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MINIREVIEW

Evolutionary Aspects of Emerging Lyme Disease in Canada

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In North America, Lyme disease (LD) is a tick-borne zoonosis caused by the spirochete bacterium *Borrelia burgdorferi sensu stricto*, which is maintained by wildlife. Tick vectors and bacteria are currently spreading into Canada and causing increasing numbers of cases of LD in humans and raising a pressing need for public health responses. There is no vaccine, and LD prevention depends on knowing who is at risk and informing them how to protect themselves from infection. Recently, it was found in the United States that some strains of *B. burgdorferi sensu stricto* cause severe disease, whereas others cause mild, self-limiting disease. While many strains occurring in the United States also occur in Canada, strains in some parts of Canada are different from those in the United States. We therefore recognize a need to identify which strains specific to Canada can cause severe disease and to characterize their geographic distribution to determine which Canadians are particularly at risk. In this review, we summarize the history of emergence of LD in North America, our current knowledge of *B. burgdorferi sensu stricto* diversity, its intriguing origins in the ecology and evolution of the bacterium, and its importance for the epidemiology and clinical and laboratory diagnosis of LD. We propose methods for investigating associations between *B. burgdorferi sensu stricto* diversity, ecology, and pathogenicity and for developing predictive tools to guide public health interventions. We also highlight the emergence of *B. burgdorferi sensu stricto* in Canada as a unique opportunity for exploring the evolutionary aspects of tick-borne pathogen emergence.

Lyme disease (LD; also called Lyme borreliosis) occurs throughout the temperate region of the Northern Hemisphere and is caused by species of the *Borrelia burgdorferi sensu lato* species complex. In Canada and the United States, *B. burgdorferi sensu stricto* (referred to here as *B. burgdorferi*) is the sole species known to be associated with human disease (1). These bacteria are maintained in nature by wild-animal reservoir hosts (rodents and other mammals as well as birds) and are transmitted by hard-bodied (ixodid) ticks which feed on these animals. LD was first recognized and began emerging in the United States in the 1970s (2). Since then, the incidence of the disease has increased in the United States to reach an estimated 300,000 cases a year at the time of writing (3). In the United States, 90% of LD cases occur in two foci, the Northeast and the upper Midwest, while in the Pacific and southern regions of the United States, human cases are less frequent (4). The geographic pattern of high-risk areas is associated with the occurrence of the tick *Ixodes scapularis* and, in part, with the distribution of certain *Borrelia* genotypes (5, 6). The emergence (or, probably more accurately, reemergence) of LD in the United States is thought to be due to reforestation following land use changes during the 20th century which led to expansions of populations of the wild-animal hosts of *I. scapularis* (7). Possibly driven by a warming climate (8), *I. scapularis* is now expanding its range into Canada from Manitoba through Ontario and Quebec to New Brunswick and Nova Scotia (9), with LD spirochetes invading newly established tick populations (10), resulting in increases in annual reported human case numbers (from <50 in 2004 to 682 in 2013 [11]). Risk modeling suggests that case numbers will increase rapidly in the coming years in Canada as *I. scapularis* invades the most heavily populated southern parts of Canada (9, 12).

The emergence of LD in North America in general, and in Canada in particular, involves the spread of a wildlife-borne zoonosis of considerable significance for human health, driven by environmental change, and, as such, is an issue of "One Health" (the interconnected and mutually dependent nature of human, animal, and environmental health [13, 14]). Understanding the mechanisms of emergence of LD, useful for prediction, therefore requires simultaneous consideration of the three components of One Health (13).

As in the United States, LD cases in Canada have three main stages: (i) early LD, (ii) early disseminated LD, and (iii) late LD (15). Erythema migrans (EM), a spreading painless erythematous skin lesion of ≥5 cm in diameter at the site of the infective tick bite, is the typical first distinct symptom of early LD (16–18). EM disappears within 1 to 2 weeks, when the spirochetes disseminate hematogenously from the skin, marking the onset of early disseminated LD. The main manifestations of early disseminated LD are neurological (radiculopathy, cranial neuropathy, and mononeuropathy multiplex), cardiac (atrioventricular heart block that can cause sudden death [19]), and cutaneous (multiple EM lesions). Neurological disease mainly affects the peripheral nervous system,

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while late LD comprises arthritis and neurological manifestations (17). Reinfection following treatment and recovery is common, particularly if individuals are reinfected with a different strain (20, 21). In up to 20% of patients, symptoms that can be debilitating persist following treatment, a condition known as posttreatment Lyme disease syndrome (PLDS) (17, 22).

