COMPARISON OF DNA SEQUENCES FROM BORRELIA BURGDORFERI FOUND IN MIDDLE TENNESSEE TO PUBLISHED STRAINS

SEAN PETERSON

To the Graduate Council:

I am submitting herewith a thesis written by Sean Peterson entitled "Comparison of DNA sequences from *Borrelia burgdorferi* found in middle Tennessee to published strains." I have examined the final paper copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Masters of Science, with major in Biology.

Chad Brooks

Dr. Chad Brooks, Major Professor

We have read this thesis and recommend its acceptance:

Andrew W. Barrass

Dr. Andrew Barrass

Karen McFarland

Dr. Karen McFarland

Acceptance for the Council:

Charles A. Pinder

Dr. Charles Pinder
Dean of Graduate Studies

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Master's degree at Austin Peay State University, I agree that the Library shall make it available to borrowers under rules of the Library. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgement of the source is made.

Permission for extensive quotation from or reproduction of this thesis may be granted by my major professor, or in his absence, by the Head of the Interlibrary Services when, in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this thesis for financial gain shall not be allowed without my written permission.

Signature 

Date 6/2/8

COMPARISON OF DNA SEQUENCES FROM BORRELIA BURGDORFERI FOUND IN MIDDLE TENNESSEE TO PUBLISHED STRAINS

A Thesis

Presented to the College of Graduate Studies

In Partial Fulfillment of the Requirements for

Master's Degree

Sean Peterson

May 2008

Acknowledgments

I would first like to thank Dr. Brooks for allowing me to use his lab and helping to guide and answer all questions. His knowledge and high expectations were the true driving force for this thesis. Additionally, my thanks go to Josh Ream for helping me convert my GPS points from minutes and seconds to degrees and decimals. Also, I appreciate Elaine Foust for providing the base map used in my GIS project. It goes without saying that Mark, Ja, Jill and Joseph were always there to lend a hand or help explain certain topics and procedures. I wouldn't be where I am at without my family who have always stood behind me and encourage me to try harder.

List of Tables

Table Page

Abstract

The rapid growth of Lyme disease has attracted much attention from the medical community. With almost 20,000 cases reported in 2006 and no known cure, Lyme disease is a serious medical concern. Currently, Tennessee is considered to be a no to low risk state for *Borrelia burgdorferi*, the Lyme disease bacterium. Oddly, compared to some northeastern states, Tennessee has a greater percentage of infected animals but an extremely smaller incidence of Lyme disease in humans. This study focuses on using DNA sequencing and clustal analysis to determine if the strain(s) found in Tennessee is a divergent strain more pathogenic *B. burgdorferi* strains found in the northeast. Six counties in northern middle Tennessee have been surveyed for *B. burgdorferi.* Specimens were processed in the lab and tested for the bacteria via DNA isolation, PCR and gel electrophoresis. Samples were then cloned into a plasmid and sent for DNA-sequencing. DNA analysis has shown that certain *B. burgdorferi* genes found in Tennessee are highly divergent while some outer surface proteins are highly conserved. These data appear insufficient to draw conclusions regarding the parasitic strategy of *B. burgdorferi* and human Lyme disease. However, these data lay the groundwork for future research to give insight

to the complex enzootic cycle and the many different animal hosts that *B. burgdorferi* utilizes in nature.

TABLE OF CONTENTS

Chapter Page

List of Figures

Figure Page

Chapter I:
Introduction

Lyme disease (LD) is the most frequently reported arthropod-borne sickness in the US and Europe and is also found in parts of Asia (9-11,24-26). In the year 2000, the Centers for Disease Control and Prevention reported more than 18,000 cases of LD in the US (13). In 2006, the number rose to 19,931 cases (14). Lyme disease, also called Lyme borreliosis, is a multisystem infection caused by the spirochete *Borrelia burgdorferi* (Bb) and can cause disease involving the skeletal, cardiovascular, and nervous system (27-29). Bb is a bacterium in the order spirochaetales due to its axial filament which provides motility and is located between the outer membrane and the cell membrane. The axial filament is made up of endoflagella which causes the filament to rotate providing either a run or tumble motion.

Bb is transmitted to humans and animals mostly by the Blacklegged (or deer) tick (*Ixodes scapularis* and *Ixodes pacificus*) and recent evidence appears to point to the American dog tick (*Dermacentor variabilis)* as another tick vector (2,5,21). Because of Bb's complex enzootic life cycle, Bb can persist in the environment of different vertebrate hosts, such as mammals, birds and even reptiles,

and is transmitted among these hosts to other ticks during feeding upon (6,23,30).

Life Cycles

All ticks are born naïve, meaning that that it has yet to become infected with Bb (6). The tick will serve as a disease-vector only after it has feed on a Bb-infected vertebrate host (12). Detailed in Figure 1, the life cycle of an *Ixodes scapularis* tick begins in spring with the eggs hatching. Larval ticks emerge from the eggs to seek their first blood meal which is the first opportunity for them to become infected and carry Bb. After their first of three blood meals, the tick molts the following year into the nymph stage and it is this point when the tick is mostly to transmit Bb to humans (6). After the second blood meal, the nymph stage will molt into the adult stage and seek larger, predominantly poor social grooming animals to undergo sexual reproduction. Gregarious animals are more likely to pick parasites off of each other and an adult tick is at least 4 times larger than a nymph.

Prior to tick feeding, the spirochetes are localized in the tick's midgut. During feeding, Bb must travel to the salivary glands where they are passed to the new host via the tick's saliva (24). At least one

gene has been associated with migration from the tick to the animal, termed ospC, meaning outer surface protein C (18). In this regard, when the ospC gene is disrupted, mutant Bb cannot infect the mouse animal model. An interesting story develops between ospC and another osp protein termed ospA. In a unique gene regulation pattern that has been well characterized but not well understood, ospA is up-regulated in the tick prior to feeding but down-regulated during tick feeding then completely "turned off" when Bb is in the animal host. In contrast, ospC's gene expression pattern is just the opposite with it being "turned on" during tick-feeding and up-regulated even more in the host (7,22). Like ospC, unsurprisingly, when the ospA gene is disrupted, Bb mutants cannot colonize the tick from the mouse animal model (34). The actual physical molecular mechanisms of ospA and ospC are not known. Once in the animal host, the biology of Bb is not well known. However, the disease manifestations are linked to the presence of Bb within those tissues. Interestingly, in non-human animals at least, for the reproductive success of Bb, Bb must find its way back to the next tick vector to be maintained in nature. Again, another outer surface protein, ospA, has been linked to the colonization of the tick from the animal host (34).

