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CONCISE COMMUNICATION

***Borrelia lonestari* Infection after a Bite by an *Amblyomma americanum* Tick**

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Erythematous rashes that are suggestive of early Lyme disease have been associated with the bite of *Amblyomma americanum* ticks, particularly in the southern United States. However, *Borrelia burgdorferi*, the causative agent of Lyme disease, has not been cultured from skin biopsy specimens from these patients, and diagnostic serum antibodies usually have not been found. *Borrelia lonestari* sp nov, an uncultured spirochete, has been detected in *A. americanum* ticks by DNA amplification techniques, but its role in human illness is unknown. We observed erythema migrans in a patient with an attached *A. americanum* tick. DNA amplification of the flagellin gene *flaB* produced *B. lonestari* sequences from the skin of the patient that were identical to those found in the attached tick. *B. lonestari* is a probable cause of erythema migrans in humans.

Borrelia burgdorferi sensu stricto, which is transmitted by ticks of the subgenus *Ixodes*, is the only established cause of Lyme disease in North America [1]. In the United States, most persons acquire Lyme disease in the northeastern or north central regions or in the Pacific Northwest; however, each year hundreds of patients from southeastern and south central states are reported to have this illness [2]. Several publications on suspected Lyme disease in southern states describe patients with a mild illness characterized by an erythema migrans rash that develops at the site of a bite by *Amblyomma americanum*, the lone star tick [3–6]. Despite the existence of *B. burgdorferi* in enzootic cycles in southern states, human *B. burgdorferi* infection acquired in this area has not been confirmed by culture from clinical specimens. In addition, serologic tests rarely indicate exposure to this organism [3, 4, 6].

A. americanum is the species of tick that most commonly bites humans in the southeastern and south central United States. It is distributed from central Texas and Oklahoma eastward across the southern states and along the Atlantic coast as far north as Maine [7, 8]. In the laboratory, *A. americanum* is an incompetent vector for *B. burgdorferi* sensu stricto. However, several studies have demonstrated that 1%–3% of *A. americanum* ticks are infected with a spirochete [9]. Although this spirochete has not yet been cultured, phylogenetic analysis of amplified DNA sequences has shown that it differs from all borrelial species known to cause Lyme disease [9, 10]. Barbour et al. [9] demonstrated by analysis of 16S rRNA sequences that it is more closely related to the relapsing fever spirochetes *Borrelia miyamotoi* sp nov in Japan and to an unnamed *Borrelia* species isolated from dogs in Florida. They proposed the name *Borrelia lonestari* sp nov. Whether *A. americanum* ticks can transmit *B. lonestari* to humans or whether *B. lonestari* causes clinical illness has not been determined.

We describe a patient with erythema migrans at the site of an attached lone star tick and at a secondary site, after recent travel to Maryland and North Carolina. Clinical specimens and the tick were evaluated for evidence of infection by *Borrelia*, using polymerase chain reaction (PCR), culture, and serologic tests.

Case Report

A 74-year-old African American man was evaluated on 24 May 1999 at New York Medical College (NYMC; Valhalla) because of an abdominal skin rash that had been present for ~4 days, as well as fatigue, cough, and right shoulder discomfort of ~1 week's duration. He denied having fever or head-

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Informed consent was obtained from the patient described in this case report.

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aches. The patient was a resident of southern Westchester County, New York. He traveled to Maryland on 6 May and then left Maryland on 6 May to travel to North Carolina, where he stayed from 7 May to 18 May. He returned to Maryland on 18 May and then left on 20 May to return to his home in Westchester County. He had walked in grassy areas in both Maryland and North Carolina. His medical history included a left nephrectomy in June 1998 for a renal cell carcinoma, prostate carcinoma diagnosed in 1997, colonic carcinoma resected in 1985, hypertension, and hypothyroidism.