Detection of antibodies to *B. burgdorferi* is the mainstay of LD diagnosis but is complicated by specificity and sensitivity issues (23). Consequently, the gold standard method of serodiagnosis is a two-tier approach comprising a screening immunofluorescence assay (IFA) or enzyme immunoassays (EIA) followed by Western blotting using standardized criteria to interpret the bands to obtain the best balance of sensitivity and specificity (24). Test sensitivity is low in early LD but increases as the disease progresses (25).

Whether or not untreated infections go on to produce disseminated LD may depend (among other factors) on the infecting strain of *B. burgdorferi* (see below). Identification of which strains of *B. burgdorferi* may cause disseminated LD and where they occur is of importance in Canada for the following reasons. First, numbers of cases of LD are currently increasing almost exponentially in Canada (11), consistent with earlier assessments of increasing risk due to tick range expansion (12). Second, there is currently no vaccine for humans, and effective public health actions resulting in tick avoidance/infection prevention, targeted at those who are at risk, are paramount for reducing the impact of emerging LD in Canada (26). As the clinical impacts of LD and postinfection immunity are strain specific (21, 27), the strain structure of *B. burgdorferi* in a particular locality will determine the extent to which a local population is at risk from disseminated LD and can suffer multiple reinfections year after year. The ability to identify which strains cause disseminated LD, and to be able to predict their occurrence in Canada by understanding any associations with environmental factors or animal hosts, would permit implementation of preventive public health actions that are "smarter" by targeting the greatest effort at protecting the populations at the greatest risk from severe LD. We also need to identify pathogenic strains for future studies that can elucidate the interactions of the human, animal, and bacterial genomes that result in infection and disease (28, 29, 30, 31).

DIVERSITY OF *BORRELIA BURGDORFERI*

The etiological agent of LD was identified in 1981 and named *Borrelia burgdorferi* (32, 33). The LD group of spirochetes consisted of >20 named or proposed species at the time of writing, with 8 in North America: *B. burgdorferi*, *B. andersoni*, *B. bissettii*, *B. californiensis*, *B. carolinensis*, *B. americana*, *B. kurtenbachii*, and *B. garinii* (although this species is found only in cliff-nesting seabird colonies in Newfoundland) (34). *B. burgdorferi* is itself a diverse species. The genome of *B. burgdorferi* comprises a linear chromosome carrying genes needed for cell maintenance and replication (the occurrence of genes encoding proteins for metabolic purposes is very limited, likely as an adaptation to a strictly parasitic life style) and a large number of circular and linear plasmids which carry the genes encoding most of the outer surface proteins (Osp), which are involved in interactions with host and vector (35). *Borrelia* species have been delineated using DNA-DNA association, 23S-5S intergenic spacer (IGS), and 16S sequences and multilocus sequence analysis (MLSA) (34, 36, 37). A number of methods of strain typing and genotyping *B. burgdorferi* have been used (38). These include determination of the chromosomal 16S–23S (*rrs-rrlA*) IGS by restriction fragment length polymorphism (RFLP) analysis, which classifies isolates into one of three groups of ribosomal sequence types (RSTs) (39), and by typing of IGS sequences (40) and analysis of sequences of the plasmid-encoded OspA and OspC (41). More recently, multilocus sequence typing (MLST) using sequences of housekeeping genes has become available (42). There is considerable correlation among the strain typing classifications obtained by analysis of IGS sequences and *ospC* alleles due to extensive linkage disequilibrium in the genome of *B. burgdorferi*. This is thought to be due to the low rates of multiplication in this organism and, relative to other bacteria, the more limited opportunities for horizontal transfer of genomic DNA and plasmids due to its vector-borne ecology (40). For this reason, it has been suggested that *ospC* is a lineage-defining gene (43), but this linkage has proved not to be absolute, and the use of sequences of multiple housekeeping genes, as used in MLST, is currently considered to be the method of choice for phylogenetic and phylogeographic analyses (34). Next-generation sequencing and analysis of single nucleotide polymorphisms (SNP) shows promise for the future (44, 45, 46, 47) but has yet to be readily applicable (mostly in terms of bioinformatics management) to larger, epidemiologically useful sample sizes for the study of *B. burgdorferi* in North America to the degree that these techniques are beginning to be applied to enterobacteriaciae (48).