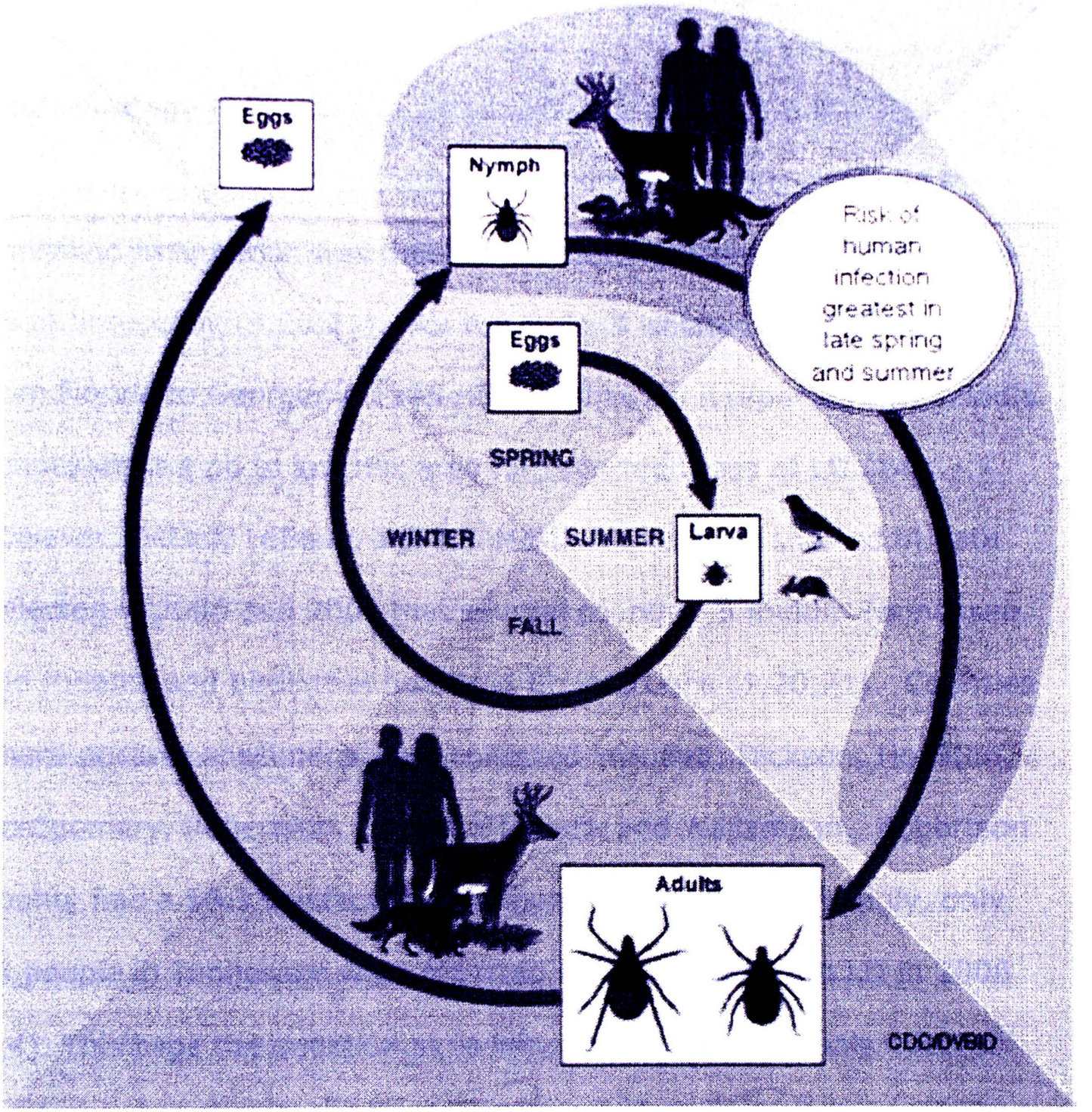


Figure 1. Life cycle of *Ixodes scapularis* with special note given to Bb-infection in the late spring through summer (image found on the CDC homepage for Lyme disease).

Epidemiology

Cases of infection are most prevalent in the northeast and the prevailing assumption was that Bb was not prevalent in the southeast US until recently (9,20,21). For example, 9 isolates were recovered from Florida to Georgia in 1995 (23). It has been reported that middle Tennessee is a no to low risk area for reported cases of LD (14). However, DeLacy LeBlanc and Jon McMahan recently showed in data collected in 2006 and 2007 that several counties in middle Tennessee had rodents and birds that harbored Bb infections (1,20,21). Counties where positive specimens were collected included: Dickson, Houston, Montgomery, Robertson, Lincoln, Stewart, and Williamson. Robertson County had a 56.3% infection rate found in voles. Surprisingly, only 15 people in Tennessee were reported to be infected with LD in 2006 (14). This begs the question as to why are so many animals infected but only a handful of people get infected with LD each year in Tennessee. It is hypothesized that the strain found in Tennessee is divergent to the one found in the northeast hindering the bacteria to survive in humans and/or cause noticeable disease in humans.

In this study, specimens collected during 2006-2007 from middle Tennessee that tested positive for Bb were DNA-sequenced to determine any genetic differences between TN Bb and other known

Lyme disease causing strains. Comparisons were focused on the 16s ribosomal subunit and four outer surface proteins (Osp), OspA, OspC, OspE and BbA68. Special note is that BbA69 does not follow the "osp" nomenclature. Previous studies have used OspA and OspC for analyzing different Bb strains throughout the world (19,32). In the white footed mouse, *Peromyscus leucopus,* Swanson reported seven variants for OspC, comparing each variant strain to the one virulent strain found in New York, B31 (31). Their findings have several implications. One possibility is that multiple ticks are introducing different strains within the same organism or one tick can have multiple Bb strains within it. Another possibility is that only one specific variant predominant over the others. This implies that there may be competition amongst the varying strains of spirochetes. In addition, they found 25% of the infected specimens show variance for the OspA and OspC proteins when compared to the B31 strain. This study indicates Bb is diverging from its strain known for high human infection in the New York area.

Chapter II:
Methods and Materials

Collection of Specimens. Rodents were caught using Sherman traps. Traps were set up along fence lines and buildings at capture sites in Dickson, Houston, Montgomery, Robertson, Stewart, and Williamson counties. These counties are considered the study area and were on farm land in either old-fields or near ponds. Traps were baited with oatmeal and peanut butter and were checked daily. Any rodent that was caught was taken back to the lab and put down with carbon dioxide. The rodents collected were then combed for ticks, which were subsequently removed and placed into individual 1.5 ml tubes. The bladder, heart, spleen, liver and ear were then dissected out and separated to later test for the presence of Bb proteins. The carcass was then placed in a zip lock bag and stored in a -80°C freezer (20,21).

Song birds were shot or caught using mist nets and processed in the field. Whole blood was collected and stored in tubes and the carcass was placed into a bag for further processing back at the lab. Once at the lab, the birds were combed for ticks which were removed and placed into separate tubes for further processing. Only the heart

and liver were removed from the birds which were also placed into separate tubes and the carcass was placed in the freezer (20,21).

At each capture site, GPS coordinates were taken. These were placed into an Excel spreadsheet to be imported into a geographic information system, ArcGIS, a software product that shows GPS points on specific map backgrounds.

DNA Isolation from Animal Tissues and Ticks. All organs were cut down to 0.5 g and digested with collagenase and proteinase K in a two part process. Exposure of the appropriate enzyme lasted for 24 hours while incubated at 34°C. Samples were then centrifuged and the supernatant was alliquoted to remove cellular debris. This liquid was then cleaned with sodium acetate and isopropanol. A DNA pellet was created by centrifuging and discarding the liquid. The pellet was resuspended in molecular grade water and was used as a DNA template in PCR (20,21). All PCR reactions were verified by gel electrophoresis. Ticks were frozen in liquid nitrogen and were crushed and treated with proteinase K. An isolation kit (Quiagen) was then used to further extract the DNA. The final product from using the kit was a DNA pellet which was then resuspended in molecular grade water and was used as a DNA template in PCR (20,21).

Testing for LD. DNA pellets were used as templates and were amplified using PCR. A specific primer set (TEC1/LD2) was used to amplify only the 16s rRNA gene sequence of Bb (15). Results were ran on a gel with a DNA ladder and a spiked well of actual Bb as a positive control (20,21).

Making a GIS Map. A base map of detailed counties was provided by Elaine Foust, a geologist from the Tennessee Division of Geology. Surrounding counties were utilized by the US census data which was given by the global information systems (GIS) center at APSU. GPS coordinates from specimens infected with LD were implemented into ArcGIS 9.2.

Calculating possible number of infected voles, mice, and birds. Once positive data was acquired by county and species, these were implemented into a spreadsheet. County square mileage and percent of rural area were utilized from City-Data.com. Square mileage of rural land was then calculated (Table1).

Table 1: Square mileage of rural land of the six counties surveyed.

County	Sq. miles	% rural	Sq. miles of rural land
Montgomery	539	25	134.75
Robertson	476	58	276.08
Stewart	458	100	458
Dickson	490	69	338.1
Houston	200	100	200
Williamson	583	30	174.9

Population densities of mice were found to be 9-14 per acre and up to 400 in thickly forested areas (16,17). Voles were reported to have a population density of 15-45 per acre in old-field habitats and up to 150 in marsh habitats (4). Songbirds were found to have a population density of 3.1-18.6 per hectares. All values were converted to square miles.

