Supplementary material

Title: Maternal Antibodies Provide Bank Voles with Strain-Specific Protection against Infection by the Lyme Disease Pathogen

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Table of Contents

Table S1. *B. afzelii* infection status of the mothers. The *B. afzelii* infection status is shown for each of the 20 females in the study, of which 13 became mothers and produced offspring. There were 7 uninfected control females that produced 22 MatAb- offspring and 6 *B. afzelii*-infected females that produced 20 MatAb+ offspring. All mothers in the uninfected control group tested positive for 0 of the 4 criteria whereas all mothers in the infected group tested positive for 3 or 4 criteria.

ID[a]	Treat[b]	Male[c]	Offspring[d]	Nymphs[e]	ELISA[f]	Ear[g]	Bladder[h]	Joint[i]	Criteria[j]	Infected[k]
V634	Control	V162	3	0/0	480	-	-	-	0	No
V635	Control	V523	3	0/3	481	-	-	-	0	No
V639	Control	VD4	4	0/4	569	-	-	-	0	No
V643	Control	V174	3	0/2	503	-	-	-	0	No
Z533	Control	Z57	3	0/1	535	-	-	-	0	No
Z536	Control	Z59	4	0/1	506	-	-	-	0	No
Z540	Control	Z109	2	0/3	513	-	-	-	0	No
V637	Control	V184	0	0/0	1104	-	-	-	0	No
Z539	Control	Z107	0	0/1	1099	-	-	-	0	No
V631	Infected	V141	5	3/3	6611	+	+	+	4	Yes
V662	Infected	V185	2	1/1	6162	-	+	+	3	Yes
V665	Infected	V242	3	2/2	3800	+	+	+	4	Yes
V666	Infected	V151	2	0/1	4135	+	+	+	4	Yes
V667	Infected	V146	4	3/4	1352	+	+	+	4	Yes
Z538	Infected	Z101	4	1/2	3530	-	+	+	4	Yes
V632	Infected	V162	0	1/2	6409	+	+	+	4	Yes
V636	Infected	V153	0	1/1	2616	+	+	+	4	Yes
V638	Infected	V208	0	1/1	7630	-	+	+	3	Yes
V641	Infected	V154	0	2/2	10913	+	+	+	4	Yes
Z534	Infected	Z108	0	1/2	8150	+	+	+	4	Yes

[a] ID is the unique identification number of each female bank vole that was mated to produce offspring.

[b] Treatment has two levels: Control and Infected. Control females were infested with uninfected nymphs whereas infected females were infested with nymphs infected with *B. afzelii* strain NE4049.
[c] Male refers to the identity of the male bank vole that sired the offspring.
[d] Offspring is the number of offspring that were produced by each female bank vole.
[e] Nymphs gives a fraction where the numerator and denominator refer to the number of engorged *B. afzelii*-infected nymphs and the number of engorged nymphs, respectively, collected for each female bank vole.
[f] ELISA absorbance values indicate the strength of the IgG antibody response against *B. afzelii*. Individuals with absorbance values > 2000 are infected with *B. afzelii* strain NE4049. Absorbance values were obtained from a commercial Lyme disease ELISA.
[g] Ear indicates whether ear tissue tested positive or negative for *B. afzelii* spirochetes using the qPCR assay.
[h] Bladder indicates whether bladder tissue tested positive or negative for *B. afzelii* spirochetes using the qPCR assay.
[i] Joint indicates whether joint tissue tested positive or negative for *B. afzelii* spirochetes using the qPCR assay.
[j] Criteria is the number of the four infection criteria for which each female tested positive for *B. afzelii*.
[k] Infected refers to whether the female is considered to be infected with *B. afzelii* (Yes) or not (No).

Table S2. B. afzelii infection status of the MatAb- offspring. Infection status of the MatAb- offspring is shown following the infectious challenge via tick bite with *B. afzelii* strain NE4049 or *B. afzelii* strain Fin-Jyv-A3. The 22 MatAb- offspring were descended from the 7 mothers that were uninfected. The 6 infection criteria for the offspring are ELISA2, Biop2, Ear, Joint, Bladder, and Culture. Offspring that tested positive for 0 or 1 of the 6 infection criteria were considered as uninfected. Offspring that tested positive for 4 or more of the 6 infection criteria were considered as infected with *B. afzelii*.

