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Outer Surface Protein C Typing of Borrelia burgdorferi in the Tick Populations of the Upper Susquehanna River Basin, New York

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Cover Page Footnote

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Abstract

Lyme disease, the most common zoonotic disease in the United States, is caused by the spirochete *Borrelia burgdorferi (B. burgdorferi)*. In order to manage and confront the notable rise in Lyme disease cases, it is crucial to cultivate a deeper understanding of *B. burgdorferi* and its genes. The outer surface protein C (*ospC*) gene is highly polymorphic and commonly used as a genetic marker due to its crucial role in establishing mammalian infection. We report novel data on the prevalence of *B. burgdorferi ospC genotypes* in the infected tick populations of the Upper Susquehanna River Basin of New York State. DNA extracted from 266 *Ixodes scapularis, the* blacklegged ticks, were tested for the presence of *ospC* gene and the positive samples were subjected to sequencing. The specific *ospC* genotype was identified for 56 positive samples which were infected with B. *burgdorferi* representing a single *ospC* genotype. A total of 12 *ospC* genotypes were identified in the 56 ticks, with genotypes I, K, and A being the most prevalent across the Upper Susquehanna River Basin with little variation among the six counties. The frequency distribution of *ospC* variants in this region is significantly different from the few previously studied regions in the Northeast. This research will have implications in the public health sector by providing assessment for Lyme disease risk in the Upper Susquehanna River Basin and insight into strain specific vaccines based on *ospC*. Further research can be done into the dispersion pattern of *B. burgdorferi* within the Upper Susquehanna River Basin, while also replicating this study for other regions.

Keywords: *Borrelia burgdorferi,* Lyme disease, *OspC*, New York, *Ixodes Scapularis*

Introduction

Lyme disease is the most common vector-borne infection and the sixth most common infectious disease in the United States. It is estimated to infect over 476,000 people per year according to the Centers for Disease Control and Prevention (CDC 2021). Over 95% of reported cases of Lyme disease in the United States are from the Northeast and upper Midwest, with New York being one of the states with the highest prevalence (CDC 2021, Roome et al., 2018). Additionally, cases of Lyme disease are on the rise due to factors such as climate change, forest fragmentation, and tick habitat expansion (Brownstein et al., 2005). In North America, Lyme disease is caused by the spirochete bacteria *Borrelia burgdorferi sensu stricto* (Brisson et al.,

2012; Burgdorfer et al., 1985), with *Borrelia mayonii* being responsible for a small fraction of Lyme disease cases (Pritt et al., 2016). The *B. burgdorferi* bacteria is carried by *Ixodes Scapularis,* or blacklegged ticks, the most common arthropod vector in the United States (Brisson et al., 2012; Rynkiewicz et al., 2017). Lyme disease is transferred when the tick feeds on a host (Pal et al., 2004) and the pathogen then begins its incubation period, which typically ranges from 3 to 30 days (Mead et al., 2017). More specifically, when infected ticks feed, the influx of blood and increased temperature induce changes in the spirochete midgut population, affecting its synthesis abilities (Tilly et al., 2008). Following bacterial growth and changes to protein expression, spirochetes migrate to the salivary glands in preparation for transmission to a host (Tilly et al., 2008).

Borrelia burgdorferi (B. burgdorferi) is a genetically diverse pathogen with many strains. The mutations between strains impact the disease's ability to infect both the host and tick which leads to a difference in their prevalence and ability to spread (Rynkiewicz et al., 2017). The outer surface protein C (*ospC*) gene, located on the cp26 plasmid, is responsible for most genetic diversity in *B. burgdorferi* (Brisson et al., 2012). This plasmid has a mutation rate of 0.011 mutation events per site (Barbour & Travinsky, 2010). Because of this gene's high diversity and low recombination rate, *ospC* is commonly used as a genetic marker to identify the different strains of *B. burgdorferi* (Norek et al., 2016; Rynkiewicz et al., 2017). The *ospC* protein is critical for establishing an infection, as well as stimulating the production of antibodies in hosts (Carrasco et al., 2015; Norek et al., 2016). Upon entering a mammalian host, the *ospC* gene is immediately expressed, which is essential for the transfer of the spirochete from the tick to the host (Rynkiewicz et al., 2017). Furthermore, the *ospC* protein likely plays a part in evading the

