Maternal transfer of neutralizing antibodies to OspA after oral vaccination of the rodent reservoir

Kathryn O'Connell ¹, Nisha Nair ², Kamalika Samanta ², Jose F. Azevedo ², Grant D. Brown ³, Christine A. Petersen ^{4,5} and Maria Gomes-Solecki ^{2,*}

¹ University of Tennessee Health Science Center Department of Comparative Medicine

² University of Tennessee Health Science Center Department of Microbiology, Immunology and Biochemistry

³ University of Iowa Department of Biostatistics

⁴ University of Iowa Department of Epidemiology

⁵ Center for Emerging Infectious Diseases

*Correspondence: Maria Gomes-Solecki, Department of Microbiology, Immunology and Biochemistry, University of Tennessee Health Science Center. 301 Molecular Science Building, 858 Madison Ave, Memphis, TN 38163 (mgomesso@uthsc.edu)

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Abstract

Lyme Disease presents unique challenges for public health efforts. We hypothesized that transfer of protective antibodies between mothers and offspring should occur after oral vaccination of C3H-HeN mice with *E. coli* overexpressing OspA. We present new evidence for maternal transfer of vaccine induced neutralizing anti-OspA IgG antibodies to mouse pups through ingestion of colostrum. Protective levels of OspA antibodies in pups were present from 2-5 weeks after birth and they persisted in some mice until 9 weeks of age. This was corroborated by detection of neutralizing antibodies in the serum of all pups at 2-3 weeks after birth and in some mice at 9 weeks of age. A clear association was found between robust antibody responses in mothers and the length of antibody persistence in the respective pups using a novel longitudinal Bayesian model. These factors are likely to impact the enzootic cycle of *B. burgdorferi* when reservoir targeted OspA-based vaccination interventions are implemented.

47 Introduction

48 The global human and economic impact of Lyme borreliosis is vast, being
 49 especially prevalent throughout the northern hemisphere ¹, and to a lower degree in
 50 parts of Central and South America, Africa, and Asia ². Lyme borreliosis is caused by
 51 some species of the spirochete *Borrelia burgdorferi* sensu lato ^{3,4}, transmitted to
 52 mammalian hosts by *Ixodes* tick vectors ⁵. The pathogen is maintained in the environment
 53 by tick-mediated infection of reservoir host species ⁶, mostly rodents, rather than through
 54 transovarial transmission between tick cohorts ⁷ or transmission between mammalian
 55 hosts ⁸. In North America, the primary reservoir host species of *B. burgdorferi* is the
 56 white-footed mouse (*Peromyscus leucopus*) ^{6,9}.

57 Antibodies to outer surface protein A (OspA) protected mice from infection with *B.*
 58 *burgdorferi* by agglutination and neutralization of this spirochete ¹⁰ within the tick midgut ¹¹
 59 which leads to blockage of transmission. We developed a reservoir targeted vaccine
 60 based in *E. coli* overexpressing OspA and showed that oral administration of such
 61 vaccines to mice induced OspA-specific IgG antibodies in blood that prevented
 62 transmission of *B. burgdorferi* from the tick vector to the murine host. This was further
 63 complemented by drastic reductions of *B. burgdorferi* from *Ixodes* nymphs used for
 64 challenge ¹². White-footed mice (*P. leucopus*) vaccinated orally with baits containing *E.*
 65 *coli* overexpressing OspA maintained sufficient levels of neutralizing OspA-specific IgG in
 66 blood for up to a year ¹³. Presence of protective levels of anti-OspA antibody in white-
 67 footed mice correlated with reductions in nymphal infection prevalence over time in a 5-
 68 year field trial¹⁴.

69 Transfer of antibodies from mother to offspring is a classical mechanism ¹⁵ by
 70 which newborns are protected from many infectious diseases. To date there is no

evidence of this type of protection against *Borrelia burgdorferi* infection. We hypothesized that maternal transfer of neutralizing anti-OspA antibodies occurred after vaccination of female mice. We immunized mice orally with the same vaccine master stock previously tested^{12,13}, and evaluated transfer of OspA-specific antibodies to offspring via transplacental and transmammary transmission. Furthermore, we developed a novel Bayesian longitudinal model to jointly evaluate mother and pup immunity, allowing a robust exploration of the potential mechanisms for maternal transfer of antibody based on the time at which we detect antibodies in pups paired with the mother's immune state.

Methods

Ethics statement. This study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health. The protocol was approved by the University of Tennessee Health Science Center (UTHSC) Institutional Animal Care and Use Committee, Animal Care Protocol Application Permit Number 19-0103.

