

been associated with HLA-DQ/DR variants (4). Although most persons lack susceptibility, high nasal carriage rates in disease-endemic areas and living conditions associated with poverty further increase infection risk for susceptible persons because acquisition is facilitated by malnutrition, overcrowding, and poor sanitation (5).

Leprosy treatment is determined according to disease severity. The Ridley-Jopling system assesses lesion quantity, neurologic involvement, and bacterial load, and the current World Health Organization system simplifies this system to facilitate clinical classification, defining paucibacillary leprosy as  $\leq 5$  skin lesions and multibacillary leprosy as  $\geq 6$  lesions (6).

Combination drug regimens for 6–24 months are highly effective. Together with efforts of the World Health Organization toward eradication, combination therapy has dramatically reduced the prevalence to current levels from previously stable levels of 10–12 million in the 1960s–1980s (7). Typical regimens include dapsone and rifampin, and clofazimine is available in the United States by investigational new drug application for multibacillary disease.

Patients undergoing treatment must be monitored for immunologic complications, such as cell-mediated reversal reaction (type 1 reaction) or interferon- $\alpha$ -mediated erythema nodosum leprosum (type 2 reaction). Reversal reactions may be especially severe and require urgent immunosuppression to avoid neurologic and vascular complications.

Leprosy is extremely rare in the United States (150 annual cases). Because transmission by prolonged close contact is more common than by casual contact, it is likely that the infection in this patient may have been acquired during childhood in a disease-endemic area, which represents the upper limit of incubation time. However, rare cases have been reported among military members, which makes it difficult to exclude the question of acquisition during military service in disease-endemic areas (8–10). Therefore, in patients with geographically appropriate foreign service or prolonged travel history, leprosy must be considered in the differential diagnosis of progressive skin lesions, particularly when lesional anesthesia is present.

## References

1. Britton WJ, Lockwood DN. Leprosy. *Lancet*. 2004;363:1209–19. [http://dx.doi.org/10.1016/S0140-6736\(04\)15952-7](http://dx.doi.org/10.1016/S0140-6736(04)15952-7)
2. Global leprosy update, 2013; reducing disease burden. *Wkly Epidemiol Rec*. 2014;89:389–400.
3. Truman RW, Singh P, Sharma R, Busso P, Rougemont J, Paniz-Mondolfi A, et al. Probable zoonotic leprosy in the southern United States. *N Engl J Med*. 2011;364:1626–33. <http://dx.doi.org/10.1056/NEJMoa1010536>
4. Blackwell JM, Jamieson SE, Burgner D. HLA and infectious diseases. *Clin Microbiol Rev*. 2009;22:370–85. <http://dx.doi.org/10.1128/CMR.00048-08>
5. Lavania M, Turankar RP, Karri S, Chaitanya VS, Sengupta U, Jadhav RS. Cohort study of the seasonal effect on nasal carriage and the presence of *Mycobacterium leprae* in an endemic area in the general population. *Clin Microbiol Infect*. 2013;19:970–4. <http://dx.doi.org/10.1111/1469-0691.12087>
6. Pardillo FE, Fajardo TT, Abalos RM, Scollard D, Gelber RH. Methods for the classification of leprosy for treatment purposes. *Clin Infect Dis*. 2007;44:1096–9. <http://dx.doi.org/10.1086/512809>
7. Noordeen SK, Lopez Bravo L, Sundaresan TK. Estimated number of leprosy cases in the world. *Bull World Health Organ*. 1992;70:7–10.
8. Bailey MS. Tropical skin diseases in British military personnel. *J R Army Med Corps*. 2013;159:224–8. <http://dx.doi.org/10.1136/jramc-2013-000124>
9. Chambers JA, Baffi CW, Nash KT. The diagnostic challenge of Hansen's disease. *Mil Med*. 2009;174:652–6. <http://dx.doi.org/10.7205/MILMED-D-02-9208>
10. Brubaker ML, Binford CH, Trautman JR. Occurrence of leprosy in U.S. veterans after service in endemic areas abroad. *Public Health Rep*. 1969;84:1051–8. <http://dx.doi.org/10.2307/4593754>

Address for correspondence: Catherine M. Berjahn, Division of Infectious Diseases, Naval Medical Center San Diego, Bldg 1, 2nd Deck, 34800 Bob Wilson Dr, San Diego, CA 92134, USA; email: catherine.berjahn@med.navy.mil