host's immune defense (Carrasco et al., 2015), making it essential for the spread and invasiveness of Lyme disease (Carrasco et al., 2015; Norek et al., 2016; Tilly et al., 2006). In a study conducted by Grimm et al. (2004) it was found that a mutant tick lacking *ospC* was not able to infect mice. This, however, had no effect on its migration through the host's body in preparation for transmission to another host (Grimm et al., 2004). This mutant's inability to cause infection, coupled with the α-helical protein structure and binding site for an unknown ligand within the *ospC* protein (although the exact molecular function remains unknown) suggests a role of *ospC* in invasive disease (Kumaran et al., 2001; Tilly et al., 2008). Additionally, a study by Xu et al. (2007) shows that expression of *ospC* increases during initial infection but decreases when the host's anti-*ospC* humoral response is triggered. This indicates that *ospC* is not only important for establishing infection, but also for evading the host's immune defense (Xu et al., 2007). However, the mechanism that *ospC* uses to establish this infection is poorly understood.

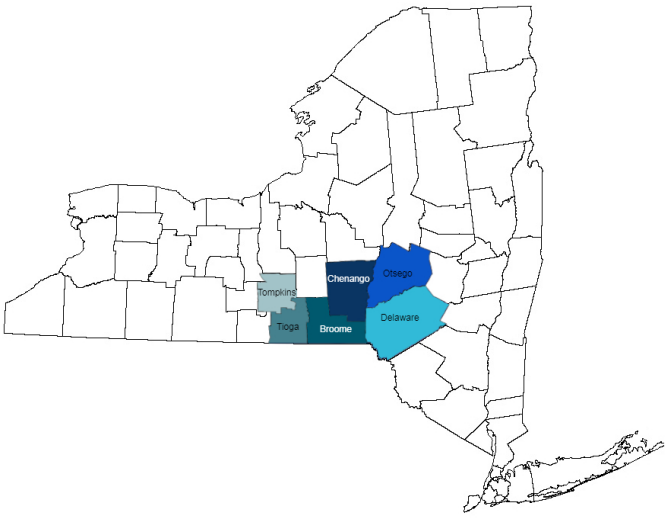
Currently, 25 *ospC* major groups, or genotypes, have been described, yet only a small subset of these genotypes are responsible for most of the *B. burgdorferi* infections detected in humans (Vuong et al., 2013). The invasiveness of a strain may depend on its *ospC* gene, especially as this gene has been shown to be necessary for establishing infection in humans. Of the strains identified in the Northeast, only five genotypes (A, B, I, K, and N) are considered highly invasive for humans (Vuong et al., 2013). While all *ospC* genotypes can establish an infection in humans, these five genotypes are known to disseminate and invade human tissues to cause a long-term infection (Dykhuizen et al., 2008; Vuong et al., 2013).

In this study, we investigate the prevalence of *B. burgdorferi ospC* strains in tick populations of the Upper Susquehanna River Basin of New York State. Understanding the

makeup of *B. burgdorferi ospC* genotypes in infected ticks will provide important information for realistic risk assessment, as the true risk of Lyme disease is associated with the prevalence of invasive *ospC* strains. Additionally, an assessment of the prevalence of invasive *ospC* genotypes informs research on Lyme disease vaccine development, as strain-specific vaccines have been shown to be more effective in providing immunity against *B. burgdorferi* (Khatchikian et al., 2015).

Figure 1

Location of Six Counties in the Upper Susquehanna River Basin in New York State



Methodology

Samples

A total of 266 samples were obtained from a larger collection of ticks collected during the summers of 2015 and 2016, and housed in the Binghamton University's Biospecimen Archive Facility. Ticks were collected using the dragging method from 13 sites in six counties across the

Upper Susquehanna River Basin (Figure 1): Broome (3 sites), Tioga, Tompkins, Chenango, Otsego, and Delaware (2 sites each).

