Maternal Transfer of Vaccine-Induced Anti-OspA Antibodies in Peromyscus spp Protects Pups from Tick-Transmitted *Borrelia burgdorferi*

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Abstract

Maternal antibody transfer provides passive immunity to offspring; however, the efficacy and duration of this protection depends on maternal antibody levels and transfer efficiency. We investigated whether oral vaccination of *Peromyscus leucopus* dams with recombinant OspA-expressing *E. coli* could induce maternal transfer of anti-OspA antibodies and protect pups from *Borrelia burgdorferi* challenge. Dams were vaccinated for different durations: (i) until breeding, (ii) until birth of pups, or (iii) until pups were 2 weeks old. Pups were challenged with *Ixodes scapularis* nymph-transmitted *B. burgdorferi* at ~ 4 weeks of age. Anti-OspA IgG were quantified in dams and pups, and *B. burgdorferi* anti-PepVF IgG were quantified in pups. Furthermore, *B. burgdorferi* burden was assessed by qPCR targeting the *flaB* gene in pups' heart and bladder tissues > 4 weeks after tick challenge. *P. leucopus* pups born to dams vaccinated until breeding, exhibited low anti-OspA antibody and were not protected from tick transmitted *B. burgdorferi* infection. However, when maternal vaccination extended until pups were born and until pups were two weeks old, significant anti-OspA antibody transfer and protection occurred. This was evidenced by absence of antibody to *B. burgdorferi* PepVF, absence of *B. burgdorferi flaB* DNA in heart and bladder tissues, and absence of *flaB* in culture from heart tissues from pups euthanized >9 weeks after birth. We show that transfer of anti-OspA antibodies from *P. leucopus* dams to offspring prevents tick transmission of *B. burgdorferi* in the major reservoir host of this spirochete in the United States.

Importance: This study contributes to our understanding of how reservoir targeted vaccines designed to block transmission of *B. burgdorferi* from infected *Ixodes scapularis* ticks may disrupt the enzootic cycle of this spirochete and reduce incidence of Lyme disease.

Key words: *Peromyscus***,** reservoir targeted oral vaccine, block transmission, *Ixodes scapularis*, enzootic cycle, OspA, mouse, *Borrelia burgdorferi*, Lyme disease

Introduction

Lyme disease, caused by *Borrelia burgdorferi* and transmitted by *Ixodes* ticks, is the most prevalent vector-borne disease in North America (1). The white-footed mouse (*Peromyscus leucopus*) serves as the primary reservoir host, maintaining *B. burgdorferi* in enzootic cycles and facilitating transmission to ticks that subsequently infect humans and other mammals (2). Unlike humans, these reservoir hosts do not develop significant disease following *B. burgdorferi* infection, yet they serve as a crucial source of spirochete acquisition for larval ticks (3). As a result, interrupting this transmission within the reservoir host has been proposed as a strategy for controlling Lyme disease (4).

Outer surface protein A (OspA) is a major target for Lyme disease vaccine development due to its essential role in *B. burgdorferi* persistence within the tick vector (5). OspA facilitates spirochete adherence to the tick midgut, allowing the bacterium to survive between blood meals (6). During tick feeding, *B. burgdorferi* downregulates OspA while upregulating OspC, facilitating migration to the salivary glands and subsequent transmission to the host (7). Vaccines targeting OspA aim to elicit antibodies that neutralize spirochetes within the tick gut, preventing their transmission before they reach the mammalian host (8). OspA-based vaccines have been explored in multiple contexts, including human vaccination (1), direct vaccination of *P. leucopus* (9), and bait-delivered reservoir-targeted vaccines that reduce pathogen burden in wild mouse populations (10).

Beyond direct vaccination, maternal transfer of immunity provides an alternative strategy to protect neonates during the critical early stages of life. (11). In the context of vector-borne pathogens, this temporary immunity may reduce early-life susceptibility to infection (12).

77 Previous studies have demonstrated that maternal vaccination with OspA results in detectable
78 anti-OspA IgG in offspring (13), though the degree to which this protects against *B. burgdorferi*
79 transmission remains unknown.

80 In this study, we investigate the extent of maternal antibody transfer in *P. leucopus* and assess
81 whether vaccination of dams with recombinant OspA-expressing *E. coli* can confer protection to
82 neonates. We test whether different durations of maternal vaccination influence antibody transfer
83 and protection against tick-mediated *B. burgdorferi* challenge. Our findings have implications
84 for reservoir-targeted vaccination strategies aimed at reducing Lyme disease risk by breaking the
85 transmission cycle of *B. burgdorferi*.

86

87 **Materials and Methods**

88 **Ethics Statement.** This study was conducted in accordance with the Guide for the Care and Use
89 of Laboratory Animals of the US National Institutes of Health. The protocol was approved by the
90 University of Tennessee Health Science Center Institutional Animal Care and Use Committee
91 (IACUC), protocol #22-0400. *Peromyscus leucopus* were sourced from the Peromyscus Genetic
92 Stock Center (PGSC) at the University of South Carolina.

