

GEOGRAPHIC DISTRIBUTION OF LYME
BORRELIOSIS IN NORTH AMERICA

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Abstract:

The research presented in this dissertation was conducted using canine serology as a tool to further the characterization of Lyme endemic regions of North America. In chapter 3, targeted tick testing in southwestern Virginia was implemented to validate canine serology showing an expansion of the Lyme endemic region from the north. All 364 ticks were morphologically and molecularly identified as *Ixodes scapularis*, and 33% tested positive for *Borrelia burgdorferi* sensu stricto by PCR. Canine serology was again utilized (Chapter 4) to describe environments where dogs, and thus humans, were most likely to be exposed to *B. burgdorferi* around the New York City Metropolitan Area. In this study environmental and social variables were organized by county and compared to prevalence of positive canine serologic tests and human case reports. The data showed that human case reports and canine antibody prevalence increased, radiating outward from areas of dense development in a manner that corresponded with higher percent forested areas. When the environmental factors were further probed, a more complete description of the habitat, which represented a higher risk of infection, corresponded more closely to canine serology than human case reports. The third study (Chapter 5) was a serosurvey of common vector-borne disease agents of dogs across Canada, namely *Borrelia burgdorferi*, *Dirofilaria immitis*, *Anaplasma* spp., and *Ehrlichia* spp. Prevalence of antibodies to *B. burgdorferi* across all samples tested was 2.5% while the other vector-borne agents had a positive prevalence less than 0.5%. The serologic prevalence of antibodies to *B. burgdorferi* in several provinces, including Nova Scotia, New Brunswick, southern Quebec, and eastern Ontario, were similar to those seen in Lyme endemic areas of the US, reaffirming the disease's endemicity in these regions of Canada. In summary, canine serology is a useful tool for mapping endemic areas of Lyme borreliosis, documenting the expansion of the endemic range, and describing the environments that support the greatest risk of infection to humans and dogs.

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CHAPTER I

INTRODUCTION

Lyme borreliosis (LB) is the most commonly diagnosed vector-borne disease of people in the United States with around 30,000 cases reported in humans each year (CDC, 2015). *Borrelia burgdorferi*, the causative agent of LB in the US, was first discovered in the Northeast by Dr. Willy Burgdorfer, but since then the identified range of both the tick vector and pathogen has expanded (Hayes et al., 1983; Ogden et al., 2006). The bacteria are transmitted by *Ixodes scapularis* in the Upper Midwest and Northeast regions of the US and southern Canada, and by *I. pacificus* on the West Coast (Diuk-Wasser et al., 2012; Lane et al., 1998). Based on existing survey data of dogs and case reports in people, the Northeast and Upper Midwest regions of the US represent an increased risk for infection with *B. burgdorferi* sensu stricto in comparison to the rest of the United States (Little et al., 2014; CDC, 2015).

Accurate diagnosis of LB can be challenging. A classic erythema migrans, or bulls-eye rash, is seen in most, but not all, human patients during acute infection (Nadelman, 2015). When infection persists and spirochetes disseminate, arthralgia, carditis, or neurologic disease may develop. Laboratory confirmation of LB in humans requires two-tiered serologic testing that can complicate clinical diagnosis (Marques, 2010). Because of the diagnostic complexities, accurate mapping of

the risk of infection based on human case reports alone is challenging, therefore active and passive surveillance for the pathogen in wildlife, ticks, and domestic animals is key to determining the endemic range (Duncan et al., 2005; Ostfeld et al., 2006; Werden et al., 2014).

Due to recent changes in climate and habitat, the range of the *I. scapularis* / *B. burgdorferi* maintenance system has spread, resulting in expansion of the historic endemic foci in the Upper Midwest and northeastern United States (Diuk-Wasser et al., 2012; Hamer et al., 2009; Ogden et al., 2006). A major driver of this expansion is the overall increase in temperatures, which has facilitated the incursion of ticks harboring the pathogen into Canada (Ogden et al., 2014). In addition to climate, habitat factors play a key role in not only supporting the tick populations, but also supporting the wildlife reservoirs for the bacteria and the ticks. In general, an increase in the density of green plants on a patch of land, especially deciduous (oak and maple) forests, correlates with higher *I. scapularis* density (Ogden et al., 2006; Guerra et al., 2002). These forested areas provide suitable habitat for the rodents and small mammals that immature *I. scapularis* prefer and white-tailed deer that support the adult ticks (Brunner et al., 2008; Kilpatrick et al., 2014). Since forested regions, rodents, white-tailed deer, and even *I. scapularis* are found in a much broader range than that of Lyme disease, there appear to be other factors that promote or hinder the maintenance of *Borrelia burgdorferi* in nature (IUCN, 2008; Nelder et al., 2016). The phenology of *I. scapularis* in the southern United States appears to be different than that of this species in the Northeast and Upper Midwest. These two populations have different host-seeking behavior, feeding preferences, and duration of attachment to hosts, and therefore, while ticks from both regions have been shown to be competent vectors, in nature, only the northern populations

are directly associated with cases of LB in humans and dogs (Oliver et al., 1993; Goddard 1992; Arsnoe et al., 2015).

Identifying ideal habitats for maintenance of the pathogen in nature allows for prediction of risk of infection within a region. Furthermore, canine serology may also provide valuable information on endemic range and high-risk habitats (Guerra et al., 2001; Duncan et al., 2005). Similar to all vector-borne pathogens, *B. burgdorferi* transmission and maintenance requires a complex assortment of interconnected environmental, climatic, and physiologic variables to be efficient (Ogden et al., 2013). Identification of these factors should allow prediction of the expansion of areas where LB is endemic and provide an opportunity to prevent future infections.

The proposed research uses canine seroprevalence to *B. burgdorferi*, along with targeted tick collection and testing, to define the range of LB in North America. Ultimately, this knowledge of the expanding endemic range will allow for more accurate diagnosis of cases of LB and describe environments that may be able to support this maintenance cycle in the future.

To better define and describe the areas where autochthonous transmission of *Borrelia burgdorferi* sensu stricto is documented to occur in North America, a series of studies were undertaken:

1) Identify *Borrelia burgdorferi* sensu stricto in resident populations of *Ixodes scapularis* in a historically non-endemic but suspected newly emergent area for Lyme borreliosis, southwest Virginia.

Based on canine serology reported over several years, it appeared the endemic range for *Borrelia burgdorferi* was expanding into southern Virginia along the Appalachian Mountains (Bowman et al., 2009; Little et al., 2014). In our study, ticks were collected from October 2012 to April 2013 by standard drag techniques from three wooded park or residential sites and subsequently tested for *B. burgdorferi* and *Anaplasma phagocytophilum*. Both tick identification and infection status were confirmed by PCR and sequencing of their respective DNA products. A total of 364 adult ticks were collected, all of which were morphologically and molecularly identified as *Ixodes scapularis*. The presence of *B. burgdorferi* sensu stricto was molecularly confirmed in 117/356 (33%) ticks tested, while only 3/364 (0.8%) ticks tested positive for *A. phagocytophilum*. This study represented the first description of *I. scapularis* along this portion of the Appalachian Region as well as documenting the southern expansion of the northern clade of *I. scapularis* (Oliver et al., 1988). These data also show that the pathogen prevalence of *B. burgdorferi* and *A. phagocytophilum* are similar to areas previously considered to be endemic for Lyme disease, further pointing to the expansion of vector and pathogen range from the Northeast.

2) Determine the environmental and social variables associated with infection with *Borrelia burgdorferi* in humans and dogs in the New York City Metropolitan Statistical Area.

Dogs have been shown to be sentinels for infection with tick-borne diseases, including *Borrelia burgdorferi* in endemic areas of the United States (Guerra et al., 2001; Mead et al., 2011; Duncan et al., 2005). In this study, the New York City Metropolitan Statistical Area (NYC MSA), a region of varying habitat and demographic diversity, was selected to determine if canine serology, along with environmental and social factors, could

identify areas where humans are at risk for infection with *B. burgdorferi*. Percent positive canine test results for *B. burgdorferi*, human case reports of LB, and social and environmental factors were all collected at a county level, and the variables were compared with canine serology and human case reports first by categorical analysis and then by multiple regression. The categorical analysis revealed a gradient of increasing prevalence radiating outwards corresponding to an increase in forested area, with the highest prevalence being 21.1%. The environmental risk factors derived from the regressions provided an accurate description of areas where dogs are at risk of infection in the area ($R^2=0.90$), but was less accurate at describing environments that correlate with human case reports ($R^2=0.74$). These data confirmed that, in endemic areas, canine serology can be utilized to describe areas where infection with *B. burgdorferi* is most likely to occur.

3) Define the extent of Lyme borreliosis expansion into Canada using serologic detection of antibodies to *Borrelia burgdorferi* in dogs

Surveillance of several common vector-borne infections in dogs has been a useful tool for monitoring changes in prevalence in the United States over the past 10 years (Bowman et al., 2009; Little et al., 2014). Veterinarians throughout Canada have begun annual screening of canine patients for antibodies to *Ehrlichia* spp., *B. burgdorferi* and *Anaplasma* spp., and to *Dirofilaria immitis* antigen, and the percent positive canine test results have been organized into a national reporting system maintained by IDEXX Laboratories, Inc. (Westbrook, ME) from 2012-2014. Test results were grouped by three-digit Canadian postal code or by major city of the veterinary hospital submitting the sample. The national prevalence of antibodies to *B. burgdorferi* was 2.5% (2,844/1115,636), while

none of the other three test analytes yielded a prevalence for past or current infection with the corresponding disease agent above 0.5%. In addition, areas of endemic or hyperendemic LB was described in Nova Scotia (15.7%), eastern Ontario (5.1%), New Brunswick (3.7%), and Quebec (2.8%). Continued surveillance of these vector-borne infections throughout Canada will be important as climate, vector range, and habitat continue to change.

SUMMARY

These studies show the utility of large scale surveillance for common vector-borne disease agents by documenting the expansion of the endemic range of LB in dogs, correlating environmental risk factors with canine serology, and documenting the incursion and endemnicity of *B. burgdorferi* in Canada. Taken together these studies provide a better description of the endemic area of LB in North America as well as the environmental factors that are key to maintaining the disease in nature. First, the population of northern clade *I. scapularis* appears to be expanding southward along the Appalachian Mountains as well as northward into Canada. Additionally, our data show that canine serology to *B. burgdorferi* is better represented by environmental risk factors than human case reports of LB. Lastly, our description of areas of endemic LB in Canada provides a base for environmental and temporal studies to describe the expansion of the maintenance system for *B. burgdorferi* north of its historic range.

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CHAPTER II

REVIEW OF LITERATURE

BORRELIA SPP. IN NORTH AMERICA

The taxonomic family *Spirochaetaceae* contains six genera of spirochetes, including *Borrelia* and *Treponema*. *Borrelia* spp. are gram-negative, spiral-curved bacteria with a unique double stranded linear chromosome approximately 900kbp and multiple linear and circular plasmids (Groshong et al., 2014). The genus contains 36 species, three of which are considered to be the causative agents for Lyme borreliosis (LB) in different parts of the world, *B. burgdorferi* sensu stricto (hereafter referred to as *B. burgdorferi*) in North America, and *B. afzelii* and *B. garinii* in Europe and Asia (Rudenko et al., 2011). These three species together with phylogenetically related, low- or non-pathogenic species are grouped together under the term *Borrelia burgdorferi* sensu lato (s.l.) complex which, in North America, also includes *B. americana*, *B. andersonii*, *B. bissetii*, *B. californiensis*, *B. carolinensis*, *B. kurtenbachii*, and Genomospecies 2 (Rudenko et al., 2011). *B. burgdorferi* s.s. is considered to be the only causative agent of LB in North America, and like the *B. burgdorferi* s.l. complex, is vectored by *Ixodes* sp. ticks. *Ixodes scapularis* is the main vector for LB in midwestern and northeastern United States and southeast Canada, while *I. pacificus* is the

predominant vector along the West Coast. Using multilocus sequence typing (MLST), *B. burgdorferi* sensu stricto has been shown to have three genetically divergent populations corresponding with the tick populations in the Northeast, Upper Midwest, and West Coast, respectively.

A second large grouping of *Borrelia* spp. includes those known as or related to the relapsing fever agents, which include *B. hermsii*, *B. miyamotoi*, *B. parkeri*, *B. lonestari*, and *B. turicatae*. These species are vectored by a wide variety of hard and soft ticks throughout the US and are considered to be distinct from the *B. burgdorferi* sensu lato complex although one of them, *B. miyamotoi*, is transmitted to people by the same *I. scapularis* vector tick. *Borrelia hermsii*, *B. turicatae*, and *B. lonestari* infections have been confirmed in dogs by blood film examination or PCR of whole blood (Whitney et al., 2007; Kelly et al., 2014; Fryxell et al., 2012). One of the main clinical differences between these relapsing fever agents and *B. burgdorferi* s.s. is the ability to detect the spirochetes by microscopy in blood samples during febrile episodes (Dworkin et al., 2008). In addition to this group, a newly described *Borrelia* sp., *candidatus B. mayonii*, has been detected in humans by PCR of whole blood or synovial fluid and visualization on blood smear for one patient (Pritt et al., 2016). While the detection of spirochetes in blood is only associated with relapsing fever *Borrelia* spp., the newly described *B. mayonii* is most genetically similar to other sensu lato *Borrelia* spp. such as *B. kurtenbachii*, *B. carolinensis*, and *B. bissettii* (Pritt et al., 2016). Future efforts to identify additional novel *Borrelia* spp. are warranted.

IXODES SPP. IN NORTH AMERICA

The taxonomic family *Ixodidae* consists of all the hard ticks, which in North America include the genera *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Ixodes*, and *Rhipicephalus*. The genus *Ixodes* contains 246 species, approximately 30 of which are found in North America. Historically, what is currently called *Ixodes scapularis* was separated into two distinct species, *I. scapularis* Say and *I. dammini* (Jackson et al., 1970; Spielman et al., 1979). Since then, the two species have been redescribed as a single species, *I. scapularis*. This consolidation was demonstrated using both interbreeding and molecular techniques (Oliver Jr et al., 1993; Wesson et al., 1993). By comparing sequences of the internal transcribed spacer (ITS) region of the gene for the 18S and 28S ribosomal subunits, Oliver et al. showed that there was more variation within populations of *I. scapularis* from the southern United States than between populations of *I. scapularis* and *I. dammini* (Wesson et al., 1993). Both molecular confirmation and successful interbreeding determined that genetically homogenous *I. scapularis* populations in the North were geographically isolated from the more genetically heterogeneous *I. scapularis* population in the South. Similar studies were also conducted with *I. pacificus*, with significant genetic difference identified between *I. pacificus* and either population of *I. scapularis*; in addition, the F1 progeny of crossbreeding experiments between *I. scapularis* and *I. pacificus* were sterile (Wesson et al., 1993; Oliver Jr et al., 1993). Therefore, at present, two distinct species are considered the main vectors for *B. burgdorferi* in the United States: *I. pacificus* on the West Coast and *I. scapularis* in the East.

Two distinct clades of *I. scapularis* have been described: the northern, American Clade and the Southern Clade. These clades remain genetically and phenotypically somewhat different although they constitute a single species. Populations of *I. scapularis* have been identified throughout the eastern US using 16S mitochondrial rDNA sequences. Current understanding of these two clades suggests that a few ticks from the original population became isolated in the Northeast, perhaps due to massive deforestation and unregulated hunting, leading to a historic decline of *I. scapularis* and subsequent homogeny (Quiet et al., 2002; Hoen et al., 2009). Regardless, the northern colony became the American Clade and served as the expanding group into the upper midwestern US and Canada (Humphrey et al., 2010, Ogden et al., 2013). Most recently, the expansion of the American Clade farther southward into Virginia was documented suggesting an actively expanding population from the Northeast, which may increase the risk of transmission of *B. burgdorferi* in these newly colonized areas (Brinkerhoff et al., 2014). The genetic differences between the clades become important in assessing the host-seeking behaviors of each population (described later) and the overall risk of transmission of *B. burgdorferi* to humans and animals.

INFECTION WITH *BORRELIA BURGDORFERI* AND DISEASE

MANIFESTATIONS

Lyme borreliosis (LB) caused by *Borrelia burgdorferi* is the most commonly diagnosed vector-borne infection in humans in the United States (Bacon et al., 2008). Infection is transmitted to the host through the bite of an infected tick (Hayes et al., 1983). In humans, a classic bulls-eye target rash, termed an erythema migrans, appears at

the site of tick attachment within 7–14 days. While this rash is clinically recognized in approximately 70% of human patients, veterinary patients infected with *B. burgdorferi* are not known to develop the lesion (Nadelman, 2015; Marques, 2010). The early clinical signs seen in humans and dogs include fever, lethargy, myalgia, and arthralgia, which can progress to more severe disease when left untreated.

If diagnosed in the acute phase, LB can be treated, but persistent damage may be caused by the migrating spirochetes, resulting in long-term damage that can be less responsive to antibiotics (Wormser et al., 2006). In disseminated infections, the spirochetes colonize a variety of tissues resulting in arthritis, carditis, or neurologic problems depending on location (Marques, 2010). In dogs, the most serious form of LB is a glomerulonephritis that results in a protein-losing nephropathy; this presentation is seen in <1% of dogs that test positive for antibodies to *B. burgdorferi* (Dambach et al., 1997; Littman, 2013). While the exact mechanism of *B. burgdorferi*-induced canine glomerulonephritis is not known, it is speculated to be related to antigen-antibody complex deposition in the glomerulus rather than tissue invasion of the spirochete (Hutton et al., 2008; Littman, 2013).

Of the other *Borrelia* spp. that make up the *B. burgdorferi* s.l. complex in the US, only *B. bissettii* and *B. kurtenbachii* are confirmed to be pathogenic to humans (Rudenko et al., 2011; Clark et al., 2014; Margos et al., 2010). Within the relapsing fever group, *B. miyamotoi* is the most recognized to cause disease in humans (Krause, et al., 2014). Infection with *B. miyamotoi* leads to similar presenting signs as classic LB including unexplained fever, headache and myalgia with or without skin rash (Lee et al., 2014). These non-specific signs in combination with the fact that *B. miyamotoi* is transmitted by

I. scapularis in Lyme disease-endemic areas make distinguishing the two infections difficult. A related spirochete, *B. lonestari*, has been implicated in relapsing fever-like disease manifestations in the South, but definitive proof of human infection has yet to be confirmed (Stromdahl et al., 2003). The wide variety of *Borrelia* spp. found worldwide and the non-specific disease manifestations induced in many patients make LB a challenging diagnosis for physicians, even in endemic areas.

Treatment

Like many tick-borne pathogens, *B. burgdorferi* can be readily treated with antibiotics if treatment is initiated promptly. For both humans and dogs, the antibiotic of choice is doxycycline. In humans, the most commonly recommended dosage is 100mg orally, twice a day for 10 days, but many doctors recommend a 14, 21, or 28-day course of antibiotics to prevent any long-term infections (Sanchez et al., 2016; Sanchez, 2015). Other effective antibiotics include amoxicillin (500mg; 3x/day; 14 days), and cefuroxime axetil (500mg; 2x/day; 14–21 days) is used for patients unable to tolerate beta-lactam or tetracycline class drugs (Sanchez et al., 2016; Little et al., 2010). In dogs, most veterinarians reserve treatment for patients exhibiting clinical signs of LB. The drugs of choice in dogs are similar to humans, but the doses are scaled to patient size. Doxycycline at 10mg/kg twice per day or amoxicillin at 20mg/kg thrice per day for 30 days are the most common treatment recommendations (Little et al., 2010; Krupka et al., 2010). If the patient does not tolerate tetracycline or beta-lactam antibiotics, azithromycin at 25mg/kg once per day for 14 days can also be effective (Krupka et al., 2010). Patients exhibiting lameness or arthritic disorders may benefit from nonsteroidal anti-

inflammatory drugs, but glucocorticoids should only be given in low doses that are not immunosuppressive.