DNA Amplification

Polymerase chain reaction (PCR) was performed to amplify a 617-bp fragment of the *B. burgdorferi ospC* gene from the DNA samples previously extracted by Roome et al. (2018). The optimized conditions of the reaction were as follows: 3 µl of 0.0003ng/µl DNA sample, 7.45 µl of dH_2O, 1.25 µl 10x PCR buffer, 0.25 µl dNTP (10mM), 0.05 µl Taq DNA polymerase, and 0.25 µl of primers (10 µM) OC6+ (5'-AAAGAATACATTAAGTGCGATATT-3') and OC623- (5'-TTAAGGTTTT TTTTGGACTTTCTGC-3') (Qiu et al., 2002). The PCR was run under conditions listed in Table 1 (Wang et al., 2014). The amplified products were run on a 1% agarose gel to test for the presence of the *B. burgdorferi ospC* gene.

Table 1

Thermocycler Program for Amplifying ospC in Borrelia burgdorferi with Primers OC6(+) and OC623(-) (Wang et al., 2014).

Number of Cycles	Temperature (C)	Time (seconds)
1	96	60
45	94	30
	54.5	30
	72	60
1	72	600

Data Analysis

Positive samples were targeted for sequencing using an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA). Bases were called using Sequencing Analysis v5.1 software (Applied Biosystems, Foster City, CA). Sequencher 5.4.6 (Gene Codes Corporation) was used to clean up the raw sequences and verify the accuracy of base calls. The corrected sequences were aligned against the sequences of known *ospC* major groups, determining *B. burgdorferi* strains from GenBank in Molecular Evolutionary Genetics Analysis (MEGA) 7 (Kumar et al., 2016). An *ospC* genotype was assigned to each sequence based on the previously described criteria (Wang et al., 2014). A median-joining network (Bandelt et al., 1999) of the Upper Susquehanna Basin sequences was constructed using Network 5.0 (Fluxus Technology Ltd, fluxus-engineering.com). MEGA X was used to construct a maximum likelihood tree (Kumar et al., 2018) of the strains sequenced along with published sequences obtained from GenBank.

Results

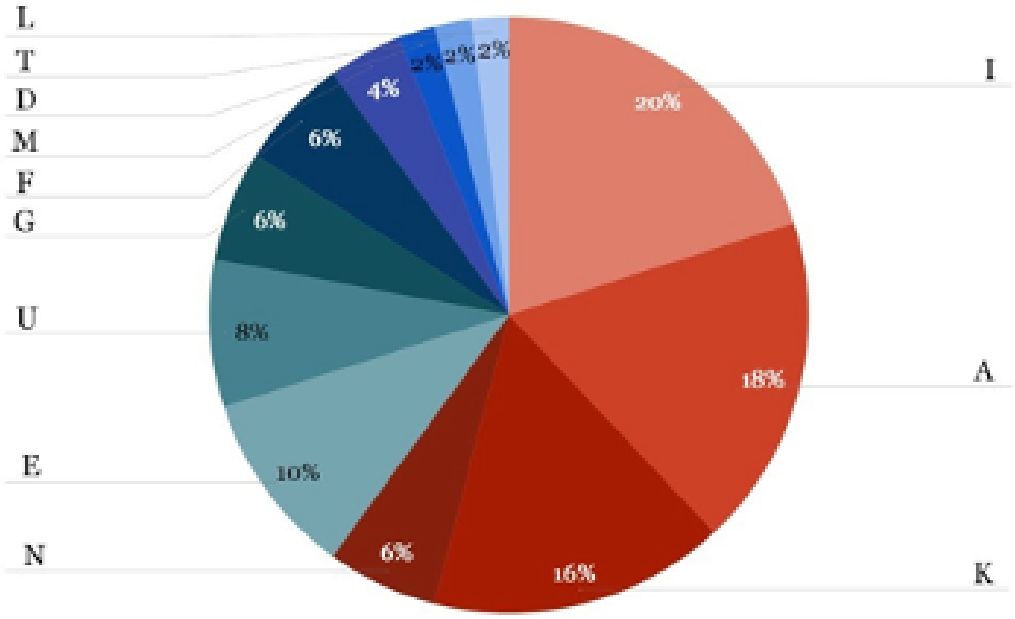
Most Common Strains in the Upper Susquehanna River Basin

