Possible *Rickettsia massiliae* infection in Greece: an imported case

Dimosthenis Chochlakis, Christine Bongiorni, Nikolaos Partalis, Yannis Tselentis, and Anna Psaroulaki

Received: April 28, 2015. Accepted: July 28, 2015
Published online: September 11, 2015
DOI: 10.7883/yoken.JJID.2015.195

Advance Publication articles have been accepted by JJID but have not been copyedited or formatted for publication.
Possible *Rickettsia massiliae* infection in Greece: an imported case.

Dimosthenis Chochlakis¹, Christine Bongiorni², Nikolaos Partalis³, Yannis Tselentis⁴, Anna Psaroulaki¹⁴

¹Regional Laboratory of Public Health, Heraklion-Crete, Greece
²Health Centre of Vamos, Chania, Crete, Greece
³A’ Unit of Internal Medicine, General Hospital of Chania, Chania, Crete, Greece
⁴Laboratory of Clinical Bacteriology, Parasitology, Zoonoses and Geographical Medicine, Medical School, University of Crete, Heraklion, Greece.

Running title: *Rickettsia massiliae* infection.

Keywords: *Rickettsia massiliae*, Spotted Fever Group, Greece

**Corresponding author:** Dimosthenis Chochlakis, e-mail: surreydimos@hotmail.com

Regional Laboratory of Public Health of Crete

School of Medicine

University of Crete – Greece

Staurakia, PC 71110

Tel: 00302810394624
Summary

Tick-borne rickettsioses are endemic in Greece, however until recently only *Rickettsia typhi* and *R. conorii* were routinely tested in human samples arriving at the National Reference Centre. During the last years, the identification of different rickettsia species in ticks led to the introduction of other spotted fever group rickettsiae in the routine analysis. Under the new scheme, *R. massiliae* is now routinely tested in human samples, hence the description of the current human case.

A 62 year old English man, permanent resident during the last years of Crete, was presented at the end of July of 2013 at the Health Centre of a small village in Chania suffering from headache, severe myalgia and weakness. No visual disturbance was observed. As he reported the symptoms began at the beginning of July and got gradually worsen with impact on daily activities. Since then, he suffered from daily fevers up to 38°C for which he was getting ibuprofen, with temporary improvement of the condition.

The patient reported a tick-bite on the 15th of June during camping at the Exmoor National Park in south west 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 at that part, with which he stated having close contact.

The clinical examination revealed an eschar at the arm, of about 5mm in diameter, and a macular rash of upper extremities. As regards the patient’s clinical signs, grade fever was recorded together 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 Cells: 7.37, Lymphocytes: 1.39), while C-reactive
protein (0.28), Erythrocyte sedimentation Rate (11 mm/h) were within normal range and the rest of the tests did not reveal any worth mentioning findings.

He was started on doxycyclin 100mg twice per day at the 9th of August and he became afebrile within 48 hours. However, seven days later the patient was feverish, but myalgia still persisted. He was therefore hospitalized at the General Hospital of Chania where ofloxacin was added on the antibiotic regimen and therapy was continued for a further week. Fifteen days past the end of treatment the patient showed a remarkable improvement with the exception of myalgias, which still persisted and for which he was prescribed painkillers.

Two serum and whole blood samples were drawn, the first at presentation at the Health Centre and the second one month later. No eschar was available at the time of clinical inspection. All samples were sent at 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 by using the QIAamp DNA blood mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. Real-time PCR targeting the \textit{gltA} gene of \textit{Rickettsia} spp was performed as previously described (1). Immunoglobulin (Ig)G and IgM antibody titers were determined for the presence of antibodies against \textit{Rickettsia} spp using a slide that could test against \textit{R. conorii}, \textit{R. siberica mongolotimonae}, \textit{R. slovaca}, \textit{R. felis}, \textit{R. massiliae} and \textit{R. typhi} as individual antigens (Fuller laboratories, California, USA), by an immunofluorescence antibody assay (IFA) that is based on the targeting of the rLPS.

At a cut-off of \( IgG \geq 1/480 \) and \( IgM \geq 1/200 \), diagnostic titers only against \textit{R. massiliae} were recorded for 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 patient at the Health Centre.
(almost a month past the tick bite). The titers revealed for each of the *Rickettsia* species tested, are given in Table 1.

