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Short Report: The Propensity of Different *Borrelia burgdorferi* sensu stricto Genotypes to Cause Disseminated Infections in Humans

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Abstract. Lineages of *Borrelia burgdorferi*, the bacterium that causes Lyme disease, can be characterized by distinct alleles at the outer surface protein C (*ospC*) locus. The lineages marked by *ospC* genotypes have been shown to be differentially invasive in different species of mammals, including humans; genotypes A, B, I, and K effectively disseminate to human blood and cerebrospinal fluid. In this report, we extend the sample of genotypes isolated from human blood to include genotypes N, H, C, M, and D, and rank each by their probability of disseminating from ticks to the blood of humans. Our results demonstrate that only some genotypes of *B. burgdorferi* present in ticks have a high propensity to disseminate in humans.

INTRODUCTION

Lyme disease, which is caused by the spirochete *Borrelia burgdorferi*, is the most prevalent vector-borne disease of humans in the United States and Europe.[1,2] *Borrelia burgdorferi* is transmitted from infected animals to feeding ticks in the *Ixodes ricinus* complex (*I. scapularis* in the northeastern and midwestern United States), and can be transmitted to another animal, including a human, during the subsequent blood meal.[3,4] However, the *B. burgdorferi* lineage transmitted from one animal species may not be infectious in humans because each mammalian species investigated, including humans, are predominantly infected with only a subset of the genetically distinct lineages found in every *B. burgdorferi* population sampled.[5–9] This idea is not without controversy.[6,10,11] A *B. burgdorferi* population that is subdivided because of specialization on different host "niches" has consequences for understanding natural population dynamics and ecology as well as practical consequences for vaccine development, treatment, and prevention. More importantly, specialization suggests that at least some of the mechanisms of infection differ among *B. burgdorferi* lineages for different animal species and by extension challenges the use of animal models to understand *B. burgdorferi* infections in humans. In this report, we combine data from human Lyme disease patients with natural tick populations to assess if humans are susceptible to the general *B. burgdorferi* population or only to a specific subset.

Lineages of *B. burgdorferi* can be classified by the allele at the highly variable outer surface protein C (*ospC*) locus. Although the function of OspC is currently unknown, it is required for the initiation of infection in mammalian hosts.[12] Most tick populations sampled in the northeastern United States contain 16 of the presently identified distinct *B. burgdorferi* lineages (called *ospC* major groups), labeled A through N, T, and U.[5,8,13] The population structure of *B. burgdorferi* is nearly clonal because the rate of horizontal transfer is astoundingly low.[14–16] Recent experiments suggest that these lineages are also serotypically different because of the OspC allele present.[11] However, differential dissemination is not necessarily caused by variation in the *ospC* gene used to mark the lineages; the results could be explained by the function of OspC or of a linked gene or genes.

Each mammalian species that is a reservoir for *B. burgdorferi* transmits only a subset of the 16 recognized genotypes found in the northeastern United States to feeding larval ticks.[5,17] For example, *Peromyscus leucopus* (white-footed mouse) transmits genotypes A, B, D, F, G, I, and K to feeding ticks, and *Blarina brevicauda* (short-tailed shrew) transmits genotypes A, D, E, F, K, and T. This study assumes that only genotypes found in ticks feeding on an animal have established a disseminated and stable infection in that animal.

Seinost and others[7] suggested that only four genotypes, A, B, I, and K, cause disseminated infections in humans. Genotypes were categorized into three groups: those that are found in nymphal ticks but not at the site of the tick bite in human skin (F and L), those found in the skin but not in the blood or cerebrospinal fluid (CSF) (C, D, E, G, H, J, M, N, T, and U), and those found in the blood and CSF (A, B, I, and K). The second group is assumed to give only a transient, localized infection, and the third group is assumed to cause a stable disseminated infection. A similar, although less resolved, pattern of human infection is visible if strains are categorized by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis of the 16S–23S ribosomal spacer region.[18,19] Three distinct RFLP genotypes (RSTs) are found in human skin at similar frequencies, RST1 at 25%, RST2 at 38%, and RST3 at 30%, but in human blood at dramatically different frequencies, RST1 and RST2 at approximately 45% each, and RST3 was found in less than 10% of human patients.[18] Thus, *B. burgdorferi* strains disseminate from the site of the initial infection to the blood with different probabilities, regardless of the genetic marker used to categorize the strains.

