Identification of *Borrelia* protein candidates in mouse skin for potential diagnosis of disseminated Lyme borreliosis

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Supplementary Information

Table S1. Mice (*Mus musculus* strain C3H/HeN) were infected with one of three *Borrelia* pathogens: *B. burgdorferi* ss strain N40, *B. afzelii* strain NE4049, and *B. garinii* strain PBi via intradermal inoculation. The initial inoculum consisted of 1000 spirochetes in 100 μL of PBS. At 40 days post-infection, the mice were treated with the dermacorticoid clobetasol over a period of two days to reactivate the spirochete population in the skin. Following the clobetasol treatment, the mice were sacrificed and the skin surrounding the clobatesol treatment was removed and tested for the spirochete load using qPCR. The data show the number of *Borrelia flagellin* gene copies relative to 10,000 *Mus musculus gapdh* gene copies. *B. afzelii* strain NE4049 establishes a much higher spirochete load in the mouse skin than the other two *Borrelia* species.

In grey, mouse skins selected for proteomic studies (Ge-LC-MS/MS).

	PCR quantification							
	Number of <i>flagellin</i> / 10 ⁴ <i>gapdh</i>							
Mouse ID #	B. burgdorferi ss	B. afzelii NE4049	B. garinii PBi					
	N40							
1	2.685	13.153	13.756					
2	28.97	11.089	30.252					
3	8.121	6.187	18.796					
4	19.312	3.9	7.483					
5	3.687	69.33	1.087					
7	4.514	2.633	3.926					
8	6.661	151.362	4.333					
9	4.472	304.39	2.687					
10	9.583	140.273	6.519					
11	42.594	23.969	5.853					
12	2.428	41.209	nq					
13	8.37	33.379	nq					
14	10.421	28.386	nq					
15	24.659	92.363						
16	6.755	37.562						
17	2.648	15.582						
18	2.309	3.066						
19	76.515	111.469						
20	72.72							
21	17.424							
22	44.545							

23	13.182		
24	21.182		
Average quantification	18.86	60.52	9.47

Supplementary Table 2: Mice (*Mus musculus* strain C3H/HeN) were infested with either 10 or 5 *I. ricinus* nymphs that were infected with *Borrelia afzelii* strain NE4049. A random sample of these nymphs had shown that 80% were infected using PCR. At 40 days post-infection, the mice were treated with the dermocorticoid clobetasol over a period of two days to reactivate the spirochete population in the skin. Following the clobetasol treatment, the mice were sacrificed and the skin surrounding the clobatesol treatment was removed and tested for the spirochete load using qPCR. The data show the number of *Borrelia flagellin* gene copies relative to 10,000 *Mus musculus gaphdh* gene copies. The spirochete load in the mouse skin was much higher for the mice infested with 10 nymphs compared to the mice infested with 5 nymphs.

	PCR quantification Number of <i>flagellin /</i> 10 ⁴ <i>gapdh</i>			
Mouse N°	10 ticks	5 ticks		
1	876	9.143		
2	602	13.653		
3	1231	9.551		
4	723	< 0.05		
5		54.338		

Supplementary Table 3: Mice (*Mus musculus* strain C3H/HeN) were infected with *B. burgdorferi* ss strain N40 via intradermal inoculation. The initial inoculum consisted of 1000 spirochetes in 100 μ L of PBS. At 40 days post-infection, the mice were treated with the dermocorticoid clobetasol over a period of two days to reactivate the spirochete population in the skin. The mice were subsequently treated with the antibiotic ceftriaxone (16 mg/kg) intradermally, twice a day over a period of 5 days. Four mice were used as control (no antibiotic treatment-Day 0) and 5 mice were killd and tested by PCR and culture at each point of kinetics (Day1 to Day5) (29 mice total).

	Da	y 0	Da	y 1	Da	y 2	Da	y 3	Da	y 4	Da	y 5
	Cult	PCR	Cult	PCR	Cult	PCR	Cult	PCR	Cult	PCR	Cult	PCR
Mouse 1	+	1.3	+	0.1	-	-	-	-	-	-	-	-
Mouse 2	+	1.3	+	0.06	-	-	-	-	-	-	-	-
Mouse 3	+	3.3	+	0.16	1	-	1	-	i	-	1	-
Mouse 4	+	1.2	+	0.11	1	-	1	_	i	-	-	-
Mouse 5			+	0.1	ı	-	ı	-	1	-	ı	-