Animals. C3H-HeN mice were purchased from Charles River Laboratories and acclimatized for one week at the specific pathogen free (SPF) environment in the LACU-UTHSC.

Bacterial strains. Glycerol stocks of *E. coli* BL21(DE3), *E. coli* BL21(DE3) transformed with pET9c-ospA and *B. burgdorferi* sensu stricto are kept at -80°C in the laboratory. Cultures of several strains of *B. burgdorferi* sensu stricto were recovered from heart and bladder tissues from white-footed mice naturally infected with *B. burgdorferi* (Bb) in 2005, 2006, 2007 and

2008. To generate a multi-strain culture for neutralization assays, 200ul of each stock was combined and grown to 1.00E07 Bb/mL in 7mL of Barbour-Stoenner-Kelly (BSK) media supplemented with 100x antibiotic mix for *Borrelia* and grown at 34°C for 2-4 weeks. *Borrelia* was enumerated by dark-field microscopy (Zeiss USA, Hawthorne, NY) and culture concentration was confirmed by qPCR using primers for *flaB* (StepOne Plus, Life Technologies, Grand Island, NY).

Production of vaccine

Vaccine was produced according to modified published protocols¹⁶. Briefly, recombinant *E. coli* were grown in Tryptone Broth Yeast (TBY) medium supplemented with 50 µg/ml Kanamycin (Kn) at 37°C, shaking at 225 rpm, until it reached an OD₆₀₀ of 0.8-0.9. The expression of recombinant OspA was induced by adding IPTG (isopropyl-β -d-thiogalactopyranoside, 0.5 - 1 mM final concentration) to the cells followed by incubation at 37°C, shaking 225 rpm, for 3 h. Non-transformed *E. coli* cells used as placebo control were grown in TBY without antibiotic. The cells were harvested by centrifugation at 4000 x g for 10 min at 4°C. Antigen expression was checked by Coomassie blue staining of constructs on polyacrylamide gels followed by immunoblot using anti-OspA monoclonal antibody 184.1. Aliquots of 1.00E10 cells/ml were resuspended in 10% glycerol-phosphate-buffered-salt solution and stored at -80°C. Aliquots were thawed at 4°C and ~1.00E09 *E. coli* cells (~300µl) were placed in a ball tipped disposable feeding needle (Fisher Scientific, Pittsburgh, PA) for oral gavage inoculation.

Immunization schedule, breeding and collection of blood

Female mice were separated into 2 groups: one group of 10 females was assigned to the

119 vaccine group and one group of 6 females was assigned to placebo. Females that received
120 oral vaccine (EcA) were labeled as mothers M1 to M10 and females that received oral placebo
121 (Ec, Ctrl) were labelled as mothers M11 to M16. A timeline diagram depicting vaccination,
122 breeding and collection of samples is shown in **Fig. 1**.

123
124 Immunization schedule: 6-week old female mice received 1 vaccine dose ($\sim 10^9$ *E. coli* cells)
125 daily by oral gavage for 2 full work-weeks (10 doses); mice rested for 1 week and on the
126 following week they received the 1st boost (1 dose/day for 5 days) and after resting another
127 week they received a 2nd boost (1 dose/day for 5 days) for a total of 20 vaccine doses per
128 mouse.

129
130 Breeding and birth: 2 weeks after receiving the last vaccine dose (8-week vaccination
131 schedule), pairs of females were co-housed with an age matched male for about 3-4 weeks.
132 After breeding each female was single housed until parturition. Parturition occurred over a
133 span of ~6 weeks due to differences in successful mating between pairs. Females started
134 giving birth to pups ~11-17 weeks after the first vaccine dose. Pups were followed for 17
135 weeks.

136
137 Collection of blood for serology and neutralization assays: blood was collected from the
138 mothers before immunization on D0, before the first boost on D21, before the second
139 boost on D35, post-vaccination before breeding ~D54, post-vaccination post-birth on
140 D133/154 (when the pups ~7 weeks old) and post-vaccination at termination ~ D203/218
141 (when the pups were ~17 weeks old). The pups were split into two cohorts. The first
142 cohort of 44 pups (Cohort A) was sacrificed for collection of blood on the day of birth

(most pups were born overnight). Cohort A included 12 control pups born to Mother 11 and from litters from Mothers 14 and 16, and 32 pups born to the first litter of vaccinated Mothers 6-10. The second cohort (n=59, Cohort B) was comprised of 33 pups born to the first litter of control Mothers 12-16, and 27 pups from the first litter of vaccinated Mothers 1-5; blood was collected via the submandibular vein on a weekly basis starting 2 weeks after birth until the pups reached 17 weeks of age.