DIAGNOSIS OF LYME BORRELIOSIS

Humans

Because symptoms of early infection are largely non-specific and flu-like, confirming a diagnosis of Lyme borreliosis (LB) may take several weeks to months. In endemic areas, the erythema migrans (EM) is considered to be diagnostic and an indication for treatment. Other available diagnostic tests include serology, polymerase chain reaction (PCR) for detection of DNA from *B. burgdorferi* within the infected tissues, and culture of the spirochetes from infected tissues (Nowakowski et al., 2001). A two tier serologic approach is currently recommended and consists of a sensitive enzyme immunoassay (EIA) followed by immunoblotting of samples that are positive or indeterminate in the first step (Sanchez et al., 2016). A PCR assay can be performed on any tissue samples, but EM or synovial fluid samples provide the most sensitive results. However, a negative PCR result does not rule out infection (Borchers et al., 2015). Culture of *Borrelia* sp. from tissues, including synovial fluid, EM, and cerebrospinal fluid, can also be attempted, but is often unrewarding (Nowakowski et al., 2001).

Veterinary Species

Diagnosis of LB in veterinary patients is predominantly limited to serology because EM is not a common finding, PCR is largely unrewarding, and culture for *B. burgdorferi* is rarely performed other than in research laboratories. Two commonly used serologic tests for detection of antibodies in dogs include the SNAP® 4Dx® Plus Test kit

(IDEXX Laboratories, Westbrook, ME) and the AccuPlex™4© (ANTECH Diagnostics, Irvine, CA); other diagnostic labs offer similar tests. In dogs and horses, a multiplex assay similar to the AccuPlex™4© test is also used in attempt to identify early or late infection by detecting antibodies to three separate outer surface proteins, OspA, OspC, and OspF (Cornell University College of Veterinary Medicine Animal Health Diagnostic Center, Ithaca, NY). The SNAP® 4Dx® Plus Test kit detects antibodies to a single specific antigen, the C6 peptide based on the VlsE lipoprotein of *B. burgdorferi*, while the AccuPlex™4© test detects antibodies to multiple antigens that are expressed in early infection, late infection, or after vaccination for *B. burgdorferi* (Levy, 2002; Stillman et al., 2014; Moroff et al., 2015). Follow-up quantitative tests are also available to confirm diagnosis in these patients.

Limitations

There are limitations with the diagnostic routes taken in both humans and veterinary patients. In humans, the most common limitation is a delay in diagnosis, as many of the early, presenting signs are non-specific, especially in those patients that do not present with an EM or tick attachment is not seen (Marques, 2010). The second limitation is the highly cross-reactive nature of serologic tests for *Borrelia* spp., which is not limited to the *B. burgdorferi* sensu lato complex, but may also be cross-reactive with relapsing fever *Borrelia* spp. (Lane et al., 1990). Since many of these borreliae have unknown pathogenicity, these positive results may confound or delay the real diagnosis. There is also significant variance between commercial assays and even between medical laboratories, with 55% of reference laboratories failing to confirm previously seropositive patient samples (Theel, 2016).

Common canine diagnostic platforms have limitations as well. While rising titers can suggest active infection, a positive result on a serologic test, such as the SNAP® 4Dx® Plus Test, does not necessarily indicate a current infection with *B. burgdorferi*. Since antibodies to *B. burgdorferi* have been shown to persist in the host for 1–3 years after effective treatment, a positive result indicates either current or previous infection and can only be accurately interpreted in light of the clinical signs (Krupka et al., 2010). There is considerable debate between the two corporate, canine serologic tests in regards to vaccination history. While the SNAP® 4Dx® Plus Test does not react with antibodies produced due to vaccination, the AccuPlex™4© test reportedly does, although all vaccinated dogs do not test positive on this assay (Levy, 2002). Each approach may have benefits and limitations, and the results should only be interpreted with vaccination history and clinical signs in mind.

Further diagnostic methods, such as PCR and culture, can provide definitive diagnosis that *B. burgdorferi* s.s. is present in the tissues, but a negative test result does not rule out LB as only small portions of tissues are used for testing and the spirochetes migrate throughout infection (Marques, 2010).

MAINTENANCE OF LYME BORRELIOSIS IN NATURE

Ticks

Maintenance of *B. burgdorferi* in nature is affected by many factors including the presence of reservoir hosts, habitat suitability, and climate; key characteristics that support the principal vector in eastern North America, *I. scapularis*. Larval *I. scapularis* emerge from the egg uninfected and acquire *B. burgdorferi* from infected reservoir hosts,

then maintain the infection transtadially with each molt (Patrican, 1997; Barbour and Fish, 1993). The larval and nymphal stages are responsible for maintaining the enzootic cycle of *B. burgdorferi*. For the system to continue within the two-year life cycle of the tick, the larvae must feed after infected nymphs have fed on a reservoir host (Kurtenbach et al., 2006). The timing of these feedings is key to maintaining the bacteria within the reservoirs and is discussed in further detail within the “Climate” section. This phenologic timing is driven primarily by climate, and, together with host preferences and questing behavior, likely plays a role in the inability of *I. scapularis* to maintain *B. burgdorferi* effectively in the South (Oliver, 1996).

Besides the climatic and environmental differences between the Northeast and the South, there are genetic differences between two distinct populations of ticks in these areas that have been described in the “*Ixodes* spp. of North America” section. Oliver et al. showed these two populations, previously *I. dammini* in the North and *I. scapularis* in the South, are in fact one species now termed *I. scapularis*, but still fall into two separate clades, the northern, American clade and the Southern clade (Oliver et al., 1993; Norris et al., 1996). These two clades appear to differ in host-seeking behavior, feeding preferences, and duration of attachment to different hosts, yet both are documented to be competent vectors of *B. burgdorferi* (Oliver et al., 1993; Goddard 1992). These differences in behavior directly impact the maintenance of *B. burgdorferi* in vertebrate reservoirs and the risk of exposure to humans and dogs. First, questing habits are significantly different between northern and southern *I. scapularis*, as indicated by the ability of researchers to flag/drag the nymphal ticks in nature. This common practice is used to estimate nymphal density and is effective at collecting significant numbers of *I.*

scapularis nymphs in the Northeast but does not provide large numbers of questing nymphs in the South, despite the nymphs being found on vertebrates commonly (Diuk-Wasser et al., 2006). Immature *I. scapularis* also use different vertebrate reservoirs for their primary feeding, which in turn alter the ability to maintain *B. burgdorferi* in the wild. In the Northeast, immature *I. scapularis* feed largely on rodents and other small mammals, whereas in the South reptiles and birds fill this role more commonly (Arsnoe et al., 2015; Kollars et al., 1999). Finally, nymphal ticks are thought to be the major vector implicated in the majority of cases of Lyme borreliosis (LB) within the Northeast, but are not reported to be a major tick parasitizing humans in the South, perhaps due to questing habits in the region (Arsnoe et al., 2015). Overall, the phenology and host-seeking behaviors of the Southern Clade *I. scapularis* perpetuates an inefficient maintenance cycle that prevents LB from becoming endemic in the South.

Similarly, the primary vector for *B. burgdorferi* on the West Coast, *I. pacificus*, has different feeding patterns than that of northern *I. scapularis*, commonly feeding on small lizards (Lane, et al., 1998; Eisen, et al., 2004). While *I. pacificus* is the vector considered to be primarily responsible for transmission of the bacteria to humans and dogs, some have suggested *I. spinipalpis* plays a role in maintaining the bacteria in reservoir rodents (Brown et al., 2006). This transmission cycle needs to be revisited as early infection and maintenance studies were performed using monoclonal antibodies that have since been shown to be cross-reactive with other *Borrelia* spp. (Shoberg et al., 1993; Bretz et al., 2000). The feeding habits of *I. pacificus* do appear to be key in limiting the expansion of the endemic area on the West Coast and minimizing the risk of infection to humans. The reliance on a vector for transmission and maintenance makes the tick a key

component to the expansion of the areas where LB is endemic as well as the risk of infection in endemic areas.

Reservoir Hosts

Animal hosts are required for maintenance of both the ticks and the spirochetes in nature, but the type, density, and diversity of animals in a region can significantly affect the prevalence of *B. burgdorferi* in a population of ticks and thus change the risk of LB in that region. American Clade *Ixodes scapularis* larvae and nymphs feed predominantly on rodents and other small mammals, but are generalists that will opportunistically feed on a wide variety of animals such as reptiles and larger mammals, including humans (Brunner et al., 2008). The major reproductive host for the adult tick is the white-tailed deer, *Odocoileus virginianus*, but again the adults can be found on a wide variety of hosts, especially if deer populations are significantly altered (Lane et al., 1991; Kilpatrick et al., 2014). Historically, the principal reservoir host for *B. burgdorferi* was thought to be the white-footed mouse, *Peromyscus leucopus*, but more recent research has shown other hosts could be more important. Although >85% of ticks fed on infected white-footed mice will acquire the pathogen, only 10% of the immature ticks collected from wildlife are from this host (Brisson et al., 2008). Both reservoir competency and tick carrying capacity ultimately determine if an animal is a suitable reservoir. When tick carrying capacity is considered, some estimates suggest *P. leucopus* is likely responsible for only 25% of the infected nymphal ticks in a particular region (Brisson et al., 2008; Ogden and Tsao, 2009). Other important vertebrate reservoirs include the masked shrew (*Sorex cinereus*), short-tailed shrew (*Blarina brevicauda*), eastern chipmunk (*Tamias striatus*),

and gray squirrel (*Sciurus carolinensis*). These 4 reservoirs all have a reservoir competence greater than 15% and serve as a more likely host for the immature ticks, which in turn allows them to infect more ticks (Brisson et al., 2004; Brisson et al., 2008). Other low competence reservoirs include the raccoon (*Procyon lotor*), Virginia opossum (*Didelphis virginiana*), striped skunk (*Mephitis mephitis*), white-tailed deer (*Odocoileus virginianus*), and various bird species, which can serve as a host to large numbers of immature ticks but are very poor at infecting naïve ticks, and therefore are not considered important reservoirs (LoGuidice et al., 2008). Small mammal reservoirs appear to infect a larger percent of ticks with higher spirochete numbers, and therefore those hosts with the most ticks constitute the primary reservoirs for the bacteria in the wild (Barbour et al., 2015).

Although in a healthy, diverse habitat, the white-footed mouse may only play a minor role in maintaining *B. burgdorferi*, in disturbed habitats this host becomes much more important. In areas where less competent reservoir hosts actively compete with *P. leucopus* or a large portion of ticks do not feed on *P. leucopus*, vertebrate host biodiversity appears to decrease the prevalence of bacteria in the feeding ticks. In contrast, with a stable white-footed mouse population, increasing the number of animals on which ticks can feed may ultimately increase the number of ticks infected thus increasing the risk of LB in that area (Brisson et al., 2008; Bouchard et al., 2013; Ogden and Tsao 2009). In the southern US and along the West Coast, larval and nymphal ticks commonly feed on lizards, a preference commonly thought to play a role in the dearth of the disease in these areas (Durden et al., 2002; Lane et al., 1998). Because of the

generalist behavior of *I. scapularis*, controlling a single reservoir is considered to be unlikely to reduce pathogen prevalence in ticks.

Birds also play a key role in maintaining populations of *I. scapularis* as well as providing a route by which the ticks can move large distances. Birds often serve as a host for immature ticks, including *I. scapularis*. While the immature ticks readily feed on birds in general, no bird species has been shown to be a particularly efficient reservoir for *B. burgdorferi*, with a reservoir competence around 10% (Brisson et al., 2008). The major impact bird hosts have is in the dispersal of ticks throughout migration routes. This migration of ticks on birds leads to the collection of a few, adventitious ticks of varying life stages in unexpected locations, but over time these small populations can become established. Migratory birds have been shown to aid in the expansion of the range of *I. scapularis* in both the midwestern United States and Canada (Schneider et al., 2015; Ogden et al., 2008). Currently, the role of birds in expansion is much clearer in Canada, as populations of *I. scapularis* throughout the country are geographically isolated from one another (Ogden et al., 2008). Due to the continuous migration of birds, adventitious ticks are found routinely. The Canadian Consensus Conference on Lyme Disease considers an area endemic only when all three feeding stages of the tick are found on an animal or in the environment for at least 2 consecutive years (Laboratory Centre for Disease control, 1991).

Habitat

Because *B. burgdorferi* spends no time outside of either a reservoir host or vector, environmental conditions favoring the maintenance of the bacteria in nature are focused on those conditions that support tick and host populations. Soil type is one key factor for

tick survival as the ticks over-winter in the topsoil. The extremes of very sandy soil and hard clay are both poor habitats for tick survival since they do not hold enough water or hold too much water, respectively (Guerra et al., 2002). The presence of dry or wet, silty loam has been shown to support significant populations of *I. scapularis* (Glass et al., 1994; Ogden, et al., 2006). This soil type can also support a healthy forest habitat, which is similarly important to tick survival (Guerra et al., 2002). In general, an increase in normalized difference vegetative index (NDVI), the density of green on a patch of land, correlates with an increase in *I. scapularis* density, but some specific forest types allow for better tick survival than others (Ogden et al., 2006). Deciduous forest, supported by oak or maple trees, have been shown to be best for supporting populations of *I. scapularis* as the leaf litter provides refuge for the ticks from the elements (Guerra et al., 2002; Ogden et al., 2006). In contrast, forested areas that consist mainly coniferous, evergreen trees do not provide an ideal habitat for ticks (Guerra et al., 2002). The risk for LB in a given area can be associated with subtle changes in the structure of the forested area. The risk for LB is highest when the suitable habitats interface with human or canine contact, which are most likely to be forest edge habitats such as trails (Brownstein et al., 2005). Fragmentation not only creates more areas of possible exposure, but also supports the increase of pathogen prevalence in ticks, presumably by removing some of the larger dilutional reservoir hosts while not affecting the white-footed mouse populations, a change that would provide a bias towards immature ticks feeding on an efficient reservoir (Allan et al., 2003). Vegetation, soil, and climate are interconnected, and extremes of any factor may affect the ability of ticks to survive in nature, ultimately affecting the risk of LB in that area.

Climate

While macroclimatic variables dictate the overall distribution of LB, microclimatic variables within each specific region can also affect tick survival and pathogen prevalence, significantly altering the maintenance of the pathogen in nature. Temperature is the main macroclimatic variable that affects tick development for each stage, such that each development phase is shorter as temperature increases, despite the fact that the range for LB is generally confined to lower average temperature areas in the northeastern and upper midwestern parts of the US (Ogden et al., 2004). Questing activity is also affected by temperature. Adults quest most actively between 5–15°C, whereas nymphs become more active at 15°C, peaking at 25°C, and declining as temperature increases further (Ogden et al., 2004). Overall survival rates at different temperatures have also been examined, with both northern and southern populations of *I. scapularis* surviving longer under conditions that simulate the climate of the Northeast (Ginsberg et al., 2014). These climatic results are thought to be the basis for southern populations of *I. scapularis* questing in nature below the cover of leaf litter. A recent, overall increase in temperature correlates with an expanding population of infected *I. scapularis* in Canada (Ogden et al., 2014). On the other hand, there also appears to be expansion of the American Clade *I. scapularis* southward. *Ixodes scapularis* collected from southwest Virginia were both novel to the area and found at altitudes that were higher than expected (Brinkerhoff et al., 2014). This increase in altitude may allow the expansion of this tick population into areas that are warmer than the traditional endemic range for LB.

Changes in temperature also affect the seasonality of feeding for each of the life stages, which in turn can alter the pathogen prevalence and maintenance within the ticks.

Because spirochaetemia is short-lived in rodents, usually lasting less than two weeks, it is important that naïve larval stages of *I. scapularis* feed within a short period after the infected nymphal stages feed (Hovius et al., 2007). When infected nymphal ticks feed in spring followed by emergence and feeding of uninfected larvae in the summer, transmission and maintenance of *B. burgdorferi* in the population is most efficient (Ogden et al., 2004; Gatewood et al., 2009). On the other hand, when nymphal *I. scapularis* feed in the fall and the larvae do not feed until the following spring/summer, transmission is inefficient (Ogden et al., 2004). This latter cycle, which results in disruption of maintenance of infection due to an inverted phenology, has been observed in field studies conducted in the southeastern US where pathogen prevalence in ticks and LB incidence in humans and dogs is much lower than that of the Northeast, which has the spring nymphs/summer larvae feeding cycle (Oliver, 1996).

Microclimate within the forest is also important for tick survival and includes air temperature, soil temperature, and relative humidity. The leaf litter provided by a thriving deciduous forest can provide a more stable environment for these variables (Guerra et al., 2002). Increased tick survival has been associated with higher relative humidity, whereas a higher air temperature, soil temperature, and low humidity are associated with decreased survival (Bertrand and Wilson, 1996). Environments with stable temperatures are considered optimal (Bertrand and Wilson, 1996). Interplay between the vector, reservoirs, habitat, climate, and pathogen form a complex web necessary for maintenance of the pathogen in nature and, ultimately, transmission to people and dogs.

PREVALENCE

The prevalence of *Borrelia burgdorferi* infection in dogs, people, horses, ticks and other animals has been established in different regions of the US and Canada. The prevalence is determined by interpreting one or more of the previously discussed diagnostic methods for a specific host.

Dogs

Two large serosurveys of pet dogs were reported using data from 2001–2007 and 2010–2012, respectively; all samples were tested with patient-side ELISAs (SNAP® 3Dx®, 4Dx®, and 4Dx® Plus; IDEXX Laboratories, Inc., Westbrook, Maine) designed to detect antibodies to *B. burgdorferi sensu stricto* (Bowman et al., 2009; Little et al., 2014). In both studies, the Northeast region had the highest reported prevalence, 11.6% and 13.3%, respectively (Table 1). The regional *B. burgdorferi* antibody prevalence for the Midwest, Southeast, and West were 4.0–4.4%, 1.0%–2.5%, and 1.4% (both studies) (Table 1). The state with the highest prevalence in the earlier study was Connecticut (18.1%; 1,846/10,209) while Massachusetts had the highest prevalence of antibodies to *B. burgdorferi* in the follow-up study (18.3%; 74,429/406,493) (Bowman et al., 2009; Little et al., 2014). Another nationwide study of 6,582 dogs was conducted utilizing a novel species-specific peptide assay (SNAP® M-A, IDEXX Laboratories, Inc., Westbrook, ME). While the prevalence differed from previous studies, the Northeast remained the focus for the majority of antibody positive test results with 22.9% (122/532) of samples testing positive (Table 1) (Qurollo et al., 2014).

Local or regional serosurveys for antibody to *Borrelia* spp. in dogs have also been reported (Table 1). States with local data on antibody presence in dogs include Alabama,

California, Connecticut, Florida, Illinois, Maine, Maryland, Massachusetts, Michigan, Minnesota, New Hampshire, New Jersey, New York, North Carolina, Ohio, Oklahoma, Oregon, Pennsylvania, Rhode Island, Virginia, Washington, Wisconsin (Wright et al., 1997; Carrade et al., 2011; Magnarelli et al., 1987; Tzipory et al., 2010; Rand et al., 2011; Rand et al., 1991; Eschner et al., 2015; Duncan et al., 2005; Daniels et al., 1993; Greene et al., 1991; Lindenmayer et al., 1991; Hamer et al., 2009; Beall et al., 2008; Gaito et al., 2014; Schulze et al., 1987; Falco et al., 1993; Magnarelli et al., 1990; Greene et al., 1988; Wang et al., 2014; Mukolwe et al., 1992; Rodgers et al., 1989; Hinrichsen et al., 2001). Two local studies utilizing PCR to detect circulating spirochetes in whole blood of dogs have also been reported (Table 1) (Fryxell et al., 2012; Wagner et al., 2012).

Similar large scale and regional studies have been carried out in Canada and document canine infection with *B. burgdorferi* in every province, although some positives are thought to represent translocation of dogs from endemic areas. Provinces with the highest prevalence in each of the two national studies reported to date are Nova Scotia, Ontario, and Prince Edward Island (Table 2) (Ourolo et al., 2014; Villeneuve et al., 2011). Additional regional surveys have been conducted in British Columbia, Nova Scotia, Ontario, Prince Edward Island, Quebec, and Saskatchewan (Table 2) (Bryan et al., 2011; Banerjee et al., 1996; Bell et al., 1992; Gary et al., 2006; Artsob et al., 1993; Artsob et al., 1992; Schurer et al., 2014).