ID1[a]	ID2[b]	Strain[c]	Nymphs[d]	ELISA1[e]	Biop1[f]	ELISA2[g]	Biop2[h]	Ear[i]	Joint[j]	Bladder[k]	Culture[l]	Criteria[m]	Infected[n]
V643	V725	NE4049	2/3	621	0	4549	18	-	+	+	+	5/6	Yes
V643	V728	NE4049	4/4	556	0	8152	32	+	+	+	+	6/6	Yes
V634	V735	NE4049	1/2	627	0	5979	46	+	+	+	+	6/6	Yes
V635	V740	NE4049	4/4	479	0	6820	20	+	+	+	+	6/6	Yes
V639	V752	NE4049	2/2	413	0	3950	129	+	-	+	+	5/6	Yes
V639	V753	NE4049	0/2	579	0	847	0	NA	NA	NA	-	0/3[o]	No
Z536	Z554	NE4049	3/3	474	0	6543	1326	+	-	+	-	4/6	Yes
Z533	Z558	NE4049	1/2	NA	0	NA	78	NA	NA	NA	NA	1/1[p]	Yes
Z540	Z562	NE4049	2/2	413	0	4579	13	+	+	+	+	6/6	Yes
V643	V727	Fin-Jyv-A3	2/2	575	0	5518	139	+	+	+	+	6/6	Yes
V634	V736	Fin-Jyv-A3	1/1	627	0	6016	163	+	+	+	+	6/6	Yes
V634	V737	Fin-Jyv-A3	2/4	603	0	8288	377	+	+	+	+	6/6	Yes
V635	V741	Fin-Jyv-A3	3/3	543	0	3602	1022	+	+	+	-	5/6	Yes
V635	V742	Fin-Jyv-A3	1/2	594	0	6451	367	+	+	+	+	6/6	Yes
V639	V754	Fin-Jyv-A3	3/3	403	0	7993	27	+	+	+	+	6/6	Yes
V639	V755	Fin-Jyv-A3	4/4	449	0	5043	45	+	+	+	+	6/6	Yes
Z536	Z556	Fin-Jyv-A3	1/3	529	0	6390	41	+	+	+	+	6/6	Yes
Z536	Z557	Fin-Jyv-A3	3/3	420	0	6575	190	+	+	+	+	6/6	Yes
Z533	Z560	Fin-Jyv-A3	2/2	488	0	5397	146	+	+	+	-	5/6	Yes
Z540	Z563	Fin-Jyv-A3	4/4	594	0	4555	2	+	+	+	-	5/6	Yes
Z536	Z555	Control	0/3	351	0	389	0	-	-	-	-	0/6	No
Z533	Z561	Control	0/4	529	0	612	0	-	-	-	-	0/6	No

[a] ID1 is the unique identification number of each mother bank vole that give birth to the offspring.
[b] ID2 is the unique identification number of each offspring bank vole.
[c] Strain refers to the strain of *B. afzelii* with which the offspring were challenged via tick bite at 35 days post-birth (PB): strain NE4049 or strain Fin-Jyv-A3.
[d] Nymphs gives a fraction where the numerator and denominator refer to the number of engorged *B. afzelii*-infected nymphs and the number of engorged nymphs, respectively, collected for each offspring bank vole.
[e] ELISA1 absorbance values indicate the level of maternally transmitted *B. afzelii*-specific IgG antibodies in the pre-infected offspring at 34 days PB. All individuals have absorbance values < 2000 indicating that they are not infected with *B. afzelii*. Absorbance values were obtained from a commercial Lyme disease ELISA.
[f] Biop1 indicates the spirochete load in the ear biopsy of the pre-infected offspring at 34 days PB. Samples were considered positive and negative if the *flagellin* gene copy number in the DNA template were > 0 and = 0, respectively.
[g] ELISA2 absorbance values indicate the strength of the IgG antibody response against *B. afzelii* in the post-infected offspring at 35 days post-infection (PI, which is 70 days post-birth). Individuals with absorbance values > 2000 are infected with *B. afzelii* strain NE4049 or Fin-Jyv-A3. Absorbance values were obtained from a commercial Lyme disease ELISA.
[h] Biop2 indicates the spirochete load in the ear biopsy of the post-infected offspring at 35 days PI (70 days PB). Samples were considered positive and negative if the *flagellin* gene copy number in the DNA template were > 0 and = 0, respectively.
[i] Ear indicates whether ear tissue tested positive or negative for *B. afzelii* at 70 days PI (105 days PB) using qPCR. Samples were considered positive and negative if the Cq value was < 38.5 cycles and > 38.5 cycles, respectively.
[j] Joint indicates whether joint tissue tested positive or negative for *B. afzelii* at 70 days PI (105 days PB) using qPCR. Samples were considered positive and negative if the Cq value was < 38.5 cycles and > 38.5 cycles, respectively.
[k] Bladder indicates whether bladder tissue tested positive or negative for *B. afzelii* at 70 days PI (105 days PB) using qPCR. Samples were considered positive and negative if the Cq value was < 38.5 cycles and > 38.5 cycles, respectively.
[l] Culture indicates whether live spirochetes were detected in at least one of the organ tissue cultures at 70 days PI (105 days PB).
[m] Criteria is the number of the six infection criteria for which each female tested positive for *B. afzelii*. These 6 criteria include: ELISA2, Biop2, ear, joint, bladder, and culture.
[n] Infected refers to whether the individuals is considered to be infected with *B. afzelii* (Yes) or not (No).
[o] Offspring V753 had 3 missing criteria (NA). This individual is uninfected because it tested positive for 0 of 3 criteria.
[p] Offspring Z558 had 5 missing criteria (NA). This individual is infected because it tested positive for 1 of 1 criterion.

Table S3. B. afzelii infection status of the MatAb+ offspring. The *B. afzelii* infection status of the MatAb+ offspring is shown following the infectious challenge via tick bite with *B. afzelii* strain NE4049 or *B. afzelii* strain Fin-Jyv-A3. The 20 MatAb+ offspring were descended from the 6 mothers that were infected with strain NE4049. The 6 infection criteria for the offspring are ELISA2, Biop2, Ear, Joint, Bladder, and Culture. Offspring that tested positive for 0 or 1 of the 6 infection criteria were considered as uninfected. Offspring that tested positive for 4 or more of the 6 infection criteria were considered as infected with *B. afzelii*.