ELISA assay

ELISA was performed according to adapted published protocols ^{13,17}. Briefly, 1 µg/mL of purified OspA ¹⁸ is used to coat flat-bottom MaxiSorp ELISA plates (eBioscience) overnight; next morning the plate is washed, blocked, incubated with the 1st antibody (mouse serum diluted at 1:100) followed by the second antibody HRP conjugated goat anti-mouse IgG (Jackson ImmunoResearch) diluted at 1:10000, TMB substrate and stop solution according to steps 16-28 of Basic Protocol 3 in ¹⁷. The plates were read in a SpectraMax ELISA reader (Molecular Devices) at 450nm.

B. burgdorferi immunoblot

Multi-strain *B. burgdorferi* was cultured in BSK media at 34° until the cell count reached 10⁸/ml; 1 ml of the culture was centrifuged, washed in PBS, further diluted to 1:20 in PBS and 25µL 4X gel loading dye containing β-Mercaptoethanol was added to 75 µL of the solution. The extract was boiled for 5 minutes at 95°C before SDS-PAGE was performed. The proteins were transferred onto PVDF by placing the gel on a Bio-Rad semi-dry transfer system. Western blot analysis was performed using a monoclonal antibody to OspA (mAb 184.1, 1:1000) and an alkaline phosphatase labelled secondary anti- antibody (1:5000).

167 Neutralization assay

168 Presence of neutralizing anti-OspA antibody was determined in serum from mothers and
 169 pups as previously described¹³. We used serum from mothers collected after vaccination
 170 and before breeding (~D54). The serum from pups was collected on the day of birth (0-
 171 24h), at 2-3 weeks after birth and at 9 weeks after birth. Briefly, a 10⁷/mL multi-strain
 172 culture of *B. burgdorferi* was incubated with heat inactivated mouse serum in the
 173 presence of guinea pig complement and incubated for 6 days at 34°C; cultures were
 174 checked on days 3 and 6 and the number of viable, motile *B. burgdorferi* were counted in
 175 a Petroff-Hausser chamber under a dark field microscope.

177 Bayesian Model

178 Rather than considering group differences and treating pup and mother analyses
 179 separately, we introduced a Bayesian model to jointly consider anti-OspA antibody levels
 180 in mothers and her pups. Specifically, we introduced a pair of auto-correlated, log-scale
 181 time-series with forcing parameters related to oral vaccine dose-timing (mothers) and
 182 birth/weaning processes (pups). Parameters were estimated separately between vaccine
 183 and placebo groups. For experimental group i , mother j , and time index t , we modeled
 184 the outcome as an auto-regressive Gaussian time series (Eq. 1).

$$185 \quad \log(Y_{ijt}) \sim N(\eta_{ijt} + \rho_1 \log(Y_{ij,t-1}), \sigma_1^2); \eta_{ijt} = \mathbf{X}_{mthr}^{(i)} \boldsymbol{\beta}_{mthr}^{(i)} \quad (1)$$

186 The design matrix $\mathbf{X}^{(i)}$ contains an intercept, a linear-time dose term (up to D54), and a
 187 linear-time change point for decay (starting at D54). Pups are coupled to these series by
 188 matching them to their respective mothers. For vaccinated group i , pup j , and time t , we
 189 defined (Eq. 2):

$$\log(Z_{ijt}) \sim N(\zeta_{ijt}, \sigma_2^2); \zeta_{ijt} = \mathbf{X}_{pup}^{(i)} \boldsymbol{\beta}_{pup}^{(i)} + \boldsymbol{\gamma}^{(i)} \mathbf{g}(\mathbf{i}, \mathbf{j}, \mathbf{t}) + \rho_2 \log(Z_{ij,t-1}) \quad (2)$$

Let $g(i, j, t)$ be zero, except on the first pup-measurement for pups not sacrificed immediately post-parturition, at which point it is the matched maternal D54 anti-OspA antibody level in blood. This set up permitted the model to directly investigate evidence for transplacental antibody transfer by allowing separate intercepts by treatment group, while simultaneously investigating the potential for post parturition transfer by coupling the pup anti-OspA level in blood to matched mothers. Vague priors were specified:

$$\boldsymbol{\beta}_\cdot \stackrel{iid}{\sim} N(0, 10), \sigma_k^2 \stackrel{iid}{\sim} \text{gamma}(1, 1), \rho_k \stackrel{iid}{\sim} \text{beta}(1, 1), \text{ and } \gamma_k \stackrel{iid}{\sim} N(0, 1).$$

The model was implemented as a reproducible R-markdown document and is referenced along with the requisite data sets in **Table S1**. The analysis presents a novel approach to joint modeling of mechanistically-related longitudinal data sets and allows us to make full use of the data from complex experiments and directly measure the effects of interest, rather than relying on informal comparisons or more basic significance tests. This approach can be extended and generalized, allowing direct application to follow-up studies. Furthermore, the model can be applied to other microbiological problems with dependent longitudinal relationships. The model was fit using the `rstan` R package interface to the `Stan` statistical software in R version 4.0.2^{19, 20}.

