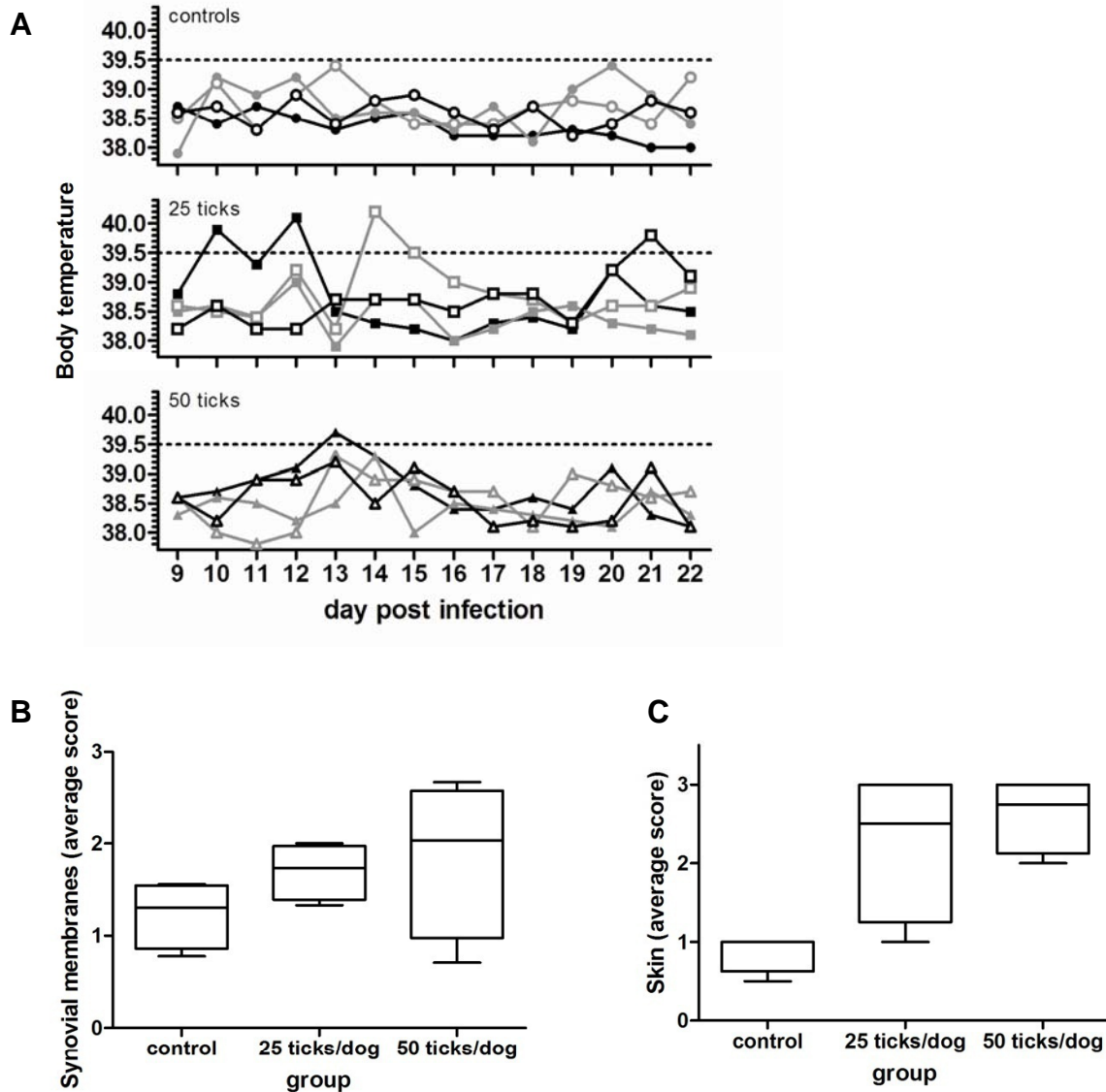


Supplementary Figure 1: Western blot and ROC-curve analysis of *B. burgdorferi* C6 and FlaB antigens. (A) Two C6 peptides were synthesized and coupled to BSA and recombinant FlaB antigen was expressed in *E.coli*. The proteins were run on 12% SDS-gels under reducing conditions and blotted to Western blot membranes. Membranes were incubated first with canine serum and then with an alkaline phosphatase conjugated anti-canine IgG antibody followed by chemiluminescence substrate. Left panel: The BSA coupled C6-P1 peptide originating from *B. burgdorferi* strain B31 (1) and C6-P2 originating from strain 297 (2) were run on the gel and incubated with a pretested positive canine serum; Right panel: FlaB antigen was run on the gel and incubated with a positive (3) and a negative (4) canine serum. (B) ROC curves were obtained for C6-P1 and FlaB by comparison of multiplex assay results with those obtained by Western blotting using whole bacteria lysates. A total of 188 canine sera were used for the analysis. Western blot results were considered positive if the 39 kDa (VlsE containing the C6 region) or 41 kDa (FlaB) bands were detected by the respective serum. Areas under the curves were 0.865 for C6-P1 and 0.682 for FlaB.



Supplementary Figure 2: Clinical data and tissue pathology in dogs

experimentally infected with *B. burgdorferi*. Dogs were either exposed to 25 (n=4) or 50 infected ticks (n=4) or were not exposed (controls; n=4) and were kept in the study until day 92 after tick exposure. (A) Body temperature was measured daily and is shown for days 9 to 22 p.i.; (B) Histological changes in synovial membranes on day 92. The average score for each dog was calculated from ten individual scores (five different joints, both sites); (C) Histological signs of skin inflammation and dermatitis on day 92 were determined in skin regions close to the tick attachment sites. The average score for each dog is the mean score from both sites.

Supplementary Table 1: Mean fluorescence intensity (MFI) values for antibodies to OspC, OspF and OspA determined by multiplex analysis in individual serum samples from dogs experimentally infected with *B. burgdorferi*.