ID1[a]	ID2[b]	Strain[c]	Nymphs[d]	ELISA1[e]	Biop1[f]	ELISA2[g]	Biop2[h]	Ear[i]	Joint[j]	Bladder[k]	Culture[l]	Criteria[m]	Infected[n]
V665	V729	NE4049	3/4	1237	0	592	0	-	-	-	-	0/6	No
V666	V734	NE4049	2/3	880	0	732	0	-	-	-	-	0/6	No
V667	V745	NE4049	3/3	644	0	522	0	-	-	-	-	0/6	No
V667	V746	NE4049	2/3	727	0	458	0	-	-	-	-	0/6	No
V631	V747	NE4049	2/3	755	0	652	0	-	-	-	-	0/6	No
V631	V748	NE4049	2/2	792	0	449	0	-	-	-	-	0/6	No
V631	V749	NE4049	2/3	713	0	369	0	-	-	-	-	0/6	No
V662	V757	NE4049	3/3	1293	0	566	0	-	-	-	-	0/6	No
Z538	Z550	NE4049	2/4	615	0	510	0	-	-	-	-	0/6	No
Z538	Z551	NE4049	0/3	532	0	428	0	-	-	+	-	1/6	No
V665	V730	Fin-Jyv-A3	2/3	1156	0	5120	62	+	+	+	+	6/6	Yes
V665	V731	Fin-Jyv-A3	1/3	1101	0	5277	547	+	+	+	-	5/6	Yes
V666	V732	Fin-Jyv-A3	3/3	682	0	6994	124	+	+	+	+	6/6	Yes
V667	V743	Fin-Jyv-A3	1/3	749	0	6379	12	-	+	+	NA	4/5	Yes
V667	V744	Fin-Jyv-A3	2/3	598	0	7055	305	-	+	+	+	5/6	Yes
V631	V750	Fin-Jyv-A3	3/4	921	0	3567	333	+	+	+	+	6/6	Yes
V631	V751	Fin-Jyv-A3	3/4	882	0	2441	614	+	-	+	+	5/6	Yes
V662	V756	Fin-Jyv-A3	1/3	1392	0	758	0	-	-	-	-	0/6	No
Z538	Z552	Fin-Jyv-A3	1/2	785	0	5556	84	+	+	+	-	5/6	Yes
Z538	Z553	Fin-Jyv-A3	2/3	524	0	3997	1022	+	+	+	+	6/6	Yes

[a] ID1 is the unique identification number of each mother bank vole that give birth to the offspring.
[b] ID2 is the unique identification number of each offspring bank vole.
[c] Strain refers to the strain of *B. afzelii* with which the offspring were challenged via tick bite at 35 days post-birth (PB): strain NE4049 or strain Fin-Jyv-A3.
[d] Nymphs gives a fraction where the numerator and denominator refer to the number of engorged *B. afzelii*-infected nymphs and the number of engorged nymphs, respectively, collected for each offspring bank vole.
[e] ELISA1 absorbance values indicate the level of maternally transmitted *B. afzelii*-specific IgG antibodies in the pre-infected offspring at 34 days PB. All individuals have absorbance values < 2000 indicating that they are not infected with *B. afzelii*. Absorbance values were obtained from a commercial Lyme disease ELISA.
[f] Biop1 indicates the spirochete load in the ear biopsy of the pre-infected offspring at 34 days PB.
[g] ELISA2 absorbance values indicate the strength of the IgG antibody response against *B. afzelii* in the post-infected offspring at 35 days post-infection (PI, which is 70 days post-birth). Individuals with absorbance values > 2000 are infected with *B. afzelii* strain NE4049 or Fin-Jyv-A3. Absorbance values were obtained from a commercial Lyme disease ELISA.
[h] Biop2 indicates the spirochete load in the ear biopsy of the post-infected offspring at 35 days PI (70 days PB).
[i] Ear indicates whether ear tissue tested positive or negative for *B. afzelii* at 70 days PI (105 days PB) using qPCR.
[j] Joint indicates whether joint tissue tested positive or negative for *B. afzelii* at 70 days PI (105 days PB) using qPCR.
[k] Bladder indicates whether bladder tissue tested positive or negative for *B. afzelii* at 70 days PI (105 days PB) using qPCR.
[l] Culture indicates whether live spirochetes were detected in at least one of the organ tissue cultures at 70 days PI (105 days PB).
[m] Criteria is the number of the six infection criteria for which each female tested positive for *B. afzelii*. These 6 criteria include: ELISA2, Biop2, ear, joint, bladder, and culture.
[n] Infected refers to whether the individuals is considered to be infected with *B. afzelii* (Yes) or not (No).

