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Failure to Identify *Borrelia burgdorferi* in Southern California Ticks by DNA Amplification

Colleagues—In the absence of erythema migrans or late systemic sequelae, the diagnosis of Lyme borreliosis after tick exposure remains difficult. Problems include the nonspecific nature of patient complaints, lack of serologic standardization, and difficulty propagating the spirochete in artificial culture medium. Magid et al. [1] recently suggested that prophylactic antibiotics given after tick exposure may be cost-effective in areas where *Borrelia burgdorferi* is hyperendemic. Knowledge of the prevalence patterns of *B. burgdorferi* infection in potential tick vectors would be useful in assessing the risk of Lyme borreliosis in exposed patients and the utility of antimicrobial prophylaxis. We used polymerase chain reaction (PCR) to screen Southern California ticks for *B. burgdorferi*.

Ixodes pacificus is the predominant vector for *B. burgdorferi* transmission to humans on the West Coast of the United States and is found throughout the coastal mountain ranges of Washington, Oregon, and California. Despite the wide distribution of this vector, well-documented cases of Lyme borreliosis remain rare in southern California. Ecologic factors including enzootic cycles, tick feeding preferences, and nonuniform distribution of infected ticks may be responsible for the low incidence of documented human infection [2-4]. To better define the epidemiology and risk of clinical infection with *B. burgdorferi* in this area, we examined ticks from multiple locations in rural San Diego County and attempted to amplify spirochetal DNA with PCR primers known to be highly sensitive and specific.

We obtained 1046 adult *I. pacificus* by dragging appropriate habitat on 32 widely dispersed rural sites during 1990-1991. From this sample, 160 specimens (5 from each site) were randomly chosen for DNA amplification studies; assays were also done on a single engorged *I. pacificus* adult that was removed from the skin of a clinic patient. *B. burgdorferi* cultures were obtained from R. A. Wirtz (Department of Entomology, Walter Reed Army Institute of Research, Washington DC). Oligonucle-

otides were synthesized and purified by Synthecell Corp. (Rockville, MD). PCR reagents (Perkin-Elmer Cetus, Norwalk, CT) were used as prescribed by the manufacturer with an automated thermocycler device. Nucleotide sequences of PCR primers were defined according to Barbour [5, 5a, 5b].

On initial PCR analysis, two pooled tick homogenates proved negative for *B. burgdorferi*. When the same homogenates were amplified in the presence of exogenous *B. burgdorferi*, bands of appropriate molecular weights for primers specific for *B. burgdorferi* were identified. Subsequent PCR analysis of all tick samples without exogenous *B. burgdorferi* added failed to detect DNA sequences of *B. burgdorferi* (as a positive control, *B. burgdorferi* alone was successfully amplified in each test).

We believe this represents the first use of PCR in screening significant numbers of competent vector ticks in southern California. Our test group ($n = 161$) represents a reasonably random sample. In adjacent Orange County, researchers tested 359 *I. pacificus* ticks by culture on BSK II medium and found only 1 to be positive (subsequently confirmed by immunofluorescent microscopy using specific monoclonal antibodies) [6]. According to the revised Centers for Disease Control and Prevention case definition [7], there have been no locally acquired cases of human Lyme borreliosis reported by San Diego County (E. Haas, Department of Health Services, personal communication). Adjacent Orange and San Bernardino Counties have reported a total of 3 patients with characteristic lesions of erythema migrans; however, the causative organism was not isolated [6].

Patients bitten by ticks are frequently concerned about developing Lyme disease. Clinicians can counsel their patients with reasonable certainty that the risk of acquiring the disease after tick exposure in this area remains low. Our data show a low prevalence of *B. burgdorferi* infection of competent tick vectors in southern California and argue strongly against routine antibiotic prophylaxis of tick-exposed patients. Future studies may be warranted as changing land use and development bring more people into contact with vectors of *B. burgdorferi*.

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Clarification of Dietary Risk Factors and Religion in a Botulism Outbreak

Colleagues—We would like to explain why, in an outbreak of botulism in Egypt caused by eating *faseikh* (salted fish), the majority of patients were Coptic Christians [1]. *Faseikh* is traditionally eaten on a national holiday, *Sham-el-Nessim*, but in 1991, *Sham-el-Nessim* and Ramadan coincided. During the month of Ramadan, most Muslims eat and drink only after sundown and avoid salty food because it makes them thirsty the following day.

Coptic Christians are under no such constraint and ate the *faseikh*, causing this group to make up 79% of the patients, even though they represent only 15% of the population. One Muslim patient in the family case-control study ate *faseikh* because she was menstruating, which exempted her from the Ramadan fast.

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Therapy with Atovaquone for *Cryptosporidium parvum* Infection in Neonatal Severe Combined Immunodeficiency Mice

Colleagues—*Cryptosporidium parvum* is a coccidial protozoan that causes protracted and severe diarrhea in immunocompromised patients, especially in patients with AIDS and in malnourished children in developing countries [1]. Currently, there is no available effective therapy for cryptosporidiosis.

The hydroxynaphthoquinone 2-[trans-4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone (atovaquone, 566C80; Wellcome Research Laboratories, Beckenham, UK) has activity against several protozoal pathogens, suggesting its potential usefulness for therapy of cryptosporidiosis. In vitro studies have demonstrated the efficacy of atovaquone against the coccidial

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protozoans *Eimeria* species and *Toxoplasma gondii* [2, 3]. In a murine model, 100% of mice that received 100 mg/kg atovaquone survived infection with 5 different strains of *T. gondii* [3]. Early animal model experiments of atovaquone therapy for cryptosporidiosis, largely unpublished, were reported in summary as equivocal [4].

The severe combined immunodeficiency (SCID) strain results from an autosomal recessive mutation in the C.B-17 inbred strain of BALB/c mice, resulting in the absence of functional B and T lymphocytes [5]. The course of cryptosporidial infection in SCID mice has been reported [6, 7]. Oral infection of 5-day-old SCID mice with 10^7 *C. parvum* oocysts resulted in cryptosporidial infection that was uniformly fatal within 7 weeks [7]. We chose this model to assess the efficacy of atovaquone for treatment of cryptosporidiosis.

C. parvum oocysts were obtained and prepared for inoculation as previously described [7]. The SCID mice were raised and housed as previously described [7]. Mice had food and water available ad libitum.

Five-day-old SCID mice were inoculated orally with 10^6 *C. parvum* oocysts in 10 μ L of PBS. Two weeks after inoculation, these mice were weaned, and littermates were randomly assigned to either the treatment or control group. The treatment group consisted of 19 mice given 0.1-mL daily intragastric doses of ≥ 100 mg/kg atovaquone (gift of S. Lefon, Burroughs Well-

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