Antibodies to	Group	Dog	Day 13 prior to infection	Day post infection					
				21	35	49	63	77	93
OspC	control	1	<i>35</i>	<i>32</i>	<i>35</i>	<i>19</i>	<i>175</i>	<i>250</i>	<i>192</i>
		2	<i>38</i>	<i>40</i>	<i>50</i>	<i>32</i>	<i>73</i>	<i>46</i>	<i>131</i>
		3	<i>7</i>	<i>30</i>	<i>51</i>	<i>23</i>	<i>39</i>	<i>25</i>	<i>26</i>
		4	<i>26</i>	<i>36</i>	<i>20</i>	<i>30</i>	<i>31</i>	<i>35</i>	<i>40</i>
	25 ticks	5	<i>20</i>	1324	5611	10434	9632	6336	5325
		6	<i>13</i>	747	8875	9964	8864	8729	6631
		7	<i>28</i>	443	9256	12590	7195	8571	4380
		8	<i>16</i>	528	3644	3375	3192	4351	5760
	50 ticks	9	<i>20</i>	2925	9206	11992	7229	7345	4963
		10	<i>19</i>	3819	9052	10527	17155	22611	20905
		11	<i>32</i>	3867	2601	6130	8189	11433	6249
		12	<i>33</i>	3692	10482	7674	7167	5262	4797
OspF	control	1	<i>77</i>	<i>85</i>	<i>131</i>	<i>81</i>	<i>323</i>	<i>496</i>	<i>401</i>
		2	<i>231</i>	<i>271</i>	<i>200</i>	<i>127</i>	<i>198</i>	<i>204</i>	<i>338</i>
		3	<i>44</i>	<i>116</i>	<i>135</i>	<i>92</i>	<i>118</i>	<i>117</i>	<i>118</i>
		4	<i>50</i>	<i>75</i>	<i>96</i>	<i>116</i>	<i>118</i>	<i>137</i>	<i>145</i>
	25 ticks	5	<i>106</i>	196	1656	4462	8736	6309	6945
		6	<i>69</i>	290	1540	3620	4142	5998	6899
		7	<i>70</i>	223	444	1356	2919	2677	5421
		8	<i>159</i>	194	358	2235	2053	4580	6020
	50 ticks	9	<i>50</i>	401	3182	3889	3421	6311	10867
		10	<i>77</i>	397	580	2235	5869	8467	10820
		11	<i>98</i>	987	2718	6282	8322	11688	17052
		12	<i>135</i>	847	1662	5364	6326	8375	7802
OspA	control	1	<i>146</i>	<i>254</i>	<i>383</i>	<i>154</i>	<i>344</i>	<i>406</i>	<i>405</i>
		2	<i>352</i>	<i>232</i>	<i>202</i>	<i>137</i>	<i>209</i>	<i>242</i>	<i>506</i>
		3	<i>79</i>	<i>91</i>	<i>110</i>	<i>77</i>	<i>95</i>	<i>155</i>	<i>136</i>
		4	<i>121</i>	<i>132</i>	<i>112</i>	<i>106</i>	<i>153</i>	<i>184</i>	<i>234</i>
	25 ticks	5	<i>115</i>	804	595	355	573	1009	624
		6	<i>86</i>	839	164	181	161	199	193
		7	<i>156</i>	5521	1063	324	170	193	221
		8	<i>71</i>	273	341	163	231	254	203
	50 ticks	9	<i>59</i>	1407	303	186	237	261	364
		10	<i>91</i>	1270	241	259	466	457	804
		11	<i>150</i>	2369	365	305	242	297	268
		12	<i>173</i>	3466	917	524	558	754	620