Humans

Lyme borreliosis has been a nationally notifiable disease in humans in the US since 1991 through the Center for Disease Control's National Notifiable Diseases Surveillance System (NNDSS). The disease is the most commonly reported vector-borne

infection in the United States and the fifth most common nationally notifiable disease (CDC, 2015). Despite being a commonly reported disease, LB is still only considered regionally endemic and 95% of all cases come from 14 states in the Northeast and Upper Midwest (CT, DE, ME, MD, MA, MN, NH, NJ, NY, PA, RI, VT, VA, WI) (CDC, 2015; Bacon et al., 2008). There are roughly 25,000–30,000 new cases each year nationwide, but the CDC estimates the actual number of new cases each year is closer to 300,000 due to under-reporting (CDC, 2013). The actual incidence of LB is 7.0–10.0 cases per 100,000 people nationally each year, but the incidence in endemic areas, such as Connecticut, Maine, New Hampshire, and Vermont, can range from 50.0–110.0 cases per 100,000 people (CDC, 2015). Outside of these hyperendemic ranges the typical incidence each year is less than 1.0 case per 100,000 in the South and West Coast.

The Public Health Agency of Canada first listed LB as a reportable disease in 2009. Since that time, the number of reported cases has increased from 144 in 2009 to 682 in 2013 (PHAC, 2102; PHAC 2013). The overall incidence of LB in Canada is 1.9 cases per 100,000 people, but again there are large variances depending on the region (PHAC, 2013). For instance, Nova Scotia has the highest incidence with 16.9 cases/100,000, while Alberta and Newfoundland & Labrador have no documented cases of local infection; all were associated with travel (PHAC, 2013). Even within the large provinces where the disease is considered endemic, the incidence may be misleading. Ontario and Quebec have an incidence of 2.4 cases/100,000 and 1.7 cases/100,000, yet the majority of those cases cluster in the southern Great Lakes region and near the US/Canada border (PHAC, 2012; PHAC, 2013).

Horses

Although joint, ocular, dermatologic, and neurologic manifestations have been reported in horses, comprehensive surveys of the entire United States or Canada are not available (Preist et al., 2012; Sears et al., 2012; James et al., 2010). The majority of studies are on horses that have already been deemed “Lyme suspect” by a veterinarian, which skews the data towards a higher prevalence (Burbelo et al., 2011; Magnarelli and Fikrig, 2005). Equine serosurveys in endemic regions for LB in the US and Canada where clinically normal horses have been tested for antibodies to *B. burgdorferi* document antibody prevalence of 8.0% (168/2100)–45.1% (37/82) (Bernard et al., 1990; Schwartz et al., 2015; Magnarelli et al., 2000; and Wagner et al., 2012). Recently, a random selection of horses in southwest Virginia were screened for antibodies to *B. burgdorferi* using the Lyme Accuplex Assay. Thirty-three percent (83/250) of horses tested positive for at least one outer surface protein (Osp), and of the horses that tested positive and were available for follow-up, 63% (40/63) remained positive for 5–17 months after the initial exam (Funk et al., 2016).

Cats

Although cats experimentally infected with *B. burgdorferi* developed an antibody response and displayed a variety of clinical signs, there have been no documented reports of clinical disease in a naturally infected cat (Gibson et al., 1995; Krupka et al., 2010). The serologic studies in cats are few and are limited to Lyme disease-endemic regions of the northeastern United States. The prevalence of antibodies to *B. burgdorferi* in healthy cats ranges from 14.0% (10/71) – 46.4% (39/84), while clinically ill or those infested

with ticks had a higher prevalence of antibody from 55.6% (5/9) – 70.8% (17/24) (Magnarelli et al., 2005; Magnarelli et al., 1990; Levy et al., 2003).

Ticks

Ticks have been evaluated by active or passive surveillance for the presence of *B. burgdorferi* sensu stricto (Table 4). In the last decade alone, surveys document *B. burgdorferi* in *I. scapularis* from Alabama, Arkansas, Connecticut, Georgia, Illinois, Indiana, Iowa, Kentucky, Louisiana, Maine, Maryland Massachusetts, Michigan, Minnesota, Mississippi, Missouri, New Hampshire, New Jersey, New York, North Carolina, North Dakota, Ohio, Oklahoma, Pennsylvania, Rhode Island, South Carolina, Tennessee, Texas, Utah, Vermont, Virginia, and Wisconsin (Table 3) (Diuk-Wasser et al., 2012; Fyxell et al., 2012; Feldman et al., 2015; Diuk-Wasser et al., 2014; Crowder et al., 2010; Hanincová et al., 2006; Ryzewski et al., 2011; Gatewood et al., 2009; Jobe et al., 2007; Jobe et al., 2006; Steiner et al., 2008; Lingren et al., 2005; Taft et al., 2005; Leydet Jr et al., 2014; Leydet Jr et al., 2013; MacQueen et al., 2012; Swanson et al., 2007; Giery et al., 2007; Anderson et al., 2006; Hamer et al., 2012; Hamer et al., 2010; Hamer et al., 2009; Stromdahl et al., 2014; Goltz et al., 2013; Walk et al., 2009; Schulze et al., 2013; Dolan et al., 2011; Schulze et al., 2006; Ullmann et al., 2005; Schulze et al., 2005; Hersh et al., 2014; Aliota et al., 2014; Prusinski et al., 2014; Tokarz et al., 2010; Moreno et al., 2005; Maggi et al., 2010; Russart et al., 2014; Wang et al., 2014; Hutchinson et al., 2015; Brown et al., 2015; Han et al., 2014; Connally et al., 2006; Mays et al., 2014; Rosen et al., 2012; Feria-Arroyo et al., 2014; Davis et al., 2015; Herrin et al., 2014; Brinkerhoff et al., 2014; Turtinen et al., 2015; Lee et al., 2014). In areas where *B. burgdorferi* is endemic or hyperendemic, prevalence of infection in nymphal and adult *I.*

scapularis typically ranges between 15% and 50% (Diuk-Wasser et al., 2012; Crowder et al., 2010; Gatewood et al., 2009; Steiner et al., 2008). *Borrelia burgdorferi* has also been reported in *I. pacificus* in California and Oregon (Table 3) (Padgett et al., 2014; Lane et al., 2010; Swei et al., 2011; Eisen et al., 2010; Crowder et al., 2010; Salkeld et al., 2014; Lane et al., 2013; Holden et al., 2006; Doggett et al., 2008).

In Canada, a large scale passive surveillance study of *I. scapularis* found the presence of *B. burgdorferi* in ticks in every province evaluated except British Columbia and Saskatchewan (Table 4) (Ogden et al., 2006). Since then, other regional studies using active collection of ticks have confirmed these results in the same provinces (Table 4) (Dibernardo et al., 2014; Gabriele-Rivet et al., 2015; Scott et al., 2016; Nelder et al., 2014; Scott et al., 2008; Morshed et al., 2006; Bouchard et al., 2013; Ogden et al., 2010).

EXPANDING DISTRIBUTION OF

BORRELIA BURGDORFERI SENSU STRICTO

Northeast Focus Expanding Southward

Although the principal reservoir host, *Peromyscus leucopus*, and primary vector, *I. scapularis*, are distributed throughout North America and most of the eastern US respectively, in the United States, laboratory-confirmed reports of LB are generally confined to states in the Northeast, mid-Atlantic, and upper Midwest (Diuk-Wasser et al., 2012). Infection with *B. burgdorferi* is most common in the Northeast, with the 14 most northeastern states reporting 95% of all human LB cases in the US (Nelson et al., 2015). Prevalence of exposure in dogs parallels that in humans, with the majority of dogs with detectable antibodies to *B. burgdorferi* identified in the Northeast (Bowman et al., 2009;

Little et al., 2014). Historically, the southern border of this region was considered to be coastal Maryland and northern coastal Virginia (Wormser et al., 2006). Indeed, until 2011, questing *I. scapularis* had not been reported inland towards the mountainous, southwestern regions of Virginia (Sonenshine et al., 1995; Amerasinghe et al., 1992; Oliver, 1988; Brinkerhoff et al., 2014). Models to predict the expansion of the endemic area southward initially predicted the expansion to occur along the mid-Atlantic coast, sparing the higher elevations of the Appalachian Mountains (Duik-Wasser et al., 2006; Duik-Wasser et al., 2012). However, recent field collections have shown an increase in *B. burgdorferi*-infected *I. scapularis*, human case reports of LB, and antibodies to *B. burgdorferi* in dogs in the higher elevations of southwestern Virginia (Brinkerhoff et al., 2014). The reason for the expansion of infected tick vectors into the higher elevations is currently unknown, but climate change allowing more favorable environmental conditions at such elevations is considered key to this phenomenon (Brinkerhoff et al., 2014).

Midwest Focus Expanding Outward

One of the major focal endemic areas for LB is the Upper Midwest of the United States, including Wisconsin, Minnesota, northern Illinois, and western Michigan. Interestingly, this population of ticks remains genetically distinct from those in the northeastern endemic range (Margos et al., 2012). Lyme borreliosis (LB) was first diagnosed in humans in the 1980's in a fairly circumscribed area of Wisconsin and western Michigan (Dryer et al., 1979; Godsey et al., 1987). In the early 1990's, annual case reports from the Centers for Disease Control and Prevention (CDC) identified the majority of cases in a focal area around eastern Minnesota and western Wisconsin

(Centers for Disease Control, 1991). Since that time, the area of documented endemicity has expanded in all directions. (Walker et al., 1998; Guerra et al., 2001; Hamer et al., 2009).

Starting with passive surveillance collections for *I. scapularis* in 1986, the Ohio Department of Natural Resources (ODNR) reported an average of 1.75 ticks per year from 1989 to 2008, followed by a sharp increase in numbers to 182 ticks collected in 2012 (Wang et al., 2014). This increase in ticks also correlated with an increase in infected ticks and seroprevalence in dogs and humans (Wang et al., 2014; CDC, 2015). Now that all three life stages of the tick, >5% prevalence in ticks, and 11.5% seroprevalence in dogs has been documented, Ohio can be considered a newly endemic area for LB, and it appears the northeastern and midwestern endemic regions of the United States will soon converge (Wang et al., 2014; Little et al., 2014).

The area of endemicity is also expanding westward. In a recent survey of ticks in North Dakota, adults and immature stages of *I. scapularis* were found in 6 northeastern counties, and 6% of the questing adults were positive for *B. burgdorferi* by PCR (Russart et al., 2014). This new finding is mirrored by data from southern Canada, reporting increased numbers of *I. scapularis* in southeastern Manitoba expanding westward (Ogden et al., 2010). As the environment, reservoir hosts, and climate continues to change, the Midwest range of LB is expected to expand further to suitable, forested habitats nearby (Wang et al., 2014).

West Coast

Lyme borreliosis on the West Coast has a distinct maintenance cycle compared to the eastern US. The differences affect both geographic distribution of the tick vectors and

transmission dynamics between reservoir hosts and to people and dogs. In this region, *B. burgdorferi* is transmitted to people and dogs by *I. pacificus* and the major reservoir is the dusky-footed woodrat (*Neotoma fuscipes*) (Brown et al., 1992). While *I. pacificus* is considered to be the main vector for the bacteria to humans, other tick species including *I. spinipalpis* and *I. jellisoni* may serve as the reservoirs maintaining the bacteria in nature (Eisen et al., 2003). Historically, this system has been considered to be the normal cycle, with *I. pacificus* primarily serving as a bridge vector since it is much less likely to feed on rodents (Lane et al., 1991; Lane et al., 1994). *Ixodes pacificus* commonly feed on reptiles such as the western fence lizard (*Sceloporus occidentalis*) and the southern alligator lizard (*Elgaria multicarinata*), which do not serve as highly competent reservoirs for *B. burgdorferi* and have borreliacidal factors within their blood that further decrease the likelihood of transmitting the bacteria (Brown et al., 2006). Furthermore, the other *Ixodes* spp. thought to be maintaining the sylvatic cycle of transmission are not known to feed on humans or dogs.

The areas where LB is endemic on the West Coast include the northern coastal mountain ranges extending up into Washington and Oregon and the foothills of the Sierra Nevada (Foley et al., 2007). The southernmost border of the area is the foothills of the Sierra Nevada near San Francisco, while the northern border extends into British Columbia, Canada (Foley et al., 2007). Unlike the endemic ranges for LB transmitted by *I. scapularis* in the US and Canada, which have expanded dramatically in recent years, the maintenance system in the West reliant on *I. pacificus* remains fairly stable. A lower prevalence of infection in ticks and a diverse and less competent reservoir population of animals likely limit opportunities for expansion to other suitable habitats along coastal

California (Clover et al., 1995; Eisen et al., 2004; Lane et al., 2005; Ogden et al., 2008). This situation is mirrored in southern British Columbia and suggests the distribution of LB on the West coast may remain relatively unchanged in the foreseeable future.

Incursion into Canada

Lyme borreliosis has become a growing concern in Canada over the last decade. While *I. scapularis* have been found along the northern shore of Lake Erie since the early 1970's (Watson et al., 1976), populations of blacklegged ticks were not studied in Canada until the early 1990's after *B. burgdorferi* was isolated from a tick in southern Ontario (Nelder et al., 2014). At this point, the National Microbiology Laboratory (NML) of the Public Health Agency of Canada (PHAC) and the Lyme Disease Association of Ontario started conducting passive surveys for *I. scapularis*, which yielded mainly ticks found attached to humans and pets (Ogden et al., 2006). Since the testing began, *B. burgdorferi* infected *I. scapularis* have been collected from humans and dogs in every province east of Alberta. British Columbia also has *B. burgdorferi* infected-*I. pacificus* which have apparently expanded northward from the historic focus in the western US. Reports of *I. pacificus* in western Canada have been documented since the mid 1990's but the endemic range of LB and number of cases does not appear to be increasing at the same pace as in the eastern provinces (Ogden et al., 2008). Accordingly, much of the current research has focused on the expansion of the range of *I. scapularis* and *B. burgdorferi* in southeastern Canada.

Expansion within Canada

Currently, the areas of southeastern Canada with the largest population of *I. scapularis* harboring *B. burgdorferi* are along the northern shores of Lake Ontario, Lake

Erie, and the St. Lawrence River. These areas are directly adjacent to areas within the United States that are considered endemic for LB, and most researchers in the field have concluded that the ticks were originally introduced by migrating birds (Scott et al., 2001). While initially these populations constituted small groups of adventitious ticks deposited by birds, the habitat of southeastern Canada was apparently suitable for establishing endemic foci due to the relatively mild climate and ample woodland habitat (Ogden et al., 2006; Barker and Lindsay 2000). These conditions have also allowed for the maintenance cycle for the agent of LB to expand throughout southeastern Canada, including Quebec, Ontario, New Brunswick, Nova Scotia, and southeastern Manitoba (Ogden 2006). More recent studies have documented that between 15% and 20% of adult ticks in these regions are infected with *B. burgdorferi* (Ogden et al., 2008; Ogden et al., 2014), which, while slightly lower than the infection prevalence in adult ticks in the northeastern endemic region of the United States, is similar to in the prevalence reported from tick vectors in the upper Midwest (Schultze, et al., 2005, Caporale et al., 2005).

Research documenting the patterns and processes responsible for expansion of endemic areas in Canada in the past decade has been used to generate predictive models for future distribution patterns. For large portions of southeastern Canada, the climate seems to be the limiting factor restricting distribution of both rodent reservoir hosts and tick vectors, and thus the maintenance cycle for the spirochetes. Climate change has the potential to rapidly increase the rate of expansion of the endemic LB range in Canada, shifting the primary limiting factor to habitat type. Current studies suggest, given the existing habitats and the continued trend of increasing temperatures, the high risk zone for LB in Quebec could move as much as 150 kilometers northward from its current

northern boundary ($45^{\circ} 0' 0''$ N) at a rate of 3.5 kilometers per year (Simon et al., 2014).

This trend would be expected to be seen throughout much of southeastern Canada and would likely also include a westward expansion similar to that seen in the northern US (Russart et al., 2014).

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Table 1: Reported seroprevalence of antibodies to *B. burgdorferi* in dogs from the US

State	Prevalence (positive/tested)	Reference
Alabama	0.0% (0/40) ^A	Qurollo et al., 2014
	0.7% (367/53,340) ^A	Little et al., 2014
	0.1% (27/18,998) ^A	Bowman et al., 2009
	1.7% (10/579) ^B	Wright et al., 1997
Alaska		
Arizona	6.7% (1/15) ^A	Qurollo et al., 2014
	0.8% (424/55,893) ^A	Little et al., 2014
	0.4% (4/992) ^A	Bowman et al., 2009
Arkansas	0.0% (0/36) ^A	Qurollo et al., 2014
	0.5% (220/42,776) ^A	Little et al., 2014
	4.0% (7/173) ^E	Fryxell et al., 2012
	0.1% (7/8,391) ^A	Bowman et al., 2009
California	2.5% (3/121) ^A	Qurollo et al., 2014
	1.6% (4,447/270,516) ^A	Little et al., 2014
	2.0% (25/1255) ^A	Carrade et al., 2011
	1.8% (540/29,454) ^A	Bowman et al., 2009
Colorado	4.1% (10/246) ^A	Qurollo et al., 2014
	1.0% (192/19,489) ^A	Little et al., 2014
	0.4% (49/11,557) ^A	Bowman et al., 2009
Connecticut	33.3% (16/48) ^A	Qurollo et al., 2014
	18.0% (33,071/183,787) ^A	Little et al., 2014
	18.1%(1,846/10,209) ^A	Bowman et al., 2009
	66.5% (103/155) ^A	Magnarelli et al., 1987
Delaware	25% (1/4) ^A	Qurollo et al., 2014
	9.5% (4,671/49,126) ^A	Little et al., 2014
	11.2% (516/4,595) ^A	Bowman et al., 2009
District of Columbia	26.7% (24/90) ^A	Qurollo et al., 2014
	8.2% (574/7,029) ^A	Little et al., 2014
Florida	1.6% (8/501) ^A	Qurollo et al., 2014
	1.0% (3,832/403,886) ^A	Little et al., 2014
	0.5% (5/1,000) ^A	Tzipory et al., 2010
	0.5% (256/54,982) ^A	Bowman et al., 2009
Georgia	3.7% (6/162) ^A	Qurollo et al., 2014
	0.8% (985/124,665) ^A	Little et al., 2014
	0.3% (77/23,333) ^A	Bowman et al., 2009
Hawaii	0.3% (6/2,360) ^A	Little et al., 2014
Idaho	3.6% (6/169) ^A	Little et al., 2014
	0.3% (1/369) ^A	Bowman et al., 2009
Illinois	6.0% (23/383) ^A	Qurollo et al., 2014
	3.0% (8,413/277,352) ^A	Little et al., 2014
	1.0% (324/31,976) ^A	Bowman et al., 2009
Indiana	9.7% (9/93) ^A	Qurollo et al., 2014
	3.5% (3,961/112,480) ^A	Little et al., 2014

	1.1% (231/20,515) ^A	Bowman et al., 2009
Iowa	11.5% (9/78) ^A	Qurollo et al., 2014
	2.9% (3,236/111,522) ^A	Little et al., 2014
	0.9% (149/17,390) ^A	Bowman et al., 2009
Kansas	0.0% (0/53) ^A	Qurollo et al., 2014
	0.5% (263/52,435) ^A	Little et al., 2014
	0.1% (6/5,473) ^A	Bowman et al., 2009
Kentucky	5.8% (4/69) ^A	Qurollo et al., 2014
	1.5% (847/56,049) ^A	Little et al., 2014
	0.2% (45/18,935) ^A	Bowman et al., 2009
Louisiana	0.0% (0/27) ^A	Qurollo et al., 2014
	0.4% (48/12,449) ^A	Little et al., 2014
	0.1% (9/11,197) ^A	Bowman et al., 2009
Maine	1.0% (46/4505) ^A	Eschner et al., 2015 ^{Vacc}
	27.6 (357/1294) ^A	Eschner et al., 2015 ^{Non}
	25% (1/4) ^A	Qurollo et al., 2014
	13.5% (29,860/221,556) ^A	Little et al., 2014
	12.7% (138/1,087) ^A	Rand et al., 2011
	11.6% (3,269/28,230) ^A	Bowman et al., 2009
	4.3% (36/828) ^C	Rand et al., 1991
Maryland	24.9% (78/313) ^A	Qurollo et al., 2014
	10.0% (27,348/273,406) ^A	Little et al., 2014
	12.6% (2,882/22,945) ^A	Bowman et al., 2009
	14.4% (24/167) ^A	Duncan et al., 2005
	2.4% (119/494) ^C	Daniels et al., 1993
	46.2% (43/93) ^{BC}	Greene et al., 1991
Massachusetts	40% (14/35) ^A	Qurollo et al., 2014
	18.3% (74,429/406,493) ^A	Little et al., 2014
	19.8% (6,729/33,915) ^A	Bowman et al., 2009
	1.4% (1/71) ^C	Daniels et al., 1993
	20.3% (611/3011) ^C	Lindenmayer et al., 1991
Michigan	0.0% (0/20) ^A	Qurollo et al., 2014
	1.2% (2,936/236,875) ^A	Little et al., 2014
	0.6% (431/67,625) ^A	Bowman et al., 2009
	0.6% (2/353) ^D	Hamer et al., 2009
Minnesota	25.0% (2/8) ^A	Qurollo et al., 2014
	8.6% (20,159/234,564) ^A	Little et al., 2014
	9.5% (7,267/76,610) ^A	Bowman et al., 2009
	36.6% (268/731) ^A	Beall et al., 2008
Mississippi	0.0% (0/16) ^A	Qurollo et al., 2014
	0.7% (43/6,643) ^A	Little et al., 2014
	0.0% (1/2,198) ^A	Bowman et al., 2009
Missouri	0.0% (0/36) ^A	Qurollo et al., 2014
	0.6% (616/108,580) ^A	Little et al., 2014
	0.2% (59/24,095) ^A	Bowman et al., 2009
Montana	0 (0/37) ^A	Little et al., 2014

