Lyme disease in South America?

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COLLEGUES—Although originally believed restricted to Europe and North America, Lyme borreliosis has recently been reported from both the Soviet Union [1] and Egypt [2]. All primary vectors appear to be members of the *Ixodes ricinus* complex: *I. dammini* in the northeastern and north central United States, *I. pacificus* in the western United States, and *I. ricinus* in Europe [3] and Asia [1]. Another member of this species complex, *I. scapularis*, has been implicated as a vector in the southeastern United States [4].

Recently, while conducting a literature review of potential tick vectors of disease in Peru, it was noted that two other species of the *I. ricinus* complex have been reported from Peru (unpublished data). The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the U.S. Navy.

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References


and other countries in South America [5]. These two species, *I. affinis* and *I. pacificus*, while not proven vectors of Lyme borreliosis, must be considered potential vectors since three members of the complex are proven vectors and one is suspected to be a vector of the disease.

As a preliminary step in the determination of the presence or absence of Lyme borreliosis in Peru, a retrospective serum survey was conducted. Serum samples of a group of 216 individuals (10 years old), chosen as a high-risk group due to their agricultural profession, were tested for the presence of antibodies to *Borrelia burgdorferi* antigen. For this survey, a commercially available ELISA kit (Cambridge Bioscience, Worcester, MA), utilizing antigen-coated microtiter plates, was used. This test kit was recently reviewed [6] and compared to Western Blot analysis, the sensitivity and specificity were 89% and 100%, respectively.

Of the 216 sera tested, 4, or nearly 2%, were positive for antibody to *B. burgdorferi* (positive sera were run a second time for reconfirmation). Due to the nature of a retrospective survey, limited clinical data were available on the positive patients. One of the four antibody-positive patients was acutely ill with hepatitis B and rapid plasma reagin-positive and weakly reactive on the fluorescent treponemal absorption test. All three others were apparently well, but one was cross-reactive for leptospirosis (1:100 dilutions to Huallaga serotype on macroagglutination test). Thus, perhaps only 2% (0.9%)...
of 216 may have actually been positive for Lyme borreliosis antibodies. While our survey is not conclusive evidence of the presence of Lyme borreliosis in South America or of the magnitude of its importance locally, it represents the first evidence of which we are aware that it may exist on this continent. Other possibilities such as the relapsing fever *Borrelia* species and the cross-reactivity seen in some patients with rheumatologic or neurologic disorders should also be considered [7-10]. Nevertheless, the presence in Peru of two species of ticks of the *L. ricinus* complex in combination with this preliminary evidence of anti-*B. burgdorferi* antibodies entices us to conduct prospective clinical surveys and in-depth studies of potential vectors.

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References


Application of the Microcapsule Agglutination Test to Serologic Studies of an Early Stage of Lyme Disease in Japan

**COLLEAGUES—**Lyme disease, a tickborne spirochetal infection, typically begins with the unique skin lesion erythema chronicum migrans (ECM). Several weeks or months later, some patients experience cardiac or neurologic disorders or arthritis. A related disease has been reported in Japan, where the vector is *Ixodes persulcatus* [2]. ELISA and indirect immunofluorescence have been widely used to detect antibodies against *Borrelia burgdorferi* [3]; however, there are disagreements about serodiagnosis of early stages of Lyme disease [4, 5]. We have previously found that the microcapsule agglutination test (MCAT) is valuable and convenient for the diagnosis of leptospirosis [6]. We describe the application of MCAT to the early diagnosis of Lyme disease.

Serum samples from patients with early Lyme disease were collected in Hokkaido, Japan, during 1989. Samples from healthy individuals were supplied by the Japanese Red Cross General Hospital, Tokyo.

*B. burgdorferi* strain IRS (ATCC 35211), originally isolated from *Ixodes ricinus* in Switzerland [7], was used for preparation of antigen. This strain was maintained in modified Kelly medium (BSK II).

Microcapsule particles sensitized with *Borrelia* antigen were prepared by a slight modification of a previously described method [6]. The optimal microcapsule suspension was added into serially diluted serum samples. The mixtures were kept overnight at 4°C, and the agglutination patterns were read the next day.

The antigen for ELISA was prepared by the method of Bidwell et al. [8]. Microtiter plates (Greiner, FRG) were coated with poly-L-lysine, washed with PBS (0.01 M phosphate buffer, 0.15 M saline, pH 7.2), and incubated with 50 μl of 1 × 10⁷/ml spirochetal cell suspension for 2 h at room temperature, followed by washing with PBS. The plates were then incubated with 1% fetal calf serum for 30 min to block the possible reactive sites. After washing with PBS, serum samples diluted 1:80 for IgM antibody and 1:200 for IgG were added to each well, and plates were incubated for 2 h. The dilutions were confirmed as appropriate from preliminary dose-response curves. After further washings, the optimal dilution of an alkaline phosphatase-labeled goat anti-human IgG or IgM was added and incubated for 2 h before addition of substrate. The absorbance at 405 nm was recorded with an automatic recorder (ETY-96; EIA analyzer).

Antibody responses against *B. burgdorferi* from 11 early Lyme disease patients whose disease was characterized by the appearance of typical ECM were assessed by MCAT and ELISA. For MCAT, the cutoff value was 160 since serum samples from 45 healthy individuals showed normal distribution with a 95th percentile titer of 109 as determined by the method of Cohen [9]. All sera from the 11 patients, including two paired sera, displayed an elevated MCAT titer compared with that from healthy individuals (figure 1). Only 1 (2%) of 45 healthy individuals had a positive titer. When cutoff levels for IgM and IgG were defined as >2 × 3 SD above the geometric mean (i.e. 0.41 for IgM and 0.82 for IgG), 8 (62%) of 13