The positive cut-off values for antibodies to OspC and OspA are ≥ 1000 and for OspF ≥ 1500 . MFI values below the positive cut-off value are shown in italics.

Supplementary Table 2: Mean fluorescence intensity (MFI) values for antibodies to C6-P1, C6-P2 and FlaB determined by multiplex analysis in individual serum samples from dogs experimentally infected with *B. burgdorferi*.

Antibodies to	Group	Dog	Day 13 prior to infection	Day post infection					
				21	35	49	63	77	93
C6-P1	control	1	<i>77</i>	<i>70</i>	<i>56</i>	<i>16</i>	<i>312</i>	<i>213</i>	<i>170</i>
		2	<i>108</i>	<i>107</i>	<i>91</i>	<i>44</i>	<i>128</i>	<i>93</i>	<i>117</i>
		3	<i>30</i>	<i>105</i>	<i>63</i>	<i>27</i>	<i>78</i>	<i>13</i>	<i>30</i>
		4	<i>65</i>	<i>42</i>	<i>26</i>	<i>13</i>	<i>83</i>	<i>65</i>	<i>56</i>
	25 ticks	5	<i>40</i>	<i>189</i>	<i>3072</i>	<i>3971</i>	<i>6326</i>	<i>6556</i>	<i>12634</i>
		6	<i>37</i>	<i>229</i>	<i>917</i>	<i>2656</i>	<i>3070</i>	<i>3491</i>	<i>4885</i>
		7	<i>163</i>	<i>334</i>	<i>2195</i>	<i>2652</i>	<i>5486</i>	<i>5398</i>	<i>6504</i>
		8	<i>58</i>	<i>707</i>	<i>2200</i>	<i>4190</i>	<i>8134</i>	<i>10578</i>	<i>11641</i>
	50 ticks	9	<i>33</i>	<i>1238</i>	<i>3757</i>	<i>3803</i>	<i>3119</i>	<i>4715</i>	<i>6204</i>
		10	<i>101</i>	<i>617</i>	<i>1768</i>	<i>2205</i>	<i>4004</i>	<i>7357</i>	<i>9074</i>
		11	<i>69</i>	<i>2452</i>	<i>5698</i>	<i>7788</i>	<i>5813</i>	<i>6861</i>	<i>7658</i>
		12	<i>83</i>	<i>1647</i>	<i>5683</i>	<i>10841</i>	<i>11840</i>	<i>13674</i>	<i>11161</i>
C6-P2	control	1	<i>61</i>	<i>73</i>	<i>57</i>	<i>23</i>	<i>260</i>	<i>158</i>	<i>102</i>
		2	<i>49</i>	<i>78</i>	<i>74</i>	<i>43</i>	<i>94</i>	<i>52</i>	<i>71</i>
		3	<i>18</i>	<i>77</i>	<i>50</i>	<i>28</i>	<i>47</i>	<i>30</i>	<i>36</i>
		4	<i>44</i>	<i>31</i>	<i>34</i>	<i>33</i>	<i>65</i>	<i>45</i>	<i>54</i>
	25 ticks	5	<i>33</i>	<i>190</i>	<i>1460</i>	<i>2307</i>	<i>5758</i>	<i>6286</i>	<i>10408</i>
		6	<i>38</i>	<i>521</i>	<i>1063</i>	<i>1576</i>	<i>1814</i>	<i>2028</i>	<i>3366</i>
		7	<i>40</i>	<i>329</i>	<i>923</i>	<i>1914</i>	<i>1958</i>	<i>2658</i>	<i>3010</i>
		8	<i>24</i>	<i>60</i>	<i>209</i>	<i>501</i>	<i>1608</i>	<i>2266</i>	<i>2915</i>
	50 ticks	9	<i>28</i>	<i>1210</i>	<i>1368</i>	<i>1279</i>	<i>1093</i>	<i>1715</i>	<i>1951</i>