Nebraska	0.0% (0/3) ^A	Quorollo et al., 2014
	2.0% (91/4,489) ^A	Little et al., 2014
	0.1% (5/4,282) ^A	Bowman et al., 2009
Nevada	0.0% (0/5) ^A	Quorollo et al., 2014
	0.6% (74/12,286) ^A	Little et al., 2014
New Hampshire	26.3% (5/19) ^A	Quorollo et al., 2014
	15.8% (20,447/129,842) ^A	Little et al., 2014
	12.9% (2,343/18,122) ^A	Bowman et al., 2009
	2.6% (4/151) ^C	Daniels et al., 1993
New Jersey	33.3% (4/12) ^A	Quorollo et al., 2014
	13.1% (38,695/295,084) ^A	Little et al., 2014
	8.9% (18/202) ^A	Gaito et al., 2014
	14.2% (2,913/20,575) ^A	Bowman et al., 2009
	34.7% (147/423) ^B	Schulze et al., 1987
New Mexico	1.6% (1/61) ^A	Quorollo et al., 2014
	0.7% (185/26,714) ^A	Little et al., 2014
	0.3% (7/2,060) ^A	Bowman et al., 2009
New York	17.1% (35/205) ^A	Quorollo et al., 2014
	9.5% (50,802/536,978) ^A	Little et al., 2014
	23.1% (104/451) ^F	Wagner et al., 2012
	7.1% (5,781/81,305) ^A	Bowman et al., 2009
	49.2% (711/1446) ^C	Falco et al., 1993
	11.5% (10/87) ^C	Daniels et al., 1993
	57.8% (242/419) ^C	Magnarelli et al., 1990
76.3% (87/114) ^{BC}	Magnarelli et al., 1987	
North Carolina	5.4% (55/1,014) ^A	Quorollo et al., 2014
	1.9% (4,837/249,170) ^A	Little et al., 2014
	1.3% (263/20,783) ^A	Bowman et al., 2009
	0.4% (4/987) ^A	Duncan et al., 2005
	3.4% (15/446) ^C	Greene et al., 1991
	1.8% (8/446) ^B	Greene et al., 1991
3.3% (33/1002) ^B	Greene et al., 1988	
North Dakota	5.4% (893/16,560) ^A	Little et al., 2014
	3.0% (136/4,558) ^A	Bowman et al., 2009
Ohio	3.7% (16/430) ^A	Quorollo et al., 2014
	0.7% (1,970/278,493) ^A	Little et al., 2014
	11.5% (41/355) ^C	Wang et al., 2014
	0.2% (140/61,138) ^A	Bowman et al., 2009
Oklahoma	0.0% (0/42) ^A	Quorollo et al., 2014
	0.6% (445/70,753) ^A	Little et al., 2014
	0.2% (19/11,549) ^A	Bowman et al., 2009
	11.7% (26/223) ^C	Mukolwe et al., 1992
	18% (47/259) ^B	Rodgers et al., 1989
Oregon	0.0% (0/35) ^A	Quorollo et al., 2014
	1.7% (312/17,893) ^A	Little et al., 2014
	0.15% (1/648) ^A	Carrade et al., 2011

	2.8% (77/2,798) ^A	Bowman et al., 2009
Pennsylvania	22.2% (45/203) ^A	Qurollo et al., 2014
	12.9% (74,481/579,657) ^A	Little et al., 2014
	9.4% (3,869/40,948) ^A	Bowman et al., 2009
	25.0% (10/40) ^A	Duncan et al., 2005
	1.2% (1/81) ^C	Daniels et al., 1993
Rhode Island	15.7% (10,001/63,797) ^A	Little et al., 2014
	14.3% (933/6,508) ^A	Bowman et al., 2009
	51.6% (143/277) ^C	Hinrichsen et al., 2001
South Carolina	5.4% (5/93) ^A	Qurollo et al., 2014
	1.0% (857/82,684) ^A	Little et al., 2014
	1.3% (148/11,562) ^A	Bowman et al., 2009
South Dakota	6.0% (270/4,497) ^A	Little et al., 2014
	0.3% (1/358) ^A	Bowman et al., 2009
Tennessee	4.4% (2/45) ^A	Qurollo et al., 2014
	0.6% (670/111,314) ^A	Little et al., 2014
	0.2% (47/18,891) ^A	Bowman et al., 2009
Texas	2.1% (20/966) ^A	Qurollo et al., 2014
	0.5% (1,935/432,919) ^A	Little et al., 2014
	0.2% (91/58,088) ^A	Bowman et al., 2009
Utah	0.0% (0/2) ^A	Qurollo et al., 2014
	1.2% (9/784) ^A	Little et al., 2014
	0.0% (0/93) ^A	Bowman et al., 2009
Vermont	33.3% (2/6) ^A	Qurollo et al., 2014
	14.8% (8,833/59,518) ^A	Little et al., 2014
	9.9% (368/3,718) ^A	Bowman et al., 2009
Virginia	20.3% (133/656) ^A	Qurollo et al., 2014
	9.7% (33,994/350,489) ^A	Little et al., 2014
	6.7% (1,924/28,787) ^A	Bowman et al., 2009
	8.7% (41/472) ^A	Duncan et al., 2005
Washington	0.0% (0/12) ^A	Qurollo et al., 2014
	1.5% (64/4,338) ^A	Little et al., 2014
	0.38% (2/528) ^A	Carrade et al., 2011
	0.0% (0/33) ^A	Bowman et al., 2009
West Virginia	0.0% (0/2) ^A	Qurollo et al., 2014
	3.5% (2,152/61,437) ^A	Little et al., 2014
	0.3% (9/2,942) ^A	Bowman et al., 2009
Wisconsin	12.1% (7/58) ^A	Qurollo et al., 2014
	11.8% (33,217/282,663) ^A	Little et al., 2014
	10.2% (6,018/59,070) ^A	Bowman et al., 2009
Wyoming	0.0% (0/1) ^A	Qurollo et al., 2014
	1.9% (7/361) ^A	Little et al., 2014
	0.0% (0/184) ^A	Bowman et al., 2009

^AC6 ELISA; ^BIIFA; ^CELISA; ^DWestern Blot; ^EFlaB PCR; ^FOspC Multiplex PCR
^{Vacc} -vaccinated dogs; ^{Non} -nonvaccinated dog

Table 2: Reported seroprevalence of antibodies to *B. burgdorferi* in dogs from Canada.

Province	Prevalence (positive/tested)	Reference
Alberta	2.1% (1/48) ^A	Qurollo et al., 2014
	0.2% (1/584) ^A	Villeneuve et al., 2011
British Columbia	1.9% (1/53) ^A	Qurollo et al., 2014
	0.0% (0/418) ^A	Villeneuve et al., 2011
	0.0% (0/88) ^A	Bryan et al., 2011
	1.7% (5/287) ^{BD}	Banerjee et al., 1996
Manitoba	2.4% (303/12,765) ^A	Herrin et al., (Unpub)
	0.0% (0/4) ^A	Qurollo et al., 2014
	1.9% (256/13,456) ^A	Villeneuve et al., 2011
New Brunswick	3.7% (60/1,631) ^A	Herrin et al., (Unpub)
	0.7% (1/151) ^A	Villeneuve et al., 2011
Nova Scotia	15.7% (33/210) ^A	Herrin et al., (Unpub)
	2.2% (15/697) ^A	Villeneuve et al., 2011
	0.0% (0/137) ^C	Bell et al., 1992
Ontario	2.3% (1,780/77,143) ^A	Herrin et al., (Unpub)
	2.4% (4/166) ^A	Qurollo et al., 2014
	0.5% (270/56,943) ^A	Villeneuve et al., 2011
	1.9% (2/108) ^A	Gary et al., 2006
	1.0% (13/1,318) ^{BCD}	Artsob et al., 1993
Prince Edward Island	0.0% (0/1) ^A	Qurollo et al., 2014
	10.0% (3/30) ^A	Villeneuve et al., 2011
	1.3% (1/75) ^A	Artsob et al., 1992
Quebec	2.8% (667/23,701) ^A	Herrin et al., (Unpub)
	0.0% (0/6) ^A	Qurollo et al., 2014
	0.6% (76/13,390) ^A	Villeneuve et al., 2011
Saskatchewan	0.5% (1/186) ^A	Herrin et al., (Unpub)
	0.0% (0/7) ^A	Qurollo et al., 2014
	2.6% (2/77) ^A	Schurer et al., 2014
	0.3% (2/582) ^A	Villeneuve et al., 2011

^AC6 ELISA; ^BIFA; ^CELISA; ^DWestern Blot; ^EFlaB PCR; ^FOspC Multiplex PCR

Table 3: Reported molecular prevalence of *Borrelia burgdorferi* sensu stricto in ticks from the U.S., 2005 – 2016.

State	Prevalence (positive/tested)	Tick Stage	PCR Target	Reference
Alabama ¹	0.0% (0/1)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
Alaska	--			
Arizona	--			
Arkansas ¹	0.0% (0/1) 53.8% (7/13) 36.1% (245/678)	Larvae Nymphs Adults	flaB	Fyxell et al., 2012
California ²	3.2% (70/2188) 0.6% (37/6036)	Nymphs Adults	flaB, 16S-23S (rRNA rrs-rrlA)	Padgett et al., 2014
	9.5% (7/74) 1.4% (1/71)	Nymphs Adults	5S-23S rRNA ITS	Lane et al., 2010
	8.1% (119/1,476)	Nymphs	5S-23S rRNA ITS	Swei et al., 2011
	4.9% (264/5431)	Nymphs	5S-23S rRNA ITS	Eisen et al., 2010
	6.8% (3/44) 1.3% (12/904)	Nymphs Adults	gyrB, rpoC, leuS, flaB, ospC, hbb, hbb	Crowder et al., 2010
	0.5% (6/1,108)	Adults	16S-23S (rrs-rrlA)	Salkeld et al., 2014
	0.0% (0/145) 0.04% (1/2,392)	Nymphs Adults	5S-23S rRNA ITS, gyrB, rpoC, leuS, flaB, ospC, hbb, hbb	Lane et al., 2013
	1.2% (2/168)	Adults	FL6/FL7	Holden et al., 2006

Colorado	--			
Connecticut¹	15.4% (79/514)	Nymphs	fliD, gB31	Feldman et al., 2015
	23.4% (253/1083)	Nymphs	16S-23S (rrs-rrlA)	Diuk-Wasser et al., 2014
	17.4% (49/281)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
	67.7% (67/99)	Adults	gyrB, rpoC, leuS, flaB, ospC, hbb, hbb	Crowder et al., 2010
	22.3% (289/1295)	Nymphs	16S-23S (rrs-rrlA)	Hanincová et al., 2006
Delaware	--			
Florida	--			
Georgia¹	0.0% (0/1)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
Hawaii	--			
Idaho	--			
Illinois¹	9.2% (30/325)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
	9.4% (3/32)	Nymphs	16S-23S (rrs-rrlA)	Rydzewski et al., 2011
	13.6% (18/132)	Nymphs	16S-23S (rrs-rrlA)	Gatewood et al., 2009
	35.5% (61/172)	Adults	ospA, -- ^a	Jobe et al., 2007 ^a
	39.4% (5/127)	Nymphs, Adults	-- ^a	Jobe et al., 2006 ^a
Indiana¹	10.4% (7/67)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
	23.5% (19/81)	Adults	gyrB, rpoC, leuS, flaB, ospC, hbb, hbb	Crowder et al., 2010
	7.7% (1/13)	Nymphs	16S-23S (rrs-rrlA)	Gatewood et al., 2009

Iowa¹	72.0% (72/100)	Adults	flaB	Steiner et al., 2008
	0.0% (0/4)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
	0.0 – 8.0% (#/#)	Larvae, Nymphs, Adults	flaB, -- ^b	Lingren et al., 2005 ^b
Kansas	--			
Kentucky¹	0.0% (0/2)	Nymphs, Adults	flaB	Taft et al., 2005
Louisiana¹	6.3% (11/174)	Adults	flaB	Leydet Jr et al., 2014
Maine¹	11.1% (2/18)	Adults	flaB	Leydet Jr et al., 2013
	42.6% (86/202)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
	42.8% (3/7)	Adults	gyrB, rpoC, leuS, flaB, ospC, hbb, hbb	MacQueen et al., 2012
	43.6% (82/188)	Nymphs	16S-23S (rrs-rrlA)	Gatewood et al., 2009
Maryland¹	58.0% (58/100)	Adults	flaB	Steiner et al., 2008
	19.4% (24/124)	Nymphs	fliD, gB3 l	Feldman et al., 2015
	16.9% (85/503)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
	18.7% (72/385)	Nymphs	16S-23S (rrs-rrlA)	Gatewood et al., 2009
	14.7% (51/348)	Nymphs	flaB, gyrB, rpoC, leuS, flaB, ospC, hbb, hbb	Swanson et al., 2007
	11.7% (12/103)	Nymphs	-- ^b	Giery et al., 2007 ^b
	69.2% (9/13)	Nymphs	flaB	Anderson et al., 2006
	60.0% (3/5)	Adults		

	12.8% (5/39)	Nymphs, Adults	flaB	Taft et al., 2005
Massachusetts¹	20.7% (18/87)	Nymphs	16S-23S (rrs-rrlA)	Diuk-Wasser et al., 2014
	12.3% (17/138)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
	0.0% (0/56)	Nymphs, Adults	flaB	Taft et al., 2005
Michigan¹	12.4% (22/178)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
	26.4% (39/148)	Larval Pool	16S-23S (rrs-rrlA)	Hamer et al., 2012
	44.3% (39/88)	Nymphs on		
	9.4% (6/64)	Host		
	49.1% (53/108)	Nymphs		
		Adults		
	9.3% (8/246)		16S-23S (rrs-rrlA)	Hamer et al., 2010
	36.6% (31/86)	Nymphs		
		Adults		
	12.7% (20/157)		16S-23S (rrs-rrlA)	Gatewood et al., 2009
		Nymphs		
	33.3% (6/18)		16S-23S (rrs-rrlA)	Hamer et al., 2009
		Adults		
Minnesota¹	25.9% (90/348)	Nymphs	flaB	Stromdahl et al., 2014
	37.5% (157/419)	Adults		
	34.7% (125/360)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
	32.1% (87/271)	Nymphs	16S-23S (rrs-rrlA)	Gatewood et al., 2009
Mississippi¹	0.0% (0/6)	Nymphs	flaB	Goltz et al., 2013
	0.0% (0/256)	Adults		
Missouri¹	0.0% (0/3)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
Montana	--			

Nebraska	--			
Nevada	--			
New Hampshire¹	0.0% (0/8)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
	60.1% (306/509)	Adults	-- [#]	Walk et al., 2009 [#]
New Jersey¹	13.0% (62/478)	Nymphs	flaB	Schulze et al., 2013
	51.5% (314/610)	Adults		
	19.5% (25/128)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
	25.6% (40/156)	Nymphs	fliD	Dolan et al., 2011
	22.9% (8/35)	Nymphs	16S-23S (rrs-rrlA)	Gatewood et al., 2009
	31.9% (30/94)	Adults	flaB	Schulze et al., 2006
	17.6% (44/250)	Nymphs	flaB, 16S-23S (rrs-rrlA), ospA	Ullmann et al., 2005
	4.4% (3/68)	Nymphs, Adults	flaB	Taft et al., 2005
	54.4% (80/147)	Adults	16S-23S (rrs-rrlA)	Schulze et al., 2005
New Mexico	--			
New York¹	23.3% (48/207)	Nymphs	fliD, gB31	Feldman et al., 2015
	28.5% (1245/4368)	Nymphs	23S rRNA	Hersh et al., 2014
	34.3% (23/67)	Nymphs	ospA	Aliota et al., 2014
	23.0% (129/561)	Adults		
	14.5% (478/3,300)	Nymphs	16S rRNA	Prusinski et al., 2014

	43.7% (3,448/7,884)	Adults		
	17.0% (232/1367)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
	22.4% (22/98) 59.6% (133/223)	Nymphs Adults	gyrB, rpoC, leuS, flaB, ospC, hbb, hbb	Crowder et al., 2010
	63.6% (182/286)	Adults	flaB, ospA	Tokarz et al., 2010
	21.3% (267/1,256)	Nymphs	16S-23S (rrs-rrlA)	Gatewood et al., 2009
	17.2% (5/29)	Nymphs	-- ^b	Giery et al., 2007 ^b
	37.3% (91/244) 36.4% (32/88)	Nymphs Adults	16S rRNA	Moreno et al., 2005
North Carolina^{1,3}	3.2% (1/31)	Nymphs, Adults	flaB	Taft et al., 2005
	0.0% (0/5)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
	0.0% (0/298) ¹ 33.5% (52/155) ³	Adults Adults	16S-23S (rrs-rrlA)	Maggi et al., 2010
North Dakota¹	0.0% (0/4)	Nymphs, Adults	flaB	Taft et al., 2005
	0.0% (0/15)	Larvae	flaB	Russart et al., 2014
	2.2% (1/45) 6.3% (2/32)	Nymphs Adults		
Ohio¹	13.5% (7/52)	Nymphs	flaB	Wang et al., 2014
	3.6% (8/221)	Adults		
Oklahoma¹	0.0% (0/5)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
Oregon²	2.1% (35/1,689)	Adults	16S rRNA	Doggett et al., 2008
Pennsylvania¹	46.0% (854/1855)	Adults	flaB	Hutchinson et al., 2015

	35.1% (114/325)	Adults	flaB	Brown et al., 2015
	17.6% (13/74)	Nymphs	flaB	Han et al., 2014
	26.8% (11/41)	Adults		
	19.7% (59/300)	Nymphs	flaB	Stromdahl et al., 2014
	35.2% (82/233)	Adults		
	23.4% (51/218)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
	19.5% (24/123)	Nymphs	16S-23S (rrs-rrlA)	Gatewood et al., 2009
	55.3 (52/94)	Adults	flaB	Steiner et al., 2008
	0.0% (0/47)	Nymphs, Adults	flaB	Taft et al., 2005
Rhode Island¹	19.5% (15/77)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
	15.8% (456/2884)	Nymphs	-- ^b	Connally et al., 2006 ^b
South Carolina¹	0.0% (0/2)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
South Dakota	--			
Tennessee¹	0.0% (0/47)	Adults	flaB, 23S rRNA	Mays et al., 2014
	0.0% (0/883)	Adults	16S rRNA	Rosen et al., 2012
Texas¹	1.6% (1/62)	Adults	flaB	Mitchell et al., 2016
	50.0% (37/74)*	Adults	flaB, 16S-23S (rrs-rrlA)	Feria-Arroyo et al., 2014
	11.1% (1/9)	Nymphs	flaB	Williamson et al., 2010
	0.0% (0/67)	Adults		
Utah²	0.0% (0/119)		fliD	Davis et al., 2015

Vermont¹	0.0% (0/16)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
	0.0% (0/16)	Nymphs	16S-23S (rrs-rrlA)	Gatewood et al., 2009
Virginia¹	32.9% (117/356)	Adults	flaB	Herrin et al., 2014
	15.8% (48/304)	Nymphs	16S rRNA	Brinkerhoff et al., 2014
	10.6% (7/66)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
	0.0% (0/7)	Nymphs	16S-23S (rrs-rrlA)	Gatewood et al., 2009
	25.0% (8/32)	Nymphs, Adults	flaB	Taft et al., 2005
Washington²	--			
West Virginia	--			
Wisconsin¹	35.7% (122/321) ^F	Adults	recA	Turtinen et al., 2015
	19.8% (95/480)	Nymphs	flaB	Stromdahl et al., 2014
	25.9% (52/201)	Adults		
	27.9% (339/1,214)	Nymphs	ospB	Lee et al., 2014
	18.7% (284/1517)	Nymphs	16S rRNA	Diuk-Wasser et al., 2012
	17.4% (234/1346)	Nymphs	16S-23S (rrs-rrlA)	Gatewood et al., 2009
	35.0% (35/100)	Adults	flaB	Steiner et al., 2008
	0.0% (0/157)	Larvae	ospB	Caporale et al., 2005
	14.3% (1/7)	Nymphs		
	4.2% (5/118)	Adults		
Wyoming	--			

^aCulture; ^bIFA

[#]Information not reported

*Results have been disputed in the literature (Norris et al., 2014; Esteve-Gassent et al., 2015; Norris et al., 2015)

¹*I. scapularis*; ²*I. pacificus*; ³*I. affinis*

Table 4: Reported molecular prevalence of *Borrelia burgdorferi* sensu stricto in ticks from Canada, 2005 – 2016.