At the time of writing, 111 sequence types (STs) of *B. burgdorferi* have been identified by MLST in North America (49) and their occurrence shows clear geographic patterns—for the most part, different STs are found in the northeastern United States, in the midwestern United States, and in California (Fig. 1), and geographic and landscape barriers currently limit gene flow between these groups (50). However, the main phylogenetic pattern of clades is not geographically defined, as illustrated in Fig. 2: clonal complexes (which mostly equate with clades in the phylogenetic tree [49]) comprise STs from multiple geographic locations, as illustrated in Fig. 2 by the color coding of STs according to the geographic location of origin described in the Fig. 1 legend. The initial population expansion of *B. burgdorferi* in North America has been estimated to have occurred thousands to a million years ago (51). The origin of the clades is unclear, and hypotheses for their occurrence include introductions from Europe and glacial-interglacial cycles altering ecological suitability for population expansion. Either way, because the main clades are not associated with geographical isolation, we speculate that clades may relate broadly to some form of ecological "isolation," which for *B. burgdorferi* may be host species association. In this scenario, successful expansions involved strains particularly adapted to hosts that were abundant at that time and whose descendants may persist today (49). There is evidence that some *B. burgdorferi* strains may be more efficiently transmitted by, or more frequently associated with, some host species than others (52, 53, 54, 55). This evidence comprises statistical associations from field and laboratory observations (including traits such as longer periods of postinfection transmissibility [52, 54]) rather than "smoking-gun," experimentally demonstrated mechanisms such as sensitivity to an alternative pathway complement (see reference 56 and below); here we use the term "host species association" to mean that certain strains are more efficiently transmitted by, or persist in, infected individuals of certain host species. However, the niche breadth of *B. burgdorferi* strains is wider than that of other *Borrelia* species: there is certainly not strong host specialization such as there is for *B. burg-*