93 **Vaccination Strategy and Experimental Design.** Dams (n=10) were assigned to three
94 experimental groups based on the duration of maternal vaccination. In the first group, referred to
95 as Breeding 1, dams received oral vaccination for 12 weeks prior to mating. The second group,
96 Breeding 2, was vaccinated for an extended period of 24 weeks, continuing until parturition. The

97 final group, Breeding 3, remained on the vaccination regimen for 36 weeks, ensuring that
98 immunization continued until their pups reached two weeks of age.

99 **Vaccine Preparation and Administration.** Dams were orally vaccinated with recombinant
100 OspA-expressing Escherichia coli (RTV pellets) produced as previously described (14),
101 inactivated by β-propiolactone. Vaccine pellets contained OspA-expressing *E. coli*
102 BL21(DE3)pLysS grown in Terrific Broth (TBY) at 37°C until OD600 ~0.8, induced with 1 mM
103 IPTG for 3 h, and harvested by centrifugation at 2000×g at 4°C. The bacterial biomass was
104 resuspended in phosphate-buffered saline (PBS) and sprayed onto feed pellets. Pellets were
105 allowed to dry between application layers.

106 Dams received RTV pellets daily for 5 consecutive days per week, with a 2-day rest period in
107 which they received regular mouse chow. Control dams received uncoated pellets. The
108 vaccination schedule was maintained based on experimental grouping (**Figure 1**).

109 **Breeding and Litter Management.** Breeding pairs were established based on high anti-OspA
110 titers [OD450>0.8) assessed by ELISA. Each vaccinated female was cohoused with an
111 unvaccinated male for 3–4 weeks, after which females were single-housed until parturition. Pups
112 remained with their dams until 24–25 days of age, at which point they underwent nymphal tick
113 challenge.

114 **Blood Collection.** Blood samples were collected via submandibular vein bleed at day 24 (pre-
115 challenge) and day 71 (terminal bleed) for pups. Samples were allowed to clot for 30 min at
116 room temperature before centrifugation at 2000×g for 10 min at 4°C. Serum was aliquoted and
117 stored at −80°C until analysis.

118 **Tissue Collection and Processing.** At necropsy on d71 (~9-10 week old pups), heart and
119 bladder tissues were harvested, transported on dry ice, and stored at −80°C. For organ culture
120 experiments, freshly excised heart tissue was immediately placed into Barbour-Stoenner-Kelly H
121 (BSK-H) medium supplemented with 6% rabbit serum (Sigma-Aldrich, St. Louis, MO) and
122 incubated at 34°C for 21 days. Culture supernatants were analyzed for: 1. Motile Borrelia
123 detection using Petroff-Hausser chamber (dark-field microscopy, 200× magnification) and 2.
124 DNA quantification via *flaB* qPCR.

125 **Tick Challenge and Borrelia Infection Monitoring.** To assess susceptibility to *Borrelia*
126 *burgdorferi* infection, pups were challenged with infected nymphal *Ixodes scapularis* ticks at ~ 4
127 weeks of age. Each pup was exposed to 5–7 infected nymphs, which were allowed to feed for 72
128 hours in a controlled containment chamber. Flat nymphal ticks were carefully transferred from
129 storage vials using fine forceps and placed at the base of the neck and upper back of gently
130 restrained pups. After placement, pups were monitored to ensure proper tick attachment before
131 being returned to their cages. After 72 hours, all engorged ticks naturally detached and were
132 collected from the bottom of the cages. Detached ticks were stored at −20°C until further
133 analysis for *B. burgdorferi* colonization via dark-field microscopy.

134 The infected nymphs used in this study were generated by feeding larval *Ixodes scapularis*
135 (Oklahoma Tick Laboratory) on a mouse infected with a multi-strain culture of *Borrelia*
136 *burgdorferi* sensu stricto. This *B. burgdorferi* culture was recovered from heart and bladder
137 tissues of Ch3-HeN mice infected with nymphal ticks collected in Massachusetts in 2020. To
138 maintain infection across generations, the multi-strain *B. burgdorferi* inoculum was first
139 introduced into C3H mice, after which successive mice were infected through tick-borne

140 transmission. Larvae were allowed to feed on infected mice, acquire *B. burgdorferi*, and molt
141 into infected nymphs, which were then used to challenge mice in a cycle that mimics natural
142 enzootic transmission of *B. burgdorferi*.

143 **ELISA for Anti-OspA and Anti-PepVF IgG.** Serum antibody responses to OspA and to *B.*
144 *burgdorferi* pepVF were assessed using an enzyme-linked immunosorbent assay (ELISA).
145 PepVF contains two peptides from *Borrelia burgdorferi* VlsE and flaB proteins in tandem,
146 separated by a 3aa glycine linker, and is used in our lab to determine *B. burgdorferi* infection
147 (15). Presence of antibody to PepVF indicates exposure to *B. burgdorferi*. Purified recombinant
148 OspA (1 μg/mL) or recombinant PepVF (1 μg/mL) was adsorbed onto Nunc MaxiSorp plates
149 (Thermo Fisher Scientific, Waltham, MA) by overnight incubation at 4°C. Following antigen
150 coating, the plates were blocked with 5% bovine serum albumin (BSA) in PBS-Tween (0.05%)
151 for 1 hour at room temperature to prevent nonspecific binding. Serum samples were diluted
152 1:100 in blocking buffer and incubated on the plates for 2 hours at 37°C. To detect antigen-
153 specific IgG, a goat anti-mouse IgG antibody conjugated to horseradish peroxidase (HRP)
154 (1:6000; Jackson ImmunoResearch, West Grove, PA) was added, followed by a 1-hour
155 incubation at 37°C. After washing, plates were developed using TMB substrate (Sigma-Aldrich)
156 for 15 minutes before stopping the reaction with 1N H_2SO_4. Optical density (OD) was measured
157 at 450 nm and blanked against the control using a SpectraMax Plus ELISA reader (Molecular
158 Devices, San Jose, CA). For Cutoff determination the positivity threshold was defined as mean +
159 3 SD of negative controls.