At examination, the patient had an oral temperature of 36.8°C. Results of musculoskeletal, neurologic, pulmonary, and cardiac examinations were normal. A 19 × 11-cm annular erythematous lesion surrounding an attached, engorged tick was present in the right lower quadrant of the abdomen, and a separate 4 × 3-cm erythematous lesion was present in the left upper quadrant (figure 1A, 1B). The patient first noticed the tick on 15 May but did not remove it because he thought it was a skin lesion. The tick was removed at NYMC on 24 May for taxonomic evaluation and microbiologic studies.

Laboratory evaluation showed normal white blood cell count and differential, hemoglobin level, and platelet count. Results of analysis of serum chemistry were normal, except for an alkaline phosphatase level of 142 U/L (normal range, 35–110 U/L). An electrocardiogram showed premature atrial contractions.

Beginning 24 May, the patient received a 14-day course of doxycycline (100 mg orally twice daily). The skin lesions resolved by 4 June. The patient reported that he had returned to his usual state of health by 16 June 1999.

Materials and Methods

Skin biopsy and culture. On 24 May 1999, a 2-mm punch biopsy sample was taken from the edge of the skin lesion in the right lower quadrant of the abdomen, farthest from the site of tick attachment. The skin sample was processed at NYMC for *B. burgdorferi* culture and PCR studies, as reported elsewhere [11].

Tick methods. The removed tick was identified as a female of the species *A. americanum* and was sent to the Centers for Disease Control and Prevention (CDC) at Fort Collins, Colorado, for further study. At the CDC, the tick was cut in half longitudinally. One half was processed for PCR, as described below, and the other half was processed for coculture with *Ixodes scapularis* embryonic cells (cell line IDE2; a gift from T. Kurtii, University of Minnesota, Minneapolis). The coculture was prepared as described by Kurtii et al. [12], was incubated at 31°C, and was checked by dark-field microscopy every week for 2 months for the presence of spirochetes.

PCR. DNA from the skin specimen and the patient's blood was extracted at NYMC by using an IsoQuick nucleic acid isolation kit (Orca Research). Because the skin specimen had been transported from the clinic in BSK-H medium (Sigma), DNA was prepared from both the skin itself and the medium fraction ("supernatant"). At the CDC, DNA from the tick was prepared by maceration of the tick in 1 mL of a guanidinium reagent (DNA STAT-60; Tel-Test) and was isolated according to the manufac-

turer's instructions. DNA samples from the patient's skin biopsy specimen, skin supernatant, and blood and from the tick were exchanged between the NYMC and CDC laboratories, and PCR was done at each site.

Nested PCR amplification with broadly-reactive flagellin gene (*flaB*) primers was done at NYMC and the CDC by using primers FlaLL and FlaRL in the first amplification and primers FlaLS and FlaRS in the second amplification, after the amplicons in the first product were diluted 1:100 with water. Primers, reaction conditions, and thermal cycling were as described elsewhere [9]. Specimens also were tested for the presence of *B. burgdorferi* DNA by PCR, with species-specific primers directed at the 23S rDNA duplication [11], the 16S-23S rDNA spacer, and *ospA* [1]. In addition, amplification of the 16S rDNA sequence was done by using broad-range eubacterial primers [13] and primers based on the *B. lonestari* 16S rDNA sequence [9].

DNA sequencing. The PCR products obtained in each laboratory from the skin-sample supernatant and the *A. americanum* tick were sequenced at the CDC after purification with a Qiagen PCR kit. Sequencing was done using a Taq DyeDeoxy terminator cycle-sequencing kit (Applied Biosystems) and was run on an Applied Biosystems Model 373A automated sequencer. Sequences were obtained from both DNA strands for each sample. The *B. lonestari flaB* sequences from skin and tick DNA have been deposited in GenBank (accession nos. AF273670 and AF273671).

