Supplemental Materials

Supplemental Methods

Tick Nucleic Acid Extraction

Adult or nymphal ticks were stored in 70% ethanol prior to processing. Each tick was processed and tested individually. DNA extraction was performed as previously described (Guy and Stanek, 1991, Rijpkema et al., 1996). Briefly, each tick was transferred into an individual 1.5 mL tube and allowed to air dry. 100 µL of 2.8-3% ammonium hydroxide was then added to each tube and the tubes were closed with an O-ring cap. After boiling at 100 ℃ for 15 minutes, the tubes were allowed to cool to room temperature and the caps were removed. Residual ammonia was evaporated from each tube by heating at 95-100 ℃ for 15 minutes and the resulting DNA was used for PCR.

Duplex TaqMan PCR for *B. burgdorferi* and *B. mayonii*

DNA extracts from ticks collected in MN were tested using a duplex TaqMan PCR targeting the oligopeptide permease periplasmic A2 gene **(***oppA2*). The total reaction volume was 25 µL reaction, containing 2.5 µL of DNA template, 1 x QUANTA PerfeCta qPCR SuperMix (Quanta Bioscience, Cat# 95064-02K), 600 nM each of forward and reverse primer Bor2F (5'-GAA GCG ACT ATT ACT CAT C) and Bor2R (5'-GGC TTT TCT AAT TTT AAC GTT), 200 nM *B. burgdorferi* specific probe Bor2-Bb-P (5'-Hex-TTC AAT ACA CAC ATC AAA CCA C-BHQ1) and a *B. mayonii*-specific probe Bor2-Bm-P (5'-FAM-TTT AAC ACG CAC ATT AAA CCG C-BHQ1). The reaction was performed on BioRad CFX96 Touch™ Real-Time PCR Detection System (BioRad, CA, USA) by using 50 cycles of thermal cycling with an initial denaturation step at 94 ℃ for 5 minutes, followed by denaturation at 94 ℃ for 15 seconds, annealing and extension at 60 ℃ for one minute.

Supplemental Table 1. Results of *Borrelia burgdorferi* sensu lato genospecies polymerase chain reaction testing of *Ixodes scapularis* ticks collected in Minnesota and Wisconsin counties, 2009-2015*‡

Collection Site County (State)	Collection Date	Adult *I. scapularis* PCR results		Nymphal *I. scapularis* PCR results	
		Borrelia mayonii **No. Pos/Total (%)†**	***Borrelia burgdorferi*** **No. Pos/Total (%)†**	***Borrelia mayonii*** **No. Pos/Total (%)†**	***Borrelia burgdorferi*** **No. Pos/Total (%)†**
Barron (WI)	October 2013	1/170 (0.6) (1 male)	68/170 (40.0)	NC	NC
Barron (WI)	June-July 2014	14/267 (5.2)[a] (11 females, 3 males)	89/267 (33.3)	3/81 (3.7)	22/81 (27.1)
Clearwater (MN)	May-June 2014	2/111 (1.8)[b] (1 female, 1 male)	54/111 (48.6)	0/3 (0)	0/3 (0)
Clearwater (MN)	April-July 2015	2/104 (1.9)[c] (1 female, 1 male)	45/104 (43.3)	1/59 (1.7)[d]	12/59 (20.3)
Eau Claire (WI)	June – July 2009	0/8 (0)	2/8 (25.0)	0/33 (0)	22/33 (66.7)
Eau Claire (WI)	June 2010	0/20 (0)	5/20 (25.0)	1/79 (1.3)	11/79 (13.9)
Houston (MN)	April-June 2014	0/102 (0)	27/102 (26.5)	NT	NT
Houston (MN)	May-June 2015	0/95 (0)	52/95 (54.7)	0/24 (0)	7/24 (29.2)
Houston (MN)	October 2015	0/23 (0)	10/23 (43.5)	NC	NC
Morrison (MN)	May-June 2014	1/123 (0.8)[e] (1 male)	63/123 (51.2)	NT	NT
Morrison (MN)	May-June 2015	4/99 (4.0)[f] (2 females, 2 males)	49/99 (49.5)	1/51 (2.0)[g]	17/51 (33.3)
Pine (MN)	May-June 2014	2/114 (1.8) (1 female, 1 male)	32/114 (28.1)	NT	NT
Pine (MN)	May-June 2015	3/108 (2.8)[h] (1 female, 2 males)	53/108 (49.1)	0/69 (0)	14/69 (20.3)
Wabasha (MN)	October 2015	0/37 (0)	23/37 (62.2)	NC	NC
	Total	29/1381 (2.1)[i] (17 females, 12 males)	570/1381 (41.3)	6/399 (1.5)[j]	83/399 (20.8)

*Abbreviations: PCR - polymerase chain reaction, Pos – positive, NC - not collected (nymphs were not found during this collection), No. – number, NT – not tested (low numbers of nymphs were collected but not submitted for PCR testing)

‡ Data for Barron (WI) and Eau Claire (WI) were previously published in Pritt et al., 2016 (Supplemental Table 5).

