Borrelia burgdorferi Infection and Cutaneous Lyme Disease, Mexico

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Four patients who had received tick bites while visiting forests in Mexico had skin lesions that met the case definition of erythema migrans, or borrelial lymphocytoma. Clinical diagnosis was supported with histologic, serologic, and molecular tests. This study suggests that Borrelia burgdorferi infection is in Mexico.

Lyme disease is the most frequently reported vectorborne infectious disease in the United States and Europe (1,2). Studies have suggested that Borrelia burgdorferi infection might be endemic to Mexico (3,4). We searched for histologic, immunologic, and molecular evidence of B. burgdorferi infection in patients with cutaneous manifestations suggestive of Lyme disease in Mexico.

The Study

From June 1999 to October 2000, 4 patients in Mexico City had clinical manifestations suggestive of Lyme disease (5,6). Two (36 and 54 years of age) had erythema migrans lesions, and 2 (9 and 34 years of age) had borrelial lymphocytoma lesions. Two reported having been bitten by a hard tick; the other 2, by a nonflying insect. Bites occurred while camping in forests: 3 near Mexico City (National Park La Marquesa) and 1 in Quintana Roo, a southern state in Mexico. All patients lived in Mexico City and had never traveled outside Mexico.

Two patients were treated for acute skin lesions (consistent with erythema migrans), malaise, and arthralgia. The skin lesion was an erythematous macula with regular, reddish edges and a pink center. One patient had a 5-cm lesion on the left forearm; the other had a 6-cm lesion on the left thigh. For the 2 other patients, a nodular erythematous cutaneous lesion (consistent with lymphocytoma), 0.5–2 cm in diameter with regular edges, developed 2 months after the bite. One patient’s lesion was on the earlobe; the other’s, on the left cheek.

Serum from each patient was tested for immunoglobulin M (IgM) and IgG against B. burgdorferi sensu lato by using a commercial ELISA (cutoff optical density 0.200 and indeterminate 0.200–0.400) (Enzygnost Borreliosis, Dade Behring, Marburg, Germany) (7). A Treponema pallidum ELISA (Abbott Murex, Wiesbaden, Germany) was performed to rule out cross-reaction with T. pallidum infection. Serum samples positive for B. burgdorferi by ELISA were further tested by Western blot (WB) by using the Marxblot assay (MarDx Diagnostics, Carlsbad, CA, USA) and Centers for Disease Control and Prevention (CDC) criteria (5).

Serum samples from the 2 lymphocytoma patients were positive for B. burgdorferi by ELISA and WB (Figure 1, panel A; Table). For the 2 erythema migrans patients, serum samples taken 2 weeks after the tick bite were negative for B. burgdorferi IgM and IgG; but 2 months later, 1 patient became seropositive, confirmed by WB (Figure 1, panel B; Table).

Histologic examination of skin biopsy specimens from each erythema migrans lesion showed a mononuclear cell infiltrate in the superficial and deep dermis; infiltrate included lymphocytes and plasma cells around the perivascular zones. Biopsy samples of lymphocytoma lesions showed dense nodular lymphocytic infiltrates in the reticular dermis with well-delineated lymphoid follicles, no atypical mitosis, B-lymphocytes (anti-CD20, DAKO, Carpenteria, CA, USA) in the germinal center (Figure 2, panel A), T-lymphocytes (anti-CD45 RO+) in the follicular zone (Figure 2, panel B), and no CD3+ cells.

DNA was extracted from the biopsy samples (Repli-g Mini Kit, QIAGEN, Valencia, CA, USA) and used for PCR amplification of a fragment of fla gene specific for B. burgdorferi sensu lato species as well as for a fragment of ospA gene, as described (8–10). DNA from a skin biopsy of a patient with systemic lupus erythematosus was used as negative control, and DNA (10 pg/μL) from B. burgdorferi sensu stricto B31 served as positive control. All procedures from DNA extraction to amplification were performed twice for each sample. Amplified products were further tested by Southern blot (SB) hybridization with probes