160 **Molecular Detection of Borrelia burgdorferi DNA by qPCR.** Total DNA was extracted from
161 heart and bladder tissues and heart culture media using the DNeasy Blood & Tissue Kit (Qiagen,

162 Germantown, MD) according to the manufacturer's protocol. qPCR was performed to quantify
163 *B. burgdorferi flaB* gene copies per mg of tissue or per mL of culture media. Quantitative PCR
164 (qPCR) was performed to quantify *Borrelia burgdorferi* flaB gene copies in DNA extracted from
165 heart and bladder tissues, as well as culture supernatants. Reactions were carried out in a 25 µL
166 volume using 2X TaqMan Master Mix (Thermo Fisher Scientific), with final primer and probe
167 concentrations of 200 nM. Each reaction contained 5 µL of DNA template, and all samples were
168 run in duplicate to ensure reproducibility. The primer and probe set used for TaqMan-based
169 detection of the *flaB* gene was as follows: Forward primer: 5'-AAGCAATCTAGGTCTCAAGC-
170 3'; Reverse primer: 5'-GCTTCAGCCTGGCCATAAATAG-3'; Probe: 5'-FAM-
171 AGATGTGGTAGACCCGAAGCCGAG-TAMRA-3'. Amplification was performed using a
172 QuantStudio 7 Real-Time PCR System (Applied Biosystems) under the following cycling
173 conditions: an initial denaturation at 95°C for 10 minutes, followed by 45 cycles of 95°C for 15
174 seconds and 60°C for 1 minute. Fluorescence was measured at each cycle, and *flaB* copy
175 numbers were determined by comparison to a standard curve generated from known
176 concentrations of *B. burgdorferi* genomic DNA

177 **Statistical Analysis.** Statistical analyses were conducted using GraphPad Prism 9.0 (GraphPad
178 Software, San Diego, CA). To assess the distribution of the data, the Shapiro-Wilk test was
179 applied to determine normality. For group comparisons, an unpaired t-test was used when data
180 followed a normal distribution, while the Mann-Whitney U test was applied to non-normally
181 distributed data. A significance threshold of $p < 0.05$ was used for all statistical tests.

182

183

184 **Results**

185 **Maternal Transfer of Anti-OspA Antibodies in Pups.** To determine the extent of maternal
186 antibody transfer, serum anti-OspA IgG titers were measured in pups when they reached D24
187 (pre-challenge). Pups were born to dams undergoing a continuous vaccination schedule. Pups
188 born from dams vaccinated until breeding pairs were formed are grouped under 1st breeding.
189 Pups born from dams vaccinated until giving birth are grouped under 2nd breeding. Pups born
190 from dams vaccinated until the pups were two weeks old are grouped under 3rd breeding. The
191 results are shown in Figure 2. Anti-OspA IgG was significantly increased in D24 pups born from
192 vaccinated dams during the 1st, the 2nd and the 3rd breeding, in contrast to pups born to control
193 unvaccinated dams. Furthermore, levels of anti-OspA IgG were significantly increased in pups
194 from the 2nd and 3rd breeding in comparison to the 1st breeding. The data suggests that longevity
195 of vaccination of the dams past birth of the pups affects the overall level of anti-OspA IgG
196 transferred from dams to pups.

197

198 **Seroconversion to *B. burgdorferi* Following Tick Challenge.** To assess whether pups were
199 protected from tick-mediated *B. burgdorferi* infection, anti-PepVF IgG were measured in serum
200 samples collected at euthanasia (~day 70), at least 4 weeks after the last day of challenge (**Figure**
201 **3.** Pups (n=8) from the 1st Breeding of vaccinated dams had equivalent anti-PepVF IgG titers as
202 control pups (n=10), confirming infection with tick-transmitted *B. burgdorferi* in both groups (p
203 = 0.7642) (Fig 3A). Although 2/3 pups from the 2nd Breeding of vaccinated dams did not have
204 anti-PepVF antibodies (Fig 3B, circle), overall differences with the control group (n=5) were not
205 significant due to the low numbers of pups in this experiment (p = 0.9432). In contrast to the

206 control group, pups from the 3rd Breeding of vaccinated dams (Fig 3C) had anti-PepVF IgG
207 levels below the cutoff (p = 0.0147), indicating absence of infection**.** These results suggest that
208 maternal vaccination that extended to- and post-parturition conferred anti-OspA passive
209 immunity to pups.