Section 1 – Creation of *I. ricinus* nymphs infected with *B. afzelii*

The nymphs used for the experimental infections were created as follows. BALB/c mice were infected with *B. afzelii* strain NE4049 or Fin-Jyv-A3 via tick bite. At 4 weeks post-infection (PI), the mice were infested with larval ticks from our *I. ricinus* colony. The engorged larval ticks were stored in individual 1.7 ml Eppendorf tubes; each tube contained a piece of moistened paper towel to ensure high humidity. The engorged larvae were kept at room temperature under ambient light conditions and were allowed to molt into nymphs. A random sample of nymphs was tested to determine the infection prevalence, which was 77.9% (67 infected nymphs/ 86 total nymphs) for NE4049 and 91.8% (67 infected nymphs/ 73 total nymphs) for Fin-Jyv-A3. Larval *I. ricinus* ticks were also fed on uninfected BALB/c mice to create uninfected control nymphs.

Section 2 – Antibody response against *B. afzelii* in the bank vole mothers

The raw data for the *B. afzelii*-specific IgG antibody response in the mother at 35 days post-infection (PI) are shown in Table S1 in the column titled 'ELISA'.

To test whether the bank vole mothers developed an IgG antibody response against *B. afzelii* at 35 days post-infection (PI), we compared this variable (the ELISA variable in Table S1) between infected mothers (n = 6) and uninfected control mothers (n = 7) using an independent two sample t-test. The IgG antibody response against *B. afzelii* was log10-transformed to improve the normality of the residuals.

The mean *B. afzelii*-specific IgG antibody response of the infected mothers (mean = 3811, 95% CI = 2692–5395) at 35 days PI was 7.4 times higher compared to the uninfected control mothers (mean = 512, 95% CI = 371–706), and this difference was significant (Figure S1; $t = -9.335$, $df = 11$, $p < 0.001$). This result shows that infected bank vole mothers developed a strong IgG antibody response against the *B. afzelii* infection.

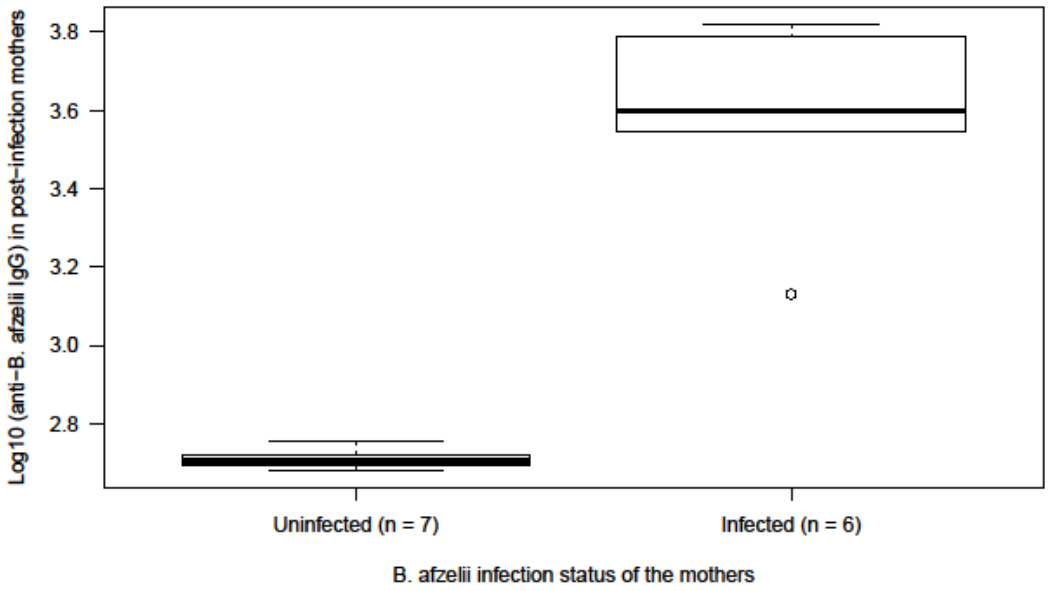

Figure S1. The *B. afzelii*-specific IgG antibody response of the infected bank vole mothers at 35 days post-infection (n = 6) was 7.4 times higher compared to the uninfected control bank vole mothers (n = 7). The strength of the *B. afzelii*-specific IgG antibody response was measured in absorbance units using a commercial Lyme disease ELISA. The infected mothers and the uninfected control mothers produced the MatAb+ and the MatAb- bank vole offspring, respectively.

Section 3 – Maternal antibody transmission differs among bank vole mothers

The raw data for the level of maternally transmitted IgG antibodies in the offspring at 34 days post-birth (PB) are shown in Tables S2 and S3 in the column titled 'ELISA1'. The raw data for the *B. afzelii*-specific IgG antibody response in the mother at 35 days post-infection (PI) are shown in Table S1 in the column titled 'ELISA'.

There were significant differences among infected bank vole mothers (n = 6) in the level of maternally transmitted IgG antibodies present in their offspring at 34 days PB (Figure S2; one-way ANOVA: $F_{12, 28} = 29.25$, $p < 0.001$). However, there was no correlation between the strength of the *B. afzelii*-specific IgG antibody response in the mother at 35 days PI and the level of maternally transmitted *B. afzelii*-specific IgG antibodies in the offspring at 34 days PB (Figure S2; r = 0.361, df =18, p = 0.118).

