

Molecular Characterization of *Borrelia* Spp. Isolates from Greater Metropolitan Chicago Reveals the Presence of *Borrelia bissettii*. Preliminary Report

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Abstract

Lyme Disease in the US is concentrated in three endemic areas: the Northeast, the upper mid-West, and the Pacific coast. In the mid-West, the range of Lyme disease has expanded to include large parts of Wisconsin and Minnesota. Despite its proximity to the mid-Western focus, Illinois, so far, has not been considered an endemic area. However, more recent data suggest that this situation may be changing. Also, the extent of borrelial diversity in the mid-West remains largely unexplored. Here, we present preliminary results on the molecular characterization of *Borrelia* isolates from rodents captured in Cook and Lake Counties, both of which are parts of the greater metropolitan Chicago area in Illinois.

We investigated the rodent reservoir present in forested areas of suburban Chicago in order to determine the frequency of infection with the Lyme disease agent(s) by culture isolation of *Borrelia* spirochetes (Picken *et al.*, unpublished). Rodent isolates of *Borrelia* were identified to the species level by genetic characterization. In total, 19 isolates were obtained over 3 years from NW Cook Co. and Lake Co. Pulsed-field gel electrophoretic analysis of *Mlu*I digested DNA from these isolates showed macrorestriction patterns similar to that of the Californian isolate, strain DN127 (PF type I), New York isolate strain 25015 (PF type II), or a variant of the latter (PF type III). Sequence data generated from the *rrf*(5S)-*rrl*(23S) intergenic spacer region of the ribosomal RNA gene cluster confirmed the identity of all the Chicago isolates studied to date as *B. bissettii*. These strains are unlike our previous *Borrelia* isolates from NW Illinois and Wisconsin. In addition, there was a predominant association of *B. bissettii* infection with pratal rodent species such as *Microtus pennsylvanicus* and *Zapus hudsonius*. The relationship of this novel enzootic focus to the established mid-Western endemic focus of Lyme disease remains to be elucidated. The geographic range and reservoir diversity of this organism may have hitherto been underestimated.

Communication

Lyme disease is a bacterial disease caused by several different species of spirochetes belonging to the genus *Borrelia* (the *Borrelia burgdorferi* sensu lato complex). It is a zoonosis which is maintained in the natural environment by cycling between ticks and several different animal species, with small rodents serving as the primary reservoir. Transmission of the disease to humans is via the bite of infected ticks. To date, at least ten different species of *Borrelia* have been isolated from the animal reservoir, or tick vectors, worldwide. However, thus far, only three species of the *Borrelia burgdorferi* sensu lato complex have been found in North America: *B. burgdorferi* sensu stricto, *B. andersonii* and *B. bissettii* (recently reviewed by Wang *et al.*, 1999). Only certain species have been isolated from humans, and are therefore known to be pathogenic for people. While in Europe several species of *Borrelia* have been isolated from human samples, in North America only *Borrelia burgdorferi* sensu stricto has been, thus far, documented as a human pathogen.

Lyme disease, which is distributed throughout vast areas of temperate zones of the northern hemisphere, is concentrated in the US in three endemic areas: northeastern, upper mid-West and along the Pacific coast. In the mid-West, Lyme disease has expanded to include large parts of Wisconsin and Minnesota. Despite its proximity to the mid-Western focus, Illinois, so far, has not been considered to be endemic for Lyme disease and the number of reported cases of this disease has been relatively low. However, more recent data indicate that the mid-Western focus may be expanding. Moreover, molecular genetic studies of mid-Western isolates, revealing the extent of borrelial diversity in the region, have received scant attention over the past decade.

In the North-East and mid-West, *Ixodes scapularis* is the primary vector of Lyme disease, whereas *Ixodes pacificus* is the primary vector in California. From a recent summary by the CDC of information on the established and/or reported presence of *Ixodes scapularis* ticks, throughout the US, it is apparent that the tick is expanding its range (Dennis *et al.*, 1998). Several foci of established or reported *I. scapularis* have been documented from Illinois and the adjacent states - Iowa and Indiana. Our earlier studies in northern Illinois, (some 80 miles west of Chicago) showed the presence of *Borrelia burgdorferi* sensu stricto infected *I. scapularis* ticks (Picken *et al.*, 1995).

Here, we present preliminary results of molecular characterization of *Borrelia* isolates from rodents captured in Cook and Lake Counties, both of which are parts of greater metropolitan Chicago.