210

211 **Pathogen Burden in Tissues (qPCR Analysis).** To directly assess pathogen dissemination to
212 tissues, we purified DNA from heart and bladder from pups euthanized > 4 weeks after tick
213 challenge (~ day 71) and performed qPCR targeting the *B. burgdorferi flaB* gene (**Figure 4**). In
214 pups from the 1st Breeding, there are no differences in *flaB* loads in heart tissues from vaccinated
215 and control groups; in pups from the 2nd Breeding, 2/3 mice in the vaccinated group did not have
216 *flaB* in heart tissues, whereas 5/5 mice in the control group had *flaB* DNA in heart tissues.
217 However, differences between the groups are not significant (p=0.3393). However, in pups from
218 the 3rd Breeding, no detectable *flaB* copies were found in bladder tissues from the vaccinated
219 group (0/6), in contrast to the pups from the control group that were all positive (6/6) for *B.*
220 *burgdorferi flaB* (p=0.0022). Absence of *B. burgdorferi flaB* DNA in pups from the 2nd and 3rd
221 Breeding of vaccinated dams correlates with absence of PepVF seroconversion, further
222 confirming the protective effect of maternal antibody transfer when dams were vaccinated until
223 parturition.

224

225 **Heart Tissue Culture of *B. burgdorferi* From Pups From the 3rd Breeding.** To further assess
226 *B. burgdorferi* persistence, heart tissues from pups born from 3rd Breeding dams were cultured in

227 BSK-H medium at 34°C for 21 days, and live spirochetes were counted using a Petroff-Hausser
228 chamber under a dark field microscope (**Figure 5A**). Only pups (4/5) from unvaccinated control
229 dams had detectable motile *B. burgdorferi* in culture, confirming that spirochetes had
230 successfully colonized cardiac tissue. The control group exhibited up to 4 million spirochetes per
231 mL, while pups from vaccinated dams had no detectable live bacteria, p=0.0152. qPCR analysis
232 of culture supernatants (**Figure 5B**) revealed that high *flaB* copy numbers were detected in
233 control heart cultures (5/5), whereas no *B. burgdorferi* DNA was present in pups from vaccinated
234 dams (0/5), p=0.0022. This indicates that dams that received vaccine until 2 weeks post-
235 parturition, produced pups that were protected from cardiac colonization by *B. burgdorferi.*

236

237 **Absence of OspA IgG in Pups Euthanized at Birth.** To determine whether maternal antibodies
238 were transferred in utero, OspA ELISA was performed on serum from pups euthanized at birth in
239 comparison to the respective dam (**Figure 6**). In contrast to the respective dam, no anti-OspA
240 IgG was detected in newborns from vaccinated dams, confirming that placental transfer of
241 antibodies was absent, and that passive immunity was most likely mediated via breastfeeding.
242 This observation aligns with previous studies demonstrating that anti-OspA IgG transfer via milk
243 is a primary mechanism of passive immunity (13).

244

245 **Anti-OspA IgG transfer from dams to the respective pup litters.** To investigate whether
246 maternal antibody depletion occurred due to antibody transfer to offspring, serum anti-OspA IgG
247 were measured in dams used for the 3rd breeding at two time points (11Jun and 14Oct) and in the

248 respective litters, on the latter timepoint, before tick challenge (**Figure 7**). We found that the
249 reduction in OspA antibody measured between the two time points in the dams corresponds to
250 the increase in OspA antibody measured in the respective litters in the latter timepoint:
251 ~0.7OD_{450} for Dam 1 (Fig 7A) and ~1.8OD_{450} for Dam 2 (Fig 7B). These findings suggest that
252 transfer of antibody from dam to pups may be dependent on the efficiency of the pups'
253 breastfeeding.

254

255 **Discussion**

256 Our study demonstrates that maternal vaccination against *B. burgdorferi* can lead to the transfer
257 of anti-OspA IgG to offspring in *Peromyscus leucopus*, though the extent of transfer and
258 protective efficacy depends on the duration of maternal immunization. Pups born to dams
259 vaccinated until birth of the pups and until two weeks postpartum exhibited significantly higher
260 anti-OspA IgG titers than those from dams vaccinated until breeding of the dams. After
261 challenge with *B. burgdorferi* infected *Ixodes scapularis* ticks, 2/3 pups from dams vaccinated
262 until birth (2nd Breeding) and all pups (6/6) from dams vaccinated until two weeks postpartum
263 (3rd Breeding), had no anti-PepVF antibody in serum, no *B. burgdorferi* DNA in bladder or heart
264 tissues, and in the 3rd Breeding we found no motile *B. burgdorferi* in heart tissue, which was
265 confirmed by qPCR. The data shows that anti-OspA antibody transferred from *P. leucopus* dams
266 to pups challenged ~ 4 weeks after birth.

267 Vaccination of *P. leucopus* dams until the breeding pairs were created (1st Breeding) did not
268 protect *P. leucopus* pups from tick-mediated infection due to absence of anti-OspA antibodies in

269 pups born more than 4 weeks after the dams were vaccinated. This was surprising given our
270 previous studies in *Peromyscus leucopus* (Meirelles Richer, 2011) and C3H-HeN mice (Phillip,
271 2021) orally vaccinated with our reservoir targeted vaccine (RTV). In the first study, we
272 vaccinated *P. leucopus* with 20-30 units of live RTV for 1-4 months and found that mice
273 maintained anti-OspA antibody for up to a year (Meirelles Richer, 2011). In the second study, we
274 found that C3H-HeN dams vaccinated with 20 units of live RTV over a period of 8 weeks,
275 produced pups 33-79 days after vaccination that maintained protective titers of OspA antibody
276 from 2-9 weeks after birth. The major difference between the former studies and the current
277 study is that in the current study we used RTV inactivated with BPL, whereas in the former
278 studies we used live RTV. This data indicates that production of anti-OspA antibody by *P.*
279 *leucopus* requires persistent vaccination, if the vaccine formulation is inactivated.