Serologic tests. Serum samples obtained from the patient during the acute (24 May) and convalescent (2 and 26 June) phases of illness were tested for antibodies to *B. burgdorferi* by an IgG/IgM ELISA (Wampole Laboratories). IgM and IgG antibodies to *B. burgdorferi* were measured separately by immunoblotting, using serum diluted 1:100. The *B. burgdorferi* antigens were from strain B31 (low passage; CDC reference collection) grown in BSK-H medium and were fractionated (15 µg per minigel) by discontinuous SDS-PAGE through 13.5% acrylamide gels; immunoblotting was done by standard procedures [1].

Results

No laboratory evidence of *B. burgdorferi* infection was found. Culture of the skin biopsy sample in BSK-H medium and coculture of the sample of tick cells failed to propagate *B. burgdorferi*. Moreover, *B. burgdorferi* sequences were not detected by PCR of DNA prepared from skin, skin supernatant, or blood, using 3 different sets of *B. burgdorferi*-specific primers. Acute- and convalescent-phase serum samples were negative for *B. burgdorferi* antibody by ELISA and immunoblotting. No IgM bands were found to be present on immunoblots for *B. burgdorferi* antigen; only a single IgG band at the position of FlaB, a 41-kDa antigen, was detected in both the acute- and convalescent-phase serum samples (data not shown).

In contrast, DNA evidence compatible with *B. lonestari* infection was obtained. Amplification of the *flaB*, using *Borrelia* genus-wide PCR primers, resulted in products of ~350 bp from both the patient (DNA from the supernatant of the skin sample) and the tick, at both the NYMC and the CDC (figure 2A). The positive control (figure 2A) was *B. burgdorferi* DNA from

