Utilizing Real-Time PCR to Detect *Borrelia burgdorferi* Infection in *Ixodes pacificus*

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ABSTRACT: Our goal was to develop a protocol using Real-Time Polymerase Chain Reaction (RT-PCR) to test field-collected *Ixodes pacificus* ticks for *Borrelia burgdorferi,* the Lyme disease bacterium, by conducting a Minimum Infection Rate (MIR) study. An acceptable protocol should yield an MIR comparable to those previously found in northern California. We used the flannel flag method to collect 1,806 adult *Ixodes pacificus* from five northern California state parks over the 2008/2009 winter season. Ticks were sorted by sex and collection location, placed in pools of five, and stored at - 80º C until tested. DNA was extracted on the Applied Biosystems 6100 Nucleic Acid PrepStation using the NucPrep® DNA Chemistry for Tissues Protocol. A positive control of heat-killed *Borrelia burgdorferi* cells was used. RT-PCR was performed on the Applied Biosystems 7500 Real-Time PCR System, using TaqMan® probe-based detection. Pools with Ct values of 40 or below were considered to be positive. Of 357 pools tested, 26 were positive for *Borrelia burgdorferi*. Assuming one positive tick per positive pool, and with a total of 1,806 individual ticks tested, the MIR in this study was 1.44%. The RT-PCR protocol developed in this study is effective in testing *Ixodes pacificus* for infection with *Borrelia burgdorferi* because the MIR in this study is comparable to infection rates found in previous studies conducted in northern California.

INTRODUCTION

Marin/Sonoma Mosquito and Vector Control District (MVCD) encompasses all of Marin and Sonoma Counties, an area of 2,100 square miles with 715,000 residents. The District's topography includes coastal regions, vast wetlands, several mountain ranges, extensive grasslands and abundant areas of oak woodlands.

There are several state parks within Marin and Sonoma Counties that offer the public varied wilderness experiences, including camping, hiking, picnicking and mountain biking. Activities in these rustic locations carry a significant risk for exposure to *Ixodes pacificus* and the western black-legged tick, the vector of *Borrelia burgdorferi* on the West Coast.

The Marin/Sonoma MVCD conducts testing of ticks for *B. burgdorferi* and sends a yearly report of the results to the California Department of Parks and Recreation. In the past, the District used the Indirect Fluorescent Antibody (IFA) technique for tick testing. In 2006 the District purchased a Real-Time PCR system for testing mosquito pools for West Nile Virus. In order to maximize utility of the PCR system, this protocol was designed for testing ticks for *B. burgdorferi.*

MATERIALS AND METHODS

Tick Collection. We collected 1,806 adult *Ixodes pacificus* by the flannel flagging method from five northern California state parks over the 2008/2009 winter season. Ticks were sorted by sex and collection location, placed in pools of five and stored at -80ºC until tested.

DNA Extraction. DNA was extracted on the Applied Biosystems 6100 Nucleic Acid PrepStation, using the NucPrep® DNA Chemistry for Tissues Protocol (Applied Biosystems, Foster City, CA). A positive control of heat-killed *Borrelia burgdorferi* cells was used (KPL, Gaithersburg, MD). Cells were rehydrated with 1 mL reagent quality water and diluted to 1:25 using 1X TE buffer.

Real-Time PCR. A 500 base-pair sequence from the flagellin gene of *B. burgdorferi* (Pahl et al. 1999) was submitted to Applied Biosystems Custom Assay Services, where a set of primers and a probe were designed (Table 1).

Table 1. Custom *B. burgdorferi* primers and probe designed by Applied Biosystems Custom Assay Services based on GenBank accession number X15660.

Forward Primer:
5' CAAACCAAGATGAAGCTATTGCTGTA 3'
Reverse Primer:
5' CTTCCTGTTGAACACCCTCTTGAA 3'
MGB Probe:
5' FAM-CAGCCTGAGCAGTTTGA-MGB 3'

The TaqMan® probe was labeled at the 5' end with a fluorescent reporter dye (FAM) and with a non-fluorescent quencher dye at the 3' end (MGB). RT-PCR was performed on the Applied Biosystems 7500 Real-Time PCR System, using TaqMan® probe-based detection (Applied Biosystems). The PCR reactions were performed in 25 µL volumes containing 2X TaqMan® Universal PCR Master Mix (Applied Biosystems), 900 nM forward primer, 900 nM reverse primer, and 250 nM probe. Five micro liters (5µL) of extracted DNA were added and the following cycling conditions applied: one cycle at 95º C for ten minutes, 45 cycles

at 95° C for 15 seconds and 60º C for one minute. Previously extracted *B. burgdorferi* DNA was used as a positive control, and a no-template control was prepared using sterile, nuclease-free water. Pools with Ct values of 40 or below were considered to be positive.

RESULTS AND DISCUSSION

Of 357 pools tested, 26 were positive for *Borrelia burgdorferi*. Assuming one positive tick per positive pool, and with a total of 1,806 individual ticks tested, the MIR in this study was 1.44%. The RT-PCR protocol developed in our study is effective in testing *Ixodes pacificus* for infection with *Borrelia burgdorferi* because the MIR we determined is comparable to infection rates found in previous studies conducted in northern California (Bissett and Hill 1987, Burgdorfer et al. 1985, Holden et al. 2006).

Future goals include using the District's recently purchased MagMax™ Express magnetic particle processor (Applied Biosystems) for DNA extraction in the tick testing protocol, developing a confirmation assay and expanding the testing program to include other tick-borne pathogens.

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