Province	Prevalence (positive/tested)	Tick Stage	PCR Target	Reference
Alberta¹	13.8% (12/87)	Nymph, Adult	ospA, 23S rRNA	Dibernardo et al., 2014
British Columbia²	0.5% (1/192)	Larval Pool	23S rRNA	Morshed et al., 2015
	4.1% (4/98)	Nymphal Pool		
	0.0% (0/13)	Adult Pool		
Manitoba¹	8.8% (15/170)	Nymphs, Adults	ospA, 23S rRNA	Dibernardo et al., 2014
	9.7% (34/349)	Adults	Multiple PCR targets	Ogden et al., 2006
New Brunswick¹	25.0% (1/4)	Adults	ospA, 23S rRNA	Gabriele-Rivet et al., 2015
	6.8% (25/366)	Nymphs, Adults	ospA, 23S rRNA	Dibernardo et al., 2014
	15.9% (24/151)	Adults	Multiple PCR targets	Ogden et al., 2006
Newfoundland¹	27.3% (9/33)	Nymphs, Adults	ospA, 23S rRNA	Dibernardo et al., 2014
	19.0% (4/21)	Adults	Multiple PCR targets	Ogden et al., 2006
Nova Scotia¹	11.8% (4/34)	Nymphs, Adults	ospA, 23S rRNA	Dibernardo et al., 2014
	15.1% (13/86)	Adults	Multiple PCR targets	Ogden et al., 2006
Ontario¹	41.3% (12/29)	Adults	flaB	Scott et al., 2016
	11.3% (32/283)	Nymphs	ospA, 23S rRNA	Nelder et al., 2014
	15.1% (873/5,763)	Adults		
	15.9% (411/2,591)	Nymphs, Adults	ospA, 23S rRNA	Dibernardo et al., 2014
	66.7% (10/15)	Adult Pool	ospA, multiple PCR targets	Scott et al., 2008

	11.1% (5/45)	Adults	Multiple PCR targets	Ogden et al., 2006
	12.9% (42/325)	Adults	Multiple PCR targets	Morshed et al., 2006
Prince Edward Island¹	9.6% (17/178)	Nymphs, Adults	ospA, 23S rRNA	Dibernardo et al., 2014
	11.1% (20/180)	Adults	Multiple PCR targets	Ogden et al., 2006
Quebec¹	13.7% (203/1,479)	Nymphs, Adults	ospA, 23S rRNA	Dibernardo et al., 2014
	3.3% (8/243)	Adults	23S rRNA	Bouchard et al., 2013
	2.3% (12/533)	Larvae	Multiple PCR targets	Ogden et al., 2010
	7.8% (18/232)	Nymphs		
	8.5% (377/4,323)	Adults		
	13.2% (128/984)		Multiple PCR targets	Ogden et al., 2006
Saskatchewan	--			

¹*I. scapularis*; ²*I. pacificus*

CHAPTER III

CONFIRMATION OF *BORRELIA BURGDORFERI* SENSU STRICTO AND *ANAPLASMA PHAGOCYTOPHILUM* IN NEWLY ESTABLISHED POPULATIONS OF *IXODES SCAPULARIS*, SOUTHWESTERN VIRGINIA¹

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ABSTRACT

To determine the prevalence of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in a newly established population of *Ixodes scapularis* in the mountainous region of southwestern Virginia, questing adult ticks were collected and the identity and infection status of each tick confirmed by PCR and sequencing. A total of 364 adult ticks were tested from three field sites. *B. burgdorferi* sensu stricto was identified in a total of 32/101 (32%) ticks from site A, 49/154 (32%) ticks from site B, and 36/101 (36%) ticks from site C, for a total prevalence of 33% (117/356). In addition, *A. phagocytophilum* was detected in 3/364 (0.8%) ticks, one from Site A and two from Site B. The prevalence of both pathogens in ticks at these sites is similar to that reported from established endemic areas. These data document the presence of *I. scapularis* and the agent of Lyme disease in a newly established Appalachian region, providing further evidence of range expansion of both the tick and public and veterinary health risk it creates.

Key Words: *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, *Ixodes scapularis*, Virginia

INTRODUCTION

In the northeastern and upper midwestern regions of the United States, *Ixodes scapularis* (Acari: Ixodidae) is the vector for both *Borrelia burgdorferi* sensu stricto (Spirochaetales: Spirochaetaceae), the causative agent of Lyme disease (LD) in the U.S., and *Anaplasma phagocytophilum* (Rickettsiales: Anaplasmataceae), the causative agent of human granulocytic anaplasmosis (HGA). Ticks harboring *B. burgdorferi* have been

documented from the northeastern and coastal regions of Virginia, and from coastal North Carolina, but there have been no published reports from the adjacent mountainous, Appalachian regions of these states (Maggi et al. 2010; Nadolny et al. 2011).

The expanding number of human Lyme borreliosis (LB) cases reported each year renders accurate determination of the geographic range where *B. burgdorferi* poses a potential infectious threat increasingly important. The present study sought to confirm the presence and determine the prevalence of *B. burgdorferi* and *A. phagocytophilum* in *I. scapularis* collected from a newly endemic region of southwestern Virginia.

MATERIAL AND METHODS

Questing adult *Ixodes* spp. ticks were collected from October 2012 through April 2013 by standard cloth drags at three sites in Giles County and Pulaski County, Virginia. Sites selected for dragging included both public recreational land and low-density private residential communities with mixed hardwood habitat. Site A is a public park bordered by woody edge habitat and a river, Site B is a wooded, low-density residential area, and Site C is a grassy and wooded area near a playground at a recreational lake. Ticks were placed in 70% ethanol at the time of collection and held at room temperature until processed for PCR.

Individual ticks were identified morphologically by comparison to standard keys (USDA Ag Handbook 485, 1976) and then dissected and total nucleic acid extracted as previously described. Briefly, internal tick contents were digested in a proteinase K lysis buffer, the DNA extracted with phenol/chloroform, resuspended in 50µl of buffer, and stored at -80°C (Halos et al. 2004). Nucleic acid extracts from each tick were individually

tested by PCR to confirm tick species, and for *B. burgdorferi* and *A. phagocytophilum* as previously described (Little et al. 1997, Stromdahl et al. 2003, Macaluso et al. 2003, Nadolny et al. 2011). Amplicons were purified using a commercial kit (Wizard® Genomic DNA Purification Kit, Promega, Fitchburg, WI) according to manufacturer's guidelines and sequenced directly (SmartSeq, eurofins genomics, Huntsville, AL).

Sequence analysis and alignment was performed using MacVector software (MacVector, Inc., Cary, NC). Sequences generated from study samples were compared with published sequences using the nucleotide Basic Local Alignment Search Tool (BLASTn, National Center for Biotechnology Information, Bethesda, MD). Differences in prevalence of *B. burgdorferi* in ticks between collection sites and tick gender were evaluated for significance with a Pearson's chi-squared test using Excel (Microsoft 2008, Redmond, WA) with significance assigned at $P < 0.05$.

RESULTS

A total of 356 adult ticks (202 female, 154 male) were collected from the three sites. All ticks were morphologically and molecularly identified as *I. scapularis* (GenBank L43862.1). *Borrelia burgdorferi* sensu stricto was detected in 32/101 ticks (31.7%) from site A, 49/154 ticks (31.8%) from site B, and 36/101 ticks (35.6%) from site C, for a total prevalence of 32.9% (117/356). Female *I. scapularis* were more likely ($P < 0.05$) to be PCR positive for *B. burgdorferi* (75/202; 37.1%) than male (42/154; 27.3%). *Anaplasma phagocytophilum* was detected by PCR in 3/356 ticks (0.8%), one from site A in a ticks in which *B. burgdorferi* was not detected, and two from site B co-infected with both agents. No *A. phagocytophilum* was detected in ticks from Site C. All

sequences showed between 99% and 100% identity with corresponding GenBank sequences (*B. burgdorferi* CP001205.1, *A. phagocytophilum* JN181070.1).

DISCUSSION

This study documents *B. burgdorferi* sensu stricto in high prevalence in questing *I. scapularis* ticks outside of the area traditionally considered endemic for Lyme borreliosis (LB), suggesting recent expansion of this disease system into the southern Appalachian region. The prevalence of these pathogens in adult ticks is comparable to those previously reported from endemic areas in the northeastern U.S. (Adelson et al. 2004, Holman et al. 2004). Geographic expansion of LB in North America has been noted by others including in Canada and the midwestern U. S. (Ogden et al. 2010; Hamer et al. 2010).

When accurate, geographic distribution patterns can aid proper diagnosis of LB in humans and dogs. However, this approach can be complicated by expanding field ecology and the presence of a number of other species in the *B. burgdorferi* sensu lato group in a given area, making identification of pathogens in field collected ticks important (Rudenko et al. 2011). *Anaplasma phagocytophilum*, an agent of both human and veterinary disease, was also detected in the ticks in the present study; both pathogenic and apparently non-pathogenic variants of *A. phagocytophilum* have been described (Massung et al. 2003).

These data confirm that populations of *I. scapularis* harboring both *B. burgdorferi* and *A. phagocytophilum* are established in southwestern Virginia; additional investigations in other areas of southern Appalachia are needed. Recent reports indicate

that *I. scapularis* ticks have also expanded their range in neighboring West Virginia, together with increased reports of LB from this region (WVDHHR, 2012). Field research documenting the expanding geographic range of these disease agents is important to support prompt diagnosis and treatment of human and canine LB.

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CHAPTER IV

***BORRELIA BURGDORFERI* IN THE NEW YORK CITY METROPOLITAN AREA, 2000-2010¹**

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ABSTRACT

Lyme borreliosis (LB) is endemic within the New York City metropolitan area, a large region with habitat and demographic diversity. Results from 175,259 canine serologic tests for specific antibodies to *Borrelia burgdorferi*, the agent of LB in the United States, were evaluated to compare infection in dogs to reported human cases and other environmental and social parameters. Initial evaluation of categorical data revealed a gradient of prevalence radiating outwards from the most densely populated areas of the region, with the highest seroprevalence in dogs (21.1%) in areas with greater than 50% forested area. Multiple regression with several environmental risk factors provided an accurate prediction of infection in dogs (adjusted $R^2 = 0.90$) but was less accurate at predicting human case reports ($R^2 = 0.74$). Analysis of canine serologic data continues to be a valuable tool for understanding transmission of zoonotic tick-borne disease agents.

Article Summary

Canine seroprevalence accurately predicted risk of infection with *Borrelia burgdorferi* for both dogs and humans in the New York City metropolitan area and can be used to identify environments that are suitable for the maintenance of *B. burgdorferi* in regions where Lyme borreliosis is confirmed to be endemic.

Keywords

Canine, Lyme borreliosis, *Borrelia burgdorferi*, 4Dx SNAP, epidemiology

INTRODUCTION

Lyme borreliosis (LB) caused by *Borrelia burgdorferi* sensu stricto is the most frequently reported vector-borne disease in people in the United States (1). Infection and

disease is common in people and dogs in the northeastern United States, with the majority (95%) of all human cases being reported from 13 northeastern states, including 9.7% from New York state alone (2). Exposure to *B. burgdorferi* in dogs parallels that in humans; the majority of dogs with detectable antibodies are identified in the Northeast, and 7.1% of pet dogs in New York state are seropositive (3, 4). Humans and dogs are both considered incidental hosts, acquiring the pathogen through the bite of an *Ixodes* spp. tick. Once infected, most human patients develop a distinctive erythema migrans rash, fever, headache, joint and muscle pain, and fatigue; if left untreated, disease may progress to more serious cardiac, articular, and neurologic conditions (5). Since its initial description in 1975, Lyme disease has continued to increase in range and incidence throughout the northeastern and upper midwestern United States and southern Canada (2, 6).

On a local level, the risk of infection with a tick-borne disease agent is directly related to exposure risk, which in turn is associated with lifestyle. For example, seroprevalence of people to *Ehrlichia chaffeensis* has been shown to be associated with a variety of risk factors, including frequency and number of tick bites, use of repellents, and even golf scores (12). Similar risk factors have been analyzed for Lyme disease and include everything from sightings of deer, a key reproductive host for the tick vector, to habitat factors such as oak trees and acorns, which provide mast to support higher rodent reservoir populations (13). Many studies focus on the deer/human interface, citing evidence that suggests increased contact with deer, such as automobile-deer collisions, correlates with increased risk of infection with *B. burgdorferi* (14, 15). A diverse and low disturbance ecosystem appears to decrease infection risk, presumably due to the presence

of less competent reservoirs which serve as dilutional hosts (16, 17). Areas with a high vegetation index (e.g. NDVI, normalized difference vegetation index), especially deciduous forests that provide ideal tick habitats, increase risk, while grasslands are typically associated with a lower risk of infection (18, 19).

A number of previous studies on *B. burgdorferi* transmission have relied on quantitative tick collection methods and pathogen testing, as well as environmental factors influencing wildlife reservoirs and vector ticks (20, 6, 21, 22). Domestic dogs have also proven to be valuable sentinels for tick-borne disease agents, allowing identification of areas where transmission is occurring (7 – 11). Pet dogs share a common habitat and environment with their owners and thus can serve as bellwethers of human infection risk. Using a comprehensive database generated by testing dogs throughout the United States, we recently described areas where canine heartworm infection and tick-borne disease exposure were common (3, 4). In the present paper, we provide a more detailed analysis of a geographically and demographically diverse area where LB is endemic in people and dogs.

METHODS

Study Area

The New York City Metropolitan Statistical Area (NYC MSA) plus 3 additional, contiguous counties in Connecticut (Litchfield, New Haven, and Fairfield) comprise the complete study area. The NYC MSA is titled the New York-Newark-Bridgeport, NY-NJ-PA Metropolitan Statistical Area, and includes a population of 20.1 million people by 2014 Census estimates, combined with the surrounding counties for a total population of

approximately 22 million people (23). The area was selected not only for the large population and data available, but also for the dramatically diverse population density and environment types, with central NYC representing an urban area, and the outer counties, including Pike, PA, more exurban with transitional counties between them. This gradient allowed the analysis of a variety of environmental and social factors within an area where LB is endemic.

Qualitative Serology

Percent positive canine test results for *B. burgdorferi* by county were obtained from a national reporting system created for veterinarians and maintained by IDEXX Laboratories, Inc. (Westbrook, ME). Briefly, veterinary practices routinely testing dogs for antibodies to *B. burgdorferi* submitted their results through a centralized system. Data were collated and tallied according to location of the veterinary practice reporting results and then sorted according to county and state (3, 4). A total of 175,259 dogs were tested over a 10 year period, 2000-2010, in the NYC MSA and adjacent counties. All qualitative testing was conducted using in-clinic SNAP®3Dx® Test kit or SNAP®4Dx® Test kit (IDEXX Laboratories, Westbrook, ME), in-clinic ELISA tests for simultaneous detection of canine antibodies to *E. canis*, *B. burgdorferi* and *Anaplasma phagocytophilum*, and *Dirofilaria immitis* antigen. The in-clinic ELISAs include a C₆ peptide-based assay that detects antibodies to *B. burgdorferi* with a sensitivity of 94.4% compared to a combination of immunofluorescence assay (IFA) and Western blot (WB) (24), a specificity of 99.6% on field samples (7), and does not react to antibodies generated by vaccination (25, 26).

Social and Environmental Variables

Variables included in the initial categorical analysis were: population density (27, 28), median household income (28), percent forested area (29), percent canine samples positive for antibody to *B. burgdorferi*, and human cases per year as reported by the CDC per 100,000 people between 2002 and 2006 (30). All variables were summarized and analyzed at the county level.

For regression, more specific environmental variables were added (Table 1), including minimum and maximum temperature and precipitation in November. November was selected to reflect a period immediately following peak questing activity of adult ticks in which egg deposition and larval development occurs. Temperature and precipitation values between months co-vary, precluding use of the same data from multiple months. We downloaded 1 km resolution November minimum and maximum temperature and precipitation from PRISM climate group (<http://www.prism.oregonstate.edu/>) for 2000-2009, and calculated the averages for this period, by county. In addition, more specific land cover types replaced percent forested area, including: open water, barren land, deciduous forest, evergreen forest, mixed forest, pasture/hay, shrub/scrub, grassland/herbaceous, cultivated crops, woody wetlands, and emergent herbaceous wetlands. These represent the available land cover classes in the US Geological Survey National Land Cover Database for 2006, derived from Landsat satellite imagery with 30 m resolution (31). For each county, we calculated % land cover type. The same dataset contains four classes of intensity of development, which we used to supplement population density, namely: no (open space, <20% impervious surfaces), low (20-49% impervious surfaces), medium (50-79% impervious surfaces), or high (80-

100% impervious surfaces) intensity development. Finally, we included the normalized difference vegetation index (NDVI) for November, derived from Moderate Resolution Imaging Spectroradiometer (MODIS) satellite data for 2000-2009, averaged by county (32).

Statistical Analysis

Initial analysis of the categorical data was performed using Excel (Microsoft 2007). The variables were split into 2 or 3 groups, and then a 2-tailed Student's T-test was performed. Variables included percent positive canine tests (0-10%, 10-20%, >20%), human case reports per 100,000 people (<10, 10-100, >100), population density (<2500, 2500-7500, >7500 person/sq mi), median household income (<\$70,000, >\$70,000), and percent forested area (<25%, 25-50%, >50%). Subsequent analyses using more specific environmental data (Table 1) were performed using StatPlus (AnalystSoft, Alexandria, VA), with significance assessed at $p < 0.05$. An initial simple regression was performed with each variable and either percent positive canine tests or human case reports. All variables that were significant by simple regression were analyzed pair-wise using a Pearson's Correlation Test; the significance of any two variables with a correlation value over 0.9 ($|r| > 0.9$) was assessed and variables that did not contribute significantly to further analysis were removed (33), then multiple backwards-stepwise regression performed on remaining significant variables. For analysis of percent positive canine tests against the environmental and social variables, five elimination steps were performed. For the analysis of human case reports, nine elimination steps were performed.

RESULTS

Percent positive canine tests for *B. burgdorferi* ranged from 1.2% in Queens County, NY to 27.3% in Putnam County, NY (Figure 1). Population-adjusted case reports of human LB followed the same general trend, ranging from 0.50 case reports/10⁵ in Orange County, NY to 438.71 case reports/10⁵ in Dutchess County, NY.

Initial categorical analysis revealed percent positive canine tests were significantly higher in counties with population density < 2,500 persons/sq mi (17.9%, $p^{AB} = 0.004$) than in counties with population density 2,500-7,500 persons/sq mi (8.0%) or > 7,500 persons/sq mi (5.1%). Percent positive canine tests did not differ significantly between counties with moderate and high population density ($p^{BB} = 0.16$). Population adjusted human case reports were also significantly higher in counties with population density < 2,500 persons/sq mi (113.4 case reports/10⁵, $p^{AB} = 0.002$), and counties with population density 2,500-7,500 persons/sq mi (10.2 case reports/10⁵, $p^{AB} = 0.002$) than in counties with > 7,500 persons/sq mi (3.4 case reports/10⁵). No significant difference was seen in percent positive canine tests ($p = 0.91$) or human case reports ($p = 0.83$) between counties with median income < \$70,000 (15.3%, 66.5 case reports/10⁵) and those with median income > \$70,000 (13.6%, 74.9 case reports/10⁵).

