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Short communication

Relapsing fever causative agent in Southern Iran is a closely related species to East African borreliae

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ABSTRACT

We obtained two blood samples from relapsing fever patients residing in Jask County, Hormozgan Province, southern Iran in 2013. Sequencing of a partial fragment of *glpQ* from two samples, and further characterization of one of them by analyzing *flaB* gene, and 16S-23S spacer (IGS) revealed the greatest sequence identity with East African borreliae, *Borrelia recurrentis,* and *Borrelia duttonii*, and *Borrelia microti* from Iran. Phylogenetic analyses of *glpQ*, *flaB,* and concatenated sequences (*glpQ*, *flab*, and IGS) clustered these sequences amongst East African Relapsing fever borreliae and *B. microti* from Iran. However, the more discriminatory IGS disclosed a unique 8-bp signature (CAGCCTAA) separating these from *B. microti* and indeed other relapsing fever borreliae. In southern Iran, relapsing fever cases are mostly from localities in which *O. erraticus* ticks, the notorious vector of *B. microti,* prevail. There are chances that this argasid tick serves as a host and vector of several closely related species or ecotypes including the one we identified in the present study. The distribution of this *Borrelia* species remains to be elucidated, but it is assumed to be endemic to lowland areas of the Hormozgan Province, as well as Sistan va Baluchistan in the southeast and South Khorasan (in Persian: Khorasan-e Jonobi) in the east of Iran.

1. Introduction

Soft tick-borne relapsing fever (STBRF) is an endemic disease in Iran, with more than 140 cases annually throughout the country during 1997–2006 (Masoumi Asl et al., 2009). In Iran, four *Borrelia* species, *Borrelia persica, Borrelia microti*, *Borrelia baltazardi*, and *Borrelia latyschewii* are known causes of relapsing fever. In the western, northwest, and foothill regions of the Alborz Mountains stretching to east in Khorasan Razavi Province, the argasid tick *Ornithodoros tholozani* is commonplace in animal shelters and adjacent human dwellings accounting for most STBRF cases attributed to *B. persica* infection (Karimi et al., 1979; Karimi, 1981; Masoumi Asl et al., 2009). However, in central and western Iran *Borrelia microti*–infected *Ornithodoros erraticus* ticks coexist with *O. tholozani,* and in southen Iran, this tick predominates in the absence of *O. tholozani* (Janbakhsh and Ardelan, 1977; Karimi, 1981). Two other *Borrelia* species have been identified in Iran, *B. latyschewii*, isolated from *Ornithodoros tartakowskyi* ticks in the southeast (Piazak Seyyed Rashti and Assmar, 2000) and *B. baltazardi* that was described in a febrile patient with thrombocytopenic purpura in Ardebil, an endemic area for STBRF *B. persica* in northwest of the country (Karimi et al., 1979; Karimi, 1981). *Borrelia baltazardi* was discriminated from *B. persica* using electron microscopy (Karimi et al., 1979) and experimental pathogenicity in animal models including adult Guinea pigs, rats, mice, and newborn rabbits (Karimi et al., 1979; Karimi, 1981; Assous and Wilamowski, 2009). This spirochete was only isolated once and attempts to maintain it in laboratory animals failed; no tick vector has been identified for this species to date (Karimi et al., 1979; Karimi, 1981). Molecular characterization of *B. persica* from Iran has been reported (Ras et al., 1996; Shirani et al., 2016), but the characterization of other Iranian borreliae remains scarce. Phylogenetic analysis using concatenated sequences of 16S rRNA, *flaB*, and *glpQ* grouped Iranian *B. microti* alongside African species, *B. duttonii, B. recurrentis*, and *B. crocidurae*, and distinct from *B. persica*, the commonly established cause of STBRF in Iran (Naddaf et al., 2012). Similarly, 16S-23S intragenic sequence (IGS) analysis conducted *in situ* using blood from relapsing fever patients in Hormozgan Province, southern Iran showed greatest similarity with East African relapsing fever borreliae, *B. recurrentis* and *B. duttonii*, but was surprisingly distinct from *B. microti* transmitted by *O. erraticus* ticks, previously believed to be the only soft tick species in this region (Naddaf et al., 2015). In this study, we further characterize the causative agents of relapsing fever amongst patients from Hormozgan Province, southern Iran by sequencing partial fragments of *glpQ, flaB,* and IGS.