## Tickborne Relapsing Fever in Southern Iran, 2011–2013

Saied Reza Naddaf, Behnaz Ghazinezhad, Mohammad Mehdi Sedaghat, Hossein Masoumi Asl, Sally Jane Cutler

Author affiliations: Pasteur Institute of Iran, Tehran, Iran (S.R. Naddaf, B. Ghazinezhad); Tehran University of Sciences, Tehran (M.M. Sedaghat); Ministry of Health and Medical Education, Tehran (H.M. Asl); University of East London, London, United Kingdom (S.J. Cutler)

DOI: <http://dx.doi.org/10.3201/eid2106.141715>

**To the Editor:** Tickborne relapsing fever (TBRF) is endemic in Iran; >1,400 cases were confirmed in 19 provinces during 1997–2006 (1). In the western, northwestern, and foothill regions of the Alborz Mountains, the Argasid soft tick *Ornithodoros tholozani* is commonplace and accounts for  $\approx 60\%$  of TBRF cases attributed to *Borrelia persica*. However, in central and western Iran, *O. tholozani* and *B. microti*-infected *O. erraticus* ticks coexist (1,2). Two other *Borrelia* species, *B. latyschewii* and *B. baltazardi*, have also been described in northeastern and northwestern Iran (3,4), but no recent human infections with these species have been documented. Cases of TBRF occurring in southern Iran have presumably been caused by *B. microti* because its tick vector, *O. erraticus*, predominates in this region.

Relapsing fever infections in Hormozgan Province in southern Iran are commonly identified during routine checks for malaria. During 2011–2013, blood samples were obtained from 14 febrile patients referred to medical centers in Jask and Rodan in Hormozgan Province

(online Technical Appendix Figure, <http://wwwnc.cdc.gov/EID/article/21/6/14-1715-Techapp1.pdf>). Informed verbal consent was obtained from all participants, and the ethical committee of Pasteur Institute of Iran approved the project. Patients seeking care had fever and >1 sign or symptom, such as headache, chills, sweating, or fatigue. Six patients reported recurrent fever and generalized muscle and joint pain. Each patient lived in a local tent, called a kapar, or in a brick or concrete-block house.

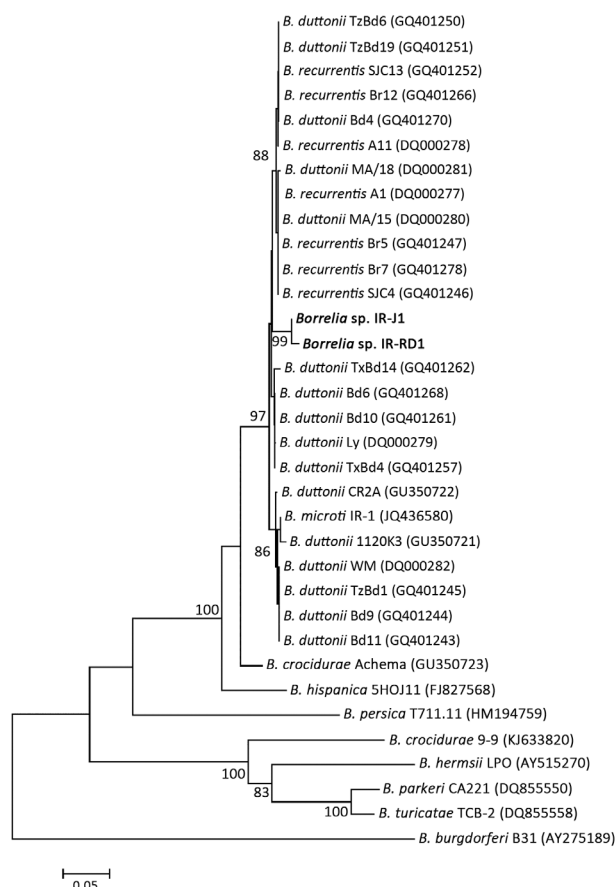
Thick and thin blood smears were prepared from blood samples, stained with Giemsa, and examined. None showed malaria parasites; however, spirochetes were observed in thick or thin smears from 3 patients (online Technical Appendix Table). Patients whose samples tested positive by microscopy were treated with 500 mg tetracycline every 6 hours for 10 days and became afebrile.

