

Figure. Geographic distribution in Guatemala of horses showing previous infections with West Nile virus (WNV), Saint Louis encephalitis virus (SLEV), or undifferentiated flavivirus as confirmed by plaque reduction neutralization test. Each location may have multiple positive horses.

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Borrelia burgdorferi in *Ixodes scapularis* Ticks, Chicago Area

To the Editor: Lyme disease is a multisystem disorder associated with skin, myocardial, musculoskeletal, and central and peripheral nervous system manifestations caused by infection with *Borrelia burgdorferi* sensu lato spirochetes (1). In the United States, the illness is caused by transmission of *B. burgdorferi* sensu stricto from the bite of infected *Ixodes scapularis* (deer) ticks found primarily in the northeastern and upper mid-western United States (2). *B. burgdorferi*-infected ticks have also been recovered in portions of northern Illinois but have not yet been reported in the Chicago region (3). In fact, a previous survey (4) of forested areas in the heavily populated regions immediately adjacent to Chicago confirmed a low risk of contracting Lyme disease. Researchers failed to recover deer ticks from 7 sampling sites and recovered only a single *Borrelia* isolate from well-described *B. burgdorferi* sensu stricto rodent reservoirs (mice, voles, chipmunks) captured from 5 sampling sites. A subsequent genetic analysis confirmed the isolate was *B. bissettii* (5), a genomic group that is likely nonpathogenic to humans in the United States.

However, the area of the Midwest where Lyme disease is endemic has continued to expand from its origin in northeastern Minnesota and northwestern Wisconsin (2,6). The Chicago metropolitan region has numerous parks and natural areas that are biologically similar to the known mid-western focus (7), and these areas support a large population of white-tailed deer. Moreover, we have seen anecdotal reports of persons with clinical signs and symptoms of Lyme disease and epidemiologic evidence that suggests local acquisition. Two ticks submitted by a resident who had hiked

in DuPage County near the east branch of the DuPage River were *I. scapularis*. We therefore began searching for questing ticks in this area and 9 other geographically diverse areas of DuPage County. The 10 sites were flagged by dragging white cotton sheets through the underbrush for 2- to 10-hour intervals during the spring of 2005. We timed these collections on the basis of information that adult deer ticks were questing in Wisconsin. Recovered ticks were placed in sealed vials and transported to the North Park University Laboratory where their identity was confirmed. The midguts were then removed aseptically, and each was placed into a separate vial that contained 2 mL modified Barbour-Stoenner-Kelly medium that could support the growth of small numbers of *B. burgdorferi* sensu stricto (8). Cultures were incubated for 6 weeks at 35°C and examined weekly for spirochetes by darkfield microscopy.

Deer ticks were not found at 8 of the DuPage County sample sites. However, 90 adult or nymphal *I.*

scapularis were collected from the remaining 2 sites, and spirochetes were recovered from 3 (3%) of the tick midgut cultures. Because of these findings and anecdotal reports of deer ticks in Cook County, we also surveyed 3 sites in Cook County during the fall of 2005. Deer ticks were not found at 2 sites, but 37 adult *I. scapularis* ticks were collected from a site in the southwestern portion of Cook County, and spirochetes were recovered from 2 (5%) cultures.

We first examined the protein profiles to identify the spirochetes. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis analyses of 4 of the 5 isolates (1 isolate lost viability before analysis) showed that they were distinctly different from the *B. bissettii* isolate recovered previously from Cook County (4), but the isolates were strikingly similar to *B. burgdorferi* sensu stricto (Figure). We then amplified an intergenic spacer region of the *rrf-rrl* portion of the rRNA from 2 isolates by a previously described method (9) and sequenced the amplified products (sequencing by

Laragen, Inc., Los Angeles, CA, USA). The sequences were identical to that of *B. burgdorferi* sensu stricto isolate B-31 (9).

The results confirmed that *I. scapularis* ticks infected with *B. burgdorferi* sensu stricto were recovered from forested areas surrounding Chicago. Additional studies to define the extent and severity of the risk are necessary, but clinicians and the public should be aware of the possibility of acquiring Lyme disease in the Chicago metropolitan region.