Results

A total of 105 C3H-HeN pups were born to the 16 mothers: 59 pups were born to mothers vaccinated with *E. coli* overexpressing OspA (EcA), and 46 pups were born to mothers that received *E. coli* placebo control (Ec, Ctrl) with an average litter size of 5.9

for EcA and 5.75 for Ctrl. Insufficient blood was collected from 2 pups born to control mothers, and as such, those pups were not included in further analysis.

215

216 **Kinetics of α OspA IgG in serum from orally vaccinated mothers over 7 months**

217 We measured anti-OspA IgG in serum from orally vaccinated C3H-HeN mothers and
218 placebo controls by ELISA (**Fig. 2**). As expected, the prevalence of mice with protective
219 levels of OspA antibody, defined by $OD_{450} \geq 0.8$, increased in EcA vaccinated mothers
220 from 20% at 21 days after receiving the first vaccine dose, to 60% after the first boost
221 (D35), to 90% after the second boost (D54). Five months after mothers received their
222 first oral vaccine dose (~D133/154), prevalence of protective levels of antibody was
223 77.8%. Two months later, 7 months after the first dose ~D203/218, prevalence of
224 protective anti-OspA antibody was 30%. Controls that received *E. coli* placebo (Ec) did not
225 develop OspA-specific IgG. Differences between EcA and Ec groups were statistically
226 significant throughout the vaccination schedule (Fig. 2A). Of the 10 mothers that received
227 the oral vaccine by gavage, Mothers 3, 4, 5, 6 and 9 developed the most robust IgG
228 response to OspA on D54 (Fig 2B).

229

230 **OspA-specific IgG was detected in serum from pups born to vaccinated mothers**

231 We measured anti-OspA IgG in serum from offspring born to the vaccinated mothers from
232 two cohorts of pups: one cohort was terminated on the day of birth to access
233 transplacental transfer of maternal antibodies and a second cohort of pups was bled over
234 a period of 4 months, weekly from week 2-17 after birth, to access transfer of
235 transmammary antibodies through colostrum and milk (**Fig. 3**). A longitudinal analysis of
236 anti-OspA IgG between EcA and Ctrl mice taken at all time points showed a clear

distinction between the two groups, except for the pups terminated on the day of birth (Fig 3A). In the cohort of pups terminated 0-24h after birth (Cohort A), overall differences in OspA-specific antibody were not significant between pups born to EcA and Ctrl mothers. However, a breakdown of data by birth mother showed there was one litter of pups born to M9 that clearly had anti-OspA antibodies in serum (Fig. 3B). In the second cohort of mice (Cohort B) we observed an increased anti-OspA IgG mean at 2 weeks post birth ($OD_{450}=1.649$, 95% CI 0.677 to 1.832) that steadily decreased until 9 weeks post birth ($OD_{450}=0.382$, 95% CI 0.019 to 0.366). Differences are significant from 2-9 weeks after birth: 2w $p=0.0002$, 3w $p=0.0001$, 4w $p=0.0010$, 5w $p=0.0006$, 6w $p=0.0024$, 7w $p=0.0249$, 8w $p=0.0144$ and 9w $p=0.0309$. When we analyzed the anti-OspA IgG by birth mother we observed significant differences in protective levels of anti-OspA IgG ($OD_{450}>0.8$) between litters born to 4 of 5 mothers (Fig. 3C). Pups born to mother M1, which never developed anti-OspA antibodies, did not have antibodies in serum. Interestingly, pups from larger litters had less total antibody to OspA in serum and pups with the highest levels of antibodies to OspA were born to the mothers of this cohort that produced the most robust response to OspA, M3, M4, M5 (Fig. 3C), which suggested a correlation between these two parameters.