280 Previous studies show that maternal IgG transfer provides passive immunity but does not
281 necessarily confer sterilizing protection against vector-borne pathogens (11, 12). The degree of
282 IgG transfer and its impact on early-life immunity can vary depending on maternal antibody
283 titers, the mechanism of transfer (transplacental vs. lactogenic), and the duration of exposure (16)
284 and neutralizing capability of the antibody (17-19). In our study, the neutralizing capability of
285 anti-OspA antibody transferred from *P. leucopus* dams to its pups is evidenced by the complete
286 absence of *B. burgdorferi* antibody in serum of pups (no exposure), as well as absence of DNA
287 or motile spirochetes in the tissues (no dissemination). This shows that for tick-transmitted *B.*
288 *burgdorferi* if *P. leucopus* is persistently vaccinated and produces sufficient anti-OspA antibody
289 to be transferred to pups, the offspring is protected to at least 4 weeks post birth. The decline in
290 maternal anti-OspA titers over time, particularly during lactation, suggests a depletion of
291 circulating IgG that could have impacted the duration of passive protection provided to pups.

292 This observation is consistent with findings in other models where maternal antibody levels
293 decrease postnatally as antibodies are transferred to offspring (11).

294 From a reservoir-targeted vaccine control perspective, our findings suggest that if maternal anti-
295 OspA antibodies reduce tick colonization efficiency or delay early-stage infection in *P. leucopus*,
296 this could have broader ecological implications by disrupting pathogen transmission cycles.
297 Further studies are needed to assess the effect of passively transferred anti-OspA antibody in
298 reduction of nymphal infection prevalence.

299

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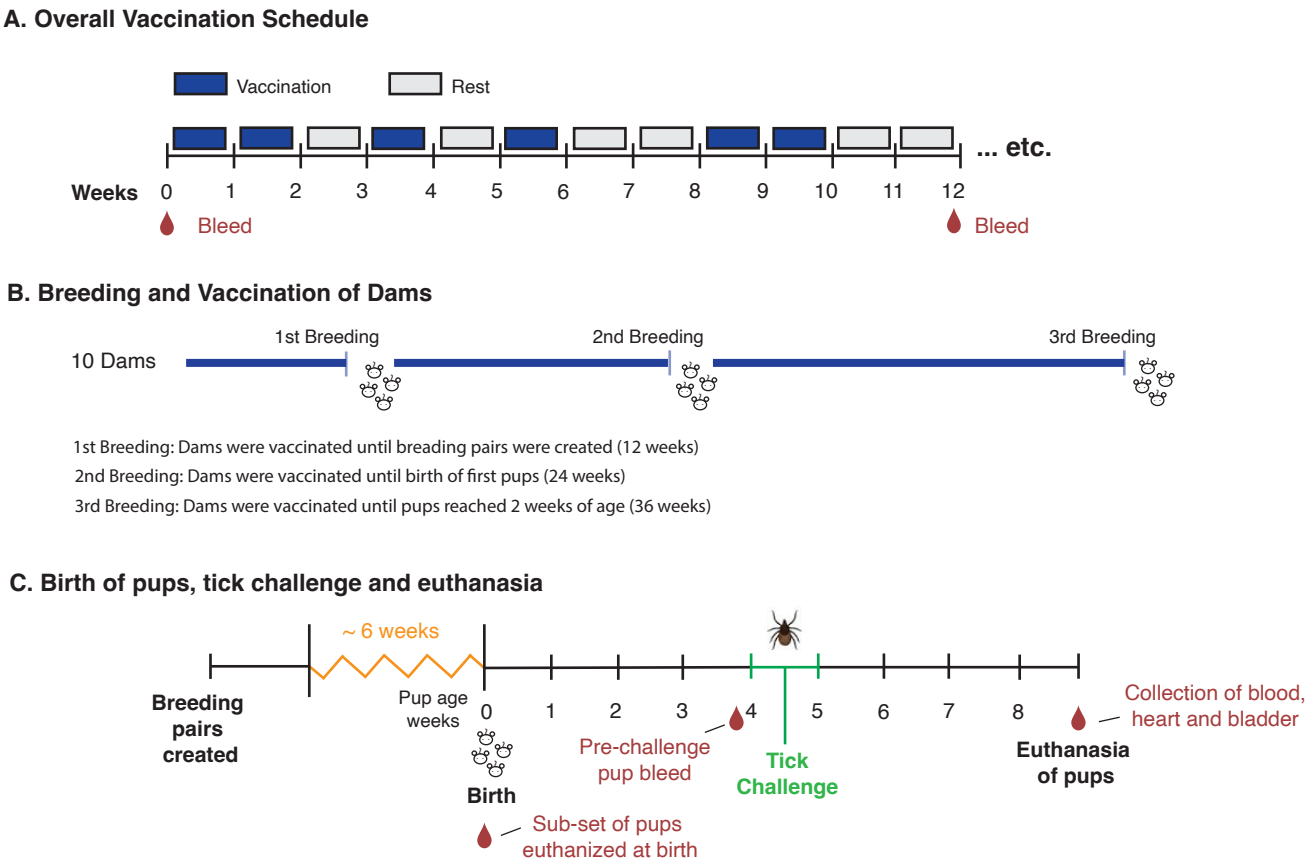