The following describes DNA-Cloning

Making the vector. Nutrient broth was made by dissolving 8g of Difco nutrient broth in 1L of deionized water and autoclaved. Ampicillin (100µg/ml) was added aseptically to the nutrient broth and *E. coli* DH5 containing pBluesript II SK was added and allowed to grow overnight at 37°C. This mixture was then spun down and the pellet was midipreped (Promega, following manufacturer's protocol) to purify the vector. The vector was digested using the restriction enzyme Xho I and phosphate groups were removed using standard dephosphorylation and ligation reactions. The vector was shrimp alkaline phosphatase treated to remove the 3' phosphate group and prevent re-ligation. This was incubated for one hour at 34°C and inactivated by heating to 65°C for 15 minutes. The vector was then

cleaned using a miniprep kit. Concentrations of nucleic acids were checked using a spectrophotometer.

Making the insert. Specimens that tested positive for Bb were used as a DNA template for a PCR reaction. Each specimen was subjected to 6 different reactions to test for the following proteins; OspA, OspC, OspE, RecA, BbA68-1 and BbA68-2. Proteins were chosen for their roles in transmission and survival from different hosts. Forward and reverse primers were designed specifically for each protein. Each reaction was 13.4 µl of molecular grade water, 4.0 µl of appropriate buffer, 1.0 µl of primer, 0.4 µl of nucleotides, 0.2 µl of high-fidelity DNA polymerase and 1.0 µl of DNA template. After the reaction was complete the insert was cleaned using a miniprep kit (Promega). The insert was then digested using Xho I and cleaned with a miniprep kit (Promega). Concentrations of nucleic acids were checked using a spectrophotometer.

Ligating insert into vector. To covalently link the PCR product (insert) to the plasmid (vector), 1.0 µl ligase was added to 4.0 µl of lygase buffer, 3.0 µl of insert and 31.0 µl of vector. This was allowed to incubate at room temperature for 15 minutes followed by a miniprep-cleaning as mentioned above.

Transformation. To transform *E. coli* with the plasmid containing the insert, 125 µl of CaCl was added to a tube with 25 µl of the plasmid and 25 µl Top10 cells. The tube was placed on ice for 5 minutes. Then it was quickly transferred to 42°C for 50 seconds. It was then immediately returned to ice for 5 minutes and 250 µl of nutrient broth was added to the tube and was allowed to incubate at room temperature for 10 minutes. 1, 10 and 100 µl were plated on nutrient agar plates and ampicillin, Xgal and IPTG treated plates and incubated at 34°C overnight. Colonies were picked with a sterile pipette tip off of the treated plates and PCR verified for completed transformation. Positives were allowed to grow in nutrient broth for 24 hours, cleaned using a miniprep kit and prepared to be sequencing. All other samples were cloned using a TOPO 10 kit (Invitrogen) following manufacturer's protocols.

Sequencing. A total of 16 samples were mailed to The University of Oklahoma and Vanderbilt University. They used BigDye Terminator chemistry which added a florescent dye, specific for each nucleotide group and was resolved on an ABI 37xl DNA Analyzer. Sequences were electronically sent back as api files. These files were then imported into FinchTV software and checked for errors. Complete sequences were blasted against Bb B31 using NCBI software.

Sequences were aligned for comparison using a specialized blast that compares two sets of sequences.

Chapter III:
Results

Though actual numbers of all animals infected is impossible to know, calculations have been made to estimate possible infected mice, voles and songbirds in six counties. The results of the calculations made to estimate possible infected mice, voles and songbirds in the tested counties in middle Tennessee are shown in Figure 3. A total of 151 mice, 19 voles and 31 birds were collected and 19 mice, 10 voles and 9 birds were found to be infected. Out of all animals caught, mice were caught 75% of the time but only account for 50% of the infected animals. Robertson County had the highest number of infected animals, 20, and it is assumed that it has the highest possible number of infected animals.

The voles that were tested from Robertson County were positive for LD at a rate of 56.3%. Voles in an old forest can be as much as a 150 strong per square mile (16,17). This means that there might be over 600,000 infected voles in this county. Dickson County had the least percent infected mice, 3.5%, while Montgomery County had the highest percent of infected birds, 40%. Songbirds were only selected from three counties with none infected in Stewart County.

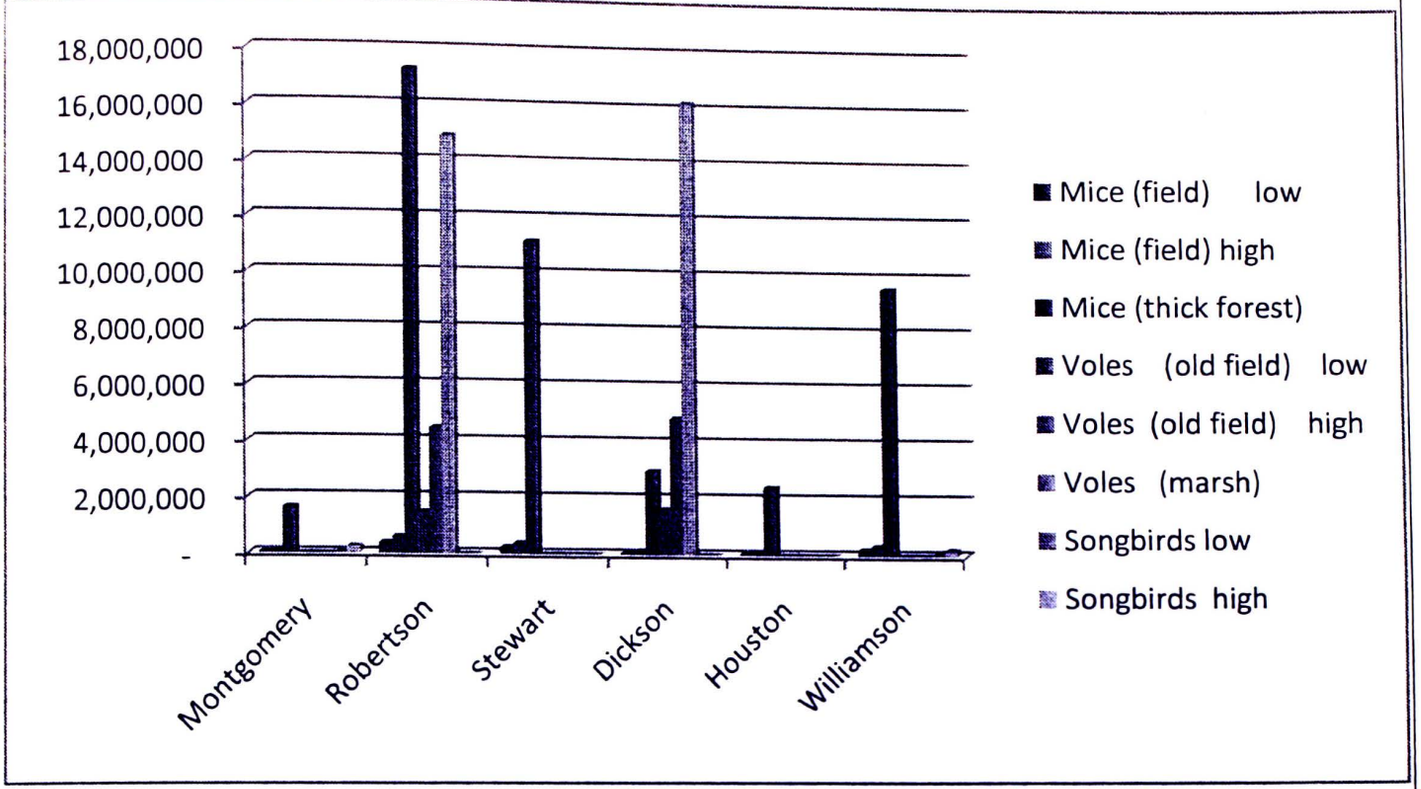


Figure 2: Possible number of infected animals in middle Tennessee counties. Estimates were generated by comparing known Bb-infection frequencies among rodents and birds in different parts of the country and the terrain type found in a given area.

