

and the EMIDA ERA-NET and INIA grant APHAEA.

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DOI: <http://dx.doi.org/10.3201/eid2012.140517>

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## Molecular Characterization of *Borrelia burgdorferi* from Case of Autochthonous Lyme Arthritis

**To the Editor:** The first Lyme borreliosis (LB) case reported to be acquired in California occurred in 1978 (1). During the past 10 years, 744 confirmed LB cases were reported in California; 419 (56.2%) were likely acquired in-state. The highest incidence of this disease occurs in northern coastal California, in locations such as Santa Cruz County (2), where

habitat supports yearlong activity of the tick vector *Ixodes pacificus* (3,4).

Existing data describe the genetic diversity of the LB agent *Borrelia burgdorferi* among ticks in California (5,6), but few instances of direct detection and genetic characterization of *B. burgdorferi* sensu stricto in samples from humans are documented in California. *B. burgdorferi* has been isolated from skin biopsy samples of 3 patients in California in whom LB was diagnosed (1). Seinost et al. genotyped strains isolated in the United States, including 7 isolates identified in California from skin, blood, or cerebrospinal fluid, but no documented exposure information was available (7). Girard et al. genotyped *B. burgdorferi* in 10- to 12-year-old stored serum samples collected from 22 northern California residents, some of whom were asymptomatic at time of collection. Of 22 PCR-positive specimens, 21 had the single laboratory type strain B31 genotype (3).

A 12-year-old resident of Santa Cruz County, California, came to the emergency department of Dominican Hospital in September 2012 with a swollen, painful right knee and mildly painful right hip. The patient's family reported that LB had been diagnosed by a local physician. Illness onset was in May 2010; symptoms consisted of recurrent knee swelling and pain lasting several days every 4–5 months and positive serologic test results for *B. burgdorferi* (not available). The patient had not traveled outside of California during the preceding 6 years. In May 2011, an IgG Western blot of the patient's serum that was processed at a commercial laboratory showed immunoreactive bands of 18, 23, 28, 30, 39, 41, 45, 58, 66, and 93 kDa. In both 2010 and 2011, the patient's family had chosen to give the patient unspecified herbal treatments instead of antibacterial drugs.

On physical examination in the emergency department, the patient's right knee was swollen; knee flexion

was reduced to 30°. The right hip was painful on rotation. Serum laboratory values included a leukocyte count of 7,000/mL, hematocrit 33%, and erythrocyte sedimentation rate of 73mm/h. Plain radiograph images of the right hip did not show any abnormalities; the radiograph of the right knee showed suprapatellar effusion (Figure). Right knee aspiration yielded 115 mL of cloudy yellow fluid; laboratory tests showed a leukocyte count of 59,750/mL and protein level 5 g/dL; no crystals were noted. Results of routine bacterial culture of synovial fluid were negative. Amoxicillin was prescribed for a suspected septic joint and was taken for 1 week. Nine months later, the patient was reportedly asymptomatic and had returned to normal activity.

Right knee synovial fluid was sent to ARUP Laboratories (Salt Lake City, UT, USA); results were positive for the *B. burgdorferi* sensu lato *recA* gene by use of a proprietary qualitative PCR procedure. At the University of California, Irvine, we thawed another aliquot of synovial fluid, which had been frozen without cryoprotectant, and

inoculated samples into BSK II medium (8). After incubation for 2 weeks at 34°C, no spirochetes were noted. We subjected another 100 mL aliquot to DNA extraction using DNeasy Blood and Tissue Kit and the QIAcube apparatus (QIAGEN, Valencia, CA, USA). We used multiplex quantitative PCR (qPCR) and primers and specific probes for the 16S ribosomal RNA genes of LB group species and for relapsing fever group species of *Borrelia* in 2 replicates as described by Barbour et al. (9). By qPCR, there were 18 gene copies of an LB group species in 1 replicate and 23 copies in the other. The qPCR results for relapsing fever group species, including *B. miyamotoi* and *B. hermsii*, which are enzootic in parts of California, were negative. We genotyped the *ospC* allele and 16S–23S intergenic spacer (IGS) using PCR amplification of each locus and direct sequencing as described by Travinsky et al. (6). Sequencing of the targeted PCR products showed that the *ospC* allele was type Hb and the IGS genotype was 13.

Two years of untreated relapsing pauciarticular arthritis of the knee and hip, a *B. burgdorferi*-positive Western blot, and laboratory detection of *B. burgdorferi* from synovial fluid by PCR in 2 different laboratories leads us to conclude that the patient had Lyme arthritis. This patient likely acquired the infection locally. The prevalence of *B. burgdorferi* in nymphal *I. pacificus* ticks (range 4%–10%) in Santa Cruz County, and >10% of the geographic area of the county is categorized as being at high acarologic risk for LB (4). To our knowledge, the combination of *ospC* allele Hb and IGS genotype 13 has been identified only in California to date (6,8). A type “H” *ospC* type was reported from synovial fluid from LB patients from the eastern United States (10), but in the absence of IGS determination, this was probably type Ha, which is more typical of that region (8). The addition of the IGS locus to *ospC* alleles

provides a precise approach to characterize genetic diversity and potential origin of *B. burgdorferi* in human tissue.

