DETECTION OF BORRELIA BURGDORFERI SEQUENCES IN A BIOPSY FROM A TENNESSEE PATIENT

JOY M. HAYNES, PRESCOTT P. LEE, REBECCA L. SEAPLANT, AND STEPHEN M. WRIGHT

Department of Biology, Middle Tennessee State University, Murfreesboro, TN 37132 (JMH, RLS, SMW)
Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614 (FPL)

ABSTRACT—*Borrelia burgdorferi* is the causative agent of Lyme disease and is transmitted by the black-legged tick, *Ixodes scapularis*. Clinical diagnosis of Lyme disease is often made on the basis of a characteristic Erythema Migrans (EM) rash. Lyme disease cases have been reported in Tennessee since 1991 when Lyme became a nationally notifiable disease. However, to date, there have been no reports of *B. burgdorferi* isolation in culture or detection of genetic sequences characteristic of *B. burgdorferi* in Tennessee. A syndrome related to Lyme disease, southern tick associated rash illness (STARI), has been described and also manifests an EM rash. The putative agent of STARI, which is not a reportable illness, is *Borrelia lonesarti*. The presence of *B. lonesarti* has been documented in the state. A skin biopsy was taken from an EM rash from a patient in East Tennessee. This study was undertaken to determine if *Borreliae* were present in the biopsy. Following nucleic acid extraction and amplification, a sequence for the *Borrelia* flagellin gene was obtained. There was 98-99% sequence identity between the biopsy and other reported *B. burgdorferi* flagellin gene sequences. This represents the first detection of genetic sequences of *B. burgdorferi* in Tennessee.

Lyme disease is the most common vector borne disease in the United States and is endemic in the northeastern United States. The causative agent is *Borrelia burgdorferi*, which was isolated in culture in 1982 (Burgdorfer et al., 1982). *Borrelia burgdorferi* spirochetes are transmitted by *Ixodes scapularis* ticks (black-legged tick, deer tick) throughout the central and eastern United States. The hallmark symptom of Lyme disease is a characteristic “bull’s-eye” rash, Erythema Migrans (EM), which occurs in nearly 80% of patients one to two weeks after *I. scapularis* feeding (CDC, 1997). Erythema Migrans rashes also have been documented at feeding sites of *Amblyomma americanum*, the Lone Star tick (Schulze et al., 1984; Masters et al., 1998; James et al., 2001). Microscopic evaluation of these tissues revealed spirochetes but this organism was not able to be cultured on medium that supported growth of *B. burgdorferi*. Genetic evaluation of this organism revealed a *Borrelia* distinct from *B. burgdorferi*, which has been provisionally named *Borrelia lonesarti* (Barbour et al., 1996). *Borrelia lonesarti* infection is believed to be responsible for a Lyme-like disease that has been designated southern tick-associated rash illness (STARI) (Burkot et al., 2001). Patient serologic testing fails to adequately distinguish between *B. burgdorferi* and *B. lonesarti* as organisms responsible for EM rashes (Kirkland et al., 1997; Masters et al., 1998).

Cases of Lyme disease, putatively attributed to *B. burgdorferi*, have been reported from nearly all states (CDC, 2004). The majority of cases in the United States occur in northeastern states. Lyme disease has been reported in Tennessee each year since Lyme disease became reportable. However, culture of *B. burgdorferi* or detection of genetic sequences has not been documented in the state. *Borrelia lonesarti* sequences have been reported from Tennessee (Stegall-Faulk et al., 2003; Stromdahl et al., 2003) and the majority of *B. lonesarti* detections have occurred in southeastern states (Burkot et al., 2001; Moore et al., 2003; Varela et al., 2004).

In 2003, a skin biopsy was taken from the margin of an EM rash from a patient residing in East Tennessee. This study was undertaken to evaluate this biopsy for the presence of *Borreliae*, and, if *Borrelia* nucleic acid was found, to determine if sequences were characteristic of *B. burgdorferi* or *B. lonesarti*.

MATERIALS AND METHODS

**Biopsy**—In April 2003, a patient presented at a clinic in East Tennessee with an EM rash. The patient resided in Greene County, Tennessee. A 2 mm skin biopsy was taken from the margin of the EM rash and retained for evaluation. No tick was available for study.

**Extraction**—Potential *Borrelia* nucleic acid was extracted from the biopsy using a ZR Genomic DNA II kit according to manufacturer instructions except digestion was allowed to proceed for 4 h at 37°C (Zymo Research, Orange, California).