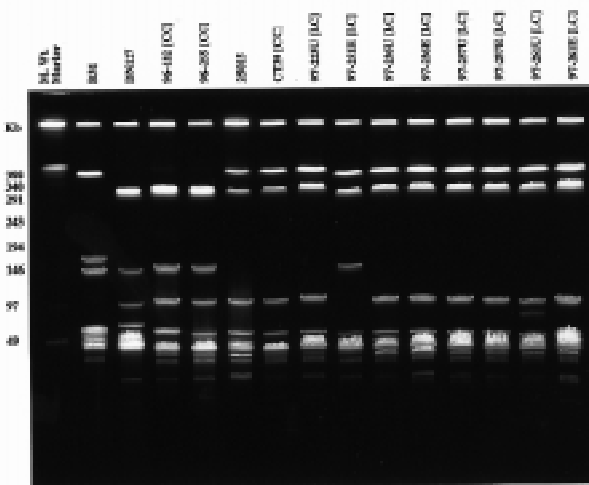
1996/1997 Chicago Isolates - *Mlu*I Macrominor Restriction Patterns

Figure 1. Pulsed-field gel of *Mlu*I-digested chromosomal DNA from 1996/1997 Chicago isolates shown in comparison to well-characterized reference strains of *B. burgdorferi* [B31] and *B. bissettii* [DN127, 25015].

Results and Discussion

In total, 19 isolates were obtained over 3 years from NW Cook Co. and Lake Co.. While only 2 isolates were obtained in 1996, 8 isolates were obtained in 1997, and 9 isolates in 1998. These were isolated from the white-footed mouse, *Peromyscus leucopus* (2 isolates), the meadow vole, *Microtus pennsylvanicus* (12 isolates), and the meadow jumping mouse, *Zapus hudsonius* (5 isolates).

Figure 1 shows pulsed-field gel electrophoresis (PFGE) analysis of *Mlu*I digested DNA from the isolates obtained in 1996 and 1997. Plasmid profile analysis of the isolates by PFGE was also performed to demonstrate their independent origin (data not shown). Three reference strains of *Borrelia* are also included on the same gel for comparison: strain B31 (type strain of *B. burgdorferi*), strain DN127 (tick isolate of *B. bissettii* from California), and strain 25015 (tick isolate of *B. bissettii* from New York). Strain CT39, isolated from NW Cook Co. in 1990, has been described previously (Picken *et al.*, 1995). It can be seen from Figure 1 that two Chicago isolates, 96-182 and 96-255, have the same pattern of high molecular mass fragments as Californian strain DN127 (PF type I). All other isolates, except strain 97-251E, have the same