† Number positive divided by the total number tested and percentage

[a] Two adults (both female) were co-infected with both agents

[b] Two adults (1 female, 1 male) were co-infected with both agents

[c] One adult female was co-infected with both agents

[d] One nymph was co-infected with both agents

[e] One adult male was co-infected with both agents

[f] Three adults (2 females, 1 male) were co-infected with both agents

[g] One nymph was co-infected with both agents

[h] Two adults (1 female, 1 male) were co-infected with both agents

[i] Eleven adults (7 females and 4 males) were co-infected with both agents

[j] Two nymphs were co-infected with both agents

Supplemental Table 2. Pairwise genetic distances of 8 concatenated housekeeping genes (4785 nucleotides) from patient isolates (MN14-1420, MN14-1539) as compared to 16 Bbsl genospecies. Genetic distances were calculated in MEGA 5.0 using the Kimura-2 model. Percent genetic similarity is calculated using the formula: 100 − (genetic distance × 100). The lowest pairwise genetic distance, highest genetic similarity for the patient isolates (MN14-1420, MN14-1539) is to *B. burgdorferi* sensu stricto (0.053 to 0.051; 94.7% and 94.9% similarity).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
1. *B. burgdorferi* B31																				
2. *B. burgdorferi* NE49	0.018																			
3. *B. burgdorferi* Z41293	0.017	0.007																		
4. *B. afzelii* VS461	0.078	0.079	0.079																	
5. *B. garinii* 20047	0.078	0.077	0.077	0.067																
6. *B. bavariensis* PBi	0.079	0.079	0.079	0.064	0.020															
7. *B. valaisiana* VS116	0.081	0.082	0.080	0.075	0.071	0.069														
8. *B. lusitaniae* PotiB2	0.078	0.078	0.078	0.069	0.068	0.066	0.074													
9. *B. spielmanii* A14S	0.086	0.087	0.086	0.067	0.078	0.076	0.082	0.078												
10. *B. bissettii* DN127	0.059	0.059	0.059	0.082	0.080	0.081	0.087	0.079	0.090											
11. *B. kurtenbachii* 25015	0.061	0.061	0.062	0.083	0.082	0.083	0.090	0.081	0.092	0.036										
12. *B. andersonii* 21123	0.057	0.057	0.056	0.082	0.082	0.083	0.089	0.082	0.091	0.066	0.067									
13. *B. sinica* CMN3	0.097	0.098	0.096	0.082	0.088	0.081	0.094	0.086	0.095	0.100	0.099	0.104								
14. *B. japonica* HO14	0.081	0.082	0.079	0.068	0.068	0.066	0.076	0.071	0.081	0.083	0.082	0.087	0.080							
15. *B. americana* SCW-41	0.053	0.054	0.051	0.085	0.083	0.082	0.086	0.082	0.091	0.060	0.063	0.056	0.105	0.086						
16. *B. carolinensis* SCW-22	0.060	0.059	0.059	0.085	0.085	0.084	0.089	0.082	0.091	0.026	0.030	0.066	0.100	0.083	0.061					
17. *B. tanukii* Hk501	0.079	0.078	0.076	0.074	0.069	0.068	0.049	0.072	0.083	0.083	0.083	0.089	0.088	0.073	0.084	0.084				
18. *B. turdi* Ya501	0.085	0.084	0.084	0.075	0.073	0.072	0.081	0.078	0.085	0.085	0.086	0.094	0.089	0.080	0.088	0.086	0.077			
19. *B. mayonii* MN14-1420	0.053	0.053	0.051	0.072	0.067	0.068	0.077	0.070	0.082	0.055	0.059	0.062	0.090	0.075	0.059	0.058	0.073	0.079		
20. *B. mayonii* MN14-1539	0.053	0.052	0.051	0.072	0.067	0.068	0.078	0.071	0.082	0.054	0.059	0.062	0.089	0.076	0.059	0.058	0.073	0.079	0.000	

References

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