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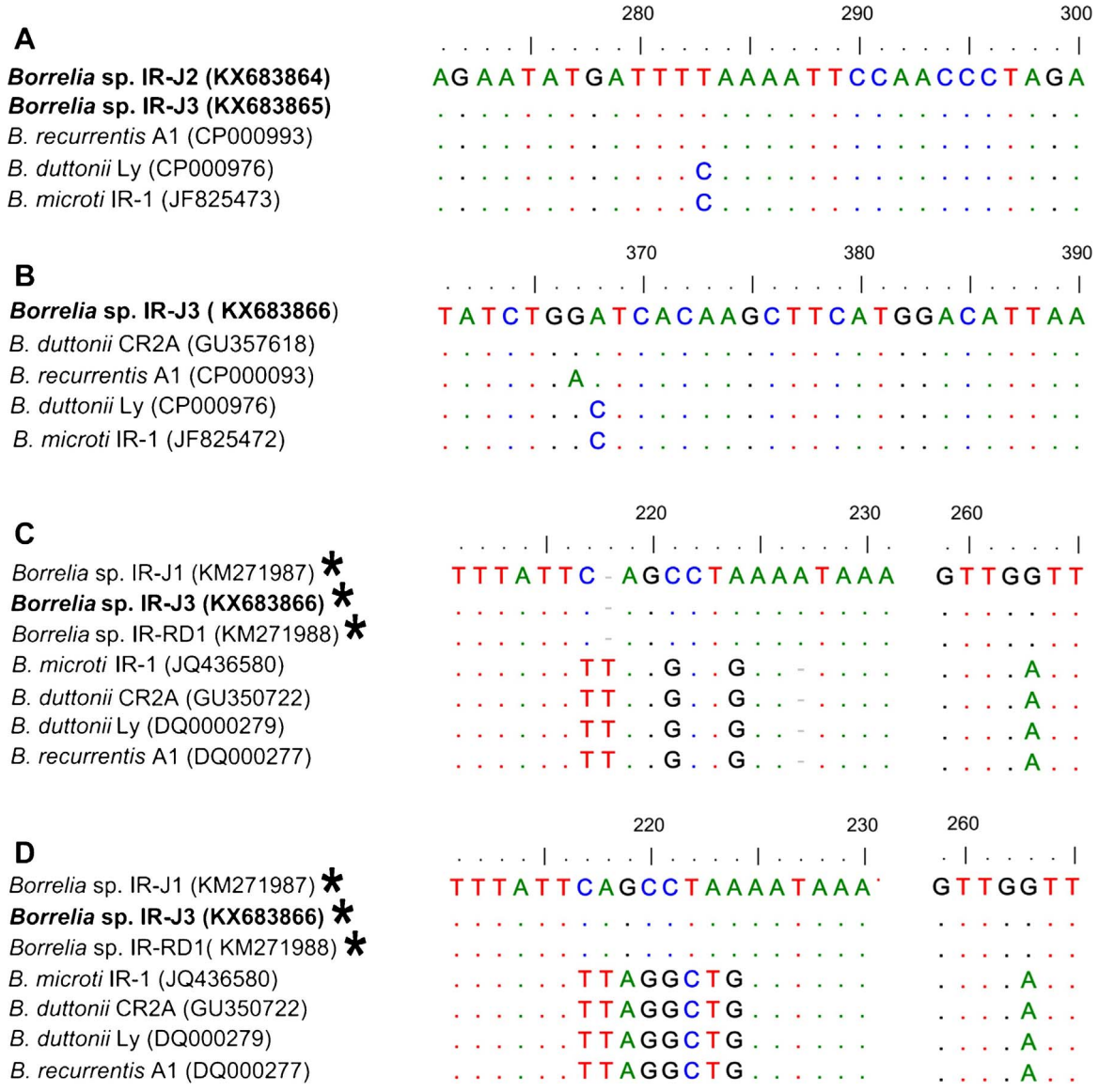

Fig. 1. Multiple sequence alignment of *glpQ* (A) and *flaB* (B) genes and IGS (C and D) of East African and Iranian borreliae. IGS (C) without gap opening and gap extension penalties, and (D) with the gap opening penalty 15, and gap extension penalty 6.66 (the template format for alignment in MEGA6). The IGS sequences from southern Iran are marked with an asterisk (*).

2. Materials and methods

Two blood samples from relapsing fever patients residing in Jask County, Hormozgan Province, south of Iran were obtained during 2013. The patients were farmers and had no history of travel to other areas of the country or abroad. Animal inoculation or *in vitro* culture of the samples was not possible as they had been kept in −20 °C for an extended period. The samples were reidentified; the human subject study had been approved by the ethical committee of Pasteur Institute of Iran (Project No. 794). These patients presented with fever, headache, and fatigue and were treated with 500 mg tetracycline orally every 6 h for ten days and became afebrile. Neither of the treated patients developed Jarisch-Herxheimer reaction or any other adverse consequences.

2.1. DNA extraction and PCR

DNA was extracted from blood samples by using the Miniprep DNA kit (QIAGEN, Hilden, Germany). Partial sequences, of the *glpQ,* and IGS were amplified with reagents and PCR conditions as previously described (Halperin et al., 2006; Cutler et al., 2010). We also amplified a partial sequence of the flagellin gene (*flaB*) gene by a nested PCR using the outer primers utilized by Assous et al. (Assous et al., 2006) and the inner primers NBOR-F 5′ tgggcatagaattaatcgtg 3′, and NBor-R 5′ tacttgttgagcaccctcac 3′designed in this study. To prevent cross-contamination, we performed DNA extraction and amplification in separate laboratories and included negative controls in each assay.

2.2. Phylogenetic analysis

The sequences generated in the present study were aligned with appropriate sequences from GenBank database. The phylogenetic trees were constructed by using the Jukes-Cantor option of the neighbor-joining method in a complete deletion procedure using MEGA6 software (Tamura et al., 2013). The robustness of the topologies was estimated through 2000 bootstrap replications.

2.3. GenBank submission

The sequences from this study were submitted to the GenBank under the accession numbers KX683864 and KX683865 for *glpQ*,