Of the 266 DNA samples extracted from *Ixodes scapularis*, 71 (26.7%) tested positive for the *ospC* gene. Sequencing revealed that 15 ticks (21.1% of positive ticks) were coinfected with multiple *ospC* genotypes, with the rest infected with a singular *ospC* genotype. The *ospC* diversity in the Upper Susquehanna River Basin is summarized in Figure 2. A total of 12 *ospC* genotypes, out of the 17 known to be common in the Northeast, were identified in the infected ticks collected in Tioga, Chenango, Tompkins, Delaware, Broome, and Otsego counties. I, A, K

and E *ospC* groups were found to be the most prevalent with frequencies of 0.2, 0.18, 0.16, and 0.1 respectively, while groups B, C, D, H, J and O were not found in any of the six counties. The three most prevalent genotypes in the Upper Susquehanna River Basin of New York were I, K, and A, compared with A, B and K reported in previous Northeast studies (Anderson & Norris, 2006; Barbour & Travinsky, 2010; Qiu et al., 2008).

Figure 2

Genetic Diversity of Borrelia burgdorferi Outer Surface Protein C (ospC) Groups in Infected Tick Populations of the Upper Susquehanna River Basin



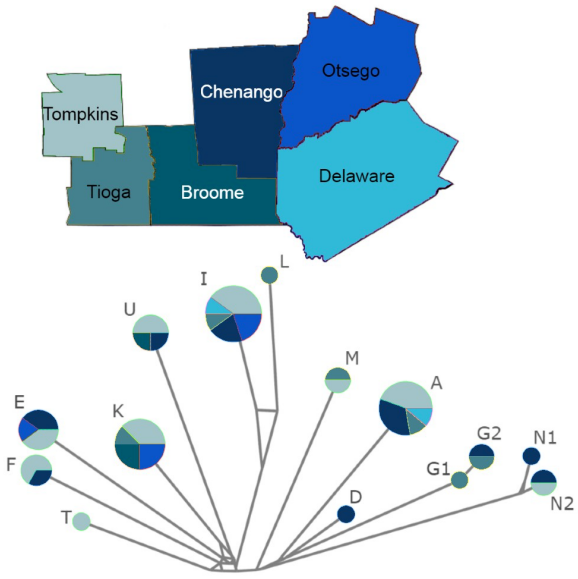
Note. The red shades represent the ospC genotypes that are likely to disseminate in the body and cause Lyme disease. The different shades of green and blue represent the non-invasive ospC genotypes which are capable of transmitting to humans but are not likely to disseminate.

Gene Diversity of Borrelia burgdorferi

The Median-joining network of the *ospC* sequences visualizes the relationship between strains reported from each county (Figure 3). As expected, most *ospC* groups were detected in all counties, suggesting insignificant differences in prevalence of *B. burgdorferi ospC* strains across the Upper Susquehanna River Basin. A majority of alleles (9 out of 14) were found in two or more counties.

Figure 3

A Median-Joining Network of outer surface protein C(ospC) Identified in the Upper Susquehanna River Basin.

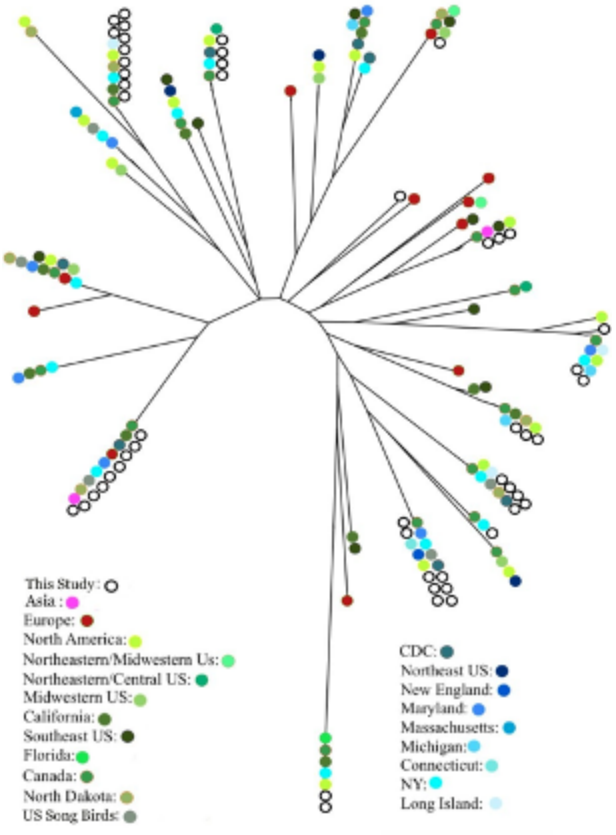


Note. Data is separated by sample site. Colors represent the proportion of a major group found in a specific county.

