Borrelia spielmanii Erythema Migrans, Hungary

To the Editor: Lyme disease is the most frequent tickborne human infection in the northern hemisphere. At least 5 species of the *Borrelia burgdorferi* sensu lato complex, *B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, *B. bissettii*, and *B. lusitaniae*, have a pathogenic role in human Lyme disease in central Europe (1–3). A sixth pathogenic strain, A14S, has been isolated from 1 Dutch (4) and 2 German patients with erythema migrans (5). This strain was also detected in 4 questing *Ixodes ricinus* ticks in Germany (6,7) and 1 in the Czech Republic (8). A14S has recently been described as a new species, *B. spielmanii* (9); its main reservoir host is probably the garden dormouse (*Eliomys quercinus*), but *B. spielmanii* could not be detected in mice or voles. Richter et al. (9) could not find ticks harboring *B. spielmanii* in 3 of 5 examined areas in Germany. They were present almost exclusively in a single area where the prevalence of infection with this genotype was 15 (6%) of 251. We describe the isolation of this novel Lyme disease spirochete from a human patient with erythema migrans in Hungary.

Since 1999, we have regularly isolated *Borrelia burgdorferi* sensu lato from skin biopsy specimens of erythema migrans and acrodermatitis chronica atrophicans taken from patients at the Center for Tick-borne Diseases, Budapest, Hungary. To identify the *Borrelia* species occurring in Hungarian Lyme disease patients, we have started to molecularly analyze cultured isolates that originate from erythema migrans of different patients. DNA was isolated from 8 bacterial pellets by using QIAamp DNA mini kit (Qiagen, Hilden, Germany). Primers BSL-F and BSL-R were used; these amplify an ≈250-bp region of the outer surface protein (osp) A gene from all Lyme disease spirochetes (10). We added 2 μL extracted DNA to a 20-μL reaction mixture composed of 1.0 U HotStart-Taq DNA polymerase, 200 μmol/L of each dNTP, 25 pmol of each primer, and 1.5 mmol/L $MgCl_2$ (HotStartTaq Master Mix, Qiagen). An initial denaturation step at 94°C for 15 min was followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s. Final extension was done at 72°C for 5 min. Amplified DNA was subjected to electrophoresis in a 1.5% agarose gel that was prestained with ethidium bromide and viewed under UV light. After purification, the dideoxy chain termination (Applied Biosystems Division, Foster City, CA, USA) was used for sequencing. Obtained sequences were checked with Chromas v.1.45 and compared to sequence data available from GenBank by using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). New sequences were submitted to GenBank.

Six sequences (DQ007298, DQ007299, DQ007300, DQ007301, DQ007302, DQ007303) showed 100% homology to *B. afzelii* Khab 625 strain (AY502599). One (DQ007297) of the remaining 2 samples showed 99.6% similarity with *B. burgdorferi* B31 (AE000790), and the other (AY995900) showed 99.21% similarity with *B. spielmanii* (AF102057).

The patient whose culture showed *B. spielmanii* was a 42-year-old woman with a homogenous erythema migrans, diagnosed on September 24, 1999. The erythema was 10 cm in diameter on the front surface of the knee at the first visit (see online Figure, available at http://www.cdc.gov/ncidod/EID/vol11no11/05-0542-G.htm). The immunoglobulin M (IgM) and IgG *Borrelia* immunoblot that applied *B. afzelii* (ACA1) antigen was negative in serum drawn on the seventh day after the appearance of erythema migrans. The patient did not remember a tick bite and had not traveled abroad during the previous 6 months. She complained of an "extremely unusual," intense, serous nasal discharge that started 3 weeks before the appearance of erythema migrans and of a moderate headache; both disappeared spontaneously 2 weeks before treatment.

