Am. J. Trop. Med. Hyg., 93(5), 2015, pp. 1070–1073
doi:10.4269/ajtmh.15-0124

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 multispacer 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*.[1] 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 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.[2] The former includes pathogens classified as *B. hispanica*, *B. crocidurae*, *B. duttonii*, and *B. recurrentis*.[2] 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.[5–7] 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 *Coxiella burnetii*,[10] *Rickettsia felis*, and *Plasmodium* spp. in this patient series.[11] A 200 μL sample of whole blood was used for DNA extraction performed using a QIAamp DNA Micro Kit according to the manufacturer protocols (Qiagen, Hilden, Germany). The samples were handled appropriately to avoid cross-contamination. The quality of the DNA handling and extraction was verified by real-time polymerase chain reaction (RT-PCR) for the housekeeping gene encoding beta-actin[12] (Table 1). *Borrelia* was then detected in the samples using a 16S rRNA gene sequence-based system, as previously described.[8,9] Two sets of negative controls (DNA of blood from a nonfebrile patient and sterile water) and a positive control (*B. recurrentis* DNA) were also analyzed in each run. All positive and negative controls demonstrated the expected results in all tests and *Borrelia* spp. were detected in four (1.6%) patients.

To confirm our results, multispacer sequence typing was performed on the four positive samples, as previously described[8] (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* (AYOU00000000.1) and *B. garinii* (AYAJ01000003.1) 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 sequencing[8,9] and the sequences (LN626647) were compared with those available in the GenBank, EMBL, and DJB 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 lusitaniae 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*.[15,16] A phylogenetic tree based on the 735-bp *flaB* gene was constructed using the MEGA software

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TABLE 1

Primers and probes used in this study

Microorganism detected	Targeted sequences	Primers (5′-3′)		Probes	Reference
		Forward	Reverse		
Borrelia spp.	16S	AGCCTTTAAAGCTTCGCTTGTAG	GCCTCCCGTAGGAGTCTGG	6-FAM CCGGCCTGAGAGGGTGAACGG-TAMRA	11
	MST2	TTTTTGCTAAAATTAACCCTTTTCA	CTCATTTTAATTTCCTTACCCCTA		11
	MST3	GCAGGTGGCTGTTAACCACT	ATGTGGGGAATGCACTCTTT		11
	MST5	CCTGAGTCGATATGGGCACT	CAACCTGACATATCTTACTCAATTCAT		11
	MST6	GGGTTCGAATCCCATTTTCT	CTCTGGGACGCCTCTTAATG		11
	MST7	TTCGCCACTGAATGTATTGC	TGCCAATGTTCTTGTTGGTC		11
	flaB	TAATACGTCAGCCATAAATGC	GCTCTTTGATCAGTTATCATTC		11
Borrelia crocidurae	*glpQ*	CCTTGGATACCCCAAATCATC	GGCAATGCATCAATTCTAAAC	6-FAM-ATGGACAAATGACAGGTCTTAC-NFQ	11
Borrelia duttonii/*Borrelia recurrentis*	*rec N*	GATGATGTAATTTCTAATGAAGGATG	TCTTTGACCAAAATTCCCCTAA	6-VIC-GCAAGTGATGAGTTTAGACGTTG TTTA-TAMRA	11
Borrelia hispanica	*rec C*	AAATTGCAACCAAGCATACAAA	TCGTCCAAATTTGATAGAGGTG	6-VIC-AGCTTAAAAAATAATATTGTCAA AGG-NFQ	11
	beta-actin	CATGCCATCCTGCGTCTGGA	CCGTGGCCATCTCTTGCTCG	6-FAM-CGGGAAATCGTGCGTGACATTA AG-TAMRA	11

MST = multispacer sequence typing.