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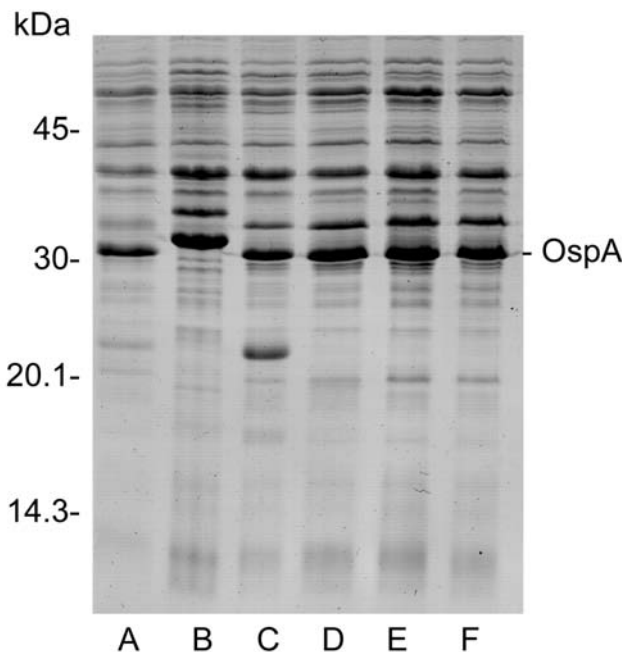


Figure. Protein profiles of *Borrelia burgdorferi* sensu stricto (lane A), *B. bissettii* (lane B), and spirochetes from *Ixodes scapularis* ticks collected from DuPage County (lanes C, D) or Cook County (lanes E, F), Illinois.

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H5N1 Influenza A Virus and Infected Human Plasma

To the Editor: Since January 2004, a total of 22 persons have been confirmed infected with avian influenza A virus (H5N1) in Thailand; 14 of these patients died. Three waves of outbreaks occurred during the past 2 years. The last patient of the third wave was a 5-year-old boy whose symptoms developed on November 28, 2005; he was hospitalized on December 5 and died 2 days later. The child resided in the Ongkharak District, Nakhon Nayok Province, 70 km northeast of Bangkok. Villagers informed the Department of Livestock after the patient's illness was diagnosed. Five dead chickens had been reported in this area from November 28 to December 1, 2005. Samples from these chickens could not be obtained, thus, no H5N1 testing was performed. The boy had fever, headache, and productive cough for 7 days before he was admitted to the Her Royal Highness Princess Maha Chakri Sirindhorn Medical Center. Clinical examination and chest radiograph showed evidence of lobar pneumonia. He was treated with antimicrobial drugs (midecamycin and penicillin G) and supportive care, including oxygen therapy. On December 7, the patient's condition worsened, and severe pneumonia with adult respiratory distress syndrome developed. Laboratory tests showed leukopenia (2,300 cells/mm³), acidosis, and low blood oxygen saturation by cutaneous pulse oximetry (81.6%). Oseltamivir was administered after his parents informed hospital staff about the boy's contact with the dead chicken. However, the boy died the same day; no autopsy was performed. On December 9, the cause of death was declared by the Ministry of Public Health to be H5N1 influenza virus.

A blood sample was collected from the patient on December 7; anticoagulation was accomplished with ethylenediaminetetraacetic acid (EDTA) for repeated biochemistry analysis and complete blood count. The plasma from the EDTA blood sample was separated 2 days later and stored at -20°C for 12 days. The sample was subsequently given to the Center of Excellence in Viral Hepatitis, Faculty of Medicine, Chulalongkorn University, for molecular diagnosis and then stored at -70°C, where specific precautions implemented for handling highly infectious disease specimens such as H5N1 influenza virus were observed. Plasma was examined by multiplex reverse transcription-polymerase chain reaction (RT-PCR) (1) and multiplex real-time RT-PCR (2), both of which showed positive results for H5N1 virus. The virus titer obtained from the plasma was 3.08 × 10³ copies/mL. The plasma specimen was processed for virus isolation by embryonated egg injection, according to the standard protocol described by Harmon (3). Briefly, 100 μL 1:2 diluted plasma was injected into the allantoic cavity of a 9-day-old embryonated egg and incubated at 37°C. The infected embryo died within 48 hours, and the allantoic fluid was shown to contain 2,048 hemagglutinin (HA) units; also, subtype H5N1 was confirmed (1,2). Whole genome sequencing was performed and submitted to the GenBank database under the strain A/Thailand/NK165/05 accession no. DQ 372591-8. The phylogenetic trees of the HA and neuraminidase (NA) genes were constructed by using MEGA 3 (4) for comparison with H5N1 viruses isolated from humans, tigers, and chickens from previous outbreaks in 2004 and 2005 (Figure). The sequence analyses of the viruses showed that the HA cleavage site contained SPQRERRKKR, which differed from the 2004 H5N1 virus by an arginine-to-lysine substitution at posi-