254

255 **Maternal and pup antibody levels unambiguously connected**

256 To further investigate the correlation between amount of anti-OspA maternal antibody and
257 the amount of antibody transferred to their respective offspring we modelled these two
258 components of maternal/fetal antibody transfer in a Bayesian Hierarchical Model
259 framework. We found clear evidence of a treatment effect in mothers and a strong
260 correlation between the amount of protective antibody in vaccinated mothers and their

261 respective offspring. Due to non-specific antibody activity, both EcA and control mothers
 262 exhibit evidence of a positive dose effect, with probabilities of an effect greater than zero:
 263 ≈ 1 in the EcA group and 0.97 in the control group. Antibody trends over time were very
 264 different between EcA and Ec, since all parameters like intercepts, dose, decay,
 265 autocorrelation, were estimated separately (**Table 1**). We estimate a 99% probability that
 266 the effect of maternal vaccination on presence of anti-OspA antibodies in the pups was
 267 higher in EcA group than Ec (**Fig. 4**). In addition, the EcA group exhibited higher
 268 autocorrelation (probability 0.88). For pups, the treatment effect is primarily seen through
 269 shifts in the intercept, capturing birth or shortly post-birth antibody activity and the D54
 270 mother OspA-specific antibody capturing exposure post-parturition. Pups exhibited an
 271 elevated baseline antibody level relative to controls (posterior probability ≈ 1), and also
 272 demonstrated a positive and elevated D54 weaning term (posterior probabilities both $\approx$
 273 1). Crucially, Ec pups exhibited significant evidence (probability 0.99) of a small, negative
 274 weaning effect. Thus, despite the presence of nonzero antibody activity in both groups,
 275 we observe unambiguous differences between vaccine-induced OspA-specific antibodies
 276 and unmodified cross-reactive antibodies in the control group. In addition, we find
 277 evidence (probability ≈ 1) that autocorrelation was higher in EcA group pups than in
 278 control group pups, and that the decay of antibody levels was slower (probability 0.85).

279

280 **Serum from vaccinated mothers and their pups neutralize *B. burgdorferi* in culture**

281 The function of neutralizing antibody (nAb) was analyzed in serum from EcA and Ctrl
 282 mothers before the females were bred on ~D54, and on the blood of their respective
 283 offspring (**Fig. 5**). nAb was measured by dark field microscopy quantification of motile *B.*
 284 *burgdorferi* in multi-strain cultures treated with serum from vaccinated and placebo

285 mothers over two time points. Due to insufficient sample to test all mothers independently,
 286 we pooled serum from the immune mothers used for the analysis of long-term kinetics of
 287 antibody to OspA (M2, M4, M5) and from the mothers used for analysis of antibody
 288 transfer at birth (M6, M7, M8, M10). All the mothers included in this analysis (M2 to M10)
 289 had levels of OspA-specific antibodies $OD_{450} > 0.8$ before breeding on D54 (Fig. 2A). We
 290 did not include M1 because this mother did not develop antibodies to OspA. We were
 291 able to keep one mother from each group used to generate the two cohorts of mice (A
 292 and B) that maintained highest levels of antibody to OspA on ~D210 (M3 and M9), to test
 293 independently. We ran an immunoblot of a multi-strain culture used for the neutralization
 294 assay against an anti-OspA monoclonal antibody and show that OspA is expressed by *B.*
 295 *burgdorferi* (Bb) in culture (**Fig S1**). We found that serum from mothers M6 to M10 that
 296 produced pups used to evaluate transplacental antibody transfer on the day of birth
 297 (Cohort A), had sufficient anti-OspA antibody to reduce the number of motile Bb in culture
 298 by about 1Log_{10} (Fig 5A); serum from the mothers that produced Cohort B pups (pooled
 299 M2, M4, M5, and individual serum from M3) used to evaluate transfer of maternal
 300 antibody through colostrum and milk, had sufficient anti-OspA antibody to reduce the
 301 number of motile Bb in culture by about 7Log_{10} (Fig 5A). Serum from M9 pups collected
 302 on the day of birth did not have enough anti-OspA antibody to neutralize motile Bb in
 303 culture (Fig 5B). Serum from 2-3 week old pups born to M2-5 all had enough anti-OspA
 304 antibody to neutralize motile Bb in culture by $1-2 \text{Log}_{10}$ (M2, M3 and M5) and by 7Log_{10}
 305 (M4) (Fig 5C). Serum from pups born to M3 still had enough anti-OspA antibody to
 306 neutralize motile Bb in culture by 1Log_{10} 9 weeks after birth (Fig 5F).

307

308

309 Discussion

310 In this study we demonstrate that specific antibodies from immunized mouse mothers
 311 were transferred to offspring born to mice orally vaccinated with *E. coli* overexpressing
 312 OspA. Immunoglobulin G specific to OspA was passed via transmammary
 313 transmission to pups through ingestion of colostrum and milk during the lactation
 314 period, shortly after birth lasting 3 weeks. Although likely, we were unable to conclude
 315 with all confidence that antibodies are not transferred via the placenta. We found a strong
 316 statistical correlation of antibody transfer between mothers that produced the most robust
 317 IgG response to OspA and their respective pups. Presence of protective levels of
 318 antibodies lasted until 4-5 weeks of age in the majority of pups but persisted in some until
 319 9 weeks. We also showed that serum obtained from vaccinated mothers and from
 320 respective offspring neutralized motility of cultured *B. burgdorferi* containing several
 321 spirochete strains demonstrating antibody function as a correlate of protection.