One possible explanation for this result is as follows. The antibody levels of the mothers were measured at 35 days PI, whereas the females were coupled with males at 2 and at 6 weeks PI. Thus the window of maternal antibody transmission occurred at 5–8, or 9–12 weeks PI depending on whether the female produced her first litter with the first or second male. This variation in the time lag between maternal infection and reproduction may explain the lack of a relationship in the IgG antibodies levels between bank vole mothers and their offspring.

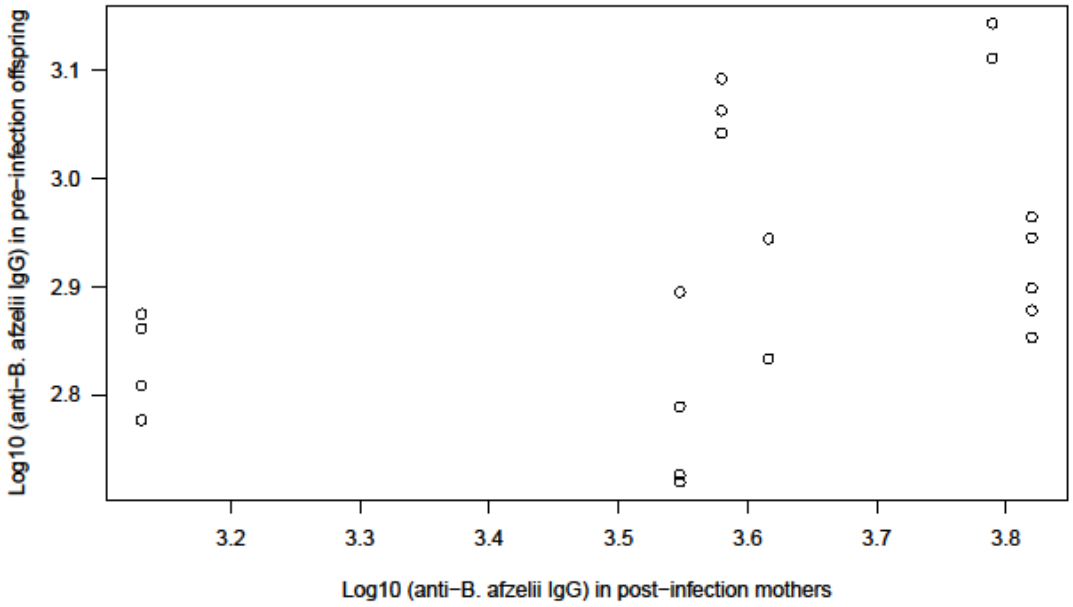

Figure S2. The relationship between the *B. afzelii*-specific IgG antibody response of the infected bank vole mothers (n = 6) at 35 days post-infection and the level of maternally transmitted *B. afzelii*-specific IgG antibodies of their MatAb+ offspring (n = 20) at 34 days post-birth was not significant. There were significant differences in the level of maternally transmitted *B. afzelii*-specific IgG antibodies among the 6 families of bank voles. In the graph, each of the 6 families is shown by a vertical cluster of data points.

Section 4 – The maternally transmitted IgG antibodies are specific for the OspC antigen

The maternally transmitted OspC-specific IgG antibody level in the offspring before the infectious challenge (34 days PB) was measured using a homemade ELISA with recombinant OspC (rOspC) proteins A3 and A10 [1]. 96-well tissue culture plates were coated overnight at 4°C with rOspC proteins A3 and A10 (1 µg of protein per well). Wells were washed three times with PBS-Tween 0.1% between each step. The plate was incubated with a BSA 2% blocking solution for 2 hours, followed by the bank vole serum samples (diluted 1:100 in 1x PBS) for 45 minutes, and the secondary antibody for 45 minutes (diluted 1:5000 in 1x PBS). The secondary antibody was a goat anti-*Mus musculus* IgG conjugated to horseradish peroxidase. After adding 100 µl of TMB, we measured the absorbance at 652 nm every 2 minutes for one hour using a plate reader (Synergy HT, Multi-detection plate reader, Bio-Tek, United States). The level of IgG antibodies against each rOspC antigen in the offspring was determined by integrating the area under the absorbance versus time curve and is measured in absorbance units.

The specificity of the maternally transmitted IgG antibodies against the OspC antigen in the naive bank vole offspring (i.e., before the infectious challenge at 35 days PB) was quantified as follows. For each offspring, we measured the ability of the maternally transmitted IgG antibodies (sampled from offspring at 34 days PB) to bind recombinant outer surface protein C (rOspC) A10 and rOspC A3 using a homemade ELISA. We calculated an OspC A10 specificity ratio for each offspring by dividing the level of IgG antibodies that bound to rOspC A10 by the level of IgG antibodies that bound to rOspC A3. Thus, the OspC A10 specificity ratio measures the ability of the maternally transmitted antibodies to recognize the maternal OspC A10 antigen compared to the foreign OspC A3 antigen. The OspC A10 specificity ratio was log10-transformed to improve the normality of the residuals.