#### Acknowledgments

We thank the staff of Dominican Hospital, Santa Cruz, California, for facilitating access to case information; the staff of the County of Santa Cruz communicable disease division for their continued support; and California Epidemiologic Investigation Services for their suggestions for this manuscript and the work of their fellow, Sharon I. Brummitt.

The project described was supported by the Preventive Health Services Block Grant from the Centers for Disease Control and Prevention. Laboratory work at University of California Irvine was supported by the National Institutes of Health grant AI-065359. This work was determined exempt by The Committee for the Protection of Human Subjects, California Office of State Health and Planning.

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DOI: <http://dx.doi.org/10.3201/eid2012.140655>

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Figure. Lateral radiograph of right knee demonstrating suprapatellar effusion without acute osseous injury (arrow).

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## Zoonotic *Baylisascaris procyonis* Roundworms in Raccoons, China

**To the Editor:** *Baylisascaris procyonis*, an intestinal roundworm that infects raccoons (*Procyon lotor*), causes fatal or severe neural larva migrans in animals and humans (1,2). Globally, ≈130 species of wild and domesticated animals are susceptible (2). Infections in humans typically occur in children who have the disorders pica or geophagia and ingest *B. procyonis* eggs in items contaminated with raccoon feces (3). Clinical manifestations include ocular disease, eosinophilic encephalitis, and eosinophilic cardiac pseudotumors; severe infection can lead to death. Since 1984, ≈24 cases of *B. procyonis*-related human neural larva migrans have been reported, mainly in the United States (1,3–5; K.R. Kazacos, pers. comm.). Despite few cases among humans, lack of effective treatment and widespread distribution of infected raccoons in close association with humans make *B. procyonis* a potentially serious public health threat (2,6). The current distribution of *B. procyonis* is poorly recorded in Asia (2,7), except for Japan (8). We describe *B. procyonis* infections among raccoons in China as part of a series of ongoing surveys of helminthic zoonoses linked to captive exotic animals in zoologic gardens (ZGs) in China.

More than 90% of raccoons in China ( $n > 320$ ) are raised as exotic ornamental animals in 18 ZGs. During 2011–2013, we collected 2×308 fecal samples (i.e., 1 repeat within each sampling) from 277 raccoons in 12 randomly selected ZGs (online Technical Appendix Figure 1, [wwwnc.cdc.gov/EID/article/20/12/14-0970-Techapp1.pdf](http://wwwnc.cdc.gov/EID/article/20/12/14-0970-Techapp1.pdf)). Samples were stored in individual plastic bags at –20°C until use. We examined raccoons ( $n =$

31) at the Sichuan ZGs twice, in June 2012 and May 2013. We identified *B. procyonis* eggs in feces using morphologic and molecular analyses (1,2,9). The nuclear first internal transcribed spacer (428 bp) and mitochondrial cytochrome c oxidase subunit 1 (*cox-1*, 938 bp) genes in each sample were PCR-amplified and sequenced. *B. procyonis* infection was confirmed by sequencing and phylogenetic analyses of both genes (7,9). We reexamined ≈60% of fecal samples to validate results. Prevalence (95% CI) was calculated for the overall population and independently for female, male, juvenile, and adult raccoons. We determined differences between the tested ZG prevalence and prevalence by sex or age of raccoons using  $\chi^2$  or Fisher exact tests in SAS (SAS Institute, Cary, NC, USA);  $p$  values <0.05 were considered significant.

Building on egg-based morphologic characterization and internal transcribed spacer 1 and *cox-1* gene-based phylogenies using neighbor-joining trees (online Technical Appendix Figure 2), we found *B. procyonis* in raccoon feces from 5/12 ZGs (42%; 95% CI 14%–70%), including 2 in the most densely populated provinces, Henan and Sichuan. More infections were found in western than central and eastern ZGs (4/6 and 1/6, respectively; Table, online Technical Appendix Figure 1) ( $p = 0.079$ ). Fecal samples of 35 raccoons (13%; 95% CI 9%–17%) tested positive for *B. procyonis*. The mean intensity of egg shedding was 5,000 eggs per gram (range 800–11,200 eggs per gram; data not shown). No significant difference was observed in the intensity of shedding by comparing sex and age of animals, and no significant differences were noted in the mean prevalence between female and male raccoons (12% versus 14%;  $p = 0.677$ ) or between adult and juvenile animals (13% versus 10%;  $p = 0.536$ ).

This investigation documents the presence and prevalence of *B. procyonis*