DNA-Sequencing Analysis

A total of 8 out of 16 DNA sequences showed some level of similarities and are shown in detail in Figures 4-9. For those that were similar, OspA and OspE showed the highest percent of homology (Figures 4-6 and 8-9). Other genetic sequences, such as a few OspA sequences, BbA68 and OspC, showed very little (<~15%) homology to known Bb strains (data not shown; there is not anything to show). Also, only one BbA68 DNA sequence amplified (a very short piece) after PCR indicating that the primers employed had little to no homology to the middle Tennessee Bb isolates (data not shown). Similarly, ospC also failed to PCR-amplify but this is not entirely surprising since ospC is known to have many genetic variations (31,33)(data not shown).

The proteins that would be translated from these variant strains do have several "new" amino acid sequences. However, it is unknown whether these changes would result in a novel protein with different functionality. None of the DNA sequence differences resulted in a stop codon indicating that the protein is still of similar length and potentially similar function.

Since the primers to clone the genes did not include the promoter elements, sequencing data did not reveal any variation that

could identify differential expression patterns from the northeastern strains. Also, a caveat with DNA sequencing is that the sequencing extension may not always extend far enough to reveal complete gene coverage. This is especially true for PCR-only DNA sequencing reactions.

Sample 19 OspA:

Identities = 772/774 (99%), Gaps = 1/774 (0%)

```
TTATTTTAAAGCGTTTTTAATTTCATCAAGTTTTGTAATTTCAACTGCTGACCCCTCTAA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TTATTTTAAAGCGTTTTTAATTTCATCAAGTTTTGTAATTTCAACTGCTGACCCCTCTAA

TTTGGTGCCATTTGAGTCGTATTGTTGTACTGTAATTGTGTTTTCTTTTGTAAACACAAG
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TTTGGTGCCATTTGAGTCGTATTGTTGTACTGTAATTGTGTTTTCTTTTGTAAACACAAG

GTCTTTAGTTTTTTTACTGTTTACAGTAATTGTTAAAGTTGAAGTGCCTGAATTCCAAGC
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GTCTTTAGTTTTTTTACTGTTTACAGTAATTGTTAAAGTTGAAGTGCCTGAATTCCAAGC

TGCAGTTTTTTTAGTAGCAGCACTACTGTCAGTGTCATTAAGTTCAACTGAAACTTCCCC
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TGCAGTTTTTTTAGTAGCAGCACTACTGTCAGTGTCATTAAGTTCAACTGAAACTTCCCC

AGATTTTGAAATATTTTTGCTTAAAGTAACAGTTCCTTCTTTAACCACCAATGTTGTTTT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AGATTTTGAAATATTTTTGCTTAAAGTAACAGTTCCTTCTTTAACCACCAATGTTGTTTT

TTCAGCAGTTAGAGTTCCTTCAAGAACATAGCCTTTTAAAACCTCTTTAGCTTTTCCAGA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TTCAGCAGTTAGAGTTCCTTCAAGAACATAGCCTTTTAAAACCTCTTTAGCTTTTCCAGA

TCCATCGCTTTTAATTCCTGTGTATTCAAGTCTGGTTCCGTCTGCTCTTGTTATTATTTT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TCCATCGCTTTTAATTCCTGTGTATTCAAGTCTGGTTCCGTCTGCTCTTGTTATTATTTT

TTCAGATACTTCACCTTTTTCATTGAATTTTTCTTCTGTTGATGACTTGTCTTTGGAAGT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TTCAGATACTTCACCTTTTTCATTGAATTTTTCTTCTGTTGATGACTTGTCTTTGGAAGT

TACTTTTTTTGATACTAGTGTTTTGCCATCTTCTTTGAAAACTTCAAGTGTGGTTTGACC
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TACTTTTTTTGATACTAGTGTTTTGCCATCTTCTTTGAAAACTTCAAGTGTGGTTTGACC

TAGATCGTCAGAAATTGTTAATTTTACTTTACTTTTGTCAGCTTTTACGTCTTCAAGTAC
|||||||||||||||||||||||||||||||||||||||||||| ||||||||||
TAGATCGTCAGAAATTGTTAATTTTACTTTACTTTTGTCAGCTTTTACGCCTTCAAGTAC

TCCAGATCCATTGTTTTTATCAGAAGTTCCTTTAAGCTCAAGCTTGTCTACTGTTGCAAT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TCCAGATCCATTGTTTTTATCAGAAGTTCCTTTAAGCTCAAGCTTGTCTACTGTTGCAAT

TAGATCGTACTTGCCGTCTTTGTTTTTTTCTTTGCTTACAAGAACTTTCATTTCACCAGG
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TAGATCGTACTTGCCGTCTTTGTTTTTTTCTTTGCTTACAAGAACTTTCATTTCACCAGG

CAAATCTACTGAAACGCTGTTTTTCTCGTCAAGGCTGCTAACATTTTGCTTACA
|||||||||||||||||| ||||||||||||||||||||||||||||||||||
CAAATCTACTGAAACGCTGTTTTTCTCGTCAAGGCTGCTAACATTTTGCTTACA
```

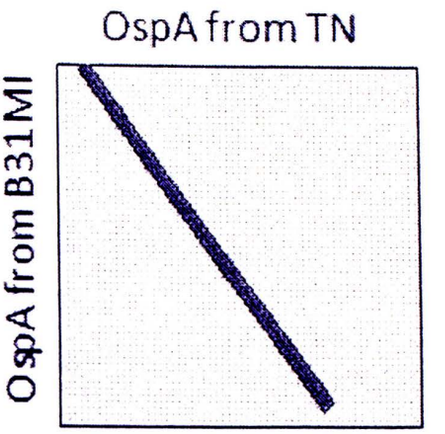
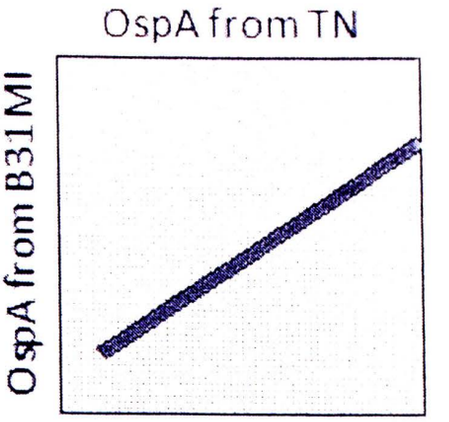


Figure 3. Sequence alignment of middle TN Bb DNA for ospA (top) compared to Lyme disease-causing strain, B31MI (bottom). The boxed regression line indicates ~99% homology between the two DNA sequences. Gray highlighted regions indicate sequence dissimilarities.

Sample 24 OspA:




```
Identities = 488/489 (99%), Gaps = 0/489 (0%)

CAAAGACGGCAAGTACGATCTAATTGCAACAGTAGACAAGCTTGAGCTTAAAGGAACTTC
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CAAAGACGGCAAGTACGATCTAATTGCAACAGTAGACAAGCTTGAGCTTAAAGGAACTTC

TGATAAAAACAATGGATCTGGAGTACTTGAAGGCGTAAAAGCTGACAAAAGTAAAGTAAA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TGATAAAAACAATGGATCTGGAGTACTTGAAGGCGTAAAAGCTGACAAAAGTAAAGTAAA

ATTAACAATTTCTGACGATCTAGGTCAAACCACACTTGAAGTTTTCAAAGAAGATGGCAA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ATTAACAATTTCTGACGATCTAGGTCAAACCACACTTGAAGTTTTCAAAGAAGATGGCAA

AACACTAGTATCAAAAAAAGTAACTTCCAAAGACAAGTCATCAACAGAAGAAAAATTCAA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AACACTAGTATCAAAAAAAGTAACTTCCAAAGACAAGTCATCAACAGAAGAAAAATTCAA

TGAAAAAGGTGAAGTATCTGAAAAAATAATAACAAGAGCAGACGGAACCAGACTTGAATA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TGAAAAAGGTGAAGTATCTGAAAAAATAATAACAAGAGCAGACGGAACCAGACTTGAATA

CACAGGAATTAAAAGCGATGGATCTGGAAAAGCTAAAGAGGTTTTAAAAGGCTATGTTCT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CACAGGAATTAAAAGCGATGGATCTGGAAAAGCTAAAGAGGTTTTAAAAGGCTATGTTCT

TGAAGGAACTCTAACTGCTGAAAAAACAACATTGGTGGTTAAAGAAGGAACTGTTACTTT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TGAAGGAACTCTAACTGCTGAAAAAACAACATTGGTGGTTAAAGAAGGAACTGTTACTTT


AAGCAAAAATATTTCAAAATCTGGGGAAGTTTCAGTTGAACTTAATGACACTGACAGTAC
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AAGCAAAAATATTTCAAAATCTGGGGAAGTTTCAGTTGAACTTAATGACACTGACAGTAG


TGCTGCTAC
|||||||||
TGCTGCTAC
```

Figure 4. Sequence alignment of middle TN Bb DNA for ospA (top) compared to Lyme disease-causing strain, B31MI (bottom). The boxed regression line indicates ~99% homology between the two DNA sequences. Gray highlighted regions indicate sequence dissimilarities.