Percent positive canine tests were significantly higher in counties with > 50% forested area (21.1%) than those with 25-50% forested area (15.3%, $p^{BC} = 0.026$) and < 25% forested area (6.3%, $p^{AC} < 0.0001$). Percent positive canine tests in counties with 25-50% forested areas were also significantly greater than those with < 25% forested area ($p^{AB} = 0.002$). Population adjusted human case reports were also significantly higher in counties with 25-50% (66.0 case reports/10⁵) or > 50% forested area (164.7 case

reports/10⁵) than in counties with <25% forested area (11.1 case reports/10⁵, p^{AB} = 0.045, p^{AC} = 0.0048), but did not differ significantly between densely and moderately forested counties (p^{BB} = 0.065).

Percent positive canine tests were significantly lower in counties with < 10 human case reports/10⁵ (8.3%) than those with 10-100 human case reports/10⁵ (13.7%, p^{AB} = 0.005) or those with >100 case reports/10⁵ (24.0%, p^{BC} < 0.0001). Similarly, human case reports of LB were significantly lower in counties with <10% positive canine test results (13.1 case reports/10⁵, p^{AB} = 0.008, p^{AC} = 0.00012) and counties with 10-20% positive canine test results (38.9 case reports/10⁵, p^{BC} = 0.0017) than counties with > 20% positive canine test results (197.0 case reports/10⁵).

By simple regression, canine percent positive tests were highly correlated with population adjusted human case reports (p < 0.00001; R² = 0.47). When compared to several environmental and social factors (Table 1), both canine percent positive tests and population adjusted human case reports significantly correlated with minimum and maximum temperature in November; NDVI for November; low, medium, and high-developed intensity; deciduous forest; and pasture/hay area (Table 1). Canine percent positive tests also correlated with population density, mixed forest area, and emergent herbaceous wetland, while human case reports correlated with shrub/scrub area (Table 1). Pearson's correlation coefficient tests identified covariance between several factors, resulting in removal of November NDVI and developed high intensity area. Remaining factors that were significant for either canine percent positive tests or population adjusted human case reports were used in subsequent multiple backwards-stepwise regressions (Table 1).

For the backwards-stepwise regressions based on percent positive canine tests, 11 factors were initially considered. After five elimination steps, remaining significant factors were human case reports per 100,000 people, population density, maximum temperature in November, deciduous forested area, mixed forest area, and precipitation in November (Table 2). Using B values for each factor and the constant (Table 2), the predicted percent positive tests generated using the regression compared closely to the actual values reported ($R^2 = 0.88$; Figure 2).

For the analysis based on human case reports, 11 factors were initially considered. After nine elimination steps, remaining significant factors were percent positive canine tests and pasture/hay area (Table 3). Again, the B values and constants (Table 3) used to predict the human case reports compared to the actual numbers reported ($R^2 = 0.63$; Figure 3).

DISCUSSION

Approximately 1 in 16 people in the United States live in the New York City metropolitan statistical area (23). In general, dog ownership follows human population trends; nationally 36.5% of households own one or more dogs although ownership rates vary somewhat geographically (34). This region proved ideal for an in depth analysis of risk factors for *B. burgdorferi* transmission due to established endemicity for Lyme borreliosis (LB) throughout the area, a robust dataset on canine seroprevalence, public availability of human case reports of LB by county, and the presence of dramatically diverse habitat factors in close geographic proximity. The large sample size, which included 175,259 test results generated by practicing veterinarians over 10 years, allowed

detailed analysis of environmental and social variables that may contribute to the risk of infection. Similar analyses attempted over larger geographic regions may be complicated by variations in tick phenology, reservoir host composition, habitat, or inclusion of data from non-endemic areas when analysis is attempted in transitional zones where maintenance cycles for *B. burgdorferi* have only recently expanded.

Key variables identified as important for predicting infection risk to dogs in this study included factors that are biologically relevant to supporting tick populations and have been shown to be important in previous studies, such as deciduous forest, mixed forest, precipitation, and temperature, and not those variables considered detrimental to tick habitat such as evergreen forest, wet habitat, and rocky or barren land (13, 15, 20, 35). Both deciduous and mixed forests provide leaf litter considered important as tick habitat, while temperature and precipitation combine to provide adequate humidity for ticks to thrive (36). Canine serology is likely a strong basis for the model because serologic testing for past or current infection with *B. burgdorferi* is routinely performed in veterinary practices on both healthy and sick animals, generating survey data that represent cross-sectional infection risk in a large portion of the canine population.

The significant variables used to predict human case reports provided less information about the type of environment which would be considered high risk for contracting LB, with the only significant factors being canine positive tests and pasture/hay area. While pasture area may represent outdoor or forest-edge activity, it is not considered ideal *I. scapularis* habitat (19). However, farmland and pastures are often converted to new housing developments and thus may serve as a focus for human population and subsequent infection risk. In comparison to canine serology, mapping risk

of infection by analysis of human case reports appeared to be less accurate in identifying high-risk areas. This shortcoming is most likely due to reliance on clinical or laboratory confirmation of disease in the human case report data as well as bias due to variations in physician visits, patient access to medical care, and reporting bias. Similar issues are thought to contribute to a dramatic underestimation of the number of cases of LB that occur each year in the United States (37).

The role of population density in creating risk for infection with *B. burgdorferi* is best considered in combination with environmental and social variables. The most densely populated areas of the region do not pose a high risk for infection due to the predominantly urban environment, and nor do rural, isolated areas that people or dogs rarely enter. Human and canine traffic in suitable habitat is key to creating risk. This crossroads phenomenon has been previously described using forest fragmentation, which presumably breaks the forest into smaller areas, allowing more human and canine exposure to forest edge habitat, and thus tick exposure (38, 39). The present study is unable to address these two competing forces at the same time, but the data did show a consistent trend to under-predict both canine positive tests and human case reports in the more populous areas, including Queens, Bronx, Hudson, Kings, and New York counties (Figure 2 & 3). These counties appear to have a higher than expected seroprevalence of canine infection with *B. burgdorferi*, supporting the interpretation that many canine and human infections are acquired when travelling outside more urban areas of the NYC MSA.

Like all analyses applied to the natural environment, the present study has limitations. The environmental and social variables used in the analysis have all been

averaged or calculated for the entire ten-year study period to reduce fluctuations that may introduce bias. This approach provides a steady value for each variable, but also constrains the results within the time period evaluated; accordingly, our results may not accurately predict cases of LB in the future due to changes in environmental and social variables over time. Moreover, the canine data is only available on a county level, and habitat characteristics can vary widely within a county, limiting the resolution of any analysis. Available data about human case reports by county were also sparse. Despite these limitations, we were able to use specific (C6) canine serology and selected environmental factors to accurately predict risk of infection in an endemic area. However, this strategy likely should be adjusted before applying to other endemic regions and would only be expected to have value in areas where autochthonous transmission of *B. burgdorferi* is confirmed to occur. Endemic areas are best identified by the presence of infected vector ticks together with laboratory-confirmed evidence of local transmission of that infection to people or dogs. In non-endemic areas, antibodies reactive to *B. burgdorferi* in dogs can be attributed to the use of non-specific assays or the result of testing dogs translocated from regions where a maintenance cycle is known to exist (3, 4, 7, 40, 41).

CONCLUSIONS

Evidence of past or current infection with *Borrelia burgdorferi* is common in dogs in the New York City metropolitan area, and most intraregional variation in canine seroprevalence can be explained by human population density, habitat type, temperature, and precipitation. Deciduous and mixed forests in the region provide ideal habitat and

microenvironments to support *I. scapularis* populations and represent areas of high risk for infection. Moreover, canine seroprevalence using specific assays for *B. burgdorferi* accurately represents risk of human LB in this endemic area, although we would not expect the same to hold true in non-endemic areas or with less specific diagnostic tests. Routine annual testing of dogs for specific antibodies to *B. burgdorferi* furthers understanding of both human and canine exposure risk and likely minimizes the bias inherent in estimating risk by human case reports alone.

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Table 1: Environmental and social variables compared to percent positive canine tests and human case reports of Lyme borreliosis

Factor	p-value for percent positive canine tests	R² for percent positive canine tests	p-value for human case reports of Lyme borreliosis	R² for human case reports of Lyme borreliosis
Canine percent positive	NA	NA	<0.00001	0.47
Human cases of LB/ 10⁵ people	<0.00001	0.47	NA	NA
Population Density	0.044	0.14	0.1012	0.09
Income	0.1646	0.07	0.1182	0.08
Minimum Temperature- November	<0.00001	0.70	0.0002	0.39
Maximum Temperature- November	<0.00001	0.55	0.0029	0.27
Precipitation- November	0.1846	0.06	0.6065	0.01
Normalized Difference Vegetation Index (NDVI)- November	0.0003	0.37	0.0126	0.20
Open Water	0.1454	0.07	0.2529	0.05
Developed- Open Space	0.3032	0.04	0.1226	0.08
Developed- Low Intensity	0.0001	0.44	0.0009	0.33
Developed- Medium Intensity	<0.00001	0.68	0.002	0.29
Developed- High Intensity	0.0022	0.29	0.0461	0.13
Barren Land	0.2574	0.05	0.4635	0.02
Deciduous Forest	<0.00001	0.78	0.0003	0.38
Evergreen Forest	0.2861	0.04	0.3632	0.03
Mixed Forest	0.0017	0.30	0.2086	0.06
Shrub/Scrub	0.2727	0.04	0.002	0.29
Grassland/Herbaceous	0.4696	0.02	0.6411	0.008
Pasture/Hay	0.0017	0.30	<0.00001	0.68
Cultivated Crops	0.1456	0.07	0.173	0.07
Woody Wetlands	0.0731	0.11	0.9843	0.000
Emergent Herbaceous Wetlands	0.0133	0.20	0.094	0.10

Table 2: Backward stepwise regression analyzing environmental and social variables against percent positive canine tests

R	R Square	Adjusted R Square	p-level > F
0.9601	0.9218	0.9014	1.364e-11
VAR	Beta	B	p-level > t
Human case reports of LB per 10⁵	0.3403	0.0263	0.0002
Population Density	0.2556	0.00001	0.0044
Maximum Temperature-November	0.2766	0.0187	0.0495
Deciduous Forest	0.8209	31.6845	0.000005
Mixed Forest	0.3797	76.9569	0.0002
Precipitation-November	0.223	0.0051	0.0021
Constant	-68.887		

Table 3: Backward stepwise regression analyzing environmental and social variables against human case reports of Lyme borreliosis.

R	R Square	Adj R Square	p-level > F
0.8706	0.7579	0.7399	4.83E-09
VAR	Beta	B	p-level > t
Percent positive canine tests	0.3395	4.4046	0.0058
Pasture/ Hay	0.6366	1316.1	5.83E-06
Constant	-39.9383		

Figure 1. Evidence of antibody to *Borrelia burgdorferi* in dogs by county in the New York City Metropolitan Statistical Area, grouped according to percent positive tests. Counties are labeled with 2 letter abbreviations and were coded as follows: 0 – 5% (light blue), 6 – 10% (blue), 11 – 20% (dark blue), and > 20% (very dark blue).

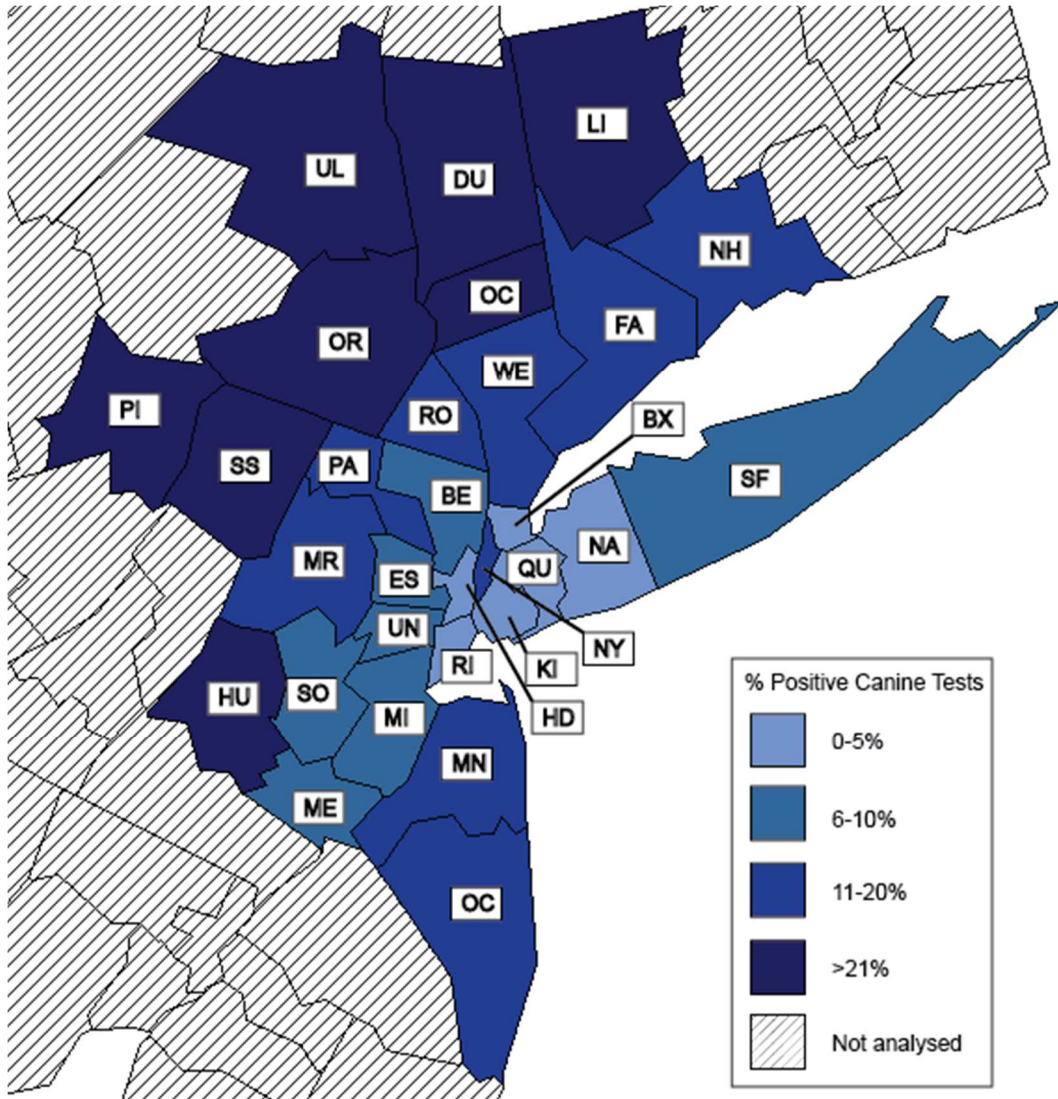


Figure 2. Predicted and observed percent positive canine tests for each county.

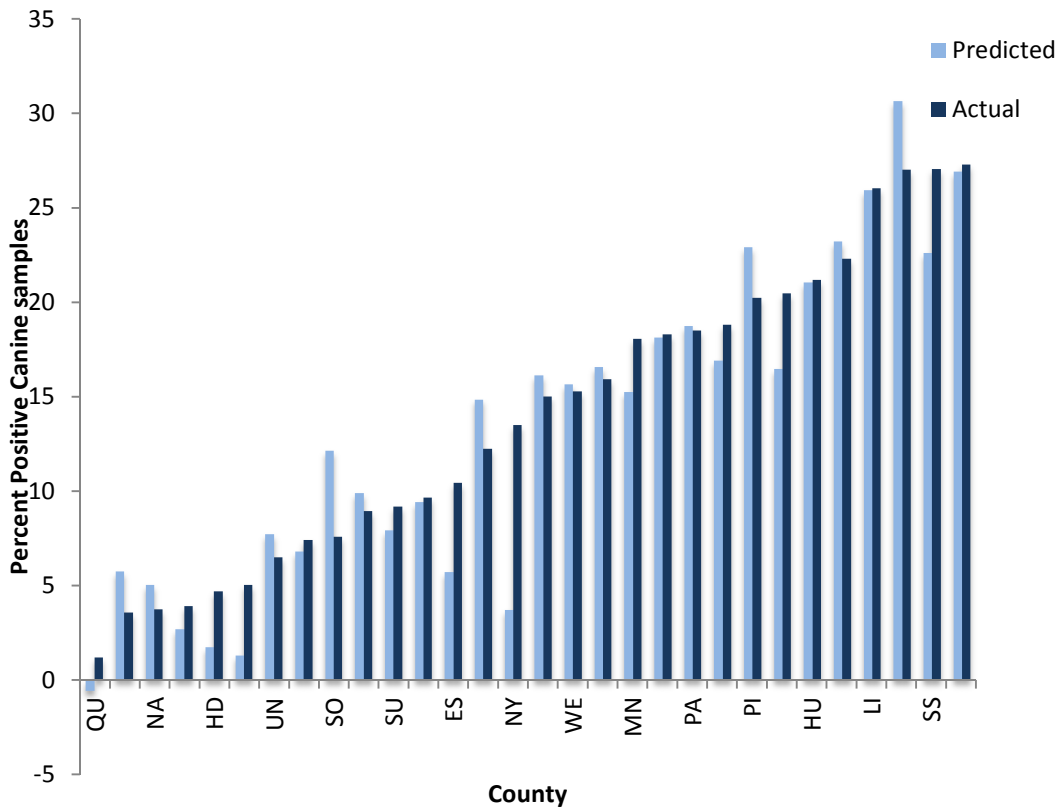
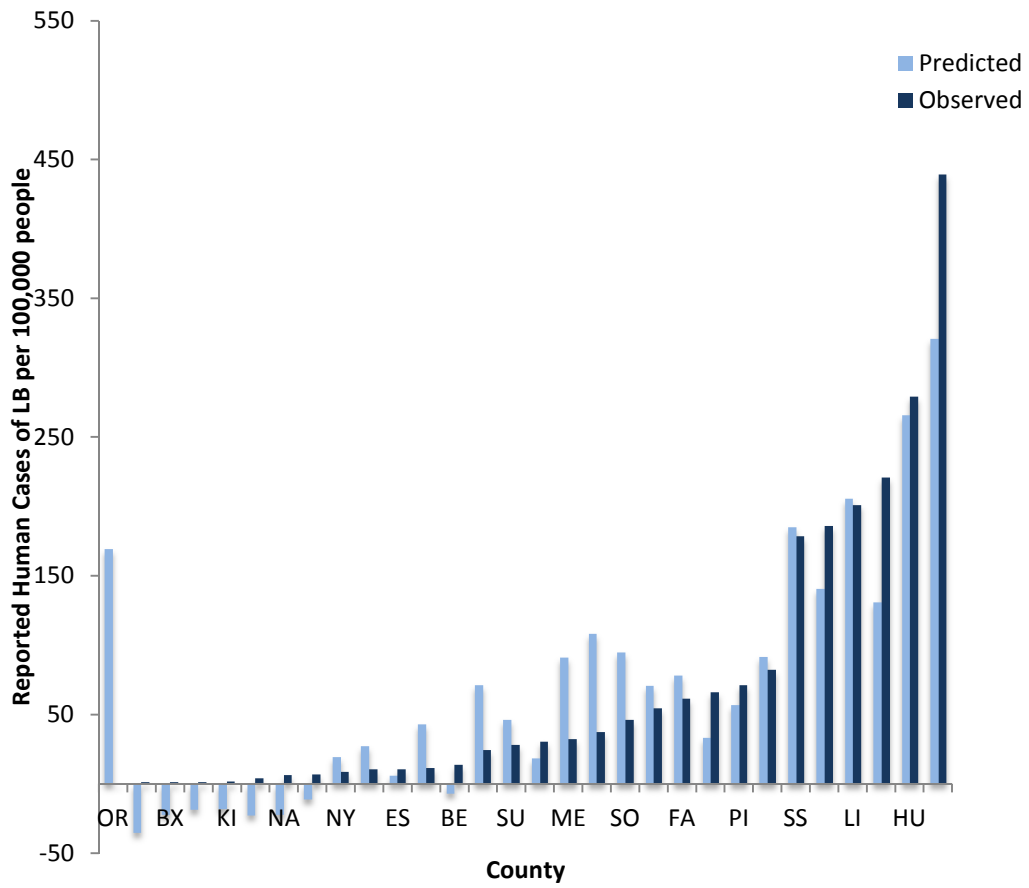


Figure 3. Predicted and observed human case reports for each county.