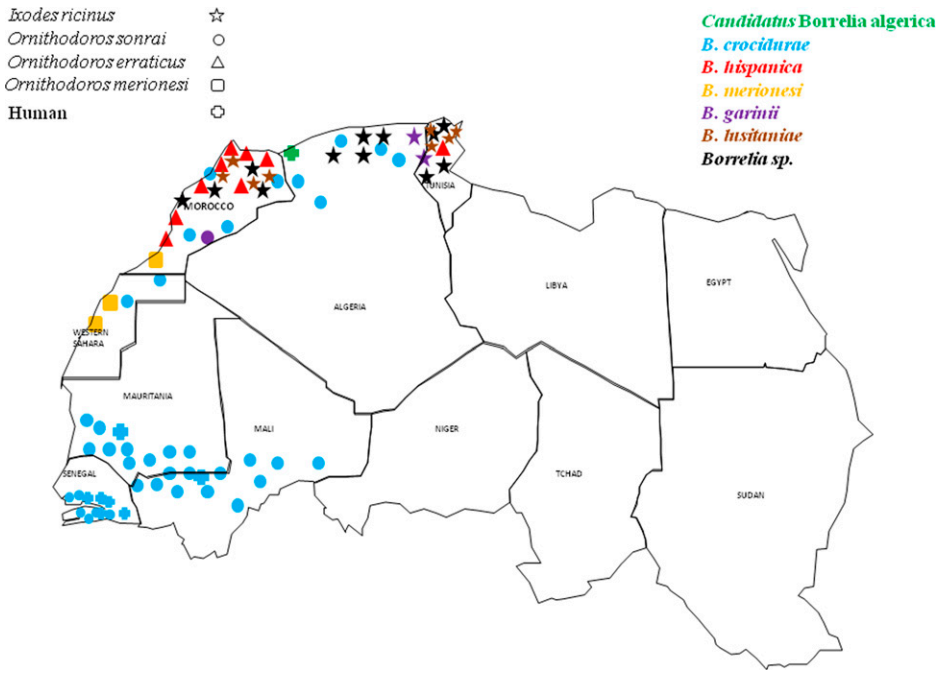

FIGURE 1. Geographical distribution of relapsing fever-causing borreliae in northwestern Africa.

(www.megasoftware.net) 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[17]; however, these cases were diagnosed serologically by *B. burgdorferi* enzyme-linked immunosorbent assay, without confirmation by western blotting.[17] 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

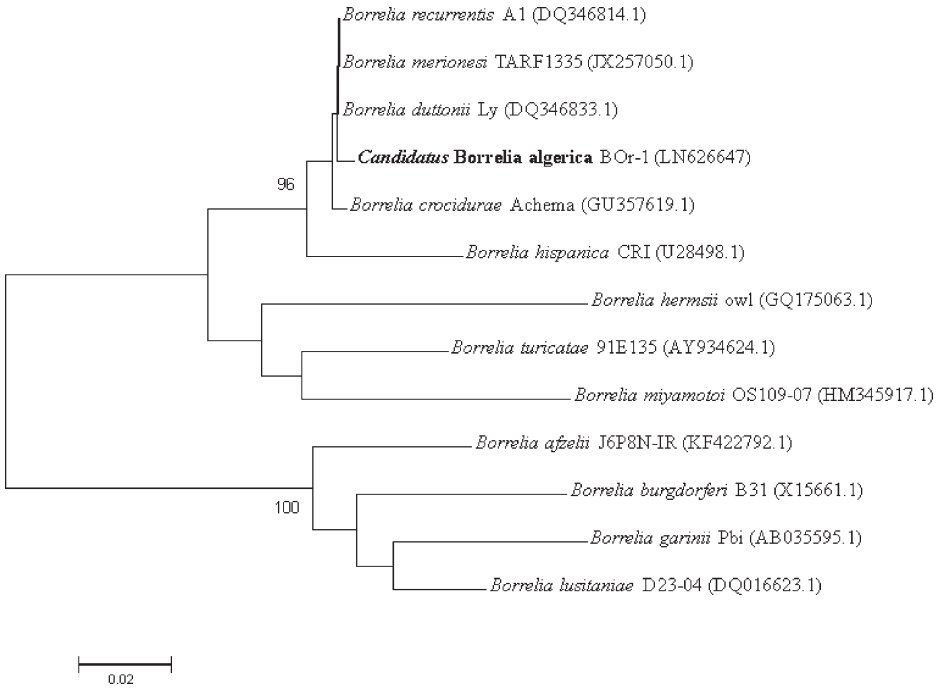

FIGURE 2. Phylogenetic tree of *Candidatus Borrelia algerica*. Bootstrap values > 95% are indicated at the nodes (the GenBank accession numbers are indicated in brackets).

surveys of arthropod populations should be conducted in north Africa to isolate and examine the geographic distribution of *Candidatus B. algerica.*

Received February 11, 2015. Accepted for publication June 3, 2015.

Published online September 28, 2015.

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