We compared the log10-transformed OspC A10 specificity ratio in the pre-infection blood sample (at 34 days PB) between the MatAb+ offspring and the MatAb- offspring using an independent two samples t-test. The OspC specificity ratio in the MatAb+ offspring was 3.07 times higher compared to the MatAb- offspring, and this difference was significant (independent two samples t-test: $t = -10.015$, $df = 39$, $p < 0.001$). This result shows that the preference of the maternally transmitted IgG antibodies for the OspC A10 antigen (compared to the OspC A3 antigen) was stronger in the MatAb+ offspring compared to the MatAb- offspring (Figure S3). For the MatAb+ offspring, the mean log10-transformed OspC A10 specificity ratio was 0.45 (95% CI = 0.40–0.50) and this was significantly greater than 0 (paired samples t-test: $t = 18.145$, $df = 18$, $p < 0.001$). This result shows that the infected mothers transmitted IgG antibodies to their offspring that were specific for OspC A10. For the MatAb- offspring, the mean log10-transformed OspC A10 specificity ratio was 0.15 (95% CI = 0.11–0.18) and this was also significantly different from 0 (paired samples t-test: $t = 7.981$, $df = 21$, $p < 0.001$). Thus, even in the absence of OspC-specific antibodies, the rOspC A10 antigen induced a stronger color reaction than the rOspC A3 antigen. This result suggests that the rOspC A10 antigen is better at binding mouse IgG or the secondary antibody compared to rOspC A3.

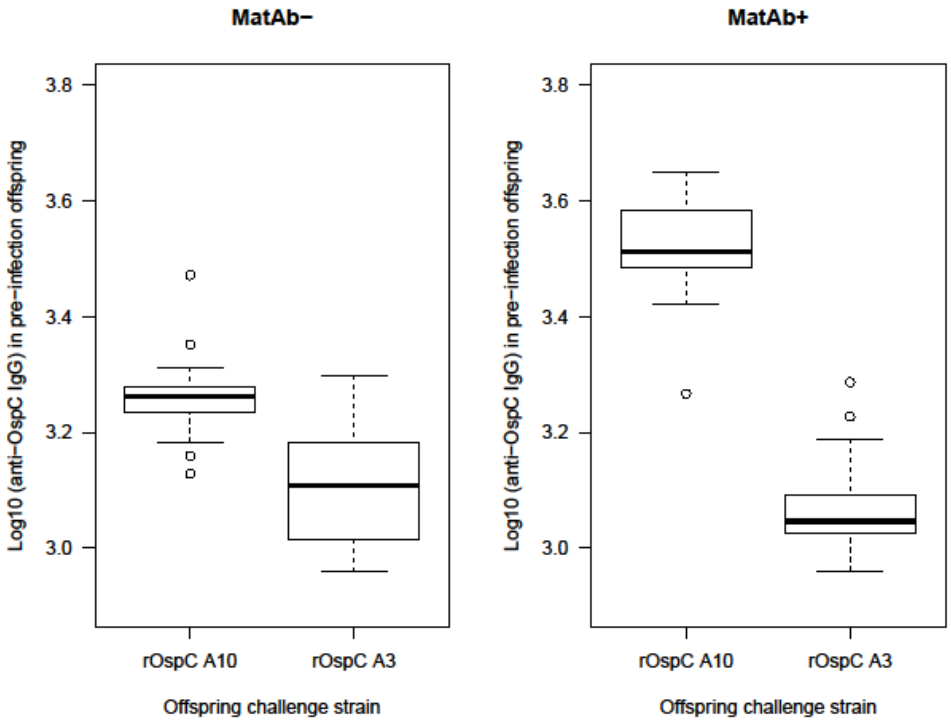

Figure S3. The ability of maternally transmitted IgG antibodies to bind *B. afzelii* OspC antigens A10 and A3 is shown for the MatAb- offspring (left panel) and the MatAb+ offspring (right panel). Blood samples were taken from the pre-infection offspring at 34 days post-birth. For the MatAb+ offspring, the ability of the maternally transmitted IgG antibodies to bind rOspC A10 was 2.82 times stronger compared to rOspC A3. For the MatAb- offspring, the ability of the maternally transmitted IgG antibodies to bind rOspC A10 was 1.40 times stronger compared to rOspC A3. The left panel of the graph suggests that our homemade ELISA is biased towards the rOspC A10 antigen because the serum samples from the pre-infection MatAb- offspring (which are not expected to have any *B. afzelii*-specific antibodies) bound more strongly to the rOspC A10 antigen compared to the rOspC A3 antigen. Despite this bias, this graph also shows that the serum samples of the pre-infection MatAb+ offspring bound much more strongly to the rOspC A10 antigen (right panel) compared to the MatAb- offspring (left panel). In contrast, the ability of the maternally transmitted antibodies to bind the rOspC A3 antigen was similar between the MatAb+ and MatAb- offspring.

Section 5 – The *B. afzelii*-specific IgG antibody levels changed dramatically after offspring were exposed to infected nymphs

For the bank vole offspring, the pre-infection blood sample was taken at 34 days post-birth (PB), whereas the post-infection blood sample was taken at 35 days post-infection (PI), which corresponds to 70 days PB. The raw data for the level of *B. afzelii*-specific IgG antibodies in the pre-infection and post-infection blood samples are shown in Tables S2 and S3 in the columns titled ‘ELISA1’ and ‘ELISA2’, respectively.