322 *B. burgdorferi* is maintained in the environment by tick-mediated infection of
 323 reservoir host species⁶, mostly rodents, rather than through transovarial transmission
 324 between tick cohorts⁷ and between mammalian hosts⁸. As a logical follow up to our
 325 previous findings we hypothesized that maternal transfer of vaccine-induced OspA-
 326 specific IgG to offspring would explain how the enzootic cycle of *B. burgdorferi* can be
 327 disrupted by oral bait immunization of field mice with OspA vaccines^{21 14}. We vaccinated
 328 C3H-HeN mothers using a standard intermittent 8-week immunization schedule for oral
 329 gavage of an *E. coli* culture overexpressing OspA and followed the IgG response for 5 months
 330 in mothers and pups. We found that maternal oral vaccination led to an exponential
 331 increase of anti-OspA IgG three weeks post first vaccine dose. This started to decline
 332 after eight weeks. This is consistent with our previous findings with the same vaccine

333 formulation and schedule ¹².

334 In the current study, we extended the schedule of blood collection until day 203-
 335 218 post first vaccine dose due to the unforeseen complication of animal work during the
 336 COVID-19 pandemic. We found that the mean of anti-OspA IgG remained above OD₄₅₀
 337 ~0.8 for about ~ 5.5 months after the 1st boost (D35) in C3H-HeN mice vaccinated by oral
 338 gavage with a live culture of *E. coli* overexpressing OspA (Fig 2A). In our previous studies
 339 ^{13,14} we determined that, unlike levels of LA-2 equivalent antibodies, an OspA ELISA
 340 $2 < OD_{450} > 0.8$ can be used to empirically establish the minimum levels of
 341 protective/neutralizing antibodies in serum from adult mice orally vaccinated with *E. coli*
 342 expressing OspA. We found that the number of mice with protective levels ¹³ of anti-OspA
 343 IgG remained above 60% for about ~3.5 months (between D35 until D154), and it
 344 remained above 30% for two additional months. This is important as vaccination of foxes
 345 with an oral baited vaccine showed that 30% vaccine antibody prevalence in immunized
 346 foxes resulted in disrupted transmission of Rabies virus ²². In addition, the strength of
 347 anti-OspA immunity differed considerably between the mothers (Fig 2B). This is also
 348 consistent with our previous findings using the same vaccine formulation and schedule ¹².
 349 Nevertheless, vaccination of *P. leucopus* using a baited formula rather than oral gavage
 350 and a different schedule of immunization ¹³ it is reasonable to expect that oral bait
 351 administration of this vaccine should result in a longer and more robust immune
 352 responses to OspA.

353 Transfer of immune elements from mother to offspring via placenta and milk is a
 354 classical mechanism by which neonates are protected from infections acknowledged for
 355 over 120 years ^{15,23,24}. When we tested for presence of anti-OspA antibodies in pups born
 356 to vaccinated mothers we found a clear difference in antibody levels at birth versus 2+

357 weeks after birth (Fig 3). After birth, antibodies to OspA remained detectable in the pups
 358 for about 2 months. Furthermore, the robustness of the maternal immune response to
 359 OspA directly correlated with the amount of antibody transferred to offspring. We
 360 observed a positive association between high levels of antibodies in the birth mother and
 361 the length of time these antibodies persisted in the offspring serum; for example, the
 362 mother with the highest level of anti-OspA antibodies produced offspring that had
 363 persistent antibodies 9 weeks after birth, indicating a more durable antibody response.
 364 Another interesting finding was that pups from larger litters had less total antibody to
 365 OspA in serum which strongly suggests a dilution effect between mother and offspring. In
 366 other words, the mother produces a fixed amount of antibody to OspA at the peak of her
 367 immune response and this is split between the offspring depending on time of suckling. At
 368 birth, the pups born to 4/5 mothers did not have antibody to OspA. However, one mother
 369 (M9) produced pups that had antibodies to OspA on the day of birth. Since most of the
 370 pups were born overnight, M9 may have been the first mother to give birth and her pups
 371 may have begun nursing much sooner than the pups born to the other mothers. Although
 372 the data is highly suggestive of absence of transplacental transfer, we cannot reach an
 373 unequivocal conclusion, given that the mothers that produced the Cohort A pups also
 374 developed less overall antibody to OspA after vaccination. This difference could be
 375 explained by the longer time these mothers took to breed and produce offspring.