CHAPTER V

CANINE INFECTION WITH *BORRELIA BURGDORFERI*, *DIROFILARIA* *IMMITIS*, *ANAPLASMA* SPP., AND *EHRlichia* SPP. IN CANADA, 2013–2014¹

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ABSTRACT

Background. Canine test results generated by veterinarians throughout Canada from 2013–2014 were evaluated to assess the geographic distribution of canine infection with *Borrelia burgdorferi*, *Dirofilaria immitis*, *Ehrlichia* spp., and *Anaplasma* spp.

Methods. The percent positive test results of 115,636 SNAP® 4Dx® Plus tests from dogs tested annually were collated by province and municipality to determine the distribution of these vector-borne infections in Canada. **Results.** A total of 2,844/115,636 (2.5%) dogs tested positive for antibody to *B. burgdorferi*. In contrast, positive test results for *D. immitis* antigen and antibodies to *Ehrlichia* spp. and *Anaplasma* spp. were low, with less than 0.5% of dogs testing positive for any one of these three agents nationwide. Provincial seroprevalence for antibodies to *B. burgdorferi* ranged from 0.5% (Saskatchewan)–15.7% (Nova Scotia); the areas of highest percent positive test results were in close proximity to regions in the United States considered endemic for Lyme borreliosis, including Nova Scotia (15.7%) and eastern Ontario (5.1%). These high endemic foci, which had significantly higher percent positive test results than the rest of the nation ($P < 0.0001$), were surrounded by areas of moderate to low seroprevalence in New Brunswick (3.7%), Quebec (2.8%), and the rest of Ontario (0.9%), as well as northward and westward through Manitoba (2.4%) and Saskatchewan (0.5%). Insufficient results were available from the westernmost provinces, including Alberta and British Columbia, to allow analysis. **Conclusion.** Increased surveillance of these vector-borne disease agents, especially *B. burgdorferi*, is important as climate, vector range, and habitat continue to change throughout Canada. Using dogs as sentinels for these pathogens can aid in recognition of the public and veterinary health threat that each pose.

Keywords

Borrelia burgdorferi, *Dirofilaria immitis*, *Ehrlichia*, *Anaplasma*, Canada, Canine

BACKGROUND

Vector-borne diseases are an emerging concern for veterinarians and physicians in much of Canada. The prevalence of vector-borne infections, including Lyme borreliosis (LB), is increasing, apparently due to changing environmental and climatic conditions (Simon et al., 2014; Ogden et al., 2014; Eisen et al., 2016). Lyme borreliosis, heartworm, anaplasmosis, and ehrlichiosis are four common vector-borne diseases that are regularly diagnosed in dogs in the United States (Bowman et al., 2009). Determining the range and prevalence of the agents that cause these diseases throughout Canada may enhance awareness of their importance, encouraging preventive measures and leading to prompt, accurate diagnosis and appropriate treatment.

Canine LB in North America is caused by infection with the spirochete *Borrelia burgdorferi* sensu stricto; other LB agents reported from people have not been identified in dogs. Disease in dogs is characterized by fever, lethargy, anorexia, and lymphadenopathy, but can progress to more severe manifestations such as arthritis and glomerulonephritis (Krukpa and Straubinger, 2010). Transmission to humans and dogs is by *Ixodes* sp. ticks; *I. scapularis* is the vector for the eastern half of Canada and *I. pacificus* the most important vector in British Columbia (Ogden et al., 2008). Ticks harboring *B. burgdorferi* have been identified throughout central and eastern Canada, including parts of Manitoba, Ontario, Quebec, Nova Scotia, and New Brunswick (Ogden et al., 2006). LB-endemic areas of Canada are defined as locations where all three life-stages of the tick (larva, nymph, adult) have been collected for 2 consecutive years and *B.*

burgdorferi infection has been confirmed in ticks or vertebrate hosts (Health Canada, 1991). LBis the most commonly reported vector-borne disease of people in the United States (CDC, 2015); approximately 25,000 cases are reported each year in the US, while in Canada, approximately 700 new cases were reported in 2015 (Public Health Agency of Canada, 2015; Hatchette et al., 2015 EID). This higher risk of infection in the US is also seen in pet dogs. Over 7% of dogs tested from 2012 – 2014 were positive for antibodies to *B. burgdorferi* in the United States (Little et al., 2014). In contrast, only 0.7% and 2.1% of dogs were reported to test positive in Canada in 2008 – 2010, respectively (Villeneuve et al., 2011; Quorollo et al., 2014).

Dirofilaria immitis, causative agent of canine heartworm disease, is considered the most important helminth infection of dogs in the United States (Bowman et al., 2009). Mosquito vectors acquire *D. immitis* microfilariae when feeding on infected dogs and transmit the third-stage larvae, which then migrate and develop within dogs (Kotani and Powers, 1982; Kume and Itagaki, 1955). The presence of adult heartworms in the pulmonary vasculature is a potential source of significant pathology (Jackson, 1974; Ishihara et al., 1978; Atwell et al., 1995). Heartworm infection has been reported in dogs in Canada since 1977, but the prevalence has remained relatively low at around 0.2% (Slocombe et al., 1993; Villeneuve et al., 2011). Because heartworm has historically been relatively uncommon in the region, most Canadian veterinary parasitologists recommend a seasonal preventive strategy. In addition, yearly testing is recommended for patients in high risk groups, including dogs who travel to endemic areas or those not receiving any preventive, or those on a preventive with poor compliance (Klotins et al., 2000 CanVetJ). Interestingly, over 77% of dogs that tested positive for infection with *D. immitis* in one

report had no travel history outside the region, supporting autochthonous infection, albeit at a low level (Villeneuve et al., 2011).

The rickettsial agents *Anaplasma phagocytophilum*, *A. platys*, *Ehrlichia canis*, and *E. ewingii* are all tick-borne bacterial pathogens infecting leukocytes of their host (Rikihisa 1991). These agents induce similar clinical signs and laboratory findings ranging from fever, anorexia, myalgia, and thrombocytopenia to severe manifestations such as epistaxis and death (Rikihisa, 1991).

Anaplasma phagocytophilum is transmitted through the bite of an *Ixodes* spp. tick, and is the causative agent of human granulocytic anaplasmosis (HGA) (Rikihisa, 2006). Previous canine serologic surveys in Canada have reported that the prevalence of dogs with antibodies to *A. phagocytophilum* is rising, with no dogs testing positive in 2006 but a prevalence ranging between 0.2% – 1.1% just five years later (Gary et al., 2006; Villeneuve et al., 2011; Qurollo et al., 2014). *Anaplasma platys*, causative agent of canine cyclic thrombocytopenia, is transmitted by *Rhipicephalus sanguineus* and infects platelets of dogs (Harvey et al., 1978; Harvey, 2006). In a previous study, 1.8% of dogs tested in Canada were reported to have antibodies to *A. platys* (Qurollo et al., 2014).

Ehrlichia canis is the causative agent of canine monocytic ehrlichiosis, and is also transmitted by *R. sanguineus*; infection causes anemia, thrombocytopenia, and, in severe cases, potentially fatal bleeding diathesis (Harrus and Waner; Neer and Harrus, 2006).

Ehrlichia ewingii is the causative agent of canine granulocytic ehrlichiosis and is transmitted by *Amblyomma americanum*. The range of *A. americanum* has dramatically expanded northward and eastward in recent decades (Springer, 2014). While *A. americanum* populations are not yet considered established in Canada, the tick is

occasionally reported from domestic dogs in Ontario with no travel history out of the region (Peregrine unpublished). Of the two, only *E. canis* has been reported in Canada previously, with 3.2% of dogs tested having antibodies to the pathogen, while 0/285 dogs tested positive for *Ehrlichia chaffeensis* or *E. ewingii* (Quorollo et al., 2014).

Evidence of past or current infection with all of these pathogens can be identified with assays commonly used for annual heartworm testing and as a screening tool for tick-borne infections, and the composite results can be evaluated on both a local and national level. For example, by reviewing the changing prevalence of antibody-positive dogs over time, previously undocumented areas of expansion of LB were detected (Bowman et al., 2009; Little et al., 2014). The present paper seeks to build on previous publications (Villeneuve et al., 2011; Quorollo et al., 2014), potentially identifying areas of recent expansion of LB as well as monitoring the overall distribution of these vector-borne infections in Canada.

MATERIALS AND METHODS

Source of data

The data collected were obtained from the SNAP[®] 4Dx[®] Plus Test kit (IDEXX Laboratories, Inc., Westbrook, Maine), an in-clinic ELISA for the simultaneous detection of canine antibodies to *B. burgdorferi*, *A. phagocytophilum*, *A. platys*, *E. canis*, and *E. ewingii*, and antigen of *D. immitis*. The results were generated from January 2013 through December 2014 by veterinarians testing patients in-clinic, mainly during routine annual wellness examination, and recording the data manually or by IDEXX SnapShot Dx[®] instrumentation. For privacy, results were provided with no patient or owner

identification, therefore travel history, confirmatory diagnostics, and clinical outcome for each result is not known.

***Borrelia burgdorferi* assay**

The analyte utilized for the *B. burgdorferi* assay is the C₆ peptide, which detects antibodies to a surface lipoprotein of *B. burgdorferi sensu stricto*. The sensitivity and specificity of the analyte is reported in the package insert to be 94.1% and 96.2% (IDEXX Laboratories, Inc., Westbrook, ME), respectively, but published studies with different populations report different values. For example, in comparison to a two-tiered, gold standard diagnostic process utilizing immunofluorescence (IFA) and Western blot (WB), the test sensitivity was 94.4% (O'Connor, 2004; Chandrashekar, 2010), and the test specificity has been reported to be 99.5% when used on field samples from dogs (O'Connor, 2004; Duncan, 2004). The C₆ analyte has also been shown to not cross-react with other *Borrelia* spp. found in the US or react to antibodies produced through vaccination (O'Connor, 2004)

Heartworm assay

The assay utilized detects *D. immitis* antigen primarily produced from the uterus of female heartworms. The sensitivity and specificity reported for the heartworm portion of the assay is 99.0% and 99.3%, respectively (IDEXX Laboratories, Inc., Westbrook, ME). Other studies have reported the sensitivity of this analyte as 84%, but that value varies with intensity of infection, with a sensitivity of 64% when only one adult, female heartworm is present and 98% when 4 or more adult heartworms are present (Chandrashekar, 2010; Atkins, 2003).

***Anaplasma* assay**

Analytes were used that detect antibodies to a peptide from the MSP2/p44 major surface protein of two distinct *Anaplasma* spp.: *A. phagocytophilum* and *A. platys*. Detection of *A. platys* was added after recognizing significant cross-reactivity (SNAP[®] 4Dx[®] Test kit insert, IDEXX Laboratories, Inc., Westbrook, ME). The reported sensitivity and specificity of the test are 90.3% and 94.3%, respectively (IDEXX Laboratories, Inc., Westbrook, ME). The sensitivity of the assay is 99.1% for *A. phagocytophilum* and 89.1% for *A. platys* when compared to IFA, while the specificities are reported as 100% and 99.8% respectively, although sensitivity and specificity against field samples may vary (Chandrashekar, 2009; Stillman 2014).

***Ehrlichia* assay**

Analytes were used that detect antibodies to the p30 and p30-1 proteins of *E. canis* and the p28 protein of *E. ewingii*. The reported sensitivity and specificity of this assay is 97.1% and 95.3%, respectively (IDEXX Laboratories, Inc., Westbrook, ME). In other studies, when compared to IFA or WB, the sensitivity was 95.7% for *E. canis* and 96.5% for *E. ewingii* (O'Connor, 2002; Stillman, 2014). The test specificity for *E. canis* has been shown to be 100% (O'Connor, 2004, 2006), while specificity for the detection of antibodies to *E. ewingii* is 93.9% (Stillman, 2014). Infection with other *Ehrlichia* spp. may induce cross-reactive antibodies leading to a positive test result on the *Ehrlichia* spp. analyte (O'Connor, 2004; Hegarty et al., 2012).

Data and statistical analysis

Data were collated by three-digit postal code of the veterinary practice where the test was performed, and then assembled into municipalities or major metropolitan areas

and provinces. Only municipalities reporting more than 30 test results were included in the study. Percent positive test results were calculated by dividing the number of dogs with a positive test result by the total number of test results reported for each agent of interest. For all samples, 95% confidence intervals were calculated using the modified Wald method (GraphPad Software, La Jolla, CA). Maps were assembled using the Canada base map and the Hatch Map function on MapViewer 7 (Golden Software, Golden, CA).

Differences in reported frequency of positive test results between municipalities and provinces were evaluated using a Chi-square test in StatPlus (Windows 7, Redmond, WA; AnalystSoft, Alexandria, VA) with significance assigned at $P < 0.0001$ as previously described (Bowman, 2009).

RESULTS

Summary

Test results were available from a total of 225 practices in 2013 and 198 practices in 2014, representing 115,636 data points from 84 different municipalities across Canada. Ontario reported the highest number of test results (77,143) followed by Quebec (23,701), Manitoba (12,765), New Brunswick (1,631), Nova Scotia (210), and Saskatchewan (186). All other provinces and territories had fewer than 30 test results reported in a single municipality.

Borrelia burgdorferi

The prevalence of antibody positive dogs nationwide was 2.5% (2,844/115,636) with provincial prevalence ranging from 0.5 – 15.7%. Over half (44/84) of the

municipalities reported 2% or greater positive test results, while 7 reported less than 0.5% positive test results. Positive test results for antibodies to *B. burgdorferi* were most common in Nova Scotia, with 15.7% of samples from this province testing positive, which was higher than the national average ($P < 0.00001$). Other provinces had percent positive test results higher than the national average, including New Brunswick (3.7%; $P = 0.001$), and Quebec (2.8%; $P < 0.00001$). Ontario had a lower overall seroprevalence than the national average (2.3%; $P < 0.00001$), but in a cluster of 11 municipalities in eastern Ontario more than 5.1% (1,335/26,081; $P < 0.00001$) of dogs tested positive.

Dirofilaria immitis

Nationwide, 0.42% (485/115,636) of dogs tested positive for heartworm antigen, and no province had percent positive test results greater than 0.5% (0-0.5%). Ontario had the highest percent positive tests (0.50%). Two municipalities had percent positive test results higher than 2%: Mirabel in Quebec (5.0%; 2/40; 95% CI 0.50% – 17.4%) and Caledonia in Ontario (4.1%; 207/5,111; 95 % CI 3.5% – 4.6%). Both of these municipalities had a higher prevalence than the national average and the rest of the respective province ($P < 0.00001$).

Anaplasma spp.

Antibody to *Anaplasma* spp. was detected in 0.29% (331/115,636) of dogs, with a provincial seroprevalence ranging from 0.0 – 0.95%. Nova Scotia and Manitoba were the only provinces that had a higher prevalence than the national average with 0.95% and 0.86% of all tests reported positive, respectively; the total number of positive tests in municipalities within these provinces that had a seroprevalence above 1.0% ranged between 2 and 12 positive tests. Percent positive test results in Ontario were significantly

lower than the national average at 0.22% ($P < 0.00001$); no municipalities in Ontario had percent positive test results over 1.0%.

***Ehrlichia* spp.**

Antibody to *Ehrlichia* spp. was identified in 0.19% of tests with a range among the provinces of 0-1.6%. Saskatchewan had the highest seroprevalence of any province (1.6%; $P < 0.00001$). A total of 4 municipalities across Canada had a reported seroprevalence higher than 1%; Saskatoon in Saskatchewan (1.6%; 3/186; 95% CI 0.33% – 4.9%), Hampton in New Brunswick (1.3%; 2/152; 95% CI 0.06% – 5.0%), and Bruce and Port Hope in Ontario (1.2%; 3/250; 95% CI 0.24% – 3.6% and 1.0%; 6/590; 95% CI 0.41% – 2.2% respectively).

DISCUSSION

The dataset in the present paper was obtained from veterinarians in practice and allowed us to determine the prevalence of four vector-borne infections throughout Canada. As reported in previous studies, the data are biased towards major population centers where the majority of dogs and dog owners reside (Little et al., 2014). While the prevalence of positive tests for heartworm antigen and antibody to *Ehrlichia* spp. and *Anaplasma* spp. were low in all provinces, there was evidence of past or current infection with at least one of these agents in every province reporting data (Table 1 and Figs. 1-4).

Percent positive tests for antibodies to *B. burgdorferi* were higher in the present study than reported in 2011 (0.72%; $P < 0.00001$), but not significantly different than more recent reports (2.1%; $P = 0.70$) (Villeneuve et al., 2011; Quorllo et al., 2014). Moderate (>1%) or high (>5%) percent positive tests in dogs were identified in areas with frequent

reports of human LB and where surveillance of ticks has confirmed the presence of *B. burgdorferi* (Werden et al., 2015; Hatchette et al., 2015; Ogden et al., 2010; Gabrielle-Rivet et al., 2015). These areas are also in close proximity to the northeastern or upper midwestern regions of the United States where LB is endemic or hyperendemic (Little et al., 2014). While the prevalence of *B. burgdorferi*-specific antibodies ranged from 0.5-15% for different provinces, there were also four municipalities with percent positive test results above 20%, the highest of which was Pictou County, Nova Scotia at 40.6% (13/32). Areas such as Pictou County, southern Quebec, and eastern Ontario appear to constitute hyperendemic foci (>5% positive tests) with a declining prevalence radiating outward. This effect is likely exaggerated by human population clusters in southern Ontario, but can also represent true foci of increased infection risk including the 11 municipalities in eastern Ontario where the seroprevalence is 5.1% versus the rest of the province with a seroprevalence of 0.87% ($P < 0.00001$).

Positive test results for heartworm antigen were most commonly seen near major population centers like Montreal and Toronto, with the rest of the municipalities reporting a prevalence of < 2% (Fig 2). This urban-centered phenomenon is common in heartworm ecology in the US as domestic dogs serve as the major reservoir for infection of mosquitoes and large cities may harbor “heat islands” that create more favorable biologic conditions for the mosquitoes as compared to the surrounding rural areas (Paras, 2014). While the total prevalence across Canada was quite low (0.42%) in the present paper, it was significantly higher than the previously described prevalence of 0.22% ($P < 0.00001$) (Villeneuve, 2011). Other studies have shown that heartworm prevalence in dogs in Canada has remained stable at approximately 0.2% over the last 30 years

(Klotins, 2000). This apparent doubling in prevalence over the last 5 years may indicate increased testing of dogs in which infection is suspected. Alternatively, it could reflect a northward expansion of mosquito vectors due to changes in climate patterns in the region (Ogden, 2014).

The analyte for *Anaplasma* spp. detects antibodies to both *A. phagocytophilum* and *A. platys*. *Anaplasma phagocytophilum* is transmitted by *I. scapularis*, like *B. burgdorferi*, and thus when mapped these two tick-borne infections often co-localize (Little et al., 2014). Some correlation between the two test results can be seen in this dataset, but it was not as strong as expected (Pearson's Correlation Coefficient $\rho=0.34$). While the municipalities with the highest *Anaplasma* spp. seroprevalence ($>2.0\%$) were associated with *B. burgdorferi* seroprevalence over 4.8% ($\rho=0.6$), the municipalities with the highest prevalence of antibodies to *B. burgdorferi* ($>10\%$) did not correspond to high *Anaplasma* spp. seroprevalence ($>1\%$) ($\rho=0.17$). *Anaplasma phagocytophilum* appears to circulate in nature at a lower level than *B. burgdorferi*, and detection of this pathogen in newly endemic areas may be difficult (Werden, 2015; Bowman, 2009; Little, 2014; Dahlgren 2015). The assays used in the present paper also detect antibody to *A. platys* and it is not possible to differentiate that response from antibody to *A. phagocytophilum*. Reports of *R. sanguineus*, the vector for *A. platys*, are rare in Canada with less than 20 ticks reported per year in Ontario, in comparison to *I. scapularis*, which averages over 1,000 submissions each year (Nelder et al 2014). Nonetheless, confirmed cases of *A. platys* in Canada have been reported as co-infections with *E. canis* and explained by travel to areas where *R. sanguineus* are more common (Al Izz 2013).