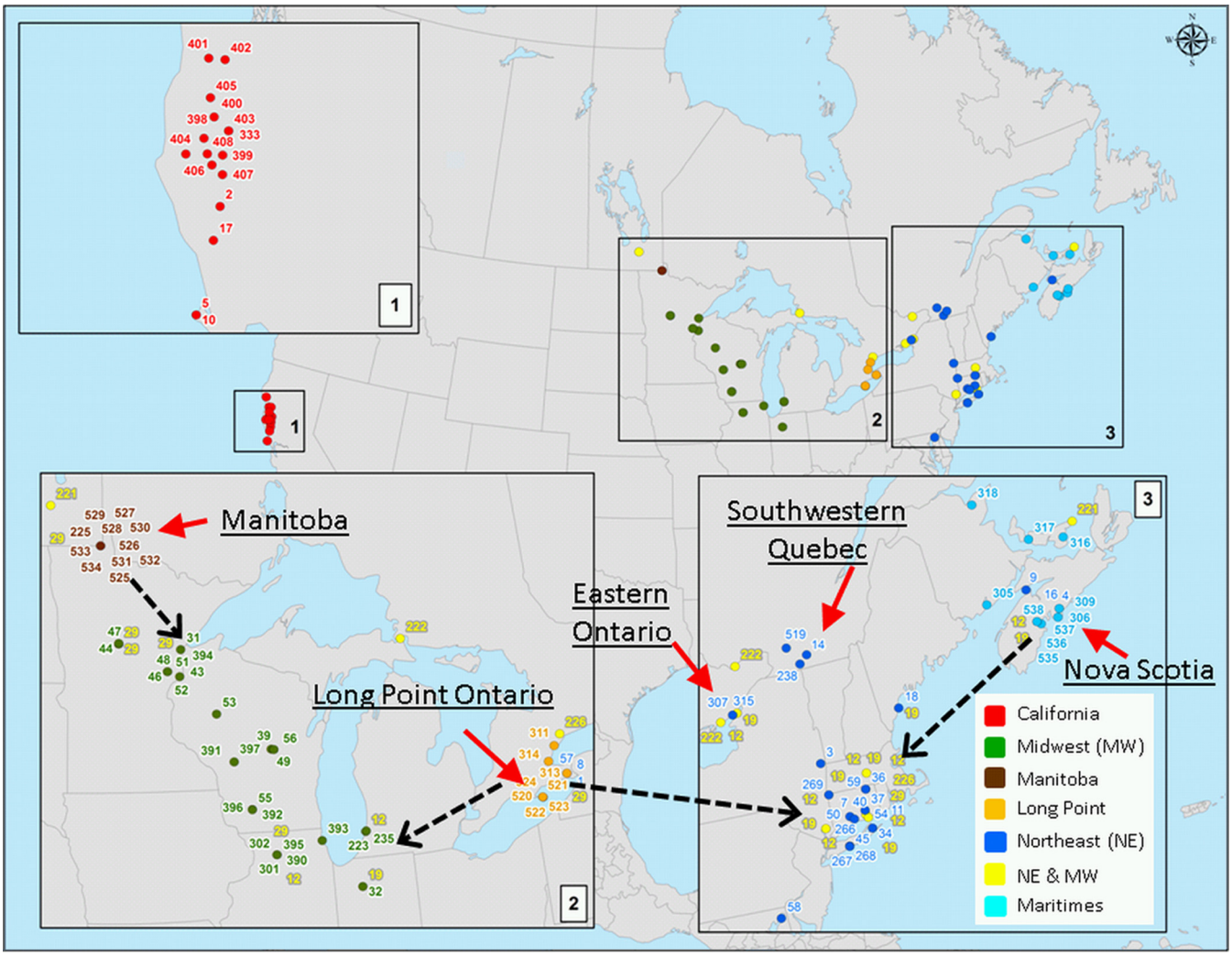

FIG 1 Locations where *B. burgdorferi* samples that have been subjected to MLST analysis have been collected in North America. The colored points indicate locations where all samples analyzed were collected, while red arrows indicate the locations of field sites where new samples in a recent study in Canada were obtained (49). The different colored points correspond to sequence types (STs) found in different geographic regions. Cyan, STs found only in the Maritimes, Canada; orange, STs found only at Long Point, Ontario, Canada; brown, STs found only in Manitoba, Canada; blue, STs found across the northeastern United States and southern Quebec, southeastern Ontario, and the Maritimes in Canada; green, STs found in the midwestern United States; yellow, STs found in both northeastern and midwestern locations of the United States and longitudinally corresponding regions of Canada; red, STs found only in California. The dashed black arrows indicate the likely locations of immediate ancestors of novel STs found in Canada. Maps were created in ArcGIS. (Adapted from reference 49.)

dorferi sensu lato species in Europe (57), and, to our knowledge, *B. burgdorferi* strains in North America remain host generalists (i.e., can infect and be transmitted from a wide range of wild-animal host species [58]).

It would be expected that the strains of *B. burgdorferi* now emerging in Canada would be the same as those occurring in the United States in locations directly to the south, assuming that migratory birds and other hosts are carrying ticks and *B. burgdorferi* from south to north each spring (59). Initial MLST-based studies of the diversity of *B. burgdorferi* in ticks collected in passive surveillance in Canada have tended to support this hypothesis (55). However, there is uncertainty as to the origin of ticks collected in passive surveillance, particularly when this involves ticks collected by veterinarians, because dogs very readily pick up ticks, including "adventitious" ticks, which may be dispersed from their location of origin (sometimes long distances of >500 km) by migratory birds or other hosts (60). More recently, we studied the diversity of *B. burgdorferi* in ticks collected from the environment or from hosts in locations in southeastern and south central Canada, where reproducing and self-sustaining populations of *I. scapularis* ticks and *B. burgdorferi* are known to be endemic and emerging (the locations of the sites of the populations are shown in Fig. 1). In that study, we found that strains in some Canadian populations (those in Manitoba and western Ontario and the Maritimes) are frequently different from those in the United States (albeit with recent ancestors immediately to the south in the United States), while in one region (extending from southeastern Ontario to southwestern Quebec), the strains are almost all the same as those in the northeastern United States (49). Details of the geographic occurrence of these strains are shown in Fig. 1. We have proposed that the occurrence of the novel strains is due to immigration of *B. burgdorferi* strains from multiple refugial pop-