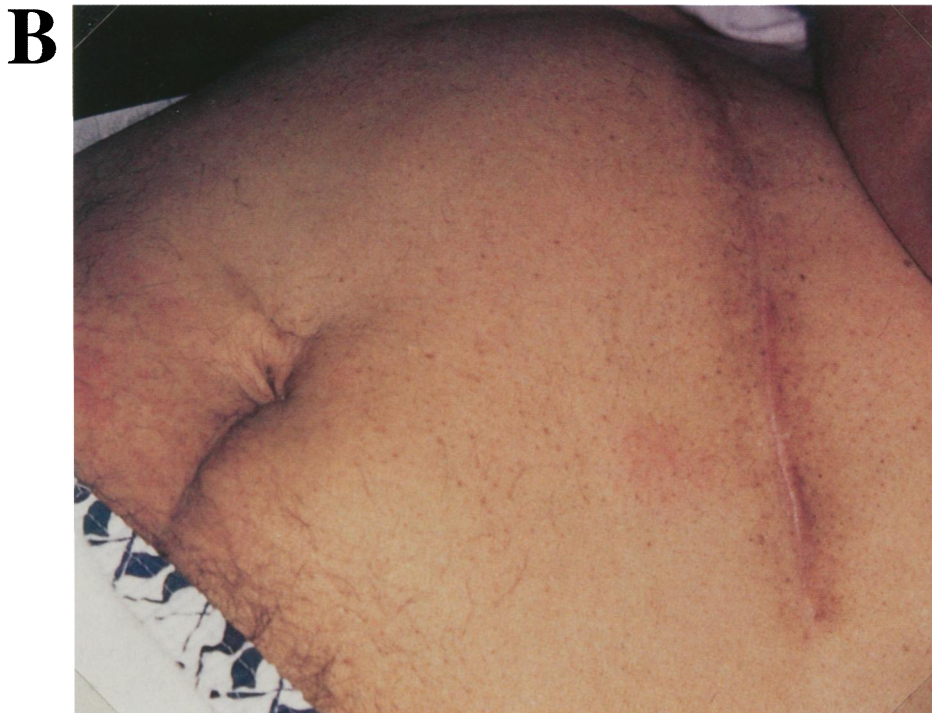
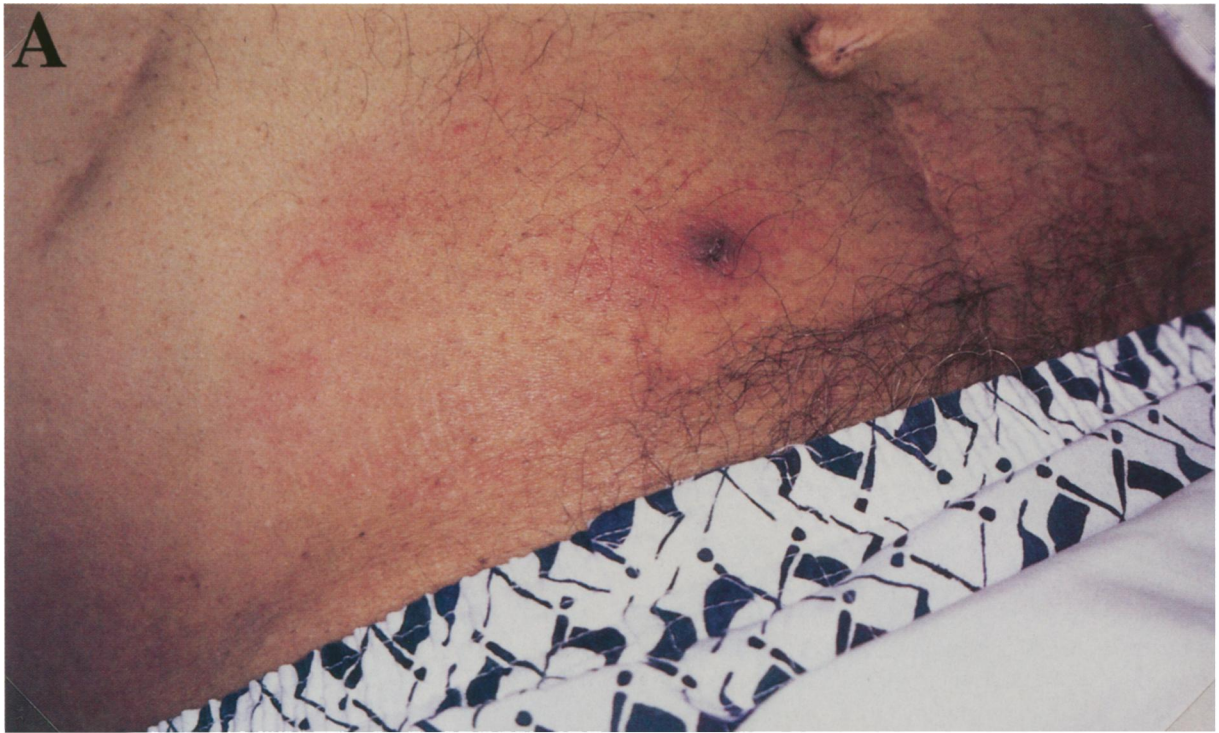


Figure 1. Erythema migrans of case patient and point of attachment of female *Amblyomma americanum* tick. *A*, Skin lesion with central clearing, in right lower quadrant of abdomen. Raised lesion marks indicate from which area the attached tick was removed. *B*, Skin lesion in left upper quadrant of abdomen, below surgical scar.