A maximum likelihood tree was constructed to investigate the relationships between the 50 *ospC* sequences first reported here and 535 previously published sequences representative of other regions of the United States and the world (Figure 4).

Figure 4

Maximum Likelihood Tree of Outer Surface Protein C Genotypes



Note. Previously published sequences are labeled by specific geographic origin when that information was available. The "North America" and "CDC" sequences belong to Borrelia burgdorferi isolated from unspecified United States locations (Earnhart et al., 2005; Anderson & Norris, 2006; Schulte-Spechtel et al., 2006; Attie et al., 2007; Haninkova et al., 2008; Margos et al., 2008; Ogden et al., 2008; Qiu et al., 2008; Girard et al., 2009; Travinsky et al., 2010; Chan et al., 2012; Margos et al., 2012; Stone et al., 2015).

Discussion

We sequenced the complete *ospC* gene of *B. burgdorferi* from 71 DNA samples extracted from *Ixodes scapularis* ticks collected from across the Upper Susquehanna River Basin during

2015 and 2016. The *ospC* genotype coinfection rate of 21.1% in these samples is consistent with the reported coinfection rates of 24-25% in tick populations of the Northeast region (Anderson & Norris, 2006; Barbour & Travinsky, 2010). The Upper Susquehanna River Basin ticks represent 12 of the 17 *ospC* genotypes previously reported from ticks collected in the Northeast region. Similar to other studied areas in the Northeast, A and K are among the three most common *ospC* genotypes in the infected tick populations of the Upper Susquehanna River Basin in New York. However, the Upper Susquehanna River Basin sample is different from other regions of the Northeast that have been previously studied (Connecticut, Massachusetts, Rhode Island, Pennsylvania, New Jersey and Maryland) in two ways: a significantly higher prevalence ($p < 0.05$) of the I genotype (20%) compared to the rest of the Northeast (0-8.1%), and an absence of the B genotype, which has been reported in 10-13.5% of infected tick populations in the aforementioned areas of the Northeast (Anderson & Norris, 2006; Barbour & Travinsky, 2010; Qiu et al., 2008). While the observed difference may be attributed to the relatively small sample size, the high frequency of I and the absence of B in this study suggests major differences in the genetic makeup of *B. burgdorferi* present between the Upper Susquehanna Basin and other studied regions of the Northeastern United States.

This study's phylogenetic analysis (Figure 4) demonstrates that the *ospC* strains from the Upper Susquehanna River Basin mainly cluster with strains from the Northeastern United States and Canada, and to a lesser extent to strains from other regions of the United States. Surprisingly, there is no clear geographic pattern to the distribution of *ospC* major groups, with some represented by *B. burgdorferi* samples reported from North America, Europe, and Asia. Such differences would primarily reflect different dynamics of blacklegged tick population expansion,

and differences in the origin of infected ticks introduced to the region, *e.g.,* by migratory birds. Further research can improve our understanding of *B. burgdorferi's* genetic variation in the United States, especially the prevalence of invasive *ospC* genotypes.

Despite Lyme disease being the most common zoonotic disease in the United States, an effective vaccine is yet to be developed. Growing research in Lyme disease immunization suggests that *ospC* may be a good candidate as a vaccine component. A study found dimeric *ospC* protein to be very effective in providing protection against Lyme disease because it exhibits epitopes that stimulate the production of antibodies (Edmondson et al., 2016).With several invasive variants of *ospC*, it is worth noting that strain-specific vaccines in different regions of the United States as well as worldwide can be more effective than a broad universal vaccine with only one or few *ospC* variants, which may be effective in only a few parts of the world. This is corroborated by a study by Khatchikian et al. (2015) which concludes that strain-specific vaccines against *B. burgdorferi* can lead to a greater public health significance. Once the most common *ospC* genotypes are known, scientists may be able to tailor a vaccine to more prevalent strains which may effectively protect more people from being infected.

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