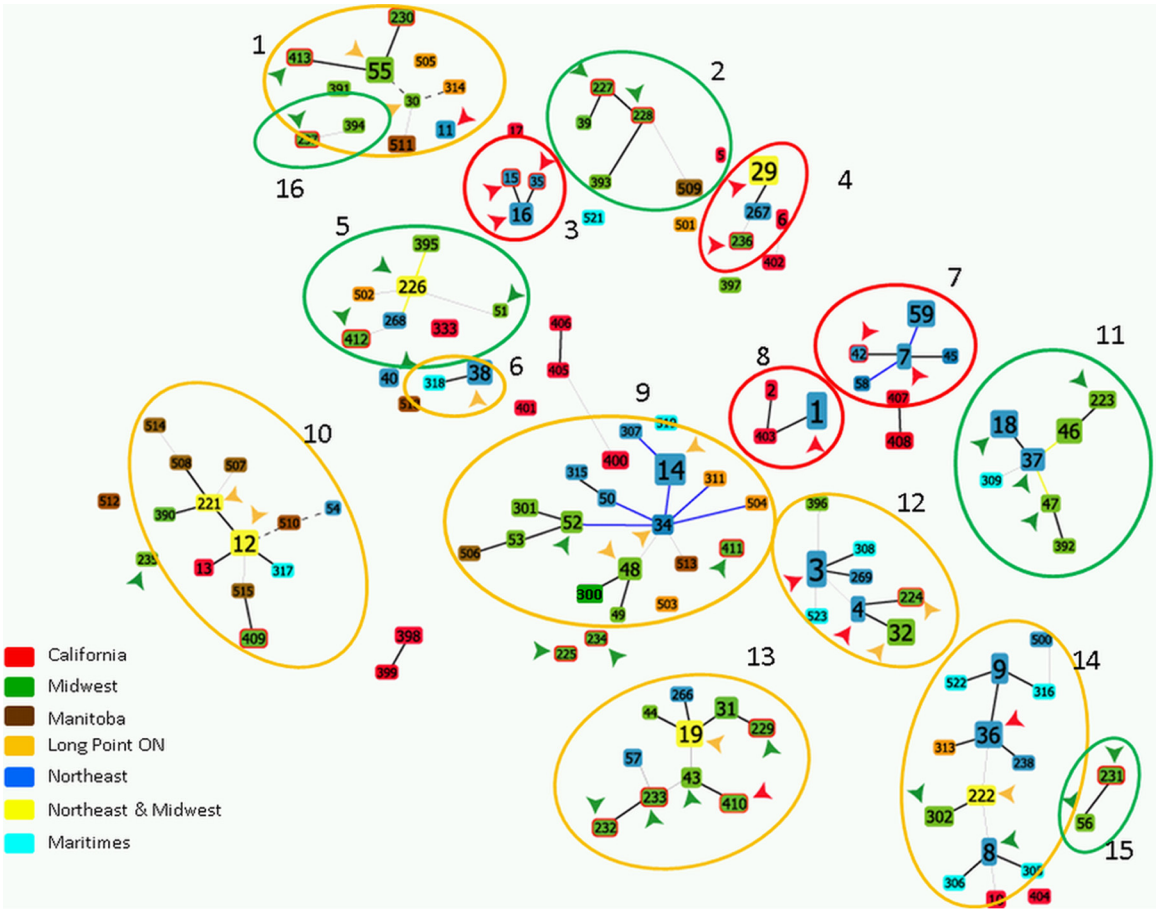

FIG 2 A population snapshot created in goeBurst of *B. burgdorferi* sequence types (STs; i.e., strains which are individually numbered) identified by MLST in samples from the United States and Canada (49). Many STs are linked (being single- or double-locus variants) into clonal complexes in which the deduced relationships are identified by the color of the lines connecting STs as follows: gray and blue lines indicate (respectively) double- and single-locus variants, while yellow and black lines indicate relationships inferred (respectively) without tiebreak rules and with tiebreak rules. STs are identified by their individual number and color coded by location of origin as follows: blue, STs common to the northeastern United States, Quebec, eastern Ontario, and the Maritimes; green, STs common to the midwestern United States, Manitoba, and western Ontario; yellow, STs occurring in both the northeastern and midwestern United States and Canada; red, STs from California; cyan STs occurring only in the Maritimes; orange, STs occurring only at Long Point Ontario; brown, STs occurring only in Manitoba. Arrows indicate pathogenicity as follows: red, STs always (to date) associated with disseminated LD; orange, STs sometimes associated with disseminated LD; green, STs not associated with disseminated LD. Red circles surround clonal complexes of STs that mostly cause disseminated LD, orange circles surround clonal complexes of STs that sometimes cause disseminated LD, and green circles surround clonal complexes of STs that do not (to our current knowledge) cause disseminated LD (27).

ulations in northern parts of the United States adjoining Canada in which studies of *B. burgdorferi* strain structure have not been conducted to date. This conclusion was reached because the novel strains in each site were mostly scattered about the phylogenetic tree and among clonal complexes (illustrated in Fig. 2) rather than comprising the distinct clades that would be expected if the new strains had arisen simply from recent founders or strains surviving in Canadian refuges (49). The increasing recognition that certain reservoir host species of *B. burgdorferi* such as the deer mouse (*Peromyscus maniculatus*) and the eastern chipmunk (*Tamias striatus*) may have survived glacial periods in northern refugia in North America may support the idea of the occurrence of multiple small refugia in the northern United States (61, 62). Even so, it is likely that strains occurring in these regions are uncommon in the United States, and they have not been detected in human cases to date (25), which could in part be due to low human population densities in the northern parts of states bordering Canada. Importantly, for Canada, where (in contrast to the United States) human population densities are highest close to the Canada-United States border, the clinical and diagnostic consequences of infection with these strains are currently unknown.

ECOLOGICAL ORIGINS AND CLINICAL AND DIAGNOSTIC IMPORTANCE OF *B. BURGDORFERI* DIVERSITY

In Europe, genospecies of the *B. burgdorferi sensu lato* species complex have almost absolute specialization for different reservoir host species and often produce a different range of LD symptoms when they infect humans (56, 63). A key molecular mechanism of this quasi-host species specialism (sensitivity to alternative pathway complement) is well established (56), although the precise mechanisms whereby different genospecies cause different types of disease in humans are not fully understood. Possible mechanisms include genospecies-specific tissue tropism, proliferation, and elicitation of pathology-causing immune responses