DNA was extracted from patients' serum samples by using the Miniprep DNA kit (QIAGEN, Hilden, Germany) and screened for borrelia DNA by using real-time PCR; negative and positive control DNA from *B. microti* or *B. persica* was also screened. *Borrelia* spp. DNA was detected in 5 (36%) of 14 serum samples (online Technical Appendix Table). Of these 5 samples, 2 were also positive by nested PCR that targeted the intergenic spacer (IGS) region (5). The 2 IGS regions were sequenced (ABI-3130XL sequencer; Applied Biosystems, Foster City, CA, USA) in both directions at the Pasteur Institute of Iran. The resulting 539- and 527-bp IGS sequences (GenBank accession nos. KM271987 and KM271988, respectively) were 97% homologous with *B. recurrentis* and *B. duttonii* from Africa (GenBank accession nos. CP000993 and DQ000280, respectively); 96% homologous with *B. microti* from Iran (GenBank accession no. JQ436580); and 92% homologous with *B. crocidurae* from Africa (GenBank accession no. GU350723). A neighbor-joining phylogenetic tree was constructed by using MEGA6 (<http://www.megasoftware.net>); the 2 IGS sequences clustered into a distinct group separate from *B. microti*, *B. duttonii*, and *B. recurrentis* genotypes (Figure).

*B. microti* was expected to be found because *O. tholozani* ticks that transmit *B. persica* are not seen in southern Iran, but *B. microti*-infected *O. erraticus* ticks have been frequently recovered from rodents' burrows in the region (6). Current molecular data from TBRF borreliae from Iran are limited to 2 isolates of *B. persica* and *B. microti* from *O. tholozani* and *O. erraticus* ticks, respectively (5,7,8). In situ IGS analysis revealed that spirochetes in our analysis had highest homology (97%) with relapsing fever agents from eastern Africa, *B. duttonii* and *B. recurrentis*, followed by *B. microti* (96%) from Iran (8). *B. microti* clustered with 1 strain (*B. duttonii*; GenBank accession no. GU350721) and apart from other *B. duttonii* IGS strains, suggesting that this strain may not be *B. duttonii*. The phylogenetic tree sepa-

rated *B. duttonii* into 4 clades, 2 of which also contained *B. recurrentis*, confirming previous observations (9) and providing further support that *B. recurrentis* represents an ecotype of *B. duttonii* rather than a species (10). Furthermore, the high level of phylogenetic similarity among borreliae from eastern Africa and Iran indicates that the borreliae in our study might represent ecotype-adapted strains. More sequencing of different genomic markers is required to substantiate or refute this possibility. Lack of GenBank data for the remaining borreliae from Iran, *B. latyschewii* and *B. baltazardi*, prevent exclusion of these species.

Although relapsing fever spirochetes from southern Iran and those from borreliae in Africa have a close phylogenetic similarity, they have different virulence levels and abilities to infect vector and host species. Consequently, deciphering the evolutionary links for these *Borrelia* spp.



**Figure.** Phylogenetic tree of *Borrelia* spp. strains isolated in Iran, 2014. Constructed on the basis of intergenic spacer sequences, the tree is drawn to scale using evolutionary distance computed using the Jukes-Cantor method in which the units reflect substitutions per site. The final dataset used 587 bp. Numbers at nodes show the level of robustness in a bootstrap test performed with 2,000 replicates; numbers <85 were removed. Scale bar indicates nucleotide substitutions over length analyzed. GenBank accession nos. for nucleotide sequences of IGS from 2 patients (in bold) are KM271987 and KM27188.

is of paramount importance and might provide valued insights into host–microbe interactions.

Our report confirms a novel *Borrelia* IGS sequence type detected in situ from 2 relapsing fever patients. This species showed greatest homology with the relapsing fever borreliae from Africa, *B. recurrentis* and *B. duttonii*, but not with *B. microti*, which is transmitted by *O. erraticus* ticks, previously believed to be the only soft tick species in this region. These findings challenge the assumption that TBRF in Iran is attributed to only *B. persica* or *B. microti*.

#### Acknowledgment

We thank Gholam Mohseni, the late technical supervisor at Bandar Abbas Health Research Station, Tehran University of Medical Sciences, for his contributions to this study.

This study was partially funded by the Pasteur Institute of Iran and by the Center for Disease Control, Ministry of Health and Medical Education, Tehran, Iran (grant no. 749).