Sample 26 OspA:

Identities = 774/774 (100%), Gaps = 0/774 (0%)

```
TGTAAGCAAAATGTTAGCAGCCTTGACGAGAAAAACAGCGTTTCAGTAGATTTGCCTGGT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TGTAAGCAAAATGTTAGCAGCCTTGACGAGAAAAACAGCGTTTCAGTAGATTTGCCTGGT

GAAATGAAAGTTCTTGTAAGCAAAGAAAAAAACAAAGACGGCAAGTACGATCTAATTGCA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GAAATGAAAGTTCTTGTAAGCAAAGAAAAAAACAAAGACGGCAAGTACGATCTAATTGCA

ACAGTAGACAAGCTTGAGCTTAAAGGAACTTCTGATAAAAACAATGGATCTGGAGTACTT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ACAGTAGACAAGCTTGAGCTTAAAGGAACTTCTGATAAAAACAATGGATCTGGAGTACTT

GAAGGCGTAAAAGCTGACAAAAGTAAAGTAAAATTAACAATTTCTGACGATCTAGGTCAA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GAAGGCGTAAAAGCTGACAAAAGTAAAGTAAAATTAACAATTTCTGACGATCTAGGTCAA

ACCACACTTGAAGTTTTCAAAGAAGATGGCAAAACACTAGTATCAAAAAAAGTAACTTCC
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ACCACACTTGAAGTTTTCAAAGAAGATGGCAAAACACTAGTATCAAAAAAAGTAACTTCC

AAAGACAAGTCATCAACAGAAGAAAAATTCAATGAAAAAGGTGAAGTATCTGAAAAAATA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AAAGACAAGTCATCAACAGAAGAAAAATTCAATGAAAAAGGTGAAGTATCTGAAAAAATA

ATAACAAGAGCAGACGGAACCAGACTTGAATACACAGGAATTAAAAGCGATGGATCTGGA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ATAACAAGAGCAGACGGAACCAGACTTGAATACACAGGAATTAAAAGCGATGGATCTGGA

AAAGCTAAAGAGGTTTTAAAAGGCTATGTTCTTGAAGGAACTCTAACTGCTGAAAAAACA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AAAGCTAAAGAGGTTTTAAAAGGCTATGTTCTTGAAGGAACTCTAACTGCTGAAAAAACA

ACATTGGTGGTTAAAGAAGGAACTGTTACTTTAAGCAAAAATATTTCAAAATCTGGGGAA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ACATTGGTGGTTAAAGAAGGAACTGTTACTTTAAGCAAAAATATTTCAAAATCTGGGGAA

GTTTCAGTTGAACTTAATGACACTGACAGTAGTGCTGCTACTAAAAAAACTGCAGCTTGG
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GTTTCAGTTGAACTTAATGACACTGACAGTAGTGCTGCTACTAAAAAAACTGCAGCTTGG

AATTCAGGCACTTCAACTTTAACAATTACTGTAAACAGTAAAAAAACTAAAGACCTTGTG
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AATTCAGGCACTTCAACTTTAACAATTACTGTAAACAGTAAAAAAACTAAAGACCTTGTG

TTTACAAAAGAAAACACAATTACAGTACAACAATACGACTCAAATGGCACCAAATTAGAG
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TTTACAAAAGAAAACACAATTACAGTACAACAATACGACTCAAATGGCACCAAATTAGAG

GGGTCAGCAGTTGAAATTACAAAACTTGATGAAATTAAAAACGCTTTAAAATAA
||||||||||||||||||||||||||||||||||||||||||||||||||||
GGGTCAGCAGTTGAAATTACAAAACTTGATGAAATTAAAAACGCTTTAAAATAA
```

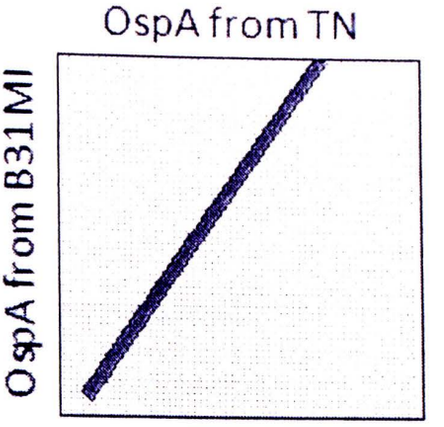
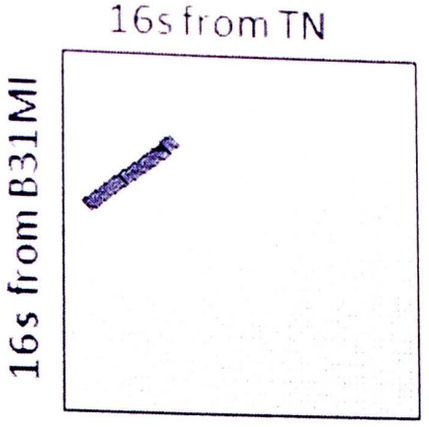


Figure 5. Sequence alignment of middle TN Bb DNA for ospA (top) compared to Lyme disease-causing strain, B31MI (bottom). The boxed regression line indicates ~100% homology between the two DNA sequences.

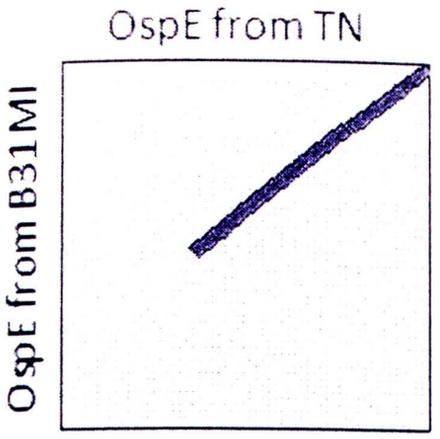
Sample 26 16s rRNA:




```
Identities = 245/293 (83%), Gaps = 3/293 (1%)

CTGGGGAGTATGCTCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGG
|||||||||||||||||||  ||||||||||||||||||||||||||||||||||||||
CTGGGGAGTATGCTCGCAAGAGTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGG

TGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCT-TC
|||||||||||||||||||| |  |||||| |||||||||||| ||||||| | |
TGGAGCATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCAGGCTTGACATATATA

TGACAATCCTAGAGATAGGACTTTCCCTTCGGGGACAGAATGACAGGTGGTGCATGGTTG
||  |    |||||||    ||  |||  |  |||    |   |||||||  ||||||||||
GGATATAGTTAGAGATAATTATTCCCCGTTTGGGGTCTATATACAGGTGCTGCATGGTTG

TCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTGT
||||||||||||  |||||  ||||||||||||||||||||||||||||||||  ||  |
TCGTCAGCTCGTGCTGTGAGGTGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTAT

TAGTTGCCAGCAT-TAA-GTTGGGCACTCTAGCAAGACTGCCGGTGATAAGTC
|||||||||||||||| ||||||||||  |||||||||||||||||||||
CTGTTACCAGCATGTAATGGTGGGGACTCAGATAAGACTGCCGGTGATAAGTC
```

Figure 6. Sequence alignment of middle TN Bb DNA for 16s ribosomal RNA (top) compared to Lyme disease-causing strain, B31MI (bottom). The boxed regression line indicates ~83% homology between the two DNA sequences. Gray highlighted regions indicate sequence dissimilarities.