Antibodies to *Ehrlichia* spp. were least commonly detected in the present study, likely due to a dearth of vector ticks in the region. As for *A. platys*, the risk for autochthonous transmission of *E. canis* by *R. sanguineus* in Canada is low, although travel cases may be diagnosed and reported (Al Izzi 2013). Similarly, *A. americanum*, the vector of *E. ewingii* and *E. chaffeensis*, is still considered rare in this area of North America (Springer 2014; Nelder et al 2014). Interestingly, the majority of positive tests for antibodies to *Ehrlichia* spp. were in southwestern Ontario, directly adjacent to the Midwest region of the United States that has now described *Ehrlichia muris*-like agent (EMLA) as a new *I. scapularis*-transmitted pathogen (Johnson EID 2015). While more research is needed, existing data suggest antibodies to EMLA may be cross-reactive with existing assays for *Ehrlichia* spp. antibodies including that used in the present paper (Hegarty et al., 2012). Although the natural maintenance cycle is not fully defined, EMLA has been identified in *I. scapularis* and white-footed mice (*Peromyscus leucopus*) (Saito 2015; Castillo 2015).

When nationwide data are collected, as in this study, there are limitations to the utility and interpretation of the data. Reporting bias, travel history, and detection method all factor into the prevalences presented (Bowman et al., 2009). In regions where low numbers of total tests are being reported, veterinarians may be using the SNAP[®] 4Dx[®] Test kit as a targeted diagnostic test rather than an annual wellness screening tool, a factor which may explain the high seroprevalence to *B. burgdorferi* reported from Nova Scotia (Table 1). Unfortunately, the current lack of data in western Canada prevents analysis in that region despite confirmation that *B. burgdorferi* is endemic in the northwestern United States and in British Columbia (Morshed et al., 2015). It should also

be noted that the low number of test results available in some areas and the low positive predictive values in low prevalence populations complicate interpretation (Peregrine 2005; Peregrine 2007).

This nationwide data can aid veterinarians in making informed decisions on annual canine wellness procedures that would be most beneficial, including acaricide use, heartworm prevention, and vaccination for *B. burgdorferi*, and when evaluated over time, the results can help document the changing distribution of vector-borne infections (Bowman et al., 2009, Little et al., 2014). Finally, these vector-borne pathogens have been documented to cause disease in humans, and mapping the risk of canine infection also describes the areas where humans are most likely to be infected (Duncan et al., 2005; Schurer JM 2014; Gaito 2014). Further prevalence studies are warranted to investigate regions with no data at present and to provide updates on the changing distribution of these infections, particularly as they become newly endemic.

CONCLUSIONS

This study serves as an update on the positive test results for common vector-borne infections in dogs, in Canada. Antibodies to *B. burgdorferi* were most commonly identified; the prevalence of infection in many provinces and the national average was higher than previously reported. While still low, percent positive *D. immitis* antigen tests were twice that reported 20 years ago, suggesting an increase in prevalence of mosquito-borne heartworm. Infections with *Anaplasma* spp. and *Ehrlichia* spp. appear to remain fairly uncommon throughout Canada. While the work described here did not control for travel or false positives, canine serology is a key tool for monitoring vector-borne

infections on a large scale and can be used to track the geographic spread of these agents and assess public health risks over time. Collectively, the data support efforts by veterinarians and physicians to protect pets and people from an increasing threat of vector-borne infections.

COMPETING INTERESTS

In the past five years, SL has received reimbursement, speaking fees, or research support from IDEXX Laboratories, manufacturer of diagnostic tests for the heartworm and tick-borne disease agents. In addition, JG and MB are employees of IDEXX Laboratories.

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AUTHORS' CONTRIBUTIONS

SL and MB conceived of the study, SL, BH, and MB coordinated its design and execution and drafted the manuscript, and JG and AP reviewed and validated the data and the manuscript. All authors read and approved the final version of the manuscript.

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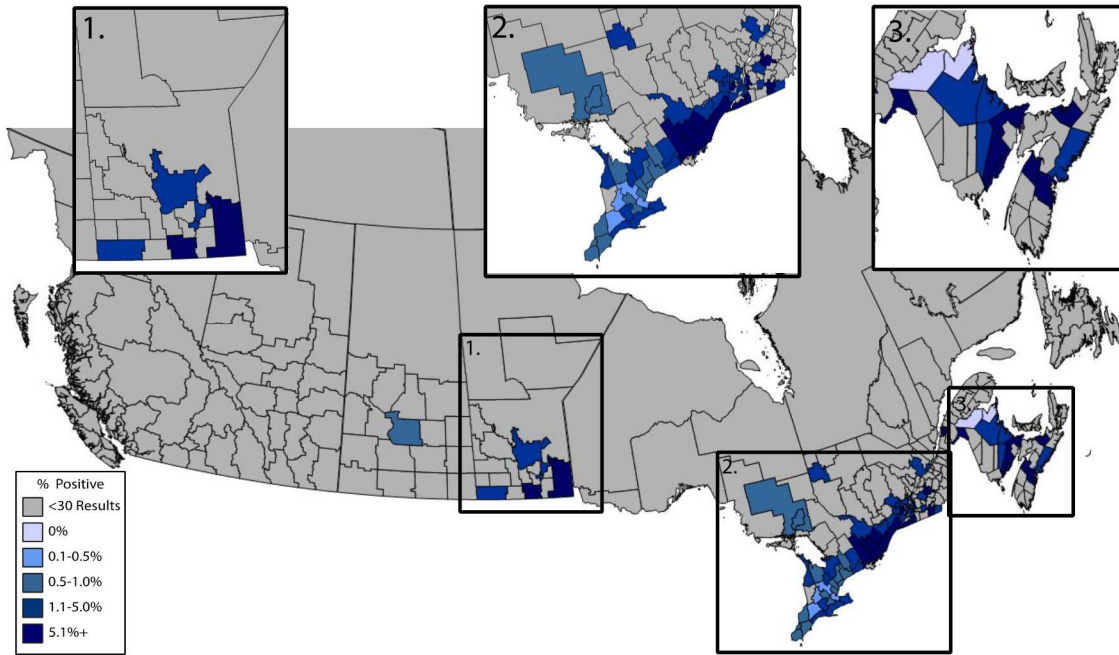
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Table 1. Vector-borne infections in dogs in Canada, 2013–2014

Province	<i>Borrelia burgdorferi</i> % (95% CI) Number positive	<i>Dirofilaria immitis</i> % (95% CI) Number positive	<i>Anaplasma</i> spp. % (95% CI) Number positive	<i>Ehrlichia</i> spp. % (95% CI) Number positive
Manitoba n = 12,765	2.4% (2.1 – 2.7) 303	0.20% (0.12 – 0.28) 26	0.86% (0.70 – 1.0) 110	0.24% (0.16 – 0.32) 31
New Brunswick n = 1,631	3.7% (2.9 – 4.7) 60	0.12% (0.01 – 0.48) 2	0.43% (0.19 – 0.90) 1	0.12% (0.01 – 0.48) 2
Nova Scotia n = 210	15.7% (11.4 – 21.3) 33	0.48% (0.01 – 2.9) 1	0.95% (0.04 – 3.6) 2	0% (0.00 – 2.2) 0
Ontario n = 77,143	2.3% (2.2 – 2.4) 1780	0.50% (0.45 – 0.55) 385	0.22% (0.19 – 0.25) 166	0.19% (0.16 – 0.22) 146
Quebec n = 23,701	2.8% (2.6 – 3.0) 667	0.30% (0.23 – 0.37) 71	0.19% (0.13 – 0.25) 46	0.16% (0.11 – 0.21) 37
Saskatchewan n = 186	0.54% (0.01 – 3.3) 1	0% (0.00 – 2.4) 0	0% (0.00 – 2.4) 0	1.6% (0.33 – 4.9) 3
National n = 115,636	2.5% (2.4 – 2.6) 2,844	0.42% (0.38 – 0.46) 485	0.29% (0.26 – 0.32) 331	0.19% (0.16 – 0.22) 219

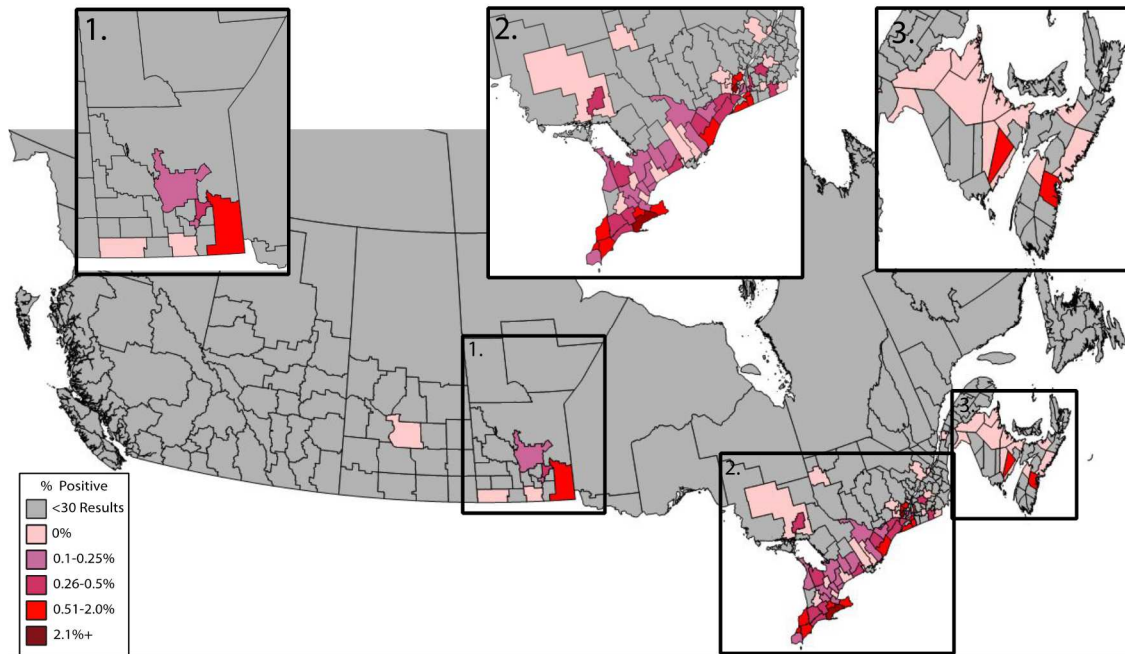
Percent positive test results, (95% Confidence Intervals (CI)), and total number positive by province for dogs tested from 2013 – 2014 for antigen of *Dirofilaria immitis* and antibody to *Borrelia burgdorferi*, *Ehrlichia* spp., and *Anaplasma* spp.

Figure 1. Percent positive antibody tests to *Borrelia burgdorferi* in dogs by municipality.



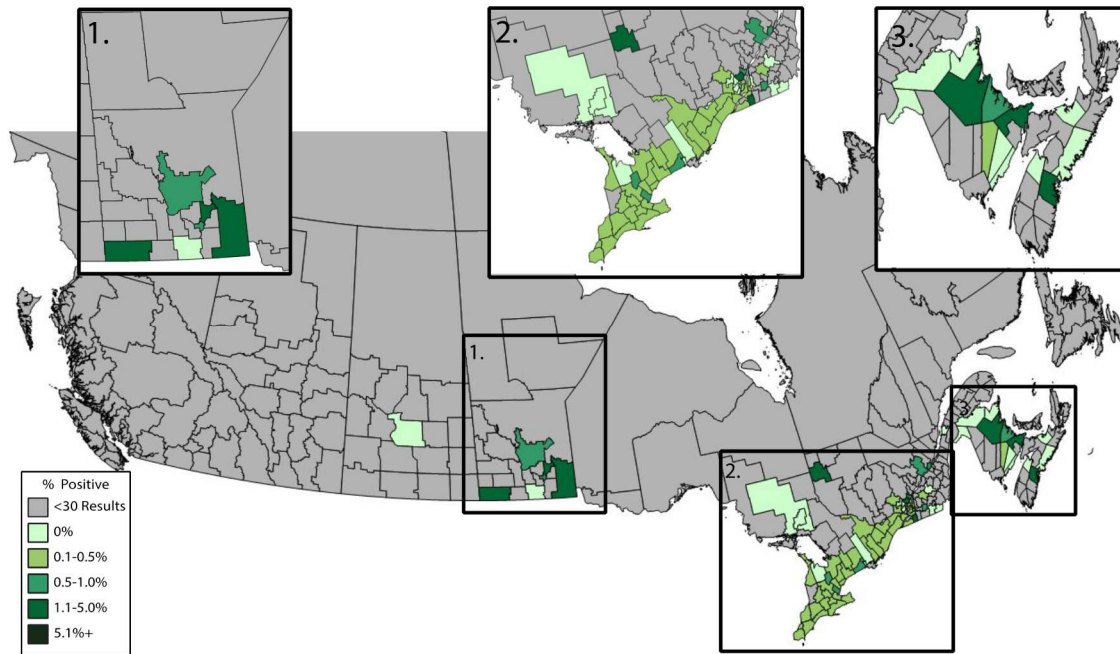
Evidence of antibody to *Borrelia burgdorferi* in dogs by municipality throughout Canada, 2013 – 2014, grouped according to percent positive tests.

Figure 2. Percent positive antigen tests of *Dirofilaria immitis* in dogs by municipality.



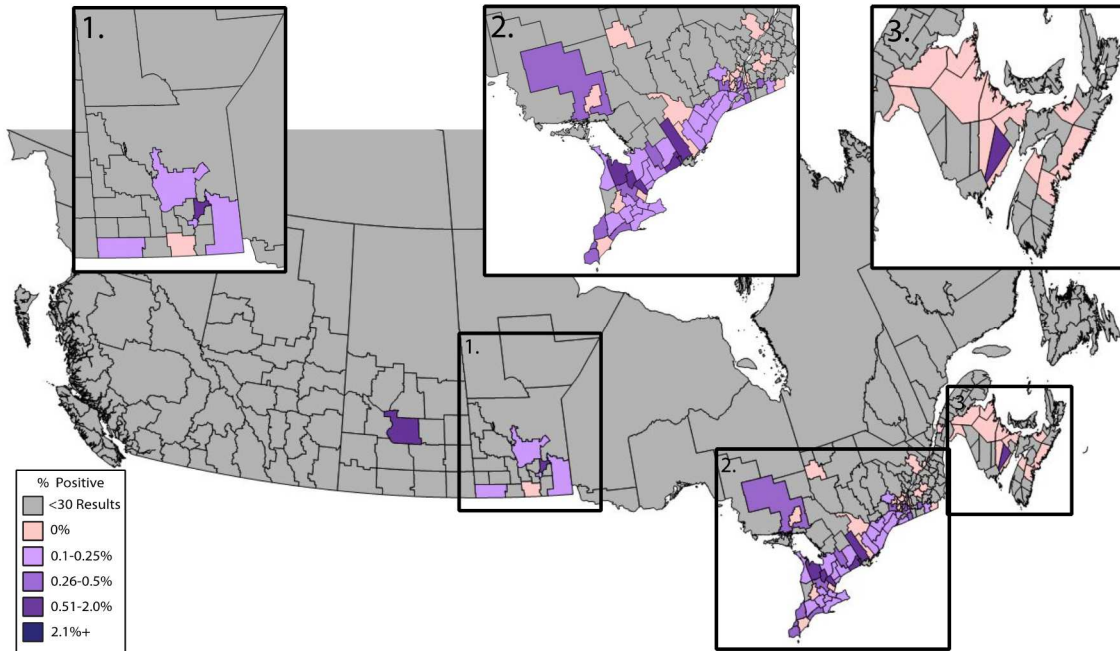
Evidence of antigen of *Dirofilaria immitis* in dogs by municipality throughout Canada, 2013 – 2014, grouped according to percent positive tests.

Figure 3. Percent positive antibody tests to *Anaplasma* spp. in dogs by municipality.



Evidence of antibody to *Anaplasma* spp. in dogs by municipality throughout Canada, 2013 – 2014, grouped according to percent positive tests.

Figure 4. Percent positive antibody tests to *Ehrlichia* spp. in dogs by municipality.



Evidence of antibody to *Ehrlichia* spp. in dogs by municipality throughout Canada, 2013 – 2014, grouped according to percent positive tests.

CHAPTER VI

CONCLUSIONS

Serologic monitoring for common vector-borne diseases of dogs, including Lyme borreliosis (LB), has been commonly pursued in veterinary practices over the last 10 years (Bowman et al., 2009; Villeneuve, 2011; Little et al., 2014; Quorollo et al., 2014). Current survey data for antibodies to *Borrelia burgdorferi* in this region show dogs in the Upper Midwest and Northeast regions of the US and Southeast Canada are more commonly infected with *B. burgdorferi* when compared to dogs from other areas (Bowman et al., 2009; Villeneuve, 2011; Little et al., 2014; Quorollo et al., 2014). The overarching goal of the research reported in this dissertation was to use canine serology as a tool to further characterize the LB-endemic regions of North America.

The goal of the first study was to determine if the range of *Ixodes scapularis* ticks harboring *B. burgdorferi* had expanded into southwestern Virginia. Changes in canine antibody prevalence to *B. burgdorferi* in the region suggested the endemic range of the pathogen had spread southward along the Appalachian Mountains (Bowman et al., 2009; Little et al., 2014). Of 364 ticks collected, all were confirmed to be *I. scapularis*. In addition, 33% of ticks tested positive for *B. burgdorferi* sensu stricto by PCR. Both the tick population and the prevalence of pathogen within them are consistent with LB-endemic areas historically considered to be limited to the Northeast. These data not only

show the expansion of *I. scapularis* and *B. burgdorferi* southward, but also highlight the utility of canine serology to inform targeted tick testing to confirm the expansion of both the bacteria and its vector.

The second study sought to define the social and environmental factors associated with human case reports and canine seroprevalence of *B. burgdorferi* infections in the New York City Metropolitan Area. Previous studies have shown that canine serology to *B. burgdorferi* correlates with human case reports and can be used to describe areas where humans are likely to become infected (Duncan et al, 2005; Mead et al., 2011). Also, environmental factors have been used to predict areas where both humans and dogs are likely to become infected, but previously, no studies have analyzed which reporting method would be most useful in describing the environments most suited for increased risk of infection (Messier et al., 2016; Guerra et al., 2001). As expected, the case reports and prevalence of positive antibody tests increased with increasing forested area, becoming progressively higher as distance from the more densely populated regions increased. Furthermore, a more complete description of environmental factors related to risk of infection, including mixed and deciduous forest, precipitation, and maximum temperature, was developed by comparing to canine serology positive tests (adjusted $R^2=0.90$). This study highlighted the utility of canine serology as an additional factor to help accurately describe the endemic areas of LB in North America.

The final study involved continued surveillance of canine vector-borne infections in Canada. Previous research in Canada has documented low seroprevalence to common vector-borne infections including *B. burgdorferi*, *Dirofilaria immitis*, *Anaplasma* spp., and *Ehrlichia* spp., but as climate and environmental factors change these infections are

expected to become more common (Villeneuve et al., 2011; Qurollo et al., 2014). Overall seroprevalence of antibodies to *B. burgdorferi* was 2.5%, while the prevalence of the other agents was below 0.5% each. These data reaffirm the endemicity of LB in parts of southern and eastern Canada, including Nova Scotia, New Brunswick, Quebec, and Ontario, which all had canine antibody seroprevalence similar to LB endemic areas in the United States. Overall, this study showed the utility of continued surveillance of vector-borne infections in dogs as vector populations in Canada continue to change over time.

In summary, continued surveillance of antibodies to *B. burgdorferi* in dogs can be beneficial to both canine and human health. Continued monitoring of canine seroprevalence to *B. burgdorferi* allows us to track the changing prevalence of infection over time as well as the geographic distribution, which we have documented provides the most accurate description of the true endemic range of the disease. Moreover, the data can also be used to describe those environments within that range that best support the bacteria, tick vector, and reservoir hosts to maintain the transmission cycle in nature. By combining all this information, we can make inferences on where the endemic range for LB may expand in the future, providing key information to aid medical professionals in the diagnosis of the disease in these areas.

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