376 Dual modelling of mothers and their corresponding pups, we observed systematic
 377 differences in antibody profiles. In addition to the unambiguous detection of immunization
 378 effects in mothers and the presence of maternal transfer of antibodies to pups (Fig 4), we
 379 validated that maintenance of antibody activity was substantially different between
 380 vaccine and placebo groups. In addition, these comparisons remained clear in the

381 presence of substantial effect heterogeneity from both mothers and pups, arising from
 382 factors such as the timing of birth of some litters, variability in maternal antibody levels,
 383 litter size variation, and numerous other factors. In addition to providing an efficient use of
 384 all the data available for these paired cohorts, this approach provides a template for how
 385 similar studies might make use of Bayesian hierarchical models. These techniques allow
 386 researchers to directly explore mechanisms under study and can be of substantial use in
 387 a wide variety of problems where complex dependence among data sets is expected.

388   The protective mechanism of action of OspA based vaccines relies on the OspA
 389 antibody ability to neutralize motile spirochetes in the nymphal tick midgut, thus
 390 preventing migration to the tick salivary gland and subsequent transmission to the next
 391 mammalian host ¹¹. When feeding on an OspA vaccinated mouse, an infected tick ingests
 392 a bloodmeal loaded with OspA antibodies that neutralize *B. burgdorferi* before it can be
 393 transmitted to the host. We and others have developed transmission blocking vaccines
 394 based in OspA ^{12,21,25}. Our reservoir targeted vaccine reduced prevalence of infected
 395 nymphal ticks by 76% after vaccination of the primary reservoir host species (*P.*
 396 *leucopus*) in a 5-year field trial ¹⁴. In the study reported here we show that 90% of
 397 vaccinated mothers produced neutralizing anti-OspA antibodies for 4-5 months after
 398 receiving the last vaccine dose. Transfer of these antibodies from mother to offspring
 399 happened through consumption of colostrum and milk and neutralizing antibodies
 400 persisted in pups through two months of age (Fig. 5). We speculate that in a field setting
 401 where adult female mice are vaccinated in April and May, transfer of neutralizing anti-
 402 OspA antibodies from mother to offspring over a 4-5 month period through late Spring
 403 and Summer may lead to a population of reservoir offspring with significant levels of
 404 neutralizing anti-OspA antibodies in blood through July and August when the uninfected

larva start to feed. This factor is likely to impact the enzootic cycle of *B. burgdorferi*. This novel observation may explain how vaccinating field mice through the summer with baits containing *E. coli* expressing OspA lead to maintenance of sufficient neutralizing anti-OspA antibodies in the offspring to prevent new reservoir host infection into the larval feeding season and may have reduced acquisition of *B. burgdorferi* by the next nymphal tick cohort the following spring¹⁴. Further experimentation will shed light on maternal transfer of anti-OspA antibody on larval and nymphal infection prevalence and will allow us to make unambiguous conclusions regarding transplacental transfer of anti-OspA antibodies.

414

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422

423 **Author contributions**

424 Conceptualization and supervision: Maria Gomes-Solecki

425 Bayesian model development, implementation, and analysis: Grant Brown

426 Experimental investigation: Kathryn O'Connell, Nisha Nair, Kamalika Samanta, Jose F.

427 Azevedo

428 Data analysis: all authors

429 Funding acquisition: Maria Gomes-Solecki and Christine Petersen

430 Writing – original draft: Maria Gomes-Solecki; and Bayesian model, Grant Brown

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432

433 **Competing interests**

434 The following authors declare potential conflicts of interest: MGS (grants from federal
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437