Our results show at least 3 distinct species of *B. burgdorferi* sensu lato in Hungary. In addition to *B. burgdorferi* sensu stricto and *B. afzelii*, known throughout Europe, we detected the recently described species *B. spielmanii* among randomly selected samples. Together with 2 previous publications (4,5), our observation also suggest that *B. spielmanii* has a pathogenic role in human Lyme disease. Although *B. spielmanii* is distributed more focally than other species of the *B. burgdorferi* sensu lato complex (9), it occurs from the Netherlands through Germany and Czech Republic to Hungary (4,5,7,8).

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Profiling *Mycobacterium ulcerans* with *hsp65*

To the Editor: *Mycobacterium ulcerans* is an emerging human pathogen responsible for Buruli ulcer, a necrotizing skin disease most commonly found in West Africa, but outbreaks have also been reported in the Americas, Australia, and Asia (1). Environmental sources of infection and mode of transmission are not completely known. *M. ulcerans* grows slowly at 32°C, requiring 6–8 weeks for colonies to be visible in primary culture. Differentiation from *M. marinum*, which also causes skin infections, is important, since *M. marinum* can usually be treated with antimicrobial agents, whereas *M. ulcerans* most often does not respond favorably to drug therapy, and treatment is usually by surgical excision (2). *M. shinshuense*, initially isolated from a child in Japan, is phenotypically and genetically related but biochemically distinct from *M. ulcerans* (3).

In the last decade, several DNA-based techniques for mycobacterial identification have been developed. Rapid molecular detection and differentiation of organisms that cause skin infections directly from tissue or exudates could be of great value for early treatment. Some techniques, especially those that include nucleic acid amplification, could be used directly on clinical samples. The accepted standard for molecular identification of mycobacteria is sequencing analysis of 2 hypervariable regions identified in 16S rRNA gene. *M. marinum* and *M. ulcerans* share identical 5′-16S rDNA and 16S-23S rRNA gene spacer sequences (4). Polymerase chain reaction (PCR)-dependent methods are based on the 16S rRNA gene (5), the *hsp65* gene (6) or the insertion sequence IS*2404* (7). Recently, a novel category of variable number tandem repeats that could distinguish *M. marinum* and *M. ulcerans* genotypes has been described (8).

Polymorphisms in the 3′-16S rDNA region discriminate *M. ulcerans* from *M. marinum* and *M. shinshuense* (5). These polymorphisms also allow the separation of *M. ulcerans* into 3 subgroups according to geographic origin and variable phenotypic differences. IS*2404* discriminates *M. ulcerans* from *M. marinum* (7). It has been used in restriction fragment length polymorphism analysis applied to a comparable number of *M. ulcerans* and *M. marinum* strains, confirming that this sequence is present in high copy numbers in *M. ulcerans* but absent in *M. marinum*. Nevertheless, an unusual mycobacterium was recently isolated that is closely related to *M. marinum* by phenotypic tests, lipid pattern, and partial 16S rDNA sequencing but presents low copy numbers of this element (9).

PCR-restriction enzyme analysis (PRA) of a 441-bp fragment of the *hsp65* gene is a rapid, easy, and inexpensive method for identifying mycobacteria (10). Devallois et al. (6) described the PRA-*hsp65* pattern of 1 *M. ulcerans* strain ATCC 33728 that originated in Japan. This isolate was considered a new species that resembled *M. ulcerans* and was named *M. shinshuense* (3).

We report here the usefulness of PRA-*hsp65* to differentiate *M. ulcerans* strains from different geographic areas. Since Buruli ulcer cases have been reported on 5 continents, we studied 33 *M. ulcerans* strains that originated from Africa (Benin, Zaire, Ghana, Congo, Angola, Côte d'Ivoire, Togo), Asia (China, Malaysia), Australia (Papua New Guinea, Australia), the Caribbean (Mexico, Surinam, French Guiana), 1 *M. shinshuense* from Japan, 1 *M. marinum* isolate and 1 IS*2404*-positive *M. marinum* isolate from France (9). All strains were identified at the Institute