INTERGENIC SPACER REGION SEQUENCES - DN127, 25015, 1996/1997 CHICAGO ISOLATES

	10	20	30	40	50	60	70	80	90	100	110
DN127	GTAAGTTATTGCCAGGGTTTTATTTTTATATTTTAAATCTTGATTTTATTTATGTTTTTAAATGTTTGTAGTTTTTTTGAATGTTTATTTAAATAACATAAAAAA										
96-182	GTAAGTTATTGCCAGGGTTTTATTTTTATATTTTAAATCTTGATTTTATTTATGTTTTTAAATG---TTAGTGTTTTTTAAATATATTTTAAATAACATAAAAAA										
96-255	GTAAGTTATTGCCAGGGTTTTATTTTTATATTTTAAATCTTGATTTTATTTATGTTTTTAAATG---TTAATGTTTTTGGATGTGTTATTTAAATAGCATAAAAAA										
25015	GTAAGTTATTGCCAGGGTTTTATTTTTATATTTTAAATCTTGATTTTATTTATGTTTTTAAATG---TTAGTGTTTTTTAAATGTTATTTTAAATAACATAAAAAA										
CT39	GTAAGTTATTGCCAGGGTTTTATTTTTATATTTTAAATCTTGATTTTATTTATGTTTTTAAATG---TTAATGTTTTTGGATGTGTTATTTAAATAGCATAAAAAA										
97-223U	GTAAGTTATTGCCAGGGTTTTATTTTTATATTTTAAATCTTGATTTTATTTATGTTTTTAAATG---TTAATGTTTTTGGATGTGTTATTTAAATAGCATAAAAAA										
97-251E	GTAAGTTATTGCCAGGGTTTTATTTTTGTTATTTTAAATCTTGATTTTATTTATGTTTTTAAATG---TTAGTGTTTTTTAAATGTTATTTTAAATAACATAAAAAA										
97-236U	GTAAGTTATTGCCAGGGTTTTATTTTTATATTTTAAATCTTGATTTTATTTATGTTTTTAAATA---TTAGTGTTTTTTAAATGTTATTTTAAATAACATAAAAAA										
97-236E	GTAAGTTATTGCCAGGGTTTTATTTTTATATTTTAAATCTTGATTTTATTTATGTTTTTAAATA---TTAGTGTTTTTTAAATGTTATTTTAAATAACATAAAAAA										
97-257U	GTAAGTTATTGCCAGGGTTTTATTTTTATATTTTAAATCTTGATTTTATTTATGTTTTTAAATA---TTAGTGTTTTTTAAATGTTATTTTAAATAACATAAAAAA										
97-257E	GTAAGTTATTGCCAGGGTTTTATTTTTATATTTTAAATCTTGATTTTATTTATGTTTTTAAATA---TTAGTGTTTTTTAAATGTTATTTTAAATAACATAAAAAA										
97-261U	GTAAGTTATTGCCAGGGTTTTATTTTTGTTATTTTAAATCTTGATTTTATTTATGTTTTTAAATG---TTAGTGTTTTTTAAATGTTATTTTAAATAACATAAAAAA										
97-261E	GTAAGTTATTGCCAGGGTTTTATTTTTGTTATTTTAAATCTTGATTTTATTTATGTTTTTAAATG---TTAGTGTTTTTTAAATGTTATTTTAAATAACATAAAAAA										
	120	130	140	150	160	170	180	190	200	210	220
DN127	TAAATATATA--TTGACATGGATTAAACAAGATATATATTTTATGTTGCGTAAACAATTTGGCAAAATAGAGATGGAAGATAAAAAATATGGTCAAAGTAATAAGAGTC										
96-182	TAAATATATA--TTGACATGGATTAAACAAGATATATATTTTATGTTGCGTAAACAATTTGGCAAAATAGAGATGGAAGATAAAAAATATGGTCAAAGTAATAAGAGTC										
96-255	TAAATATATA--TTGACATGGATTAAACAAGATATATATTTTATGTTGCGTAAACAATTTGGCAAAATAGAGATGGAAGATAAAAAATATGGTCAAAGTAATAAGAGTC										
25015	TAAATATATA--TTGACATGGATTAAACAAGATATATATTTTATGTTGCGTAAACAATTTGGCAAAATAGAGATGGAAGATAAAAAATATGGTCAAAGTAATAAGAGTC										
CT39	TAAATATATA--TTGACATGGATTAAACAAGATATATATTTTATGTTGCGTAAACAATTTGGCAAAATAGAGATGGAAGATAAAAAATATGGTCAAAGTAATAAGAGTC										
97-223U	TAAATATATA--TTGACATGGATTAAACAAGATATATATTTTATGTTGCGTAAACAATTTGGCAAAATAGAGATGGAAGATAAAAAATATGGTCAAAGTAATAAGAGTC										
97-251E	TAAATATATA--TTGACATGGATTAAACAAGATATATATTTTATGTTGCGTAAACAATTTGGCAAAATAGAGATGGAAGATAAAAAATATGGTCAAAGTAATAAGAGTC										
97-236U	TAAATATATATA T TTGACACGGATTAAACAAGATATATATTTTATGTTGCGTAAACAATTTGGCAAAATAGAGATGGAAGATAAAAAATATGGTCAAAGTAATAAGAGTC										
97-236E	TAAATATATATA T TTGACACGGATTAAACAAGATATATATTTTATGTTGCGTAAACAATTTGGCAAAATAGAGATGGAAGATAAAAAATATGGTCAAAGTAATAAGAGTC										
97-257U	TAAATATATATA T TTGACACGGATTAAACAAGATATATATTTTATGTTGCGTAAACAATTTGGCAAAATAGAGATGGAAGATAAAAAATATGGTCAAAGTAATAAGAGTC										
97-257E	TAAATATATATA T TTGACACGGATTAAACAAGATATATATTTTATGTTGCGTAAACAATTTGGCAAAATAGAGATGGAAGATAAAAAATATGGTCAAAGTAATAAGAGTC										
97-261U	TAAATATATA--TTGACACGGATTAAACAAGATATATATTTTATGTTGCGTAAACAATTTGGCAAAATAGAGATGGAAGATAAAAAATATGGTCAAAGTAATAAGAGTC										
97-261E	TAAATATATA--TTGACACGGATTAAACAAGATATATATTTTATGTTGCGTAAACAATTTGGCAAAATAGAGATGGAAGATAAAAAATATGGTCAAAGTAATAAGAGTC										