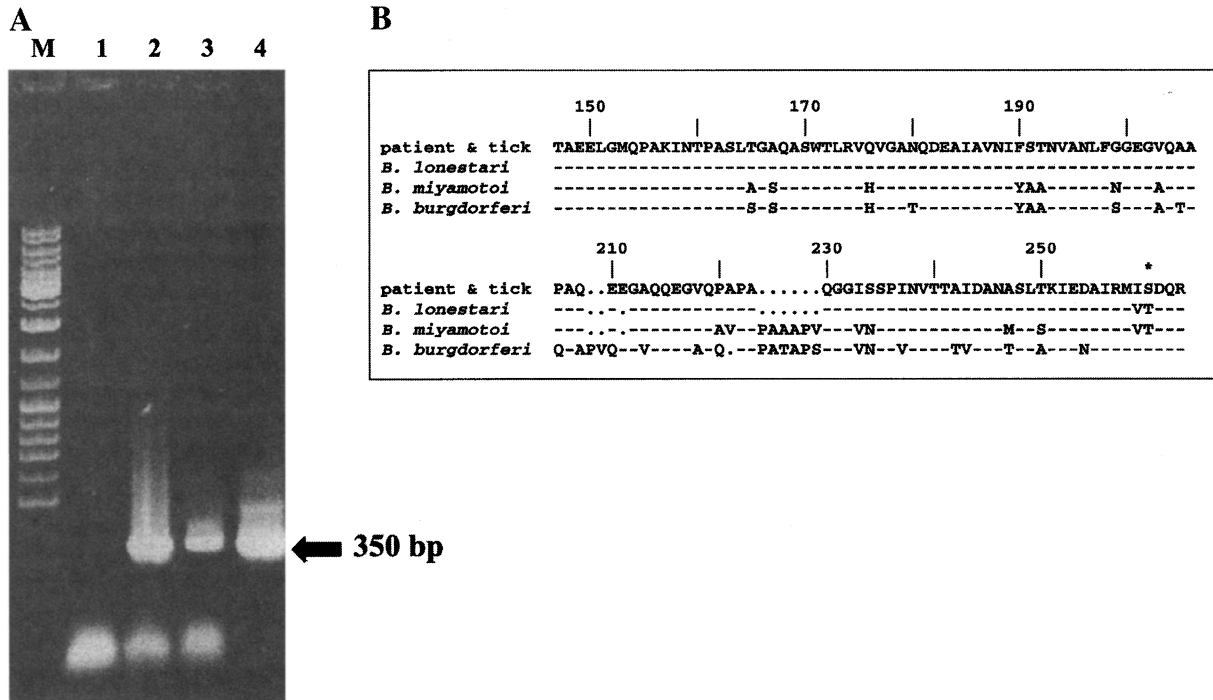


Figure 2. Analysis of DNA from case patient and tick. *A*, *flaB* DNA amplified by polymerase chain reaction (PCR). Amplicons were separated by electrophoresis on a 1% agarose gel and were detected by ethidium-bromide staining. Lane *M*, Molecular weight markers, in 100-bp intervals; lane 1, negative control (water); lane 2, case patient DNA from supernatant of a skin biopsy sample; lane 3, tick DNA; lane 4, positive control (*Borrelia burgdorferi* B31 DNA). *B*, Deduced amino acid sequence of portion of *flaB* DNA amplified from both patient skin-sample supernatant and the attached tick. Sequences are compared with the equivalent regions of *flaB* from *B. lonestari* in ticks from New Jersey, *B. miyamotoi* HT31, and *B. burgdorferi* B31. GenBank accession nos. for the reference sequences are AAA99141, BAA07832, and CAA33696, respectively. *Amino acid position that showed sequence heterogeneity in the uncloned amplicons. Serine was deduced from the AGT sequence of tick DNA amplified at both New York Medical College (NYMC; Valhalla) and the Centers for Disease Control and Prevention (CDC; Fort Collins, Colorado) and from patient skin-sample supernatant DNA amplified at NYMC. Threonine was deduced from the ACT sequence of skin-sample supernatant DNA amplified at the CDC. Nos. correspond to the amino acid positions of FlaB in *B. miyamotoi*.

cultured spirochetes, not from skin. During 1991–1997, we detected *B. burgdorferi* DNA in 65% (192 of 294) of skin biopsy specimens from patients with erythema migrans. It was not uncommon for *B. burgdorferi* DNA to be detected in a skin-sample supernatant but not in the corresponding macerated skin extract (I.S., unpublished data). No amplicons were detected with DNA produced from the macerated skin sample, from the patient’s blood, or from the negative controls that lacked DNA.

