TICK CHECK: The prevalence of Lyme disease causing ticks in Wisconsin

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Abstract

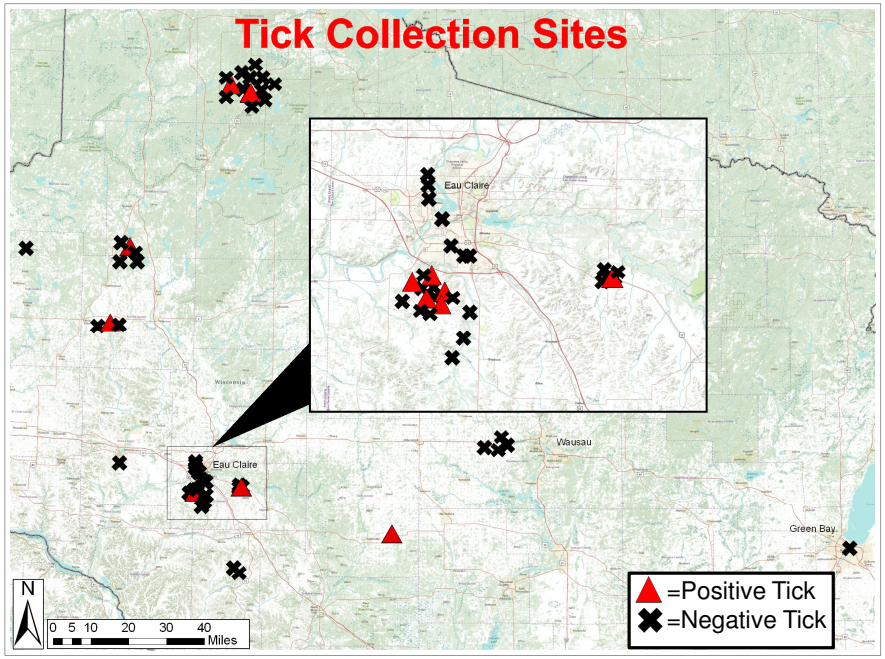
Little is known about the prevalence of Lyme disease infected ticks in Wisconsin. This study was undertaken to detect the presence of Lyme disease causing bacteria, *Borrelia burgdorferi* (*B. burgdorferi*), in deer ticks (*Ixodes scapularis*). A rapid Real Time PCR assay was developed and used to amplify a portion of the *recA* gene from *B. burgdorferi* DNA found in deer ticks in Wisconsin from 2009-2010. A tick was presumed to harbor *B. burgdorferi* bacteria if the amplified DNA was a 222 base pair product and had a melting temperature of 82°C. **We found that 17.7% of the 62 deer ticks presumably harbored the *B. burgdorferi* bacteria. Ticks testing positive for bacteria were found in Eau Claire, Granton, Fall Creek, and Drummond, Wisconsin.** This type of assay could potentially assist physicians in the diagnosis and treatment of Lyme disease cases.

Background

Deer ticks are the main vector that transmit *B. burgdorferi* from small mammals to humans. Once the bacteria is transmitted to humans, it can cause severe symptoms that can include bull's-eye shaped rashes, fever, headache, and joint pain (4). The prevalence of *B. burgdorferi* in deer ticks has been well studied in many areas around the world, and it has been reported as high as 43% in some endemic areas on the East Coast of the United States (7). However, no studies have examined the prevalence of *B. burgdorferi* in deer ticks in Wisconsin. As the number of Lyme disease cases increase in Wisconsin (3), it has become increasingly important to understand the ecology of the disease.

To determine if a deer tick harbored *B. burgdorferi*, a rapid real time polymerase chain reaction (RT-PCR) was utilized to amplify a portion of the *recA* gene of *B. burgdorferi*. Similar procedures have been used in some labs around the world because of the effectiveness of RT-PCR as a rapid diagnostic tool for detecting bacteria in ticks(5). If the amplification of the *recA* gene resulted in a 222 base pair product that denatures at 82°C, the tick was presumed to harbor the *B. burgdorferi* bacteria. Infected and non-infected ticks were mapped to show the distribution of the infected deer ticks across Wisconsin.

Tick Collection Sites

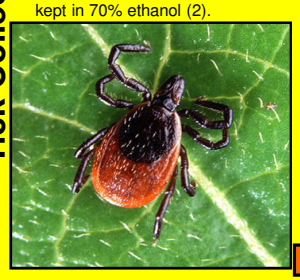


This map was made with the help of Joel Jahnke.

Materials and Methods

Tick Collection

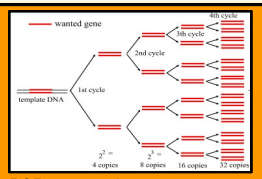
Collection was made, in part, by Christina Weimer, Biology 306 classes, and other UW- Eau Claire Biology Department members. Collected ticks were kept in 70% ethanol (2).



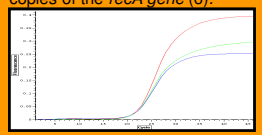
DNA Extraction

Ticks were bisected and then homogenized in a buffer solution. A detergent (SDS) and Proteinase (Proteinase K) were added to the solution to remove membrane lipids and proteins. The solution was kept in a 56°C water bath overnight to allow for maximal Proteinase activity. Phenol and chloroform were added to the DNA containing supernatant and gently mixed to extract remaining proteins and lipids from the aqueous solution. DNA was precipitated from the aqueous top layer with 100% ethanol. The ethanol solution was spun in a centrifuge for 15 minutes to pellet the DNA out of solution. The ethanol was removed, and the DNA pellet was dissolved in water. DNA extracts were stored in -20°C.

RT-PCR

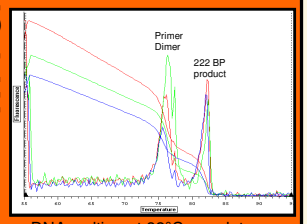


PCR was used to create more copies of the *recA gene* (6).



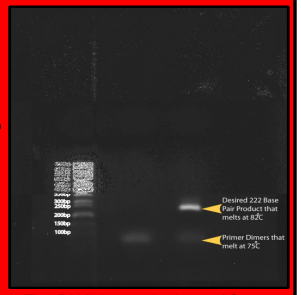
Using SYBR Green, a fluorescing dye that binds to double stranded DNA, we were able to monitor the amount of DNA being amplified.

RT-PCR



DNA melting at 82°C correlates with the melting temperature (T_m) of the ***recA* gene product**. DNA melting at 75°C was the melting of primer dimers. **Primer dimers** are commonly seen in most PCR procedures.

Gel Electrophoresis



The DNA that melted at 82°C was run on a gel to confirm that is was the desired 222 base pair product.

Conclusions and Future Research

- 17.7% of ticks were presumed to harbor *B. burgdorferi* bacteria.
- More ticks will need to be assayed for a more comprehensive view of the prevalence.
- Presumptive positive ticks should be verified with additional primers.
- Sequence the 222 base pair product for confirmation.

References and Acknowledgements

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