Figure 2. Intergenic spacer region sequences - DN127, 25015, 1996/1997 Chicago isolates.

macrorestriction pattern as strain 25015 from New York (PF type II). Strain 97-251E has a variant of this type (PF type III). All 1998 isolates possessed the PF type II macrorestriction pattern (PF gels not shown). The closely similar macrorestriction pattern findings indicate that the analyzed isolates belong to the species *B. bissettii*.

To further confirm the identity of the isolates, sequence data was generated from the *rrf* (5S)-*rrl* (23S) intergenic spacer region (IGSR) of the ribosomal RNA gene cluster. Analysis and comparison of IGSR sequences from many *Borrelia* spp. isolates has demonstrated that these sequences can be used to identify and classify *Borrelia* isolates to the species level. The results for the 1996/1997 Chicago isolates and relevant reference strains are shown in Figure 2. The sequences generated are closely similar to those of *B. bissettii* reference strains DN127 and 25015, thus confirming the identity of these isolates as *B. bissettii*. Comparison of the sequences from the 1998 isolates gave similar results (data not shown). These findings indicate that all of the Chicago rodent isolates investigated to date belong to the species *B. bissetti*.

The isolates obtained from the Chicago area are unlike our previous isolates from NW Illinois and Wisconsin, which were identified as *B. burgdorferi* sensu stricto (Picken *et al.*, 1995). This suggests that although the isolates are genetically closely-related to Californian and New York strains of *B. bissettii*, they are native to the region, and form a discrete isolated enclave within certain geographic confines of the northern suburbs. There was also a predominant association of these strains with pratal rodent species (17 of 19 isolates) such as *M. pennsylvanicus* and *Z. hudsonius*. This may signal the involvement of a stenoadaptive tick species with a preference for graminicolous rodents as the vector responsible for maintenance and transmission of the spirochete. The relationship of this new *B. bissettii* enzootic focus to the established midwestern hyperendemic focus of Lyme disease/*B. burgdorferi* sensu stricto infection in Minnesota and Wisconsin remains to be elucidated. Pathogenicity studies of these recent Chicago isolates using a rabbit model demonstrated persistence of the organisms in skin for at least 25 days (Picken *et al.*, 1999).

In Conclusion, *Borrelia* are present in the rodent reservoir of forested areas in suburban Chicago. The strains of *Borrelia* isolated to date appear most similar to Californian (DN127) and New York (25015) tick isolates of *B. bissettii*. This organism appears to be emerging as the second most important group of *Borrelia* found in North America. The geographic range and reservoir diversity of the spirochete may have hitherto been underestimated.

Experimental Procedure

During Summer and Autumn (April to November) of 1996, 1997, and 1998, rodents were live-captured from forested areas in suburban Chicago using baited Sherman traps (Picken *et al.*, unpublished). Ear snips and urinary bladders from euthanized animals were cultured in BSK-II medium. Cultures were checked periodically for the presence of spirochetes by dark-field microscopy (Picken *et al.*, 1995). Isolates with the suffix U were derived from urinary bladder cultures, whereas isolates with the suffix E were derived from ear snips.

Positive cultures identified by this means were sub-cultured into fresh BSK-II medium and grown to high cell density. To identify *Borrelia* isolates to the species level, spirochetes were harvested by centrifugation and processed into agarose blocks for PFGE. The restriction enzyme *Mlu*I was used to digest chromosomal DNA in agarose and the fragments were

separated using a Biorad CHEF DRII pulsed-field apparatus (Picken *et al.*, 1995).

For the further genetic characterization of isolates, a 223 base pair intergenic spacer fragment from the ribosomal RNA gene cluster of the *Borrelia* genome was amplified by PCR and sequenced (Picken, 1992) (GenBank submission numbers: AF263524-34). The sequences obtained were compared to known sequences from well-characterized reference strains of various *Borrelia* spp.

Acknowledgements

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