Blood-Borne Candidatus Borrelia algerica in a Patient with Prolonged Fever in Oran, Algeria

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Abstract. To improve the knowledge base of Borrelia in north Africa, we tested 257 blood samples collected from febrile patients in Oran, Algeria, between January and December 2012 for Borrelia species using flagellin gene polymerase chain reaction sequencing. A sequence indicative of a new Borrelia sp. named Candidatus Borrelia algerica was detected in one blood sample. Further multiplexer sequence typing indicated this Borrelia sp. had 97% similarity with Borrelia crocidurae, Borrelia duttonii, and Borrelia recurrentis. In silico comparison of Candidatus B. algerica spacer sequences with those of Borrelia hispanica and Borrelia garinii revealed 94% and 89% similarity, respectively. Candidatus B. algerica is a new relapsing fever Borrelia sp. detected in Oran. Further studies may help predict its epidemiological importance.

Relapsing fever borreliae are arthropod-borne pathogens causing mild to deadly septicemia and miscarriage.1 In Africa, cultured representatives include tick-borne Borrelia crocidurae, Borrelia duttonii, and Borrelia hispanica transmitted by Ornithodoros soft ticks and louse-borne Borrelia recurrentis.5 Borreliae are fastidious bacteria responsible for various febrile presentations, most commonly malaria-like symptoms.1,2 Borreliae have been documented in patients with tick-borne relapsing fever, however little is known regarding Borrelia in north Africa.2 Borrelia crocidurae has been detected in patients with a 2.5% prevalence in Ornithodoros sonrai ticks.3 While Lyme group Borrelia garinii was recently detected in Ixodes ricinus ticks, collected from El Ghora, Algeria.4 In addition, at least 10 different relapsing fever-causing borreliae have been documented in Africa, including five different borreliae in humans and five different borreliae in nonhuman hosts.5 The former includes pathogens classified as B. hispanica, B. crocidurae, B. duttonii, and B. recurrentis.6 Although relapsing fever-causing Borrelia may form one genetic species, they differ in their vector, host range, and disease spectra protein profile by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.4,5 Accordingly, molecular tools can be used to discriminate these different Borrelia.8,9 Here, using such molecular tools, we detected sequences indicative of a new Borrelia sp. named Candidatus Borrelia algerica in a blood sample from a patient with prolonged fever in Oran, Algeria.

We studied 257 blood samples collected from febrile patients in Oran between January and December 2012. Interviews, sampling (3–4 mL blood in ethylenediaminetetraacetic acid [EDTA] tubes) and a medical examination were performed on each individual with a fever (an axillary temperature > 37.5°C) and a questionnaire was completed by each patient. We have previously reported the presence of Borrelia relapsing fever, however little is known regarding Borrelia in north Africa.2 Additional borreliae have been documented in Africa, including five different borreliae in humans and five different borreliae in nonhuman hosts.2 The former includes pathogens classified as B. hispanica, B. crocidurae, B. duttonii, and B. recurrentis.6 Although relapsing fever-causing Borrelia may form one genetic species, they differ in their vector, host range, and disease spectra protein profile by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.4,5 Accordingly, molecular tools can be used to discriminate these different Borrelia.8,9 Here, using such molecular tools, we detected sequences indicative of a new Borrelia sp. named Candidatus Borrelia algerica in a blood sample from a patient with prolonged fever in Oran, Algeria.

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To confirm our results, multiplexer sequence typing was performed on the four positive samples, as previously described13 (Table 1). Only one blood sample resulted in positive amplification and sequencing of the two spacers (GenBank LN626643 and LN626644). Concatenation of the spacer sequences indicated that this Borrelia sp. had 97% similarity with B. crocidurae, B. duttonii, and B. recurrentis. Moreover, the in silico comparison of these spacer sequences with those of B. hispanica (AYAO00000000.1) and B. garinii (AYAOQ000100003.1) revealed 94% and 89% similarity, respectively, indicating a new Borrelia species, that we named Candidatus B. algerica. Candidatus B. algerica DNA was then tested by a second RT-PCR assay targeting the glpQ gene for B. crocidurae, the recN gene for B. duttonii/B. recurrentis and the recC gene for B. hispanica, as previously described.9 The results of all assays were negative, providing further evidence of a new species. Finally, Candidatus B. algerica DNA was tested by flaB gene PCR sequencing8,9 and the sequences (LN626647) were compared with those available in the GenBank, EMBL, and DDB databases using the gapped BLASTN 2.0.5 program in the National Center for Biotechnology Information server. Candidatus B. algerica showed 99.6% sequence similarity with B. duttonii (CP000976.1) and 99.3% similarity with B. crocidurae (GU357619.1) (Figure 1).