Sequencing of 4 PCR products, from amplicons produced independently in each laboratory from DNA of the skin-sample supernatant and of the tick, revealed that this portion of *flaB* was identical in host and vector samples, with the possible exception of the amino acid marked by an asterisk in figure 2*B*. Sequences differed only slightly from those obtained elsewhere for *B. lonestari* in *A. americanum* ticks from New Jersey and Texas [9]. All sequences in this study contained an extra AGA at positions 330–332, compared with the published *B. lonestari* sequences (GenBank U26704-5) and 2 nucleotide differences near the 3’ end of the amplicons. The deduced amino

acid sequence contains an extra glutamic acid as a consequence of the AGA insertion at amino acid position 211 (figure 2*B*). In this study, we found 2 conservative amino acid changes near the carboxy terminus, an isoleucine followed by serine, compared with valine-threonine in the *B. lonestari* sequences in ticks from New Jersey. Alignment of the deduced amino acid sequences obtained from the skin biopsy sample and the tick with the equivalent regions of FlaB from other *Borrelia* species confirms that the sequence does not differ significantly from the *B. lonestari* sequence (figure 2*B*).

Discussion

The clinical and laboratory data from this study strongly suggest that *A. americanum* ticks transmit *B. lonestari* to humans and cause an illness that is indistinguishable clinically from early stage Lyme disease. First, there was a temporal relationship between the bite of an *A. americanum* tick and erythema migrans at the site of the tick bite and at a remote region. Second, we identified *B. lonestari flaB* sequences in both

the tick and a skin sample obtained from the periphery of the skin lesion. Third, laboratory evidence (culture, PCR, and serologic tests) does not support a diagnosis of infection by *B. burgdorferi*, although the skin lesion resolved after standard antibiotic therapy for Lyme disease.

Curiously, our repeated attempts to amplify 16S rDNA fragments both from the biopsy specimen and from the tick extract, using broad-range eubacterial and *B. lonestari*-specific primers, were unsuccessful. The reason for this failure is unclear, although the relatively larger PCR product expected from such amplification (~1400 bp vs. 350 bp) and a low number of *Borrelia* genome equivalents in the samples may account for this observation.

The validity of using *flaB* sequences to establish *Borrelia* taxonomy has been demonstrated elsewhere [14]. Furthermore, in numerous instances, sequence-based identification of a microbe that had not been cultured provided the initial evidence for disease causation. Another tickborne illness, human monocytic ehrlichiosis, is a pertinent example [15]. The seminal observations from gene sequencing of *Ehrlichia chaffeensis* provided the impetus for the eventual development of culture methods and specific serum antibody tests.

An important question is how many of the other reported cases of erythema migrans or erythema migrans-like illness in southern states and elsewhere in the United States are due to *B. lonestari*. A Lyme disease-like illness after a bite from an *A. americanum* tick has been reported in New Jersey [8]. Of 3 series of patients with an illness involving a rash who were from the southern United States, 9%–14% recalled a preceding tick bite that was consistent with that of *A. americanum* ticks [3, 4, 6]. Because only adult female *A. americanum* ticks are likely to be identified by patients, these proportions should be regarded as minimum estimates. Masters et al. [5] have described 17 selected patients with erythema migrans-like illness who were from Missouri, all of whom had a preceding bite from an *A. americanum* tick. Theoretically, *B. lonestari*-infected *A. americanum* may be sympatric with *B. burgdorferi*-infected *I. scapularis*, particularly in states along the eastern seaboard. This coexistence has been documented in New Jersey [2,8]. In such areas, 2 different *Borrelia* species may cause erythema migrans, and not all erythema migrans will indicate Lyme disease.

A specific serologic test for evidence of exposure to *B. lonestari* is not yet available. We are working to develop and validate such a test, because it will be an important tool in determining the etiology of erythema migrans in individual patients and in understanding the epidemiology of *B. lonestari* infections.

In the United States, *B. lonestari* may be a new tickborne pathogen of humans. This case report suggests that efforts to assess the public health importance of *B. lonestari* infection are warranted. This assessment should include concerted clinical

and epidemiologic studies and field investigations of vector ticks and their hosts.

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