(64, 65, 66). In North America, there is evidence that different strains of *B. burgdorferi* vary in their capacity to elicit an immune response in humans that is detectable, in the early stages of infection, by current methods of serodiagnosis (i.e., the two-tiered test comprising EIA followed by Western blot analysis interpreted by CDC-recommended criteria [1, 24]). Studies in mouse models suggest the possibility that some strains cause at least some pathology associated with disseminated infection, despite a failure to elicit a serological response detectable by two-tier method; one RST-3 strain of *B. burgdorferi* (determined by RFLP analysis of 16S-23S rRNA gene spacer sequences) produced some evidence of carditis in C3H mice but did not elicit a detectable immune response (67). While the two-tiered serological test has good sensitivity in later LD, sensitivity is low in very early disease (i.e., at the EM stage), and up to 20% of patients may test negative in very early disseminated LD (25). To date, there have been no discoveries of specific determinants of pathogenicity or virulence factors of *B. burgdorferi* such as those represented by the toxin secretion and invasion-encoding pathogenicity islands of many other pathogenic bacteria (68, 69, 70), and it is thought that most of the pathogenic effects are due to local inflammation induced by the bacterium and effects of autoantibodies (71, 72). The capacity of *B. burgdorferi* strains to systemically disseminate in humans has been considered equivalent to pathogenicity for this species complex (1, 73). The molecular determinants of "pathogenicity" of *B. burgdorferi* are therefore those that permit generalized dissemination in humans and subsequent persistence, implying that there are likely multiple determinants with complex functions in pathogenicity (74, 75). Studies have demonstrated that the pathogenicity of North American *B. burgdorferi* in humans, defined by whether disease manifestations are limited to the initial EM stage or instead progress to hematogenous dissemination and subsequent development of disseminated disease symptoms, can vary with infecting strain. The plasmid-borne, highly polymorphic *ospC* gene and the 16S-23S (*rrs-rrlA*) IGS have been the most commonly used genetic markers for identifying U.S. strains of *B. burgdorferi* that cause disseminated or localized LD (1, 65, 73, 76, 77, 78). Strains exhibiting restriction fragment length polymorphism in the 16S-23S rRNA intergenic spacer that are designated RST-1 and RST-2 types or that belong to major *ospC* allele groups A, B, H, I, and K are more frequently associated with hematogenous dissemination early in the course of LD, while RST-3 types and strains carrying *ospC* major groups other than A, B, H, I, and K (particularly T and U) are more likely to cause EM lesions alone and nondisseminated infection (1, 65, 73, 76, 77, 78). Different RST groups are also associated with different rates of antibiotic-refractory Lyme arthritis (79), indicating that knowledge of the infecting strain genotype may be important for predicting disease outcome and treatment planning.

Both RST and *ospC* typing methods have proved to be useful tools for classifying *B. burgdorferi* strains by their tendency to disseminate in humans. However, RST typing has limited discriminatory power for this purpose (6, 80), and the suitability of *ospC* typing may also be restricted, since the highly variable *ospC* gene is subject to strong selection by the host immune system (38, 41, 42). While associations of genotypes with dissemination are clear, specific genetic determinants facilitating dissemination remain unknown, although it could be argued that *ospC* could be a direct determinant of dissemination as it is mechanistically involved in *B. burgdorferi*-host interactions during the early phases of infection (81, 82, 83). A recent study showed that pathogenicity is often (genetically) predictable by examination of the housekeeping gene sequences used in MLST for phylogenetic analyses and that these sequences predicted pathogenicity better than *ospC* sequences (27). In Fig. 2, a population snapshot created in goeBurst of *B. burgdorferi* MLST sequence types (STs) from the United States and Canada is shown in which many STs are linked into clonal complexes. Some of these clonal complexes (which mostly correspond to clades in the MLST phylogenetic tree [49]) comprise a mix of STs that have been shown to date to be pathogenic (i.e., produce disseminated LD) with STs that are not pathogenic (i.e., do not produce disseminated LD). However, half of the clonal complexes comprise STs that are either all pathogenic or all nonpathogenic (Fig. 2 [27]). Associations of pathogenicity (as a phenotype) and MLST strain types are unlikely to be mechanistic as the housekeeping genes amplified and sequenced in MLST are not surface expressed and are not involved in interactions with the host. However, the fact that some MLST strain types are particularly associated with pathogenicity is highly significant because it suggests that pathogenicity in humans, who are not involved in the ecology of *B. burgdorferi*, is a phenotype associated with different lineages of the bacterium. In turn, this implies that the bacterial genetic determinants of pathogenicity in humans have origins in the evolution of the bacterium, driven by the ecological factors that have shaped its phylogeny. Because geographic isolation has not been a factor involved in the divergence of the phylogenetic groups (and clonal complexes; Fig. 2) that also appears to describe *B. burgdorferi* pathogenicity (50), alternative ecological factors, including those relating to associations with reservoir host species, may have been influential.