Figure 1. Western Blot (WB) immunoglobulin G results from cutaneous cases. A) WB with serum from patient 1, showing erythema migrans. B) WB with serum from patient 4, showing lymphocytoma; a strong positive and a weak positive control were included.
specific for *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii*, as described (9). DNA from the 4 biopsy samples was positive for *B. burgdorferi* sensu lato fla gene by PCR and confirmed as *B. burgdorferi* sensu stricto by SB (online Appendix Figure, available from www.cdc.gov/EID/content/13/10/1556-appG.htm; Table). All DNA biopsy samples were negative by SB with the probes specific for *B. garinii* and *B. afzelii*. We were able to amplify the OspA gene for only 1 case of erythema migrans, by using PCR and SB tests (data not shown).

The PCR products of the fla gene from 3 patients and of the ospA gene from 1 patient were sequenced by using a commercial kit (GenomeLab DTCS-Quick Start Kit, Beckman Coulter, Inc., Fullerton, CA, USA) with the sequencer from Beckman Coulter, Inc., according to manufacturer’s instructions. We used the DNAMAN program (Lynnon Corporation, Vaudreuil-Dorion, Quebec, Canada) to align the sequences with the reported sequences of the *B. burgdorferi* sensu stricto B31 strain (online Appendix Figure). For the 2 erythema migrans cases, we found 3 base substitutions (online Appendix Figure, panel C), 1 of which was not conserved, leading to a change in amino acid (G for R in the 75 aa); these 2 sequences had 99% homology with the sequence of *B. burgdorferi* fla gene of isolate B31 (BLAST program) (11). For the lymphocytoma case, we found 2 base substitutions, the same as those of the erythema migrans cases, including the nonconserved base substitution (online Appendix Figure, panel C).

Regarding the ospA gene in the erythema migrans case, the sequence showed 1 base substitution that was not conserved, leading to a change in the amino acid 5 (L for I). The sequence of this case had 99% homology with the plasmid Ip54 gene of B31 strain sequence (11) (online Appendix Figure, panel D).

The 3 adult patients received doxycycline 200 mg/day for 3 weeks; the child received amoxicillin 50 mg/kg a day for 3 weeks. For all patients, lesions were gone at the end of the treatment and had not recurred 3 years later.

**Conclusions**

Erythema migrans is the diagnostic marker for Lyme disease associated with *B. burgdorferi* infection (5,6). Histologic data from our 2 erythema migrans cases agreed with data reported for other erythema migrans cases (5). Moreover, the 2 erythema migrans cases were positive for *B. burgdorferi* sensu stricto fla gene and 1 for ospA gene; the 3 cases had a high degree of homology to the sequences of strain B31. In addition, 1 case met CDC criteria for sero-positivity to *B. burgdorferi* infection (5).
Borrelial lymphocytoma is a rare clinical entity reported mostly in Europe (12–14) and sporadically in the United States (15). In this study, histologic and immunohistochemical data from the 2 lymphocytoma cases agreed with data from previous cases. These results were not specific enough to be considered diagnostic; however, germinal centers are present in 80% of borrelial lymphocytoma cases (12). Serum samples from 2 patients were positive by WB, which fulfills CDC criteria (5). In 1 case, fla gene was amplified and sequenced, showing high homology with the fla gene from *B. burgdorferi* sensu stricto strain B31 (11). Few reports describe genotyping of *B. burgdorferi* species in borrelial lymphocytoma. In Slovenia, *B. afzelii* and *B. bissettii* were identified (13); in Germany, *B. garinii* was identified (14). In our lymphocytoma patients, we identified *B. burgdorferi* sensu stricto. That the 2 borrelial lymphocytoma cases occurred in patients who had visited the same national park suggests that *B. burgdorferi* is endemic to that area.

This study documents *B. burgdorferi* infection in Mexican patients. Relevant epidemiologic data are 1) cases occurred after visiting forest areas, 2) patients reported having been bitten by a nonflying insect, 3) cases occurred during the summer-fall season, 4) no patient reported having traveled to another country, and 5) all skin lesions resolved after treatment with an antimicrobial drug. Our results suggest that *B. burgdorferi* infection occurs in Mexico and that continuous surveillance for Lyme disease in Mexico should be mandatory.

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