Figure 1. Study timeline. (A) Oral vaccination schedule of adult female mice. Pellets coated with the vaccine were exchanged for regular feed at the end of vaccination weeks. Regular feed was provided during rest periods. Controls mice were fed uncoated control pellets following the same schedule. Mice were bled at the begining and end of each vaccination cycle and anti-OspA antibody titer was measured by ELISA. The vaccination cycle was repeated until vaccinated mice reached a minimum anti-ospA antibody titer of OD (blanked) > 3. B) Timeline for breeding of dams. C) Birth of pups, challenge with B. burgdorferi infected ticks and collection of tissues after euthanasia.

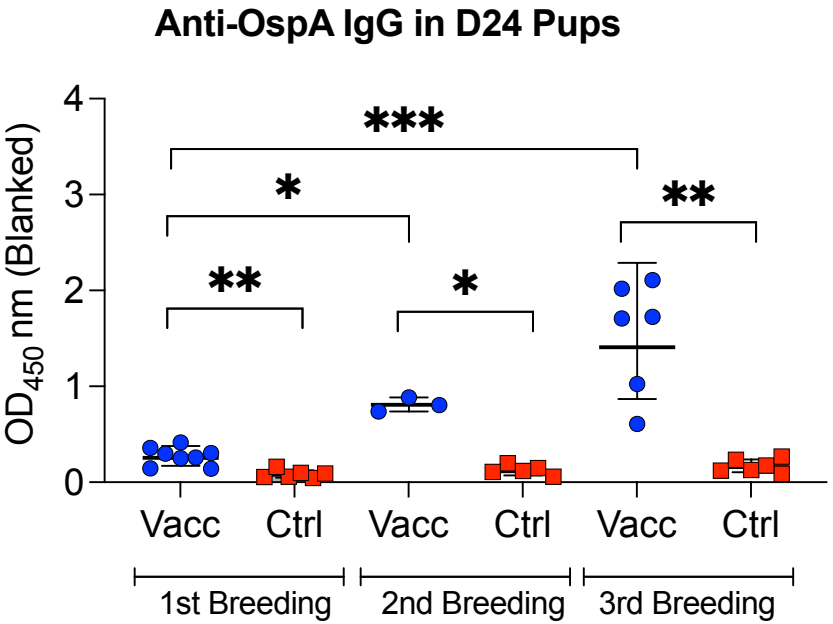

Figure 2. Maternal transfer of anti-OspA IgG antibodies to pups. ELISA quantification of anti-OspA IgG in sera from D24 pups born to dams vaccinated for different durations. Optical density (OD) at 450nm (blanked) is shown for each group. 1st Breeding, pups from dams vaccinated until breeding; 2nd Breeding, pups from dams vaccinated until birth; 3rd breeding, pups from dams vaccinated until pups reached two weeks of age. Each point represents an individual pup, with lines indicating group means ± SD. Statistical analysis by Unpaited t test or Mann-Whitney test depending of normal distribution of data, *p<0.05, ** p<0.005, *** p<0.0005.

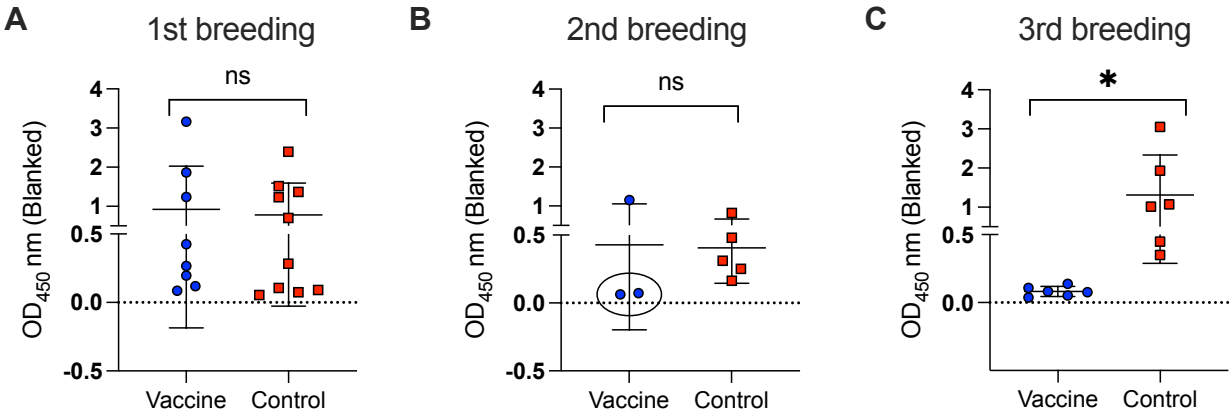

Figure 3. Anti-PepVF antibody responses in terminal bleeds. (A–C) ELISA quantification of anti-PepVF IgG in terminal sera from pups born to dams vaccinated for different durations. Pups were bled > 4 weeks following tick challenge. Optical density (OD) at 450 nm (blanked) is shown for each group. (A) Pups from dams vaccinated until breeding. (B) Pups from dams vaccinated until birth. (C) Pups from dams vaccinated until pups reached two weeks of age. Each data point represents an individual pup, with lines indicating group means ± SD. Statistics by Unpaired t test or Mann Whitney test depending normal distribution of data: ns, not significant, * $p<0.05$.

