Enlarging Borrelia burgdorferi DNA directly from ticks has proven challenging, and researchers often must culture the bacteria from the tick to identify whether it is a carrier of Lyme disease causing agent. Culturing is a time consuming and resource intensive process that can delay important tasks. An alternative method that may solve the problem of Borrelia in the lab is the polymerase chain reaction (PCR) to directly detect the Borrelia burgdorferi DNA. The purpose of our study is to develop and validate a PCR protocol to screen for Lyme disease, Ixodes scapularis, for the presence of Borrelia burgdorferi bacteria, the causative agent of Lyme disease. Development of a reliable PCR protocol to quantify the incidence of Lyme disease carrying ticks will allow us to determine deer population size correlates with the fraction of ticks infected with B. burgdorferi. A solid PCR assay can also lead to more reliable testing for Lyme disease in humans and other organisms. Information on Lyme carrying incidence can provide useful public health information to communities to warn people where they are most likely to be exposed to Lyme disease. Ticks will be collected, DNA extracted, DNA will be subjected to two rounds of PCR using a "flanking primer" method. A correct product indicates the presence of B. burgdorferi bacteria and a tick capable of causing Lyme disease. Positive and negative results will be recorded to determine if deer populations influence the rate of I. scapularis infection rates. Currently we are working on optimizing the protocol using purified DNA from B. burgdorferi cultures.

**Background**

Lyme disease is the most widespread tick-borne illness in Europe and the United States (Qiu et al. 2002). Lyme disease was first discovered in the 1970s when a mysterious arthritic-like disease began afflicting individuals of all ages and races (Ernst et al. 1977). Ten years later, Lyme disease was linked to a particular bacterium B. burgdorferi (Caporale and Kocher 1994). B. burgdorferi is a highly motile, spirochetal bacteria that causes Lyme disease in small mammals, domesticated animals, and humans (CDC). The increase in Lyme disease cases is thought to be due to the increase in deer populations observed in the northeastern United States (Caporale and Kocher 1994). Lyme disease presents with a variety of symptoms including erythematous annular rash (common "bulls eye" rash), flu-like symptoms, neurological complications, and arthritis (Borde et al. 1997). Detection of the B. burgdorferi in the B. burgdorferi antibody are both common methods to diagnose (Schmidt 1997). Antibody assays can be unreliable if taken too soon after infection has taken place (Schmidt 1997). The polymerase chain reaction (PCR) works by denaturing and amplifying Borrelia burgdorferi DNA in a given sample either from an infected tick or an infected individual (CDC). Blood, skin lesions, stool samples (Schmidt 1997). The sensitivity of PCR depends greatly on the ability of primer set to bind to B. burgdorferi DNA. Humans and their domesticated animals can deal with the effects of Lyme disease for long periods of time before an accurate diagnosis can be given. It is in the best interest of individuals to have effective means to test for Lyme disease and to know when ticks are carrying or are ticks are particularly abundant. It is hypothesized that areas in Essex County, Massachusetts with high populations of deer will be home to high populations of B. burgdorferi infected ticks. Ticks will be collected from areas with high and low deer populations and tested with a two step PCR protocol described below.

**Methods**

**Field Protocol**

- **Tick Collection**: Tick collection will be performed at four sites in the Essex County, MA area: Longhills reservation, Gordon College, Appleton Farm, and Ipswich River Audubon Sanctuary. Areas will be picked to represent regions high and low deer populations to gather ticks, which chases will be run along routes and chases will be checked every 20 minutes for ticks. Any ticks gathered will be placed individually in expanded tubes, labeled with their location, and placed in the 4°C freezer until DNA extraction.

**Laboratory Protocol**

The purpose of the laboratory protocol is to develop a method to screen ticks for the presence of B. burgdorferi, the causative agent of Lyme disease. The polymerase chain reaction (PCR) works by amplifying small amounts of DNA with the use of template-specific primer sets. Primers were chosen by comparing two known strains of B. burgdorferi DNA. Primers were selected from regions where little or no differences occurred (See figure 1). Two primer pairs, an exterior and interior set were designed.

- **One-step PCR**: A one-step PCR procedure is desirable because contamination from other forms of DNA is less likely (Caporale and Kocher 1994). A nested PCR procedure more sensitive; however, contamination is more likely because the PCR tube is opened twice (Caporale and Kocher 1994).

**PCR reactions were done in 50 µl final volume, with 0.2 mM dNTPs, 1 mM each primer, and 5 units OneTaq in Standard PCR reactions were done in 50 µl final volume, with 0.2 mM dNTPs, 1 mM each primer, and 5 units OneTaq in Standard PCR in Lambda H3 Ladder.**

- **Cycling parameters used were**: 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 7 min.

- **GEL ELECTROPHORESIS**

PCR products were run on a 3% or 1.5% agarose gels to separate DNA fragments. Outer primers should yield a single band at 781 bp. Using the internal primers, a 392 base pair band should be amplified from within the 781 base pair amplicon.

**PCR Primer Selection**

<table>
<thead>
<tr>
<th>Primer Set</th>
<th>Sequence</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exterior Forward</td>
<td>5'-GTCGATGAATGATTACGGGCG-3'</td>
<td>50 bp</td>
</tr>
<tr>
<td>Exterior Reverse</td>
<td>5'-AGGCTGCAGTGCCAATAATGGT-3'</td>
<td>50 bp</td>
</tr>
<tr>
<td>Interior Forward</td>
<td>5'-GTCGATGAATGATTACGGGCG-3'</td>
<td>50 bp</td>
</tr>
<tr>
<td>Interior Reverse</td>
<td>5'-AGGCTGCAGTGCCAATAATGGT-3'</td>
<td>50 bp</td>
</tr>
</tbody>
</table>

- **PCR Products were run on a 3% agarose gel and a Lambda H3 Ladder.**

**Results**

Any samples from the one-step PCR procedure are expected to band at approximately 800 base pair region because the size of the amplified product is 781 base pairs. Samples run in the nested PCR procedures should show a strong banding at the 400 base pair region because the amplified product is 392 base pairs. False banding may occur at the 800 base pair region should any one-step PCR product not anneal to the nested set of primers. For accurate reporting of B. burgdorferi infections, any negative samples should be run through the one-step PCR and nested PCR procedures again. Running negative samples a second time will ensure that faulty primers, human error, denatured enzymes, or error in cycling were not the cause of a negative test (Schmidt 1997).

Using the developed PCR procedure to diagnose and illustrate how deer populations affect the spread of B. burgdorferi infection rates will be useful information for surrounding communities because ticks are known to use deer as vehicles to spread into new areas (Caporale and Kocher 1994). If using taylor very closely compact areas need helpful information on B. burgdorferi infection rates, larger regions and suburban communities can be surveyed to give the medical community and the public more information on the likelihood of contracting Lyme disease.

**Discussion**

It is our goal, that through this research more is known about the bacteria B. burgdorferi and how deer contribute to its spreading. Further research needs to be undertaken to determine if a PCR procedure can be developed into a diagnostic method to screen for Lyme disease cases in humans. The development of a highly accurate and sensitive PCR procedure could readily offer the method of testing for Lyme disease. The incorrect use of the PCR protocol to diagnose human cases of Lyme disease from cerebral spinal fluid, urine, blood, or skin lesion although licensed procedures are not sensitive enough for a high level of accuracy (Schmidt 1997). Pirenne et al. 1997). The unique primes that we have designed, if they prove their sensitivity and specificity, could provide a more accurate and easy method of testing humans for Lyme disease.

**References and Acknowledgements**