Horizontal gene transfer contributes to genetic diversity in *B. burgdorferi sensu lato*, although, apart from some specific loci, rates of horizontal gene transfer are generally low (84). However, recombination within *B. burgdorferi sensu lato* species has been shown to be 50 times higher than recombination between species (46). Furthermore, in *B. burgdorferi sensu stricto*, most recombination occurs within clades (49). The clades are not geographically defined or isolated, so this may support a hypothesis of host species association of clades, because both the donor strain and the recipient strain must be capable of infecting the same host for recombination to occur in that host or in a tick that subsequently feeds on that host. Current evidence suggests that host species association is mechanistically possible even if that does not mean that strains of *B. burgdorferi* are specialists but may be evolving toward specialism and may have done so in the past (52, 53, 54, 55). Together, these findings support the use of MLST as a strain typing tool useful for exploring the host species associations and pathogenicity of *B. burgdorferi*.

There are currently recognized ecological drivers of diversification of tick-borne pathogens such as *B. burgdorferi*, including drivers of host specialism. To persist in nature, tick-borne pathogens must be transmissible from ticks to hosts and the pathogen must disseminate in the host to locations where uninfected ticks can then acquire the pathogen and, following successful feeding and molting, subsequently transmit the pathogen to another host. In so doing, the pathogen must mechanistically negotiate multiple host environments, evade the innate and acquired immune response, and not kill or debilitate the host, which would prevent onward transmission to ticks (56). There are four key phases to host infection and onward transmission from the host (reviewed

in reference 85): (i) initial infection in the skin of the host at the site of inoculation by an infected tick, which requires evasion of the host innate immune response, potentially via exploitation or binding of vector and/or host molecules (56); (ii) dissemination from the site of infection, which requires motility, binding of host receptors on vascular surfaces, immune evasion, and systemic dissemination during a short period of spirochetemia (86, 87, 88); (iii) persistence of infection to maximize the number of uninfected ticks the infected host can infect, which requires evasion of the host innate and acquired immune response (89, 90, 91, 92); and (iv) transmission from the host to a feeding uninfected tick, which likely requires either persistence in the dermis or some form of redissemination back to the dermis, where the spirochetes can be picked up by ticks (93). Although all of these processes are important determinants of whether or not, and how efficiently, a wild-animal species acts as a reservoir host for a particular strain, processes i to iii are important for infection in humans, and processes ii and iii are likely important in determining the capacity of different strains to cause disseminated LD.

Observed differences in transmission of different strains from reservoir host species in North America (which may underpin field-observed associations of strains with host species) in experimental settings have involved differences in the lengths of the periods postinfection during which the strain is efficiently transmitted from the infected hosts to ticks. Some strains are transmitted almost lifelong by *Peromyscus leucopus* (the white-footed mouse) following infection, while others are transmitted for only a short period postinfection and infection may be eliminated, presumably by the host immune response (51, 54). Those demonstrated to be transmitted almost lifelong by *P. leucopus* were of the RST-1 type of strains that are also known to often cause disseminated LD in humans, while those strains that were transmitted only for short periods were of the RST-3 type of strains that are less associated with disease manifestations involving dissemination of the bacteria (51, 54). Therefore, in some circumstances, RST-1 type strains may predominate in locations where *P. leucopus* mice are dominant hosts for immature ticks. However, short-lived infections were not a general characteristic of the RST-3 strain used because when mouse species other than *P. leucopus* were infected with this strain, efficient transmission to ticks persisted for long period postinfection (54). This could mean that hosts other than mice may be preferred hosts for the RST-3 strain used in these experiments. Therefore, different strains, which show different degrees of pathogenicity in humans, may have adapted to some extent for longer-term persistence in, and transmission from, different host species. This provides experimental support for the hypothesis that the phenotypic characteristics of strains of *B. burgdorferi* that determine their pathogenicity in humans may have their origins in adaptations to host species that advantage their transmissibility and thus persistence in nature.