Sample 1 OspE:

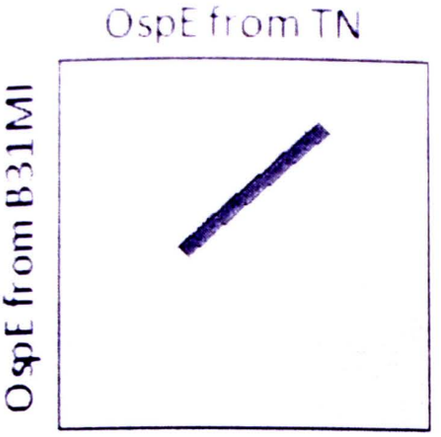


Identities = 269/301 (89%), Gaps = 7/301 (2%)

```
CTGGACAGACCTAGGAGATTTAGTTGTAAGAAAACAATAAGATGGTATTGATACGGGTTT
||||  |||||  ||||||||||||||||||||||||  ||  ||||||||||||||||||||
CTGGGCAGACTTAGGAGATTTAGTTGTAAGAAAAGAAAAAGATGGTATTGATACGGGTTT

AAACGCTGGGGGACATTCGGCTACATTCTTTTCATTAAAAGAATCATAAGTTAATAACTT
|||||||||||||||||||||||||||||||||||||||  ||||||||  ||  ||||||||||
AAACGCTGGGGGACATTCGGCTACATTCTTTTCATTAGAAGAATCAGAAATTAATAACTT

TATGAAAGCAATGACTAAAGGCGGATCATTTAAAACTAGTTTGTATTATGGATATAAGTA
|||  ||||||||||||  ||||||||||||||||||||||||||||||||||||||||  |
TATAAAAGCAATGACTGAAGGCGGATCATTTAAAACTAGTTTGTATTATGGATATAATGA

CGAACAAAGTATATGCAAATGGCATCCAAAACAAAGAGATCATAACAAAAATAGAAAGTA
||||  ||||||  ||       ||||  |||||  ||||||||||||||||  |||||||  |
CGAAGAAAGTAAAT-------GCATTCAAAATAAAGAGATCATAACAAAGATAGAAAAAA

TTAATGGTGCTGAACATATTGCGTTTTTAGGAGATAAAATTAATAACCGTGTGGGGGGAGA
||||||  |  |||||  |||||  |  ||||||||||||||||||||||  |||  |||||||||
TTAATGATACTGAATATATTACATTTTTAGGAGATAAAATTAATAACAGTGCGGGGGGAGA
```

Figure 7. Sequence alignment of middle TN Bb DNA for ospE (top) compared to Lyme disease-causing strain, B31MI (bottom). The boxed regression line indicates ~89% homology between the two DNA sequences. Gray highlighted regions indicate sequence dissimilarities.

Sample 10 OspE:




```
Identities = 164/192 (85%), Gaps = 0/192 (0%)

CTGGGGAGACCTAGCAGATTTAGTTGTAGGAAAACAATAAGATGCTTTCGATTCGGGTTA
|||||  ||||  |||  ||||||||||||||  |||||  ||  ||||||  |  |  |||  ||||||
CTGGGCAGACTTAGGAGATTTAGTTGTAAGAAAAGAAAAAGATGGTATTGATACGGGTTT

AAACGCTGGGGGGACATTCTGCTACATTCTTTTCATTAGAAGAAGCATAATTTAATACCTT
||||||||||||||||||  ||||||||||||||||||||||||  ||  ||  ||||||  |||
AAACGCTGGGGGGACATTCGGCTACATTCTTTTCATTAGAAGAATCAGAAATTAATAACTT

TATGAAATCTGTGACTAAAGGCGGATCATTTAATACTAGTGTGTATTATGGATATGAGAA
|||  |||  |   |||||  ||||||||||||||||  ||||||  ||||||||||||||  |   |
TATAAAAGCAATGACTGAAGGCGGATCATTTAAAACTAGTTTGTATTATGGATATAATGA

CGAACCAAGTAA
||||   ||||||
CGAAGAAAGTAA
```

Figure 8. Sequence alignment of middle TN Bb DNA for ospE (top) compared to Lyme disease-causing strain, B31MI (bottom). The boxed regression line indicates ~85% homology between the two DNA sequences. Gray highlighted regions indicate sequence dissimilarities.

Chapter IV:
Discussion

Several Tennessee counties were surveyed for this project. Therefore, figure 10 is a GIS map created to help visualize where the capture sites were and how many animals tested positive for Bb at each site. Because of multiple points at one location, points have been translocated to show all points. Fairly equal numbers of animals were collected from each site, but the GIS map shows that some counties contain more Bb-infected animals than others. Maps such as this one gives insight to the distribution of Bb-infected animals and could afford better understanding to the spread of Lyme disease. Additionally, GIS mapping could be employed to better understand other diseases at the human level and/or wildlife level.

Bb was found to be in middle Tennessee in 38 specimens. Voles seemed to be the most susceptible to Bb with approximately 52% being found infected (20,21). Therefore, there could be as many as 91 million Bb-infected mice, voles, and songbirds in the six counties surveyed (based on statistical estimates). Additionally, Bb has also been found in chipmunks, shrews and deer which were not surveyed in this study (14). The numbers reported here are only estimates and

could be much higher since only rural square miles were used for each county. A larger sample size is needed to increase the accuracy of the estimates of Bb-infected animals.

DNA sequence analysis of the Bb isolates found in middle Tennessee revealed that Bb is present in Tennessee. This observation is major focus of this thesis since the most health professionals dismiss Lyme disease in Tennessee as a nonoccurrence. However, the Bb which is found in middle Tennessee shows some genetic variations when compared to northeastern Bb strains. All the ospE genes were approximately 85% similar which is believed to be important for Bb-survival in the animal host (3). However, like ospA and ospC, the exact role(s) is not well understood, but recent evidence supports that ospE and BbA68 are important proteins for Bb colonization of the animal host by allowing Bb to circumvent part of the animal host innate immune system called the complement cascade (8). With this in mind, BbA68 DNA sequencing was difficult since a majority of the middle Tennessee isolates would not PCR amplify (all but one would not amplify) indicating that the primers employed had little to no homology to the BbA68 gene harbored by those Bb isolates (data not shown). Since BbA68 is the only known human factor H binding protein which is needed to inactivate the human complement cascade and, in theory,

allow Bb-infection of people, this could very well be the "answer" as to why clinicians do not see more cases of human Lyme disease in Tennessee. Further DNA sequencing is necessary to better characterize the Tennessee Bb isolates which should give insight as to the evolutionary forces behind BbA68 divergence away from aiding human infection.