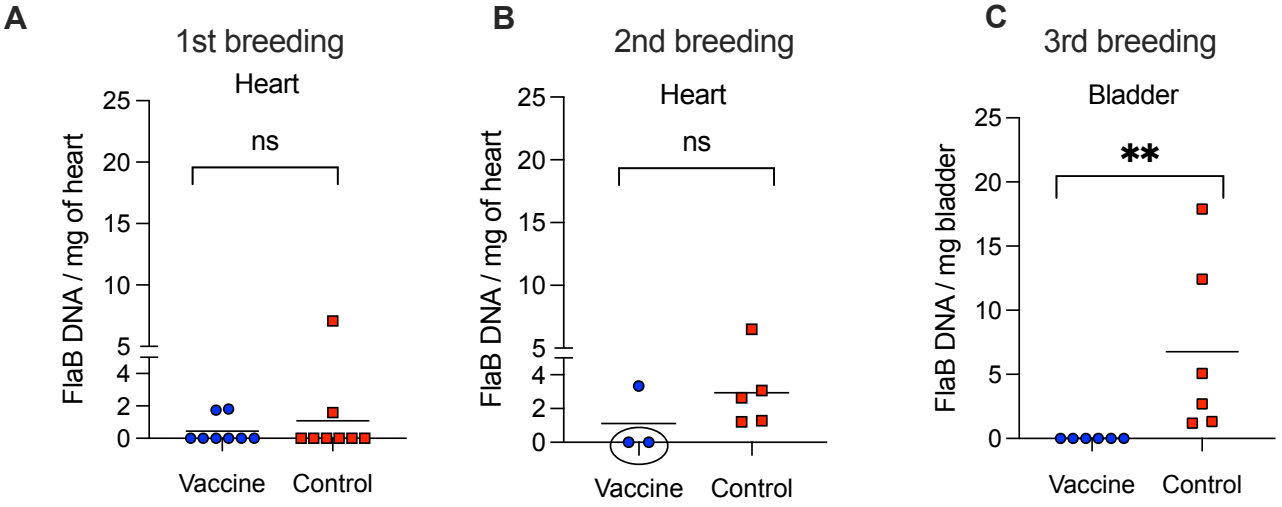

Figure 4. Borrelia DNA detection in heart and bladder tissues of pups from dams vaccinated for different durations. (A–C) qPCR quantification of *Borrelia burgdorferi flaB* gene in tissues collected from pups > 4 weeks post tick challenge. (A) *flaB* qPCR in heart tissue from pups born to dams vaccinated until breeding; (B) *flaB* qPCR in heart tissue from pups born to dams vaccinated until birth; (C) *flaB* qPCR in bladder tissue from pups born to dams vaccinated until pups reached two weeks of age. Each data point represents an individual pup, with lines indicating group means. Statistics by Mann Whitney test: ns, not significant, **p<0.005.

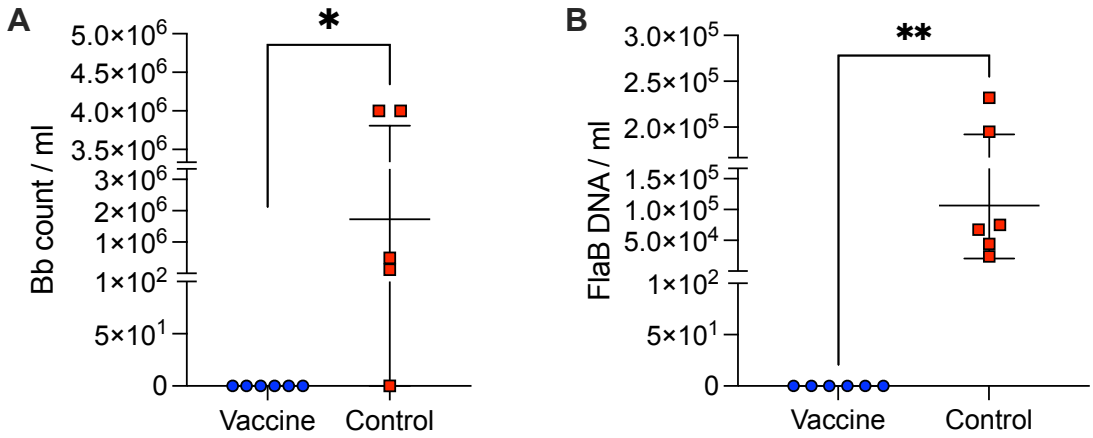

Figure 5. *Borrelia burgdorferi* burden in heart cultures from pups born to dams vaccinated until two weeks postpartum (3rd Breeding). Quantification of *B. burgdorferi* in heart tissue cultures from pups at > 4 weeks post-challenge. (A) Enumeration of motile *B. burgdorferi* in heart tissue cultures using a Petroff-Hausser counting chamber under a dark field microscope. (B) qPCR quantification of *B. burgdorferi* flaB gene in heart culture media. Each data point represents an individual pup, with lines indicating group means ± SD. Statistics by Mann Whitney test, *$p<0.05$ and ** $p<0.005$.