However, one cannot assess the lineages transmitted by feeding ticks to determine which lineages can establish stable infections in humans. A strain found in human blood is assumed to disseminate and consequently cause stable infections in humans. However, this idea is also controversial because it has been shown that one lineage that transiently invaded the blood stream did not maintain a stable infection in mice.[20] Regardless, only those genotypes regularly found in

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human blood have been found in human CSF, suggesting that dissemination to blood and establishing a disseminated infection are tightly correlated.

In this report, we present data suggesting that the criteria used to define "invasive" genotypes described by Seinost and others,[7] any genotype found in human blood is invasive, should be reconsidered. Recent studies have found genotypes in human blood samples other than those observed by Seinost and others,[7] suggesting that at least eight of the 16 genotypes found in the northeastern United States are invasive in humans by these standards.[10,11] In understanding the importance of a genotype as a human pathogen, a more important measure is the degree to which these genotypes differ in their ability to disseminate and establish stable infections in humans. In this report, we develop a metric to measure the relative human invasiveness of *B. burgdorferi* genotypes with the assumption that those lineages that are highly invasive will also form long-term stable infections.

MATERIALS AND METHODS

Human blood isolates. Blood was obtained from patients presenting with erythema migrans at the Lyme Disease Practice of the Westchester Medical Center (Valhalla, NY) between 1997 and 2002. One aliquot of blood specimen was inoculated into 20 aliquots of complete Barbour-Stoenner-Kelly medium without antibiotics or gelatin and cultured at 33°C for up to 12 weeks.[21] First passage cultures were frozen at −80°C; approximately 1 μL of this stock was used directly to determine *ospC* genotype by PCR and reverse line blot (described below). *Borrelia burgdorferi* genotypes do not differ in their ability to grow in culture.[22] All patients are assumed to have acquired their infections in Westchester County, New York, or adjacent counties in the lower Hudson valley. Six of these isolates were previously reported by Seinost and others[7] and are not included as new data. The study was reviewed and approved by the human subjects institutional review board, and the volunteers provided written informed consent.

Determination of *ospC* genotype. The *ospC* genotype was determined using PCR and reverse line blotting (RLB). Each culture was screened for *B. burgdorferi* by PCR targeting the *ospC* locus.[5] Positive samples (66 of 67 tested) were subjected to RLB to determine the *ospC* allele present. The methodology and oligonucleotides used for RLB were described previously.[5,7,8] The RLB results from 16 *B. burgdorferi* isolates were confirmed by sequencing of the PCR amplicon with 100% concordance.

Tick samples. The proportion of blood infections in which genotype i is found is a function of the frequency at which humans are exposed to genotype i through a tick bite, and the probability genotype i will disseminate to the blood given that a person is exposed. To determine the probability genotype i will disseminate given human exposure, we must know both the proportion of blood infections with genotype i, as well as the frequency at which humans are exposed to genotype i through a tick bite. In this report, we assume that the frequency humans are exposed to each genotype through a tick bite is proportional to the frequency at which each genotype is found in the local *I. scapularis* population. As previously stated, most patients with a disseminated *B. burgdorferi* infection seen at Westchester Medical Center acquired the Lyme bacteria in the southern counties of New York. Therefore, we used the published genotype frequencies found in eight *I. scapularis* populations located in southern New York[5,8,23] to estimate the frequency at which humans are exposed to each *ospC* genotype.