Geographic variations in tick seasonality likely interplay with host species-strain associations driven by immune or other mechanisms: the more separated are the seasons of activity of infecting nymphal ticks and infection-acquiring larval ticks, the longer the host must remain alive and infective (56). Greater seasonal synchrony of nymphal and larval *I. scapularis* ticks in the upper Midwest of the United States (94) is associated with greater *B. burgdorferi* diversity (27). A recent key finding is that many of the *B. burgdorferi* strains occurring in the more western parts of Ontario and Manitoba and in the Maritimes are different from those found to date in the United States (49) (Fig. 1 and 2). Strains occurring in southern Quebec and eastern Ontario are nearly all the same as those occurring in the northeastern United States, so understanding the pathogenicity of strains in the northeastern United States is more relevant to this region of Canada. However, while the strains here may be the same, we are seeing skewing of strain frequencies compared to those seen in the northeastern United States due to founder events and other processes associated with recent invasion of the bacterium (59). Therefore, we need a greater understanding of which strains in Canada are pathogenic and where they occur. The possible existence of host species association of *B. burgdorferi* strains would mean that the occurrence of different strains may be predictable by the occurrence of particular host species and perhaps also of lineages of key *Peromyscus* sp. rodent reservoir hosts. These lineages have a geographic pattern in North America that is much more complex (95, 96) than that currently considered for *B. burgdorferi* phylogeography (49, 50, 51). This pattern has already proven to be compellingly associated with the phylogeographic pattern of zoonoses other than *B. burgdorferi* (e.g., Sin Nombre virus [97]) and could be similarly associated with *B. burgdorferi*, given recent evidence indicating that exposure of rodent hosts to *B. burgdorferi* infections may reciprocally shape their populations (30). The emergence of *B. burgdorferi* in North America, and particularly in Canada, driven by climate and habitat change, provides an intriguing opportunity to explore the evolutionary aspect of a key emerging vector-borne zoonosis, and evolutionary aspects are recognized as a neglected but critical factor in understanding how interactions among environment, pathogens, and animal hosts determine risks for human health (98, 99).

HOW TO STUDY AND PREDICT THE ECOLOGICAL ORIGINS AND CLINICAL AND DIAGNOSTIC IMPORTANCE OF *B. BURGDORFERI* DIVERSITY

Prospective studies are clearly needed to study the associations of novel *B. burgdorferi* strains in Canada with disseminated LD and their capacity to be detected in diagnostic tests and are also needed to elucidate their ecological origins to allow spatiotemporal prediction of their occurrence. The tools for this are at our fingertips. Apart from the currently available molecular phylogenetic methods of MLST, other methods such as genomic SNP analyses for cultured *Borrelia* strains may help to develop sufficiently sensitive typing tools to obtain information about relevant samples from infected patients and assess strain associations with clinical observations (27). A downside to this is that samples for such study are not usually collected in the course of routine diagnosis, and this will mean designing prospective studies. Systematic studies of wild animals and ticks collected from the field will be essential for a number of reasons. First, using clinical samples from infected people diagnosed using current diagnostic tests to search for novel strains may be equivalent to confining our search to what is visible under the lamppost that currently illuminates human LD cases. Second, in order to develop predictive methods that will help us to understand risk in the environments to which humans are exposed, samples from these environments must be collected and the pathogenic potential of strains occurring there must be characterized. Collection and analysis of relevant samples from ticks and animal hosts is routinely conducted in Canada using well-established field and laboratory protocols (100), and culture of pure strains from mixed infections is very feasible (101) when

next-generation approaches (see, e.g., reference 47) for analysis of mixed infections are not available. Differences in disease severity and dissemination properties among *B. burgdorferi* genotypes have been experimentally tested and corroborated for selected strains in C3H/HeJ mice (67, 102), indicating that mouse pathogenesis studies are very useful for prediction of disease outcomes in humans. Therefore, methods for exploring associations between strains occurring in the field and pathogenicity in humans already exist. Wild-rodent host lineages can be identified using analysis of standard mitochondrial gene targets (96) and host community structure, which may be to some extent estimated by analysis of remote-sensed habitat or environmental data or ground-level landscape analysis (103, 104, 105, 106) and may indicate occurrences of different strains. With current geomatics technology, this information can be readily synthesized into risk maps that permit identification of the populations at risk from different strains by public health end users (12).

CONCLUSIONS

LD continues to emerge in the United States and is now emerging in Canada. Novel strains of *B. burgdorferi* occur in Canada, and these may originate from as-yet-unexplored refugia in the very northernmost parts of the United States bordering Canada. Increasingly, it is recognized that strains of *B. burgdorferi* differ in their capacity to disseminate and cause disease of different severities in humans. Humans do not take part in the natural transmission cycle of *B. burgdorferi*, and pathogenicity in humans is most likely to have evolved as a consequence of interactions with natural reservoir hosts. The potential association of pathogenicity of *B. burgdorferi* strains with different lineages, each in turn associated with different reservoir host species, raises the intriguing possibility that the occurrence of pathogenic *B. burgdorferi* strains may be predictable in ways that are practicable for public health end users. The tools to explore this hypothesis already exist, so such an endeavor would be practical and feasible. The emergence of LD in Canada due to the spread of the tick vector, and *B. burgdorferi*, represents a public health challenge requiring exploration of the ecological and evolutionary origins of genetic diversity of *B. burgdorferi* and its epidemiological and pathological consequences for practical public health purposes. But it also represents an interesting and unique opportunity to explore the evolutionary aspects of the emergence by invasion of a tick-borne pathogen.

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