLPS.

At a cut-off level of IgG $\geq 1/480$ and IgM $\geq 1/200$, titers against *R. massiliae* alone were recorded from the first (IgG 1/3840, IgM –) and second (IgG 1/480, IgM –) serum samples. The absence of IgM antibodies may be due to the delayed presentation of the patient at the Health Center (almost a month past the tick bite). Titers determined for each of the *Rickettsia* species tested are shown in Table 1.

Furthermore, DNA was also tested for *Anaplasma* species by real-time PCR and for antibodies against *A. phagocytophilum* (Focus Diagnostics, Cypress, CA, USA) by IFA; none of the PCR amplifications was positive either for *Rickettsia* or for *Anaplasma* species, while results were negative on IFA against *A. phagocytophilum* as well.

R. massiliae is a spotted fever group rickettsia first described in 1993 (2). The first isolation in humans dates back to 1985, although it was not fully identified until 2005 (3). It is mostly associated with ticks of the *Rhipicephalus* species, while lately, there have been reports of the presence of the pathogen in the *Ixodid* tick species (4) and possibly in *Haemaphysalis punctata*

Received April 28, 2015. Accepted July 28, 2015. J-STAGE Advance Publication September 11, 2015.

DOI: 10.7883/yoken.JJID.2015.195

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Table 1. Antibody titers regarding G and M immunoglobulins tested against *Rickettsia* species by indirect immunofluorescence

Antibody tested	<i>Rickettsia</i> species						
	<i>R. conorii</i>	<i>R. typhi</i>	<i>R. felis</i>	<i>R. sibirica mongolotimonae</i>	<i>R. massiliae</i>	<i>R. slovaca</i>	
IgG (1/)	240	240	480	960	3,840	240	1st sample
IgM (1/)	–	–	–	–	–	–	
IgG (1/)	60	–	–	–	480	120	2nd sample
IgM (1/)	–	–	–	–	–	–	

–: a negative result.

(5). The impact of the presence of the pathogen in these later species in terms of human infection needs to be clarified, although both species are known to be capable of biting humans as well.

To date, 4 cases of *R. massiliae* infection in humans have been described, 3 of which were reported in Europe (3,6,7) and 1 in Argentina (8). In Greece, 3 *Rickettsia* species of the spotted fever group have been involved in human disease: *R. conorii* (9), *R. sibirica mongolotimonae* (10), and *R. aeschlimanii* (11).

Contrary to the case described herein, all 4 cases (Table 2) (3,6–8) described to date were diagnosed by molecular means. Notably, when diagnosing on the basis of IFA, the possibility of cross-reactions among species should be taken into consideration; for this reason, we cannot definitely exclude the possibility of an infection from a different species in our case. In such cases, the use of Western blotting could prove of great assistance in order to reach a definitive outcome at the species level, which is important particularly in epidemiological studies.

All patients recorded tick bite, as was the case in our patient as well. Although we did not collect ticks from Exmoor National Park, in the past at least one study has investigated the presence of *Ixodes ricinus* in the area surrounding the park (12).

In the past *R. massiliae* has been detected in *Rhipicephalus* species in Greece (13,14), although the current case perhaps corresponds to an imported one in Crete; nevertheless, it should still be regarded as the 5th case to be described in the literature.

The introduction of testing for more spotted-fever group *Rickettsiae* in routine diagnosis may prove of great usefulness where results from PCR may not be available and/or conclusive. In any case, the potential limitation of cross-reactions among different *Rickettsia* species may be overcome with Western blotting, wherever this is possible.

Conflict of interest None to declare.

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Table 2. Clinical characteristics of the human cases due to *R. massiliae* infection described so far

Country	Mo/yr	°C	Chill	Malaise	Night sweat	Myalgia	Headache	Rash	Necrotic eschar	Vision loss	Occipital swelling	Hepatomegaly	Lymphadenopathy	Disturbed hepatic enzyme	Therapy	Ref.
Italy	June/1985	y						Maculopapular (palms; soles)	Right ankle			Slight			Tetracycline (13d)	3
Argentina	July/2005	y	y	y				Palpable purpuric (trunk; palms; soles)	Tache noir					y	Doxycycline, prednisolone	8
France	May/2007	y			y		y	Maculopapular (palms; soles)	Buttocks, thighs	Bilateral chorioretinitis					Doxycycline, ofloxacin, methyl-prednisolone	7
Italy	May/2012	y					y		Scalp		y	y			Doxycycline	6
Greece ¹⁾	July/2013	y		y		y	y	Macular (upper extremities)	Arm						Ibuprofen, Doxycycline, ofloxacin	

¹⁾: probable imported case from the United Kingdom.
y: present.