Relative invasiveness: a measure of genotype dissemination. We calculate the metric of relative invasiveness in humans of genotype i as the proportion of blood isolates that are genotype i divided by the probability of exposure to genotype i, i.e., the proportion of genotype i found in ticks. Relative invasiveness values above 1 indicate that genotype i is at a higher frequency in the human blood than in tick populations; the opposite is true for values below 1. Assuming that genotype i reaches human skin in proportion to its frequency in tick populations,[24] one would expect the same proportion of genotype i in human blood and in ticks if all genotypes disseminated with the same probability (i.e., relative invasiveness would equal 1). However, the tick population from which each patient was exposed is unknown (and likely unsampled). Additionally, the genotype frequency distributions differ significantly (in a statistical sense) among the populations,[8,25] potentially biasing our measures of the relative invasiveness for each genotype. Thus, we calculated the relative invasiveness for each genotype from each of the eight populations separately and report the median and range for this metric. We ranked the genotypes according to the invasiveness metric calculated from each of the tick populations. Some genotypes were not assayed for in some of the tick populations; relative invasiveness values of these genotypes were not calculated from these populations.

RESULTS

Strains isolated from blood. We determined the *ospC* genotype for 63 isolates cultured from human blood using PCR-RLB.[5,8] Of the 63 cultures, three contained mixed infections, two with genotypes A and B, and one with genotypes A and K, giving a total of 66 genotypes observed in blood (Table 1). As found by Seinost and others,[7] A, B, I, and K are the most commonly found genotypes. Additionally, we found 1–4 isolates of genotypes C (2), H (4), N (4), and M (1).

Relative invasiveness. We calculated a metric of relative invasiveness in humans for genotype i by dividing the proportion of human blood and CSF cultures with genotype i by the proportion of genotype i in each of eight tick populations in southern New York.[5,8,23] The median and range of these val-

TABLE 1

Numbers of each genotype of *Borrelia burgdorferi* found in blood and cerebrospinal fluid from the northeastern United States

Genotype	Seinost*	Alghaferi†	Earnhart†	New*	Total
K	16	1	4	21	42
A	10	0	5	19	34
B	3	1	1	8	13
N	0	4	3	4	11
I	3	0	0	7	10
H	0	1	0	4	5
C	0	0	1	2	3
M	0	0	0	1	1
D	0	1	0	0	1
Total	32	7	15	66	120

* From patients in the New York City metropolitan area.[7]
† From patients in the Baltimore, Maryland, metropolitan area.[10,11]

ues are shown in Table 2. Values that differ from 1 indicate that the frequency in human blood differs from that found in ticks.

The frequency data from tick populations were not pooled because of the noted differences in *ospC* frequencies among tick populations.[8,25] Some of the genotypes were not assayed in all populations (particularly type C) and rare genotypes were not found in one or two of the tick populations with small sample sizes. For these genotypes, we could not calculate the relative invasiveness, giving differing numbers of populations from which relative invasiveness for a genotype could be calculated (Table 2). Table 2 includes relative invasiveness results calculated with and without the outliers. Outliers were always extremely large values caused by dividing by an unusually low genotype frequency in ticks. We identified extremely large outliers for only three genotypes (A, I, and K). The relative invasiveness for each of these three genotypes was high regardless of the tick population used to calculate the value. The last column in Table 2 is the rank of the relative invasiveness metric, calculated without outliers included, averaged across all tick populations. Genotype A was the most invasive when calculated from three populations (rank = 1), second most invasive in two populations and third most in one population (average = 1.5). Genotype M was the least invasive in six of the seven populations for which we calculated a value for genotype M. The rank order of the genotypes, as well as the value for the relative invasiveness, stay relatively constant among the tick populations, indicating that the variation among tick populations does not substantially affect the results.

DISCUSSION

We analyzed *B. burgdorferi* collected from human Lyme disease patients in conjunction with *B. burgdorferi* in natural populations of ticks to assess the degree to which genotypically distinct lineages are host species specialists, concentrated in a few host species. Our data suggest that each *B. burgdorferi* lineage, categorized by *ospC* genotype, has a different propensity to infect humans and disseminate to the circulatory system. Nevertheless, these data suggest that *B. burgdorferi* genotypes cannot be classified as specialists or as invasive or non-invasive in humans. Rather, it is likely that each genotype can infect any natural animal reservoir species, but the probabilities that this will occur differ dramatically among genotypes.