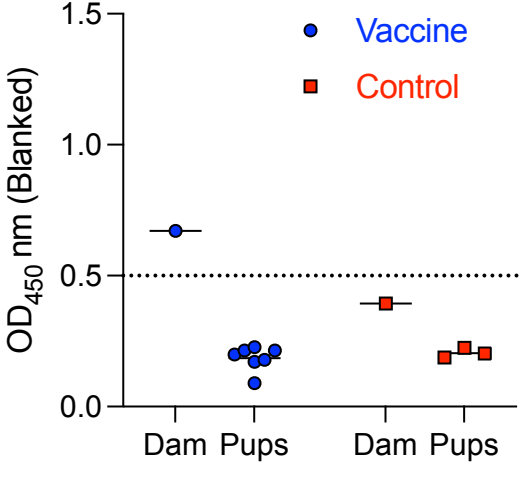

Figure 6. Anti-OspA IgG in pups and respective dam, at birth before breastfeeding. ELISA quantification of anti-OspA IgG in sera from pups sacrificed at birth, before the opportunity for breast milk intake. Pups were delivered from dams vaccinated until two weeks postpartum (3rd Breeding). Optical density (OD) at 450 nm (blanked) is shown. Each data point represents an individual pup, with lines indicating group means. The cutoff value (dashed line) represents the mean + 3 SD of negative controls.

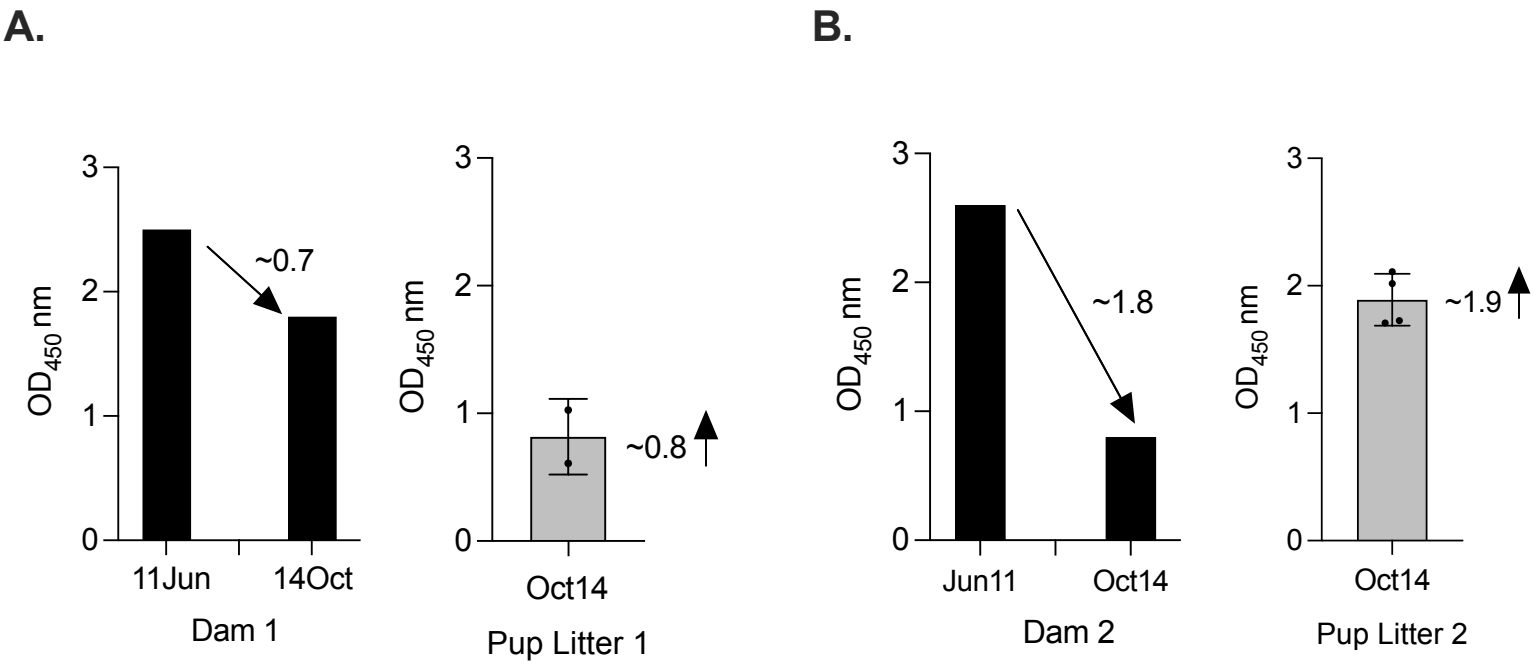

Figure 7. Anti-OspA IgG transfer from dams to the respective litters. ELISA quantification of anti-OspA IgG in sera from two dams used in the 3rd breeding and the respective litters. Sera from dams were colected at two time-points ~ 4 months apart. Sera from the respective litters were collected on the latter time point, before tick challenge. Each data point represents an individual pup, with lines indicating group means ± SD.