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Table 1: Parameter Estimates and Comparisons				
Parameter Estimates				
Parameter	Cohort	Mean (95% CrI)	P(x>0)	P(x<0)
EcA Intercept	Mothers	-1.44 (-1.81, -1.05)	0	1
EcA Linear Dose Effect	Mothers	2.38 (1.82, 2.93)	1	0
EcA Decay Term	Mothers	-3.9 (-5.21, -2.58)	0	1
Control Intercept	Mothers	-1.6 (-2.25, -0.96)	0	1
Control Linear Dose Effect	Mothers	0.94 (-0.08, 1.96)	0.97	0.03
Control Decay Term	Mothers	-2.48 (-4.88, -0.07)	0.02	0.98
EcA Autocorrelation	Mothers	-0.85 (-1.02, -0.67)	0	1
Control Autocorrelation	Mothers	0.03 (-0.4, 0.46)	0.56	0.44
EcA Intercept	Pups	-1.94 (-2.17, -1.7)	0	1
EcA Decay Term	Pups	-0.34 (-0.78, 0.09)	0.06	0.94
Control Intercept	Pups	0.4 (0.2, 0.6)	1	0
Control Decay Term	Pups	0.2 (0.01, 0.5)	1	0
EcA Autocorrelation	Pups	0.69 (0.61, 0.77)	1	0
Control Autocorrelation	Pups	0.27 (0.19, 0.36)	1	0
EcA Pup Maternal Exposure Term	Mothers/Pups	1.39 (1.08, 1.71)	1	0
Control Pup Maternal Exposure Term	Mothers/Pups	-0.01 (-0.02, 0)	0.01	0.99
EcA vs. Control (Ec) Comparisons				
Comparison	Cohort	Mean (95% CrI)	P(x>0)	P(x<0)
Intercept	Mothers	0.16 (-0.59, 0.91)	0.67	0.33
Linear Dose Effect	Mothers	1.44 (0.26, 2.6)	0.99	0.01
Decay	Mothers	-1.42 (-4.16, 1.35)	0.15	0.85
Autocorrelation	Mothers	1.09 (0.79, 1.39)	1	0
Intercept	Pups	0.37 (-0.24, 0.98)	0.89	0.11
Decay	Pups	0.2 (-0.14, 0.48)	0.88	0.12
Autocorrelation	Pups	0.42 (0.3, 0.54)	1	0
Maternal Exposure Term	Mothers/Pups	1.41 (1.09, 1.72)	1	0

Fig. 1. Diagram of the immunization schedule, breeding, parturition and bleeding of mothers and offspring. OV, oral vaccination; D, day after first vaccine dose; w, weeks.

Fig. 2. Kinetics of OspA-specific antibodies in orally vaccinated and placebo mothers over ~7 months. We measured anti-OspA IgG by ELISA in serum from 10 mothers orally vaccinated *E. coli* expressing OspA (EcA, M1-M10) and 6 non-vaccinated placebo controls (Ec, M11-M16). Mice were bled before (D0) and after immunization (D21, D35), before breeding (D54) after the pups were born ~D140 (7 wk old pups) and ~D210 (17 wk old pups). A, Comparison of anti-OspA IgG (OD₄₅₀>0.8) between EcA and placebo Ec groups. Differences between EcA and Ec groups are statistically significant by unpaired t test with Welch's correction, D21 $p=0.0038$, D35 $p=0.0260$, D54 $p=0.0003$, D140 $p=0.0147$, D210 $p=0.0488$. B, Longitudinal log scale trajectory of antibody response over time, each line represents one mother, M5 was not bled on D140.

3A.

3B.

Pup Cohort A: 0-24h after birth, by Mother

3C.

Pup Cohort B: 2w-9w, by Mother

Fig. 3. Kinetics of OspA-specific antibodies in offspring born to orally vaccinated mothers at birth and over 4 months post birth. A, Longitudinal log scale trajectory of anti-OspA IgG measured in serum from both cohorts of pups: Cohort A pups analysed on the day of birth, and Cohort B pups analysed 2 to 9 weeks after birth. B and C, Anti-OspA IgG in both cohorts of pups grouped by mother. Statistics by the Unpaired t test with Welch's correction between EcA and Ec Ctrl, * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$ and **** $p < 0.0001$. EcA, oral vaccination with *E. coli* expressing OspA; Ec Ctrl, placebo control; M1-M10 EcA vaccinated mothers; w, weeks.

Estimated Group Trajectories Mothers and Pups (95% Cr-I)

Figure 4. Bayesian hierarchical model showing median anti-OspA antibody levels in EcA vaccinated mothers, control mothers, and pups from each group. Each group median is given along with 95% posterior credible intervals (Cr-I). Differences in antibody curves between vaccinated and unvaccinated mothers and their offspring are immediately apparent. Legend: EcA, oral vaccination with *E. coli* expressing OspA; Ec, oral vaccination with *E. coli* placebo; Cr-I, Credible Intervals.

A.

B.

C.

D.

Figure 5. Anti-OspA neutralizing antibody (nAb) function against *B. burgdorferi*. Number of motile *B. burgdorferi* (Bb) after treatment of a multistrain culture with A) pooled serum from mothers M6 M7 M8 M10, pooled serum from mothers M2 M4 M5 and individual serum samples from mothers M3 and M9; B, pooled serum from all pups born to M9 on the day of birth (Cohort A); C, pooled 2-3w serum from Cohort B pups born to M2 M3, M4 and M5 (serum was pooled according to birth mother); and D, pooled serum from all pups born to M3 collected 9 weeks after birth (Cohort B). Live Bb were counted under a dark field microscope.