		10	<i>29</i>	<i>365</i>	<i>1758</i>	<i>2207</i>	<i>3468</i>	<i>4680</i>	<i>5180</i>
		11	<i>47</i>	<i>585</i>	<i>583</i>	<i>1419</i>	<i>1404</i>	<i>1869</i>	<i>2302</i>
		12	<i>60</i>	<i>582</i>	<i>2292</i>	<i>3971</i>	<i>4848</i>	<i>7257</i>	<i>6409</i>
FlaB	control	1	<i>570</i>	<i>224</i>	<i>348</i>	<i>297</i>	<i>478</i>	<i>778</i>	<i>673</i>
		2	<i>741</i>	<i>477</i>	<i>986</i>	<i>719</i>	<i>590</i>	<i>1419</i>	<i>2519</i>
		3	<i>39</i>	<i>229</i>	<i>396</i>	<i>370</i>	<i>256</i>	<i>192</i>	<i>176</i>
		4	<i>122</i>	<i>172</i>	<i>253</i>	<i>277</i>	<i>251</i>	<i>292</i>	<i>355</i>
	25 ticks	5	<i>333</i>	<i>762</i>	<i>1898</i>	<i>6241</i>	<i>7095</i>	<i>5907</i>	<i>5786</i>
		6	<i>47</i>	<i>1488</i>	<i>2238</i>	<i>3088</i>	<i>3492</i>	<i>4967</i>	<i>6557</i>
		7	<i>115</i>	<i>574</i>	<i>2457</i>	<i>6932</i>	<i>8559</i>	<i>6317</i>	<i>6580</i>
		8	<i>177</i>	<i>695</i>	<i>1726</i>	<i>5232</i>	<i>9204</i>	<i>13150</i>	<i>13040</i>
	50 ticks	9	<i>294</i>	<i>1415</i>	<i>5788</i>	<i>8910</i>	<i>7395</i>	<i>6775</i>	<i>6676</i>
		10	<i>143</i>	<i>1428</i>	<i>4743</i>	<i>9856</i>	<i>13899</i>	<i>13323</i>	<i>10577</i>
		11	<i>253</i>	<i>4207</i>	<i>6256</i>	<i>15629</i>	<i>12861</i>	<i>13830</i>	<i>10886</i>
		12	<i>227</i>	<i>4116</i>	<i>9922</i>	<i>14993</i>	<i>15890</i>	<i>15380</i>	<i>12580</i>

The positive cut-off values for antibodies to C6-P1 and FlaB are ≥ 1500 and for C6-P2 ≥ 1000 . MFI values below the positive cut-off value are shown in italics.

Supplementary Table 3: Quant[®] C6 ELISA antibody values (U/ml) for individual serum samples from dogs experimentally infected with *B. burgdorferi*.

Group	Dog	Day 13 prior to infection	Day post infection					
			21	35	49	63	77	93
control	1	34	12	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	10
25 ticks	5	0	26	256	338	349	355	143
	6	0	28	70	288	172	253	175
	7	0	47	177	279	246	252	386
	8	0	40	100	252	253	269	230
50 ticks	9	0	101	253	327	183	185	106
	10	0	57	66	242	379	305	168
	11	0	153	183	257	219	231	272
	12	26	44	201	312	304	384	370

The positive cut-off value for antibodies to C6 in this ELISA is ≥ 30 U/ml. Values below the positive cut-off value are shown in italics.