The level of *B. afzelii*-specific IgG antibodies increased dramatically from the pre-infection blood sample to the post-infection blood sample for the MatAb- offspring (left column in Figure S4) and for the MatAb+ offspring that were challenged with strain Fin-Jyv-A3 (bottom-right panel in Figure S4). This result indicates that all of these offspring acquired *B. afzelii* following the infectious challenge. One MatAb+ offspring (V756) was protected from infectious challenge with strain Fin-Jyv-A3 (bottom-right panel in Figure S4). The level of *B. afzelii*-specific IgG antibody response decreased from the pre-infection blood sample to the post-infection blood sample for the MatAb+ offspring that were challenged with strain NE4049 (top-right panel in Figure S4). This result indicates that the MatAb+ offspring were protected against the infectious challenge with strain NE4049 and that the level of maternal antibodies in these offspring waned over time.

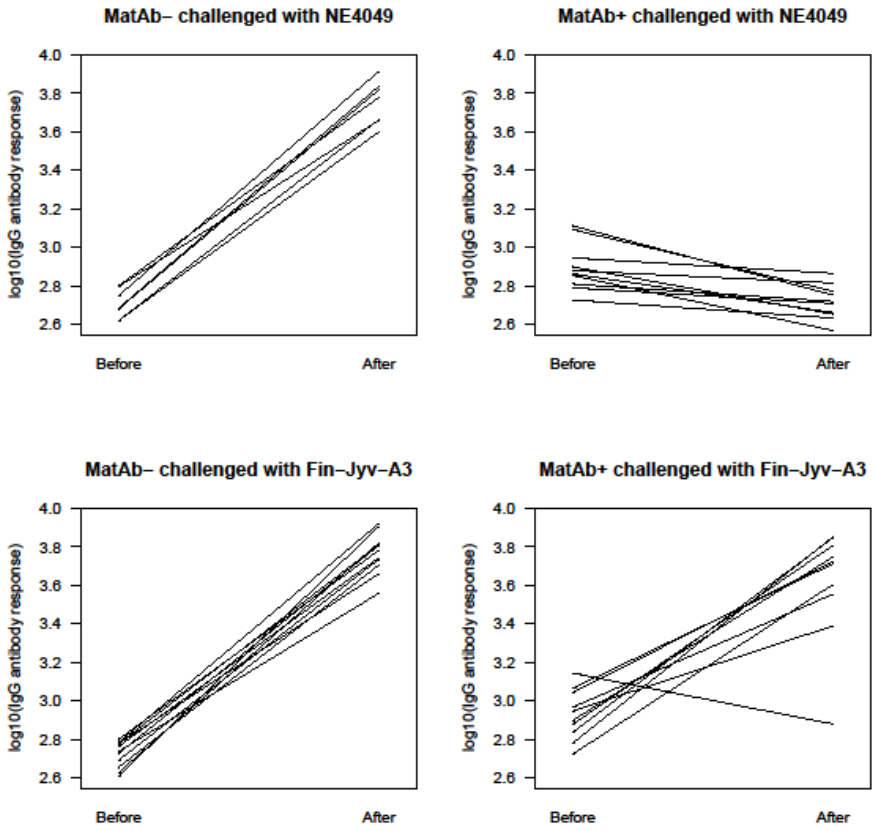

Figure S4. Interaction plot that shows the level of *B. afzelii*-specific IgG antibodies in the offspring before and after the infectious challenge. The pre-infection blood sample was taken at 34 days post-birth; the post-infection blood sample was taken at 35 days post-infection, which corresponds to 70 days post-birth. The MatAb- (left column) and MatAb+ (right column) refer to the offspring without and with maternal antibodies against *B. afzelii* strain NE4049, respectively. The offspring were either challenged with strain NE4049 or strain Fin-Jyv-A3. The MatAb- offspring (left column) were equally susceptible to both strains. The MatAb+ offspring were protected against the maternal

strain (NE4049; top-right panel) but not the new strain (Fin-Jyv-A3; bottom-right panel). The top-right panel also shows that the level of maternal IgG antibodies in the MatAb+ offspring waned over time.

Section 6 – The *B. afzelii* spirochete load in the ear tissue changed dramatically after offspring were exposed to infected nymphs

For the bank vole offspring, the pre-infection ear tissue biopsy was taken at 34 days post-birth (PB), whereas the post-infection ear tissue biopsy was taken at 35 days post-infection (PI), which corresponds to 70 days PB. The raw data for the spirochete loads in the pre-infection and post-infection ear tissue biopsies are shown in Tables S2 and S3 in the columns titled 'Biop1' and 'Biop2', respectively.

The *B. afzelii* spirochete load in the ear tissue increased dramatically from the pre-infection ear biopsy to the post-infection ear biopsy for the MatAb- offspring (left column in Figure S5) and for the MatAb+ offspring that were challenged with strain Fin-Jyv-A3 (bottom-right panel in Figure S5). This result indicates that all of these offspring acquired *B. afzelii* following the infectious challenge. One MatAb+ offspring (V756) was protected from infectious challenge with strain Fin-Jyv-A3 (bottom-right panel in Figure S5). The *B. afzelii* spirochete load in the ear tissue was zero for the pre- and post-infection ear biopsies of the MatAb+ offspring that were challenged with strain NE4049 (top-right panel in Figure S7). This result indicates that the MatAb+ offspring were protected against the infectious challenge with strain NE4049. Figure S5 also shows that there is no vertical transmission of *B. afzelii* from mothers to their offspring.