**DNA Amplification**—The biopsy extract was subjected to two rounds of amplification. The first set of primers, derived from a conserved region of the *Borrelia* flagellin gene, was designed to amplify a fragment of 499 bp for *B. burgdorferi* or 476 bp for *B. lonesarti*. The primer sequences were: forward, 5′ CAAAAATTTAATAACACCAGCAT; reverse, 5′ GCAATCGAGTCATCAATTAGGCATTGCAGA (Integrated DNA Technologies, Coralville, Iowa). Following the first amplification, nested PCR was done using internal conserved sequences of the flagellin gene to verify *Borrelia* DNA was present. Expected fragment sizes were 319 bp for *B. burgdorferi* or 298 bp for *B. lonesarti*. Nested primer sequences were: forward, 5′ TAAATGCGAAGGAAATTTTGT; reverse, 5′ GCTATTATATATAGAGA-
CAT (Integrated DNA Technologies). Other reagents for
PCR included Taq polymerase, 10× buffer and dNTPs
(Promega, Madison, Wisconsin). Negative dH2O controls
were included in all reactions. PCR products were visualized
on a 1.25% agarose gel (NuSieve, FMC BioProducts, Rock-
land, Maine) stained with ethidium bromide.

Cloning and Sequencing—The PCR amplicon was column
cleaned (Quantum Prep, BioRad, Hercules, California). The
clean product was ligated into pGEM-T (Promega) and
transformed into Escherichia coli JM109 (Promega). Trans-
formants were visually inspected for beta-galactosidase activity
and plasmids were obtained by standard alkaline lysis. Bi-
directional sequencing was done on an Open Gene Automated
Sequencer using a Dye Primer Cycle Sequencing Kit (Visible
Genetics, Toronto, Ontario, Canada) according to manufac-
turer instructions. Forward primer M13Cy5.0 was provided in
the kit. The reverse primer was Sp6Cy5.0, sequence: 5'
ATTAGGTGACACTATAGAATAC (Integrated DNA
Technologies).

RESULTS

Nested amplification of the biopsy resulted in successful
recovery of Borrelia DNA. As seen in Fig. 1, an electrophoretic
gel containing the PCR product, the nested amplified biopsy
extract appears in lane 4. When compared with lane 3 (B.
burgdorferi positive control) and lane 5 (B. lonestari positive
control), the amplified biopsy product migrates with the B.
burgdorferi control while the B. lonestari control is slightly
lower on the gel (expected fragment size 298 bp).

To ensure that the biopsy contained DNA from B.
burgdorferi, the nested PCR product was sequenced. The
GenBank accession number assigned to this sequence is
AY964065. Sequence identity was determined by a nucleotide
BLAST search against the GenBank DNA database. The B.
burgdorferi sequence from the biopsy demonstrated the great-
est homology (99%) with B. burgdorferi strain B31, differing at
only 2 of 319 nucleotides. Sequence identity with all B.
burgdorferi flagellin sequences in GenBank was 98–99%.

DISCUSSION

This investigation determined that an EM biopsy con-
tained DNA characteristic of B. burgdorferi. This is the first
report of a B. burgdorferi sequence in Tennessee. Previous
investigations have only found DNA from B. lonestari (Stegall-
Faulk et al., 2003; Stromdahl et al., 2003).

It may be that some cases reported as Lyme disease based
solely on the presence of the EM rash may actually represent
STARI. Clinical diagnosis of Lyme disease usually does not
involve PCR analysis of skin biopsies, although this has been
reported as the most sensitive diagnostic method (No-
wakowski et al., 2001). Others in the southeastern United
States have investigated biopsies of EM sites for evidence of B.
burgdorferi but, in most cases, culture results were negative
(Kirkland et al., 1997; Felz et al., 1999). It is possible that in
those reports, B. lonestari was present but not able to be
cultured or detected. In regard to those studies, it is
particularly intriguing that the patients presenting with EM
were residents of Georgia, North Carolina and South
Carolina. These states, bordering Tennessee, represent a region
of the country where B. lonestari is most commonly found.

Another explanation for the rarity in detecting B.
burgdorferi in Tennessee may have to do with the vector
presence. While I. scapularis is established throughout most of
the eastern portion of the United States, there are pockets
where I. scapularis is not found, including much of Tennessee
(Dennis et al., 1998). If the vector of B. burgdorferi were scarce,
so, too, its pathogen would be expected to be rare. The vector
of B. lonestari, A. americanum, is widely distributed through-
out the southeastern United States (Childs and Paddock,
2003). A tick collection study conducted during 2002–2003 in
middle Tennessee recovered 8199 ticks. Amblyomma amer-
icanum represented over 91% of collected ticks; no I. scapularis
were found (Ludyjan-Ybarra, 2004). Documentation of B.
burgdorferi in Tennessee by this study may lead to more
accurate diagnoses of Lyme disease or STARI in the state.

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LITERATURE CITED


