Analysis of Borrelia in Lipotena cervi (Deer Keds) as a Model of Infection in the White Tailed Deer Population of New York State

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Abstract
Lyme disease or Lyme borreliosis is the most prevalent tick-borne disease in America, and the etiological agent of Lyme disease is the Borrelia spirochete. Transmission of Lyme disease in North America is through the deer tick, which takes blood meals from infected white-tailed deer. The purpose of this study is to determine Borrelia cervi (ked flies), which also take blood meals from the white tail deer, carry this bacteria. PCR analysis was used to detect the presence of the B. burgdorferi flagellin B (flaB) gene in DNA from ked flies. Of 40 ked flies tested, 8 were positive for the flaB gene, yielding an infection rate (20%) that is very similar to that found in ticks taken from the regional deer population (25%). Gel electrophoresis displayed that the PCR product was approximately 30 base pairs smaller than the predicted size for B. burgdorferi. This is also consistent with variation in the flaB gene among strains of this bacteria and may indicate a unique strain of B. burgdorferi in ked flies. It is possible that the strain of Borrelia cycling in ked flies may be different from that in regional deer tick populations.

Introduction
Borrelia burgdorferi is a microaerophilic spirochete and the etiological agent for Lyme borreliosis (Lyme Disease). Lyme Disease is the number one tick-borne disease in America and is transmitted by xodes scapularis (common deer tick) (1). Three major genospecies worldwide are: B. burgdorferi sensu stricto, B. garinii and B. afzelii, with sensu stricto being the most common species in the Northeast of the United States (2). The predominant strains in the Northeast of the United States include B31, N40, J01 and 297.

In endemic areas 20% of deer ticks are infected with this bacteria (3) and the major reservoirs are Odorcellus virginianus (white tailed deer) and Peromyscus maniculatus (the white footed mouse). Infection begins at the site of the tick bite and requires attachment for 24-48 hrs to transmit the disease. Later it spreads into circulation and infects other organs causing arthritis, carditis and neurological diseases (1). The flagellum (encoded by the flaB gene) is expressed throughout the life cycle of the bacterium and serves as the primary means of its mobility. Additionally, it gives the bacteria shape as it wraps around the protoplasmic body (2). The genome of Borrelia is the largest of any known bacterium with one chromosome, and depending on the strain as many as 16 to 21 linear and circular plasmids (2). Two plasmids are found in all strains and have extremely conserved structures, and the deletion of other plasmids have no effect on the bacteria. Of the many strains found in the Northeast, the four major ones (B31, N40, J01 and 297) vary widely in their number of plasmids as well (4). Moreover, multiple recombination events cause the plasmid DNA to form a highly variable mosaic of genes (4). It is probable that this genomic variability contributes considerably to the ability of Borrelia to infect hosts and in their propensity to infect Numanas (5).

The purpose of this study was to determine if Lipotena cervi (ked flies) are also a reservoir for Borrelia, and if so, to determine which strain infects these flies. Ked flies have a unique life cycle that includes winged and wingless forms of the mature fly. The life cycle begins when a female releases a single puparium in the coat of a deer, which then drops to the ground and matures into a winged ked fly. Winged ked flies infest white tailed deer, take a blood meal and drop their wings. Thus, these flies spend the duration of their life on the host.

DNA was extracted from ked flies using a Qiagen DNeasy Blood and Tissue Kit using standard methods. Primers were designed to amplify a predicted 233 bp product from the flaB gene of B. burgdorferi sensu stricto B31 (Genbank Accession Number X15661.1). Polymerase chain reaction (PCR) was performed in 20 µl reactions with 20ng DNA extracted from ked flies, 25µM dNTPs, 5µl each upper and lower flaB primers (forward primer 5’TTCTACATGATAGGCGGCA 3’ and reverse primer 5’GACGCTTTGAGGCCTTGAAG 3’), 1 unit of Taq DNA polymerase (Fisher Bioreagents Bohemia, New York). Reaction conditions were 95°C/30 seconds followed by 30 cycles of 55°C/30 seconds, 55°C/30 seconds and 72°C/1 minute with a final extension of 72°C/5 minutes. The approximate length of the PCR products were determined by gel electrophoresis on a 2.0% agarose gel with SYBR Safe using Track it 1 kb DNA ladder (Life Technologies, Grand Island, NY). For these studies, genomic DNA from B31 was kindly provided by John F. Bruno M.D., Ph.D. (Stony Brook University) to use as a positive control.

Town, County       Number
Sampled
Saratoga, Erie    5
Holland, Erie    3
Wales, Erie    2
Franklinville, Cattaraugus 2
Farnersville, Cattaraugus 5
Hutch, Cattaraugus 1
Lyndon, Cattaraugus 2
Hume, Allegany 1
West Almond, Allegany 1
Amity, Allegany 1
Clarksville, Allegany 1
Scio, Allegany 1
Bolivar, Allegany 1
Beinart, Allegany 1
Eagle, Wyoming 3
Java, Wyoming 1
Arcade, Wyoming 6

Figure 1: Regional map of Western New York showing towns from which ked flies were collected in an tick-containing Borrelia positive ked flies.

Figure 2: Gel electrophoresis showing the presence of B. burgdorferi DNA in ked flies. Lane 1 - Track it 1kb DNA Ladder Lane 2 - B. burgdorferi PCR performed with a representative ked fly sample Lane 3 - B. burgdorferi sensu stricto B31 DNA (predicted 233 bp ampiclon) Lane 4 - Negative control without DNA

Figure 3: An amino acid aligment of various strains of Borrelia. B. burgdorferi strain B31 (Accession # X15661.1) in black; B. burgdorferi sensu stricto K10 (Accession AL42881.1) in green; B. lantestarii (Accession AY850064.1) in red. Bright green boxes highlight sequence differences.

Figure 4: The predicted sizes of amplicons for flaB PCR performed in this study. B. burgdorferi strain B31 (Accession # X15661.1) in black; B. burgdorferi sensu stricto K10 (Accession AL42881.1) in green; B. lantestarii (Accession AY850064.1) in red.

Methods

Results

Conclusions

- Using PCR analysis, it was determined that 20% (8/40) of ked flies sampled were positive for Borrelia burgdorferi. This infection rate matches that of ticks in the area sampled.
- The resulting amplicons from PCR were consistently 20-30 base pairs smaller than the amplicons of the positive control.
- DNA and amino acid sequence analysis shows that the flaB gene size varies among species.
- This data suggests that a new strain, or possibly new species, is circulating in the ked fly population of Western New York.

References