Borrelia bastardiae is a species within the complex Borrelia burgdorferi sensu lato and is by far the predominant Borrelia species detected in I. ricinus ticks in Tunisia and Morocco.13,14 Borrelia miyamotoi also belongs to the relapsing fever borreliae group and may cause relapsing fever and Lyme disease-like symptoms throughout the Holarctic region of the world, because of its widespread prevalence in the tick vector I. ricinus.8,14 A phylogenetic tree based on the 735-bp flaB gene was constructed using the MEGA software.

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## Table 1

Primer and probes used in this study

<table>
<thead>
<tr>
<th>Microorganism detected</th>
<th>Targeted sequences</th>
<th>Primers (5'-3')</th>
<th>Probes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Forward</td>
<td>Reverse</td>
<td></td>
</tr>
<tr>
<td><strong>Borrelia spp.</strong></td>
<td>16S</td>
<td>AGCCCTTTAAAGCTTCGTGTAAG</td>
<td>GCCTCCCGTAGGAGTCTGG</td>
<td>6-FAM CCGGCTGAGAGGGTGAAACGG-TAMRA</td>
</tr>
<tr>
<td></td>
<td>MST2</td>
<td>TTTTGTCTAAATTAACCCTTTCA</td>
<td>CTCATTAAATTCCTTACCCCTA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MST3</td>
<td>GCAAGTGCTGTTAACACT</td>
<td>ATGTGGGAATGCACCTCTTT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MST5</td>
<td>CCTAGTGCGAATGGGCTC</td>
<td>CAACTTGACATATCTTACTCAATTC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MST6</td>
<td>GGTTTCGATCCATTTTCTC</td>
<td>CTCGGGAGCCCTTTAAATG</td>
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</tr>
<tr>
<td></td>
<td>MST7</td>
<td>TCCGCACCTGAATGTATGTC</td>
<td>TGCCCATTTCTTTGTC</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>flaB</strong></td>
<td>TAAATCGTCAAGCCATAATGC</td>
<td>GCTCTTTGATCGTTACATT</td>
<td></td>
</tr>
<tr>
<td><strong>Borrelia crocidurae</strong></td>
<td>gplQ</td>
<td>CTTGGAATACCCAAAATATCC</td>
<td>GGCAATGCAATAATCTAAAC</td>
<td>6-FAM-ATGGACAAATGACAGGTCTTAC-NFO</td>
</tr>
<tr>
<td><strong>Borrelia duttoni</strong></td>
<td>rec N</td>
<td>GATGTGTAATTTCTAATGAGGGATG</td>
<td>TCTTTGACACAAAAATCCCTAA</td>
<td>6-VIC-GCAAATGAGTGTTAGACGTTGTTT-TAMRA</td>
</tr>
<tr>
<td><strong>Borrelia recurrentis</strong></td>
<td>rec C</td>
<td>AAATTGCACAACAGCATACAA</td>
<td>TCCTTCAATTTGATAGAGGTG</td>
<td>6-VIC-AGCTTAAAAATAATTTGTCAA AGG-NFO</td>
</tr>
<tr>
<td><strong>Borrelia hispanica</strong></td>
<td>beta-actin</td>
<td>CATGCCATCTCGTGCTGGA</td>
<td>CCGTGGACATCTTTGCTGC</td>
<td>6-FAM-CGGGAAATCGTGACATTA AG-TAMRA</td>
</tr>
</tbody>
</table>

MST = multispacer sequence typing.
and revealed that Candidatus B. algerica clustered with relapsing fever borreliae, differing from B. recurrentis and B. duttonii (Figure 2).

We believe that our results are accurate, as all molecular assays have previously been evaluated and are routinely used in our reference center. Furthermore, all negative controls were negative in each molecular assay. Lyme disease has been previously suspected in 21 Algerian patients; however, these cases were diagnosed serologically by B. burgdorferi enzyme-linked immunosorbent assay, without confirmation by western blotting. Antigenic cross-reactions between Lyme-disease-group and relapsing-fever-group borreliae may suggest that these infections could have been caused by other Borrelia spp. of the relapsing fever group.

In conclusion, we have determined that Candidatus B. algerica is a new relapsing fever Borrelia sp. detected in Oran. Clinicians and microbiologists need to be aware of these data to further predict its epidemiological importance. Further
surveys of arthropod populations should be conducted in north Africa to isolate and examine the geographic distribution of *Candidatus B. algerica*.

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