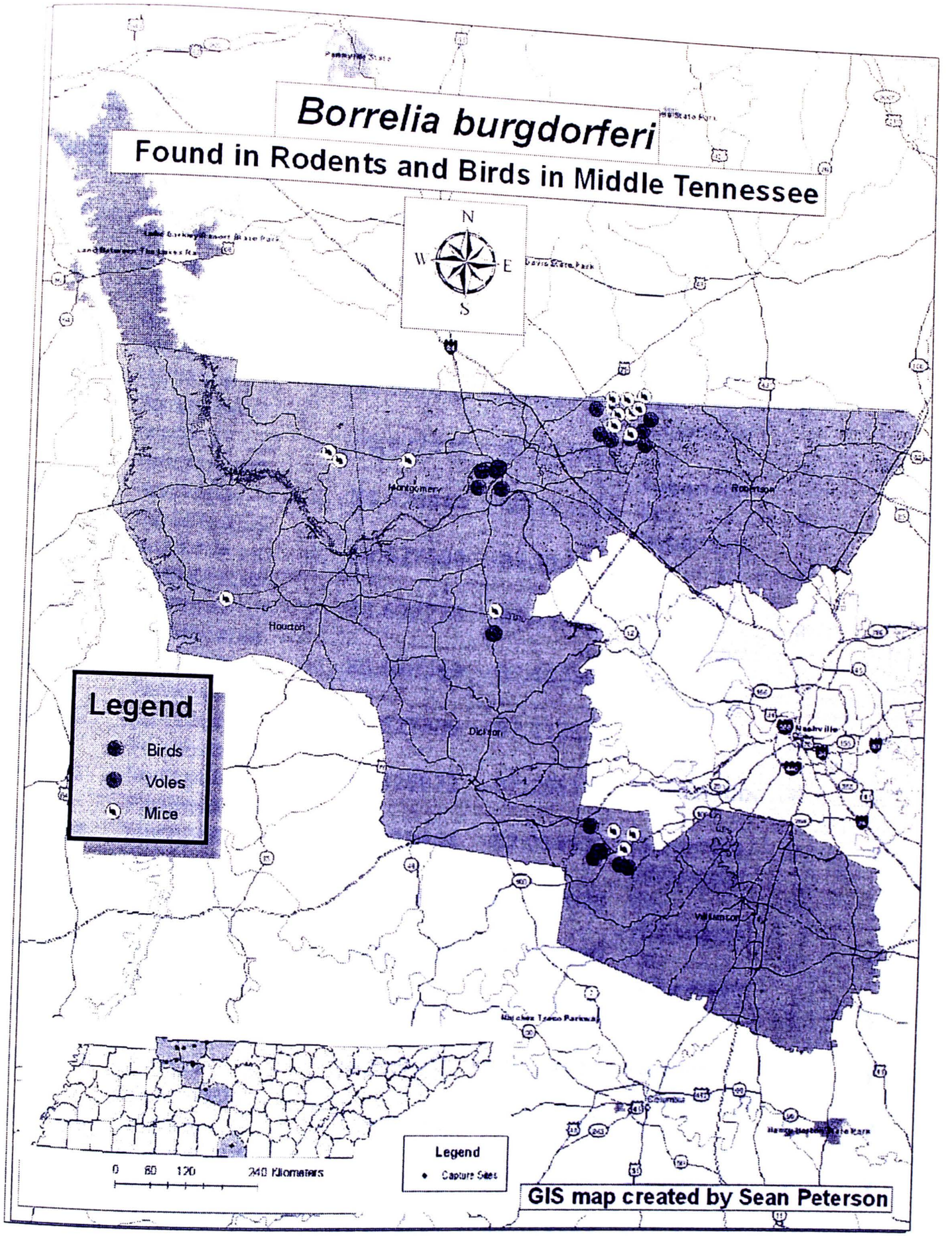


Figure 9. Map of capture sites and animals infected with Bb.

Appendix

Table 2. Bb-infected mice with GPS locations

Code	Species	Latitude	Longitude	Location	PosB	Posb
MuF1	Peromyscus leucopus	36.627444	-87.113083	Robertson Co.	y	y
Mu2	Peromyscus leucopus	36.627361	-87.113306	Robertson Co.	y	y
MuF5	Peromyscus leucopus	36.627361	-87.113000	Robertson Co.	y	y
MuF10	Peromyscus leucopus	36.627083	-87.113167	Robertson Co.	n	y
MuF6	Peromyscus leucopus	36.627500	-87.112667	Robertson Co.	y	y
MuM9	Peromyscus leucopus	36.626944	-87.113222	Robertson Co.	y	y
MuF14	Peromyscus leucopus	36.627500	-87.112417	Robertson Co.	y	y
MuM22	Peromyscus leucopus	36.627278	-87.111083	Robertson Co.	n	y
MuM24	Peromyscus leucopus	36.626389	-87.112028	Robertson Co.	n	y
MuF26	Peromyscus leucopus	36.627389	-87.113194	Robertson Co.	n	y
MuF73	Peromyscus leucopus	36.325972	-87.355194	Dickson Co.	n	y
MuM84	Peromyscus leucopus	36.293444	-87.354639	Lincoln Co.	y	y
MuM106	Peromyscus leucopus	36.026667	-87.907028	Williamson Co.	n	y
MuM107	Peromyscus leucopus	36.027000	-87.907389	Williamson Co.	y	y
MuF110	Peromyscus leucopus	36.026944	-87.907444	Williamson Co.	y	y
MuM119	Peromyscus leucopus	36.539083	-87.674444	Stewart Co.	y	y
MuM122	Peromyscus leucopus	36.535000	-87.528250	Montgomery Co.	n	y
MuF123	Peromyscus leucopus	36.528583	-87.654111	Stewart Co.	y	y
MuM140	Reithrodontomys humulis	36.320278	-87.845472	Houston Co.	y	y

Positive data for mice. PosB indicates positive for the genus and Posb is positive for the species *Borrelia burgdorferi.*

Table 3. Bb-infected voles with GPS locations

Code	Species	Latitude	Longitude	Location	PosB	Posb
MvF1	Microtus ochrogaster	36.533111	-87.361444	Robertson Co.	y	y
MvM2	Microtus ochrogaster	36.533083	-87.361639	Robertson Co.	y	y
MvF3	Microtus ochrogaster	36.533250	-87.361583	Robertson Co.	y	y
MvF6	Microtus ochrogaster	36.533250	-87.361361	Robertson Co.	y	y
MvF7	Microtus ochrogaster	36.533139	-87.361556	Robertson Co.	y	y
MvF9	Microtus ochrogaster	36.533056	-87.361583	Robertson Co.	y	y
MvM10	Microtus ochrogaster	36.025778	-87.907167	Robertson Co.	y	y
MvM11	Microtus ochrogaster	36.027000	-87.907389	Robertson Co.	y	y
MvF15	Microtus ochrogaster	36.025639	-87.907333	Robertson Co.	y	y
MvM18	Microtus ochrogaster	36.293111	-87.354806	Dickson Co.	y	y

Table 4. Bb-infected birds with GPS locations

Code	Species	Latitude	Longitude	Location	PosB	Posb
B3	Cardinalis cardinalis	36.533250	-87.361583	Montgomery Co.	y	y
B5	Mimus polyglottos	36.533028	-87.361611	Montgomery Co.	n	y
B6	Baeolophus bicolor	36.533250	-87.361361	Montgomery Co.	n	y
B7	Baeolophus bicolor	36.533139	-87.361556	Montgomery Co.	n	y
B12	Turdus migratorius	36.027000	-87.907389	Williamson Co.	y	y
B15	Sialia sialis	36.025639	-87.907333	Williamson Co.	y	y
B23	Cardinalis cardinalis	36.026750	-87.906722	Williamson Co.	y	y
B24	Poecile carolinensis	36.026389	-87.906500	Williamson Co.	y	y
B25	Poecile carolinensis	36.026528	-87.906583	Williamson Co.	y	y
B28	Thryothorus ludovicianus	36.026556	-87.906528	Williamson Co.	n	y

Table 5. Percent of infected specimens in middle Tennessee.

County	Num. of infected			% infected		
	Mice	Voles	Birds	Mice	Voles	Birds
Montgomery	1	0	4	4.8	NA	40
Robertson	11	9	0	24.4	56.3	NA
Stewart	2	0	0	9.5	NA	0
Dickson	1	1	0	3.4	50	NA
Houston	1	0	0	4.8	0	NA
Williamson	3	0	5	21.4	NA	26.3
Total	19	10	9	12.6	52.6	29.0

Table 6. Approximation of possible infected mice, voles, birds.

County	Possible infected							
	Mice (field) low	Mice (field) high	Mice (thick forest)	Voles (old field) low	Voles (old field) high	Voles (marsh)	Birds low	Birds high
Montgomery	36960	57493	1642667	NA	NA	NA	43282	259636
Robertson	388721	604677	17276473	1490832	4472496	14908320	NA	NA
Stewart	251246	390827	11166476	NA	NA	NA	0	0
Dickson	67154	104461	2984607	1622880	4868640	16228800	NA	NA
Houston	54857	85333	2438095	0	0	0	NA	NA
Williamson	215877	335808	9594514	NA	NA	NA	36959	221709
Total	1014814	1578599	45102832	3113712	9341136	31137120	80241	481345

Reference List

1. Aberer, E. and P. H. Duray. 1991. Morphology of *Borrelia burgdorferi*: structural patters of cultured borreliae in relation to staining methods. J. Clin. Microbiol. 29:764-772.