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

*Rickettsia massiliae* is a spotted fever group rickettsia first described in 1993 (2), while its first isolation from human dates back to 1985 although it was fully identified in 2005 (3). It is mostly associated with ticks of the Rhipicephalus species, while lately there have been reports for the presence of the pathogen in Ixodid tick species (4) and possibly in *Haemaphysalis punctata* (5). The impact presence of the pathogen in these later species in terms of human infection needs to be clarified, although both these species are known as capable of biting humans as well.

To date, four cases of *R. massiliae* infection in humans have been described, three of which were reported in Europe (3, 6, 7) and one in Argentina (8). In Greece, three *Rickettsia* species of the Spotted Fever Group have been implicated in human disease, *R. conorii* (9), *R. sibirica mongolotimonae* (10) and *R. aeschlimanii* (11) so far.

Contrary to the case described herein, all four cases (Table 2) (3, 6-8) described so far have been diagnosed by molecular means. It should be pointed out that whenever diagnosis is based on IFA, the possibility of cross-reactions among species should be taken under consideration; that is why 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 be proven of great assistance in order to come to a definitive outcome at the species level, which is important especially in epidemiological studies.
All patients recorded tick bite, as was the case in our patient as well. Although we did not manage to collect ticks from the Exmoor National Park, in the past there has been a study investigating the presence of *Ixodes ricinus* at the surrounding area of the Park (12).

Although *R. massiliae* has been detected in *Rhipicephalus* species in the past in Greece (13, 14), the current case perhaps corresponds to an imported one in Crete; nevertheless, it still counts for the 5th bibliographically described case.

Introducing the testing of more SFG Rickettsiae in routine diagnosis may prove of great usefulness where PCR may not be available and/or conclusive. In any case, the possible limitation of potential cross-reactions among different Rickettsia species may be overcome through the use of Western blotting, wherever of course this is possible.

**Conflict of interest**

The authors declare no conflict of interest.
References


Table 1: Antibody titers regarding G and M immunoglobulins tested against *Rickettsia* species by indirect immunofluorescence. “-” indicates a negative result.

<table>
<thead>
<tr>
<th>Antibodies tested</th>
<th>R. conorii</th>
<th>R. typhi</th>
<th>R. felis</th>
<th>R. sibirica mongolotimonae</th>
<th>R. massiliae</th>
<th>R. slovaca</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG (1/)</td>
<td>240</td>
<td>240</td>
<td>480</td>
<td>960</td>
<td>3840</td>
<td>240</td>
</tr>
<tr>
<td>IgM (1/)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IgG (1/)</td>
<td>60</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>480</td>
<td>120</td>
</tr>
<tr>
<td>IgM (1/)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

1st sample

2nd sample
<table>
<thead>
<tr>
<th>Country</th>
<th>Month/year</th>
<th>°C</th>
<th>Chills</th>
<th>Malaise</th>
<th>Night sweats</th>
<th>Myalgia</th>
<th>Headache</th>
<th>Rash</th>
<th>Necrotic eschar</th>
<th>Vision loss</th>
<th>Occipital swelling</th>
<th>Hepato-megaly</th>
<th>Lymphadenopathy</th>
<th>Disturbed hepatic enzymes</th>
<th>Therapy</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italy</td>
<td>June/1985</td>
<td>y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Maculopapular (palms; soles)</td>
<td>Right ankle</td>
<td>Slight</td>
<td></td>
<td></td>
<td></td>
<td>Disturbed hepatic enzymes</td>
<td>Tetracycline (13d)</td>
</tr>
<tr>
<td>Argentina</td>
<td>July/2005</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Palpable purpuric (trunk; palms; soles)</td>
<td>Tache noir</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Doxycycline, prednisolone</td>
</tr>
<tr>
<td>France</td>
<td>May/2007</td>
<td>y</td>
<td></td>
<td>y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Maculopapular (palms; soles)</td>
<td>Buttocks, thighs</td>
<td>Bilateral chorio-retinitis</td>
<td></td>
<td></td>
<td></td>
<td>Doxycycline, ofloxacin, methyl-prednisolone</td>
<td>7</td>
</tr>
<tr>
<td>Italy</td>
<td>May/2012</td>
<td>y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Scalp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Doxycycline</td>
</tr>
<tr>
<td>Greece*</td>
<td>July/2013</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Macular (upper extremities)</td>
<td>Arm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ibuprofen, Doxycycline, ofloxacin</td>
</tr>
</tbody>
</table>

Table 2: Clinical characteristics of the human cases due to *R. massiliae* infection described so far. *: probable imported case from the United Kingdom. y: present.