TABLE 2
Relative invasiveness metric for *Borrelia burgdorferi* genotypes*

Genotype	Median (range)	Median (range)†	Average rank‡
A	2.6 (1.7–8.2) (n = 7)	2.5 (1.7–3.4) (n = 6)	1.5
I	2.8 (1.9–11.5) (n = 6)	2.1(1.9–4.2) (n = 5)	1.8
K	2.1 (1.9–7.2) (n = 8)	2.0 (1.9–3.0) (n = 7)	2.1
B	1.0 (0.8–1.4) (n = 7)	1.0 (0.8–1.4) (n = 7)	3.9
N	1.1 (0.8–2.3) (n = 7)	1.1 (0.8–2.3) (n = 7)	4.0
H	0.7 (0.3–1.1) (n = 8)	0.7 (0.3–1.1) (n = 8)	4.8
C	0.3 (0.2–0.8) (n = 3)	0.3 (0.2–0.8) (n = 3)	5.3
M	0.2 (0.1–0.3) (n = 7)	0.2 (0.1–0.3) (n = 7)	6.1
D§	~0	~0	NA
E,F,G,J, L,T¶	0	0	NA

* n = the number of populations in which a genotype was observed. Some of the genotypes were not assayed for in some populations, resulting in fewer than eight populations used to create the metric.
† Outliers removed.
‡ The rank of relative invasiveness is determined within each tick population for each genotype. The ranks were subsequently averaged across all tick populations. NA = not applicable.
§ Not found in blood from the New York samples, but found in blood of patient from the Baltimore region (Table 1).
¶ Common in ticks but not found in blood.

The probability that a human will acquire a disseminated *B. burgdorferi* infection is a function of the probability of dissemination and the probability of exposure, both of which differ considerably among genotypes in humans (Tables 1 and 2). Our findings suggest that the probability that genotypes A, I, and K will disseminate, given that they are present in ticks, is roughly twice that of genotype B and N; the remaining genotypes detected in our patient sample lag far behind. These latter genotypes appear to disseminate rarely in northeastern patients even though they are readily found in ticks in the lower Hudson Valley,[23] although this may change with increased sampling. We propose that all genotypes can enter the bloodstream with some probability but, for many, that probability is low. Genotypes A, B, I, K, and N all appear to disseminate readily into human blood. The predominance of genotypes A and K in human blood compared with other highly invasive genotypes (Table 1) is likely caused by a higher human exposure rate of these genotypes given their abundance in natural tick populations.

The metric we report, the relative invasiveness of each genotype, shows the change in genotype frequency from ticks to human blood. The range of relative invasiveness values observed in this study indicates that a filter that discriminates among genotypes exists between ticks and the human circulatory system, preferentially rejecting certain genotypes and commonly allowing others through. Genotypes A, I, and K are found in human blood twice as frequently as they are found in tick populations (range = 1.7–4.2×), whereas genotypes B and N are found at approximately the same frequencies in ticks and human blood (range = 0.8–2.3×) (Table 2). These data indicate that the probability of dissemination from tick to blood is greater for some genotypes than for others.

Alternatively, the frequency of human exposure may be greater for genotypes A, I, and K than expected given their frequencies in ticks, leading to their overrepresentation in human blood. This could occur in a number of scenarios. First, the tick populations sampled were not those from which human patients acquired their infections, potentially biasing our metric of relative invasiveness. For this reason, we calculated this metric using eight tick populations displaying significantly different *ospC* frequency distributions and the relative invasiveness metric was robust to these variations; the ranks of the invasiveness of the genotypes were relatively constant among the populations. Thus, although *ospC* frequency distributions differ among locations and from year to year, the predominance of certain genotypes (A, K, and I, and B and N) in human blood overwhelms the effects of this variation.