2. Agre, F. and R. Schwartz. 1993. The value of early treatment of deer tick bites for the prevention of Lyme disease. Am. J. Dis. Child. 147:945-947.

3. Akins, D. R., M. J. Caimano, X. Yang, F. Cerna, M. V. Norgard, and J. Radolf. 1999. Molecular and evolutionary analysis of *Borrelia burgdorferi* 297 circular plasmid-encoded lipoproteins with OspE- and OspF-like leader peptides. Infect Immun. 67:1526-1532.

4. Banfield, A. 1974. The mammals of Canada. University of Toronto Press 438.

5. Barbour, A. G. 1996. Does Lyme Disease occur in the South - a survey of emerging tick- borne infections in the region. Am. J. Med. Sci. 311:34-40.

6. Barbour, A. G. and S. F. Hayes. 1986. Biology of *Borrelia* species. Microbiol. Rev. 50:381-400.

7. Brooks, C. S., P. S. Hefty, S. E. Jolliff, and D. R. Akins. 2003. Global analysis of *Borrelia burgdorferi* genes regulated by mammalian host-specific signals. Infect. Immun. 71:3371-3383.

8. Brooks, C. S., S. R. Vuppala, A. M. Jett, A. Alitalo, S. Meri, and D. R. Akins. 2005. Complement regulator-acquiring surface protein 1 imparts resistance to human serum in *Borrelia burgdorferi*. J Immunol. 175:3299-3308.

9. Burgdorfer, W. 1991. Lyme borreliosis: ten years after discovery of the etiologic agent, Borrelia burgdorferi. Infection 19:257-262.

10. Burgdorfer, W. 1993. How the discovery of *Borrelia burgdorferi* came about. Clin. Dermatol. 11:335-338.

11. Burgdorfer, W. 1986. Discovery of the Lyme disease spirochete: a historical review. Zentralb. Bakt. Hyg. 263:7-10.

12. Burgdorfer, W., J. F. Anderson, L. Gern, R. S. Lane, J. Piesman, and A. Spielman. 1991. Relationship of Borrelia burgdorferi to its arthropod vectors. Scandinavian Journal of Infectious Diseases - Supplementum 77:35-40.

13. Centers for Disease Control. 2004. Lyme disease - United States, 2001-2002. Morbid. Mortal. Weekly Rep. 53:365-369.

14. Centers for Disease Control and Prevention. 2007. Lyme disease--United States, 2003-2005. MMWR Morb. Mortal. Wkly. Rep. 56:573-576.

15. Cyr, T. L., M. C. Jenkins, R. D. Hall, E. J. Masters, and G. A. McDonald. 2005. Improving the specificity of 16S rDNA-based polymerase chain reaction for detecting Borrelia burgdorferi sensu lato-causative agents of human Lyme disease. J. Appl. Microbiol. 98:962-970.

16. Dalquest, W. 1941. Ecological relationships of four small mammals in western Washington. Journal of Mammalogy 22(2):170-173.

17. Drake, J. 1958. The brush mouse *Peromyscus boylii* in southern Durango. Michigan State University Museum Publications: Biological Series 1(3):97-132.

18. Grimm, D., K. Tilly, R. Byram, P. E. Stewart, J. G. Krum, D. M. Bueschel, T. G. Schwan, P. F. Policastro, A. F. Elias, and P. A. Rosa. 2004. Outer-surface protein C of the Lyme disease spirochete: a protein induced in ticks for infection of mammals. Proc. Natl. Acad. Sci. U. S. A 101:3142-3147.

19. Hayes, R. B., K. Husgafvel-Pursiainen, and H. Vainio. 2005. Molecular epidemiology: convergence between toxicology and epidemiology. Mutat. Res. 592:1-2.

20. LeBlanc DeLacy. 2006. Reservoir competence of North American bird and rodent species for the Lyme disease spirochete, *Borrelia burgdorferi*. Austin Peay State University Press .

21. McMahan Jon. 2007. Survey of Middle Tennessee mammalian and tick populations for tick-borne diseases: *Borrelia burgdorferi*, *Borrelia hermsii*, and *Anaplasma phagocytophila*. Austin Peay State University Press .

22. Ojaimi, C., C. Brooks, S. Casjens, P. Rosa, A. Elias, A. Barbour, A. Jasinskas, J. Benach, L. Katona, J. Radolf, M. Caimano, J. Skare, K. Swingle, D. Akins, and I. Schwartz. 2003. Profiling of temperature-induced changes in *Borrelia burgdorferi* gene expression by using whole genome arrays. Infect. Immun. 71:1689-1705.

23. Oliver, J. H., Jr., F. W. Chandler, Jr., A. M. James, F. H. Sanders, Jr., H. J. Hutcheson, L. O. Huey, B. S. McGuire, and R. S. Lane. 1995. Natural occurrence and characterization of the Lyme disease spirochete, Borrelia burgdorferi, in cotton rats (Sigmodon hispidus) from Georgia and Florida. J. Parasitol. 81:30-36.

24. Steere, A. C. 1994. Lyme disease: a growing threat to urban populations. Proc. Natl. Acad. Sci. U. S. A 91:2378-2383.

25. Steere, A. C. 2001. Lyme disease. N. Engl. J Med 345:115-125.

26. Steere, A. C. 1989. Lyme Disease. N. Engl. J. Med. 321:586-597.

27. Steere, A. C., T. F. Broderick, and S. E. Malawista. 1978. Erythema chronicum migrans and Lyme arthritis: epidemiologic evidence for a tick vector. Am. J. Epidemiol. 108:312-321.

28. Steere, A. C., A. Gibofsky, M. E. Patarroyo, R. J. Winchester, J. A. Hardin, and S. E. Malawista. 1979. Chronic Lyme arthritis:clinical and immunogenetic differentiation from rheumatoid arthritis. Ann. Intern. Med. 90:896-901.

29. Steere, A. C., R. T. Schoen, and E. Taylor. 1987. The clinical evolution of Lyme arthritis. Ann. Intern. Med. 107:725-731.

30. Swanson, K. I. and D. E. Norris. 2007. Detection of Borrelia burgdorferi DNA in lizards from Southern Maryland. Vector. Borne. Zoonotic. Dis. 7:42-49.

31. Swanson, K. I. and D. E. Norris. 2008. Presence of Multiple Variants of Borrelia burgdorferi in the Natural Reservoir Peromyscus leucopus Throughout a Transmission Season. Vector. Borne. Zoonotic. Dis.

32. Theisen, M., B. Frederiksen, A. M. Lebech, J. Vuust, and K. Hansen. 1993. Polymorphism in *ospC* gene of Borrelia burgdorferi and immunoreactivity of OspC protein: implications for taxonomy and for use of OspC protein as a diagnostic antigen. J. Clin. Microbiol. 31:2570-2576.

33. Wilske, B., U. Busch, V. Fingerle, S. Jauris-Heipke, V. Preac Mursic, and G. Will. 1996. Immunological and molecular variability of OspA and OspC. Implications for Borrelia vaccine development. Infection 24:208-212.

34. Yang, X. F., U. Pal, S. M. Alani, E. Fikrig, and M. V. Norgard. 2004. Essential Role for OspA/B in the Life Cycle of the Lyme Disease Spirochete. J Exp. Med 199:641-648.

Vita

Sean Peterson grew up in New York and completed his high school education there in 1997. He joined the Army in 1998 and spent three years in an infantry unit on Ft. Campbell. In 2001 he was honorably discharged and joined the Tennessee National Guard for one year and started his college career at Hopkinsville Community College. After two years he received an A.A. He then transferred to Austin Peay and graduated with a B.S. in education in 2006. He started his Master's degree the next semester in Biology. He plans on graduating in the summer of 2008. After that he will teach in New York and work on his Ph. D., hopefully in genetics.