#### References

- Masoumi Asl H, Goya MM, Vatandoost H, Zahraei SM, Mafi M, Asmar M, et al. The epidemiology of tick-borne relapsing fever in Iran during 1997–2006. *Travel Med Infect Dis.* 2009; 7:160–4. <http://dx.doi.org/10.1016/j.tmaid.2009.01.009>
- Aghighi Z, Assmar M, Piazak N, Javadian E, Seyedi Rashti MA, Kia EB, et al. Distribution of soft ticks and their natural infection with *Borrelia* in a focus of relapsing fever in Iran. *Iran J Arthropod-Borne Dis.* 2007;1:14–8.
- Karimi U. Relapsing fever and its epidemiology [in Farsi]. Tehran (Iran): Pasteur Institute of Iran; 1981.
- Karimi Y, Hovind-Hougen K, Birch-Andersen A, Asmar M. *Borrelia persica* and *B. baltazardi* sp. nov.: experimental pathogenicity for some animals and comparison of the ultrastructure. *Ann Microbiol (Paris).* 1979;130B:157–68.
- Oshaghi MA, Rafinejad J, Choubdar N, Piazak N, Vatandoost H, Telmadarraiy Z, et al. Discrimination of relapsing fever *Borrelia persica* and *Borrelia microti* by diagnostic species-specific primers and polymerase chain reaction-restriction fragment length polymorphism. *Vector Borne Zoonotic Dis.* 2011;11:201–7. <http://dx.doi.org/10.1089/vbz.2009.0170>
- Janbakhsh B, Ardelan A. The nature of sporadic cases of relapsing fever in Kazeroun area, southern Iran. *Bull Soc Pathol Exot Filiales.* 1977;70:587–9.
- Ras NM, Lascola B, Postic D, Cutler SJ, Rodhain F, Baranton G, et al. Phylogenesis of relapsing fever *Borrelia* spp. *Int J Syst Bacteriol.* 1996;46:859–65. <http://dx.doi.org/10.1099/00207713-46-4-859>
- Naddaf SR, Ghazinezhad B, Bahramali G, Cutler SJ. Phylogenetic analysis of the spirochete *Borrelia microti*, a potential agent of relapsing fever in Iran. *J Clin Microbiol.* 2012;50:2873–6. <http://dx.doi.org/10.1128/JCM.00801-12>
- Cutler SJ, Bonilla EM, Singh RJ. Population structure of East African relapsing fever *Borrelia* spp. *Emerg Infect Dis.* 2010;16:1076–80. <http://dx.doi.org/10.3201/eid1607.091085>
- Lescot M, Audic S, Robert C, Nguyen TT, Blanc G, Cutler SJ, et al. The genome of *Borrelia recurrentis*, the agent of deadly louse-borne relapsing fever, is a degraded subset of tick-borne *Borrelia duttonii*. *PLoS Genet.* 2008;4:e1000185. <http://dx.doi.org/10.1371/journal.pgen.1000185>

Address for correspondence: Sally Jane Cutler, School of Health, Sport, and Bioscience, University of East London, Water Lane, Stratford, London E15 4LZ, UK; email: s.cutler@uel.ac.uk

## Reducing the Risk for Waterborne Nosocomial Neonatal Legionellosis

Joseph S. Cervia

Author affiliation: Hofstra–North Shore/Long Island Jewish Health System School of Medicine, Hempstead, New York, USA

DOI: <http://dx.doi.org/10.3201/eid2106.141779>

**To the Editor:** I read with interest the report by Wei et al. (1) regarding 2 cases of neonatal legionellosis associated with infant formula prepared with hospital tap water. Two hospitals were involved, and water samples from both were positive for *Legionella pneumophila* bacteria that had molecular profiles indistinguishable from those for bacteria from the infected neonates. As Wei et al. (1) and others have established, control of waterborne pathogens, such as *Legionella* spp., in health care institutions remains a work in progress.

Recently, leading medical centers have recognized the efficacy and cost-effectiveness of performing certain measures to ensure the safety of hospital water. These measures include routine microbial analyses of tap water and use of waterborne pathogen prevention and control measures such as hot water flushing of plumbing; use of chlorination, chlorine dioxide, monochloramine, copper–silver ionization, or ultraviolet light; ozonation; and point-of-use water filtration. Each method has advantages and disadvantages related to ease of implementation, cost, maintenance issues, and short- and long-term effectiveness. Randomized controlled trials comparing the efficacy of these strategies are lacking, but the availability of guidance for using waterborne pathogen prevention and control strategies has resulted in substantial declines in health care–associated legionellosis (2). Efforts at waterborne pathogen detection and control are complicated by the role of biofilm, comprising microbes embedded in the polymeric matrix attached to internal plumbing surfaces, which protects waterborne pathogens from adverse environmental conditions, including antimicrobial agents and systemic controls (e.g., ultraviolet light, metals, acid pH) (2,3).