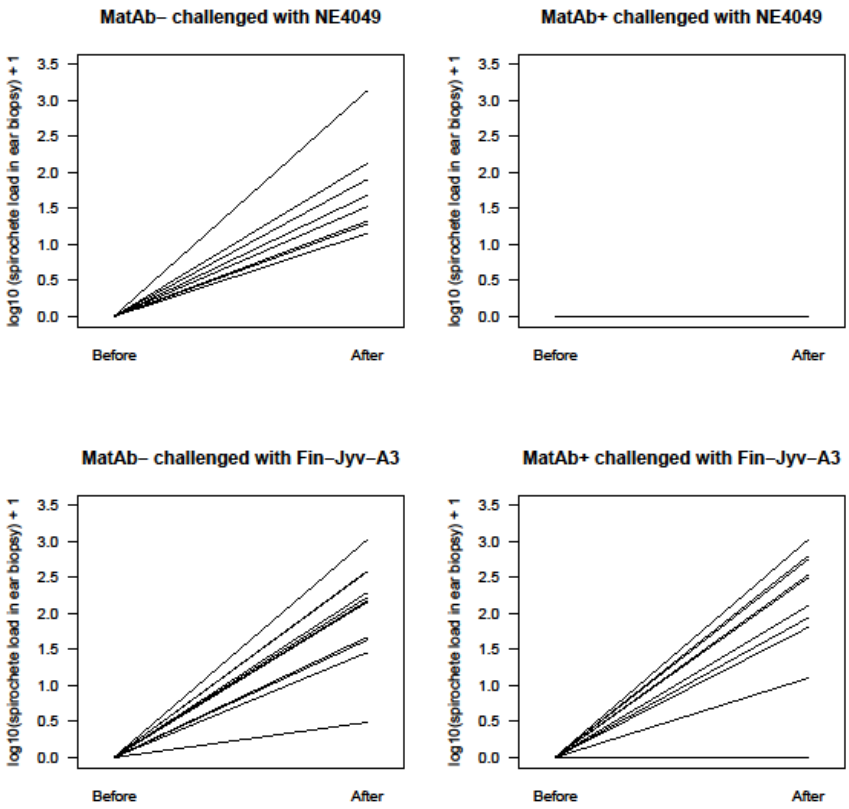

Figure S5. Interaction plot that shows the ear biopsy spirochete loads of the offspring before and after the infectious challenge. The pre-infection ear biopsy was taken at 34 days post-birth; the post-infection ear biopsy was taken at 35 days post-infection, which corresponds to 70 days post-birth. The MatAb- (left column) and MatAb+ (right column) refer to the offspring without and with maternal antibodies against *B. afzelii* strain NE4049, respectively. The offspring were either challenged with strain NE4049 or strain Fin-Jyv-A3. The MatAb- offspring (left column) were equally susceptible to both strains. The MatAb+ offspring were protected against the maternal strain (NE4049; top-right panel) but not the new strain (Fin-Jyv-A3; bottom-right panel). Figure S7 shows that there is no vertical transmission of *B. afzelii* from mothers to their offspring.

Section 7 – Culture of tissue biopsies to detect live *B. afzelii* spirochetes

The tissue biopsy culture confirmed the detection of live spirochetes in most animals (Table S3 and Table S4). Live spirochetes were found for 0% (0/9) of the MatAb+/NE4049 offspring, 66.6% (6/9) of the MatAb+/Fin-Jyv-A3 offspring, 75% (6/8) of the MatAb-/NE4049 offspring, and 72.7% (8/11) of the MatAb-/Fin-Jyv-A3 offspring. In summary, live spirochetes were recovered from tissue samples for most of the infected animals (71.4% = 20/28), but for none of the uninfected animals (0.0% = 0/9).

Section 8 – The *ospC*-specific qPCR to confirm identity of infecting strain

Strain NE4049 carries *ospC* allele A10 and strain Fin-Jyv-A3 carries *ospC* allele A3. The *ospC*-specific qPCR confirmed that all animals were infected with the expected strain (Table S3 and Table S4). Spirochetes carrying *ospC* allele A10 were detected in 0% (0/9) of the MatAb+/NE4049 offspring (i.e. because these individuals were protected from the infectious challenge with strain NE4049), 0% (0/10) of the MatAb+/Fin-Jyv-A3 offspring, 100% (8/8) of the MatAb-/NE4049 offspring, and 0% (0/11) of the MatAb-/Fin-Jyv-A3. In contrast, spirochetes carrying *ospC* allele A3 were detected in 0% (0/9) of the MatAb+/NE4049 offspring, 100% (10/10) of the MatAb+/Fin-Jyv-A3 offspring, 0% (0/8) of the MatAb-/NE4049 offspring, and 100% (11/11) of the MatAb-/Fin-Jyv-A3 offspring. In summary, the *ospC* allele corresponding to the expected strain was always found in the tissues of the experimentally infected bank vole offspring.

References

1. Jacquet M., Durand J., Rais O., Voordouw M.J. 2015 Cross-reactive acquired immunity influences transmission success of the Lyme disease pathogen, *Borrelia afzelii*. *Infection Genetics and Evolution* **36**, 131-140. (doi:10.1016/j.meegid.2015.09.012).