Second, all of the tick populations used in this study were sampled from state parks, yet most Lyme disease cases are contracted peri-domestically.[26,27] The frequency of *ospC* genotypes in semi-pristine state parks may differ substantially from the frequency distribution found around suburban homes, thus biasing our estimates of relative invasiveness. For this to occur, the *ospC* frequency distribution in all suburban areas in southern New York would need to be biased toward A, B, I, and K, even though the surrounding areas contained

all genotypes at relatively even frequencies.[8] This could occur if the animal community composition differed in suburbs, as it likely does,[28] and each animal species acts as a different niche for *ospC* genotypes.[5] However, this scenario necessitates the assumption that animal species are infected with different genotypes, which supports the hypothesis that humans would also be infected by different genotypes.[7] Additionally, mice, which are the most prevalent small animals around suburban homes, also transmit genotypes D, F, and G in addition to A, B, I, and K,[5] yet these genotypes are rarely found in the blood of patients. Regardless, genotypes A, B, I, and K cause most human infections in the northeastern United States and thus should be the primary target for vaccines, prevention measures, and treatments.

Relative invasiveness indicates that there are significant differences among genotypes in their ability to cause disseminated human infections. An alternative explanation is that genotypes with relatively high invasiveness are actually able to persist in human blood for more time than the less "invasive" genotypes and are thus more likely to be sampled. In the field, genotype A appears to produce a persistent infection in *P. leucopus*, but genotype E does not.[5] Experimentally, both genotypes A and E can disseminate into mouse blood,[20] but only genotype A forms a stable, persistent infection; genotype E is eradicated from all mice. It is conceivable that genotype E was rarely seen in the study of Brisson and Dykhuizen[5] because it did not persist from the infection in late spring to the late summer sampling. Thus, the strains characterized in the current study as having high relative invasiveness may only produce a spirochetemia of greater duration in humans. However, only genotypes A, B, I, K, and N have been found in CSF, suggesting that these types do disseminate in humans with a higher probability than other genotypes.[7,11]

Nymphal ticks transmit most infections to humans,[21] yet our statistic uses both nymphal and adult tick populations. However, genotype frequencies do not differ among nymphal and adult ticks except for genotypes K and N. Genotype K is slightly lower in adult ticks ($\chi^2 = 4.9$, $P \sim 0.025$), and the frequency of genotype N is substantially higher ($\chi^2 = 10.1$, $P \sim 0.001$). Consequently, if most human infections are caused by nymphal ticks, the relative invasiveness of genotype K may be slightly lower than we report and that of genotype N may be dramatically higher, putting genotype N on par with genotypes A, I, and K as one of the most invasive genotypes.

Although genotype N disseminates with a relatively high probability, it was not detected in the 32 human patients with blood or CSF infections examined by Seinost and others,[7] even though three patients with erythema migrans had genotype N in their skin. Assuming that the true frequency of type N in the blood or CSF of humans in the northeastern United States is 4 in 66 (Table 1), we expect that in a random sample of 32 patients none will be infected by genotype N 13.5% of the time ($1 - (4/66)^{32} = 0.135$). Thus, it is likely that genotype N was missed by chance from the small sample of 32 patients. With the much larger sample of 120 patients, the probability of not identifying a highly invasive genotype has become exceedingly low. However, human invasive genotypes not present in the northeastern United States may be found in patients from other Lyme disease foci such as Europe, the Midwest, or the west coast of the United States.[29]

The impact of an *ospC* genotype on human health is the result of both the invasiveness of that genotype and the frequency of human exposure. Genotypes A and K, in addition to being highly invasive in humans, are also the most common genotypes found in tick populations in the northeast United States, resulting in the preponderance of genotypes A and K found in human blood (76 of 120 [63%] [Table 1]). Genotypes I and N, and also highly invasive in humans, are less common in tick populations than A and K. Other Lyme disease foci, such as the mid-Atlantic, upper Midwest, Pacific coast, and Europe, may have a different frequency distribution of genotypes, which will result in a proportional difference in the frequency of genotypes found in human blood.[10,11] Vaccines to control the Lyme disease epidemic may need to differ by region depending on the invasive genotypes that are common in local tick populations.

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