Prevention of legionellosis in health care settings offers a clinically beneficial and cost-effective alternative to intermittent case detection and outbreak control. For example, it has been demonstrated that, even in the absence of a recognized outbreak, hospital units caring

# Tickborne Relapsing Fever in Southern Iran, 2011–2013

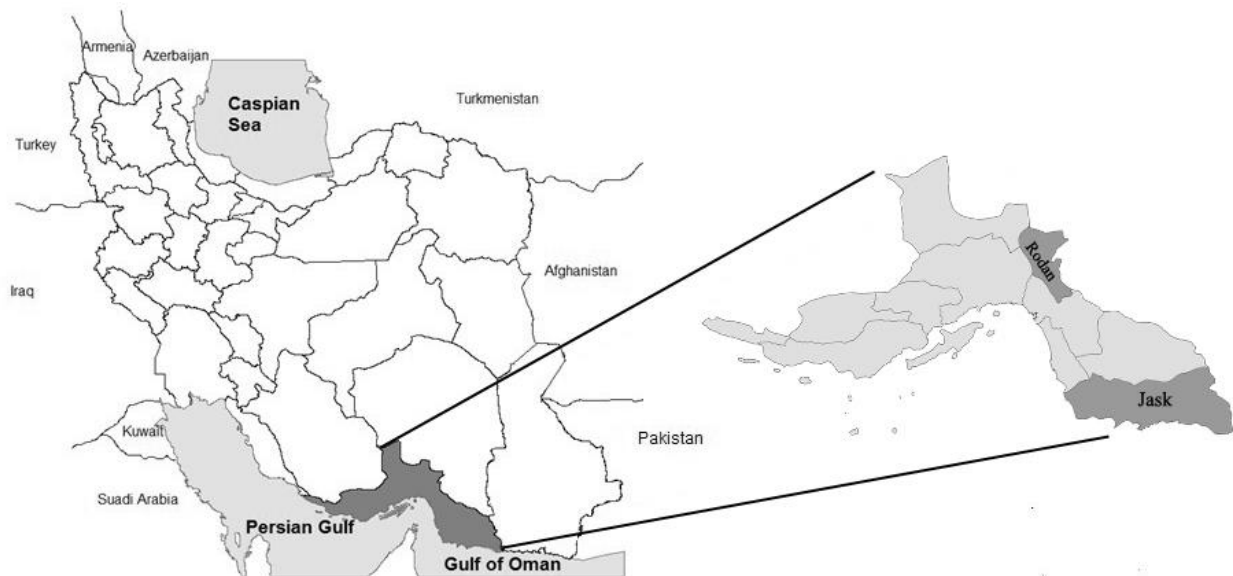
## Technical Appendix

**Technical Appendix Table.** Characteristics and clinical and laboratory findings for patients having positive results for TBRF by rt-PCR, Jask and Rodan Counties, Iran, 2011–2013\*

Patient no.	Sex	Age, y	Occupation	Area, Province	Relapses	Microscopy (count/10 high power fields)	rt-PCR	IGS nested PCR
1	M	22	Unknown	Jask, Hormozgan	Yes	Positive† (2.3)	Positive	Positive
2	M	59	Farmer	Jask, Hormozgan	Yes	Positive† (1)	Positive	Negative
3	M	28	Farmer	Rodan, Hormozgan	Not reported	Positive† (5.2)	Positive	Positive
4	M	18	Unknown	Jask, Hormozgan	Not reported	Negative	Positive	Negative
5	F	25	Housewife	Rodan, Hormozgan	Yes	Negative	Positive	Negative

\*TBRF, tickborne relapsing fever, rt-PCR, real-time PCR, IGS, intergenic space;

†Blood from all family members of spirochetemic patients were examined for spirochetes; all had negative results.



**Technical Appendix Figure.** Study site (Jask and Rodan Counties) in Hormozgan Province, south Iran, from which patients were recruited.