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Maternal transfer of neutralizing antibodies to *B. burgdorferi* OspA after oral vaccination of the rodent reservoir

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ABSTRACT

Lyme Disease presents unique challenges for public health. Transfer of protective antibodies between mothers and offspring should occur after vaccination of mice. We present new evidence for maternal transfer of oral vaccine induced neutralizing anti-OspA IgG antibodies to mouse pups mainly through ingestion of colostrum. We found a strong statistical correlation of antibody transfer between mothers that produced the most robust IgG response to OspA and their respective pups. OspA-specific antibody was detected as early as 24 h after birth and protective levels of antibodies lasted until ~5 weeks of age in the majority of pups but persisted in some mice until 9 weeks. This was further supported by detection of neutralizing antibodies in serum of all pups at 2–3 weeks after birth and in some offspring adult mice at 9 weeks of age. A clear association was found between robust antibody responses in mothers and the length of time antibody persisted in the respective pups using a novel longitudinal Bayesian model. These factors are likely to impact the enzootic cycle of *B. burgdorferi* if reservoir targeted OspA-based vaccination interventions are implemented.

1. Introduction

The proteins presented on the outer surface of *B. burgdorferi* change as the spirochete transits between the tick vector and the mammalian host. In unfed ticks, the spirochetes produce outer surface protein A (OspA) while *B. burgdorferi* resides in the tick midgut [1]. OspA was proposed as a vaccine candidate for Lyme disease after passive transfer of anti-OspA antibodies [2,3] and active immunization with OspA recombinant protein [4] protected mice from challenge with strains of cultured *B. burgdorferi*. Additional studies showed that *B. burgdorferi* was nearly eliminated from infected nymphal ticks feeding on OspA vaccinated mice [5]. Thus, when a nymphal tick feeds on an OspA vaccinated mammal, anti-OspA antibodies present in the tick bloodmeal neutralize most live spirochetes in the tick midgut which results in blockage of transmission of the spirochete from the tick vector to the host [6,7]. In addition, OspA antibodies also reduce acquisition of *B. burgdorferi* by uninfected larval ticks feeding on infected mice [8]. Hence, OspA is the ideal target for development of transmission-blocking vaccines to prevent Lyme disease. Vaccines based in recombinant OspA have been approved for use in humans [9] and dogs [10].

The risk of contracting Lyme disease is proportional to the probability of the host being bitten by an infected tick [11]; this risk correlates significantly with infected *I. scapularis* nymph density [12–14] and with nymphal infection prevalence [15,16]. Control measures aimed at disrupting transit of *B. burgdorferi* to and from the vector tick can reduce exposure to Lyme disease risk [17]. We developed a reservoir targeted vaccine using recombinant *E. coli* as an adjuvanted carrier to recombinant OspA [18] and showed that oral administration of such vaccines to mice induced OspA-specific IgG antibodies in blood that drastically reduced *B. burgdorferi* from the tick vector and prevented transmission to the murine host [19]. Although other species such as chipmunks and shrews are competent reservoirs for *B. burgdorferi* [20], the white-footed mouse (*Peromyscus leucopus*) is the reservoir species that infects most larval ticks [21]. White-footed mice vaccinated orally in a

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laboratory setting maintained sufficient levels of OspA-specific IgG in blood for up to a year [22]. We targeted white-footed mice for disruption of *I. scapularis* infection in a large field study using a bait vaccine based on *E. coli* delivered OspA. We found that vaccination of wild white-footed mice resulted in a drastic reduction of nymphal infection prevalence in ticks flagged on the site treated with the vaccine for 5 consecutive years [17].

Transfer of antibodies from mother to offspring is a classical mechanism [23] by which newborns are protected from many infectious diseases. Maternal IgG antibodies against the relapsing fever spirochete *Borrelia duttonii* were found to be transferred to mice [24]. Thus, it was reasonable to expect that maternal transfer of neutralizing anti-OspA antibodies should occur after vaccination of female mice. We immunized mice orally with the same vaccine master stock previously tested [17,19,22], and evaluated transfer of OspA-specific antibodies to offspring via transplacental and transmammary passage. In addition, we developed a novel Bayesian longitudinal model to jointly evaluate mother and pup immunity.

2. Methods

2.1. 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 UTHSC Institutional Animal Care and Use Committee, protocol #19-0103.

2.2. Bacterial strains and culture conditions

Glycerol stocks of *E. coli* BL21(DE3)pLysS (NEB, Ipswich, MA) transformed with pET9c-*ospA* [19] were used to produce the vaccine. Untransformed *E. coli* BL21(DE3)pLysS were used as placebo controls. Multi-strain cultures of *B. burgdorferi* sensu stricto recovered from heart and bladder tissues from white-footed mice naturally infected with *B. burgdorferi* (Bb) in 2005, 2006, 2007 and 2008 are kept as glycerol stocks in the laboratory. We have sequenced DNA purified from ticks that fed on mice infected with this multi-strain culture and found it contains the following OspC variants: A, D, E, F, I, J, K, M, Q, T, X [25]. To generate a multi-strain culture for neutralization assays, 200ul of each stock (2005 to 2008) was combined and cultured in 7 mL of Barbour-Stoenner-Kelly (BSK) media supplemented with 100x antibiotic mix for *Borrelia* at 34 °C for 2–4 weeks until a cell density of 10^7 Bb/mL was reached. All bacterial glycerol stocks are stored at −80 °C.

2.3. Production of vaccine

Vaccine was produced according to published protocol [18] bypassing the lyophilization step [25]. Briefly, pET9c-*ospA*-transformed and untransformed *E. coli* BL21(DE3)pLysS were cultured in TBY media supplemented with 50 μg/ml Kan, and grown at 37 °C, 220 rpm, for about 3 h to reach an OD_{600} = 0.8. Protein expression was induced with 1 mM IPTG/ml during 3 h to reach OD_{600} = 1 (~10^9 *E. coli* cells/ml). The cells were harvested by centrifugation at 4000*g* for 10 min at 4 °C and the biomass was resuspended in 20% glycerol/phosphate buffered salt solution (Gibco, Grand Island, NY) in 1% of the initial volume. Cell suspensions in aliquots of 1 mL were snap frozen in a dry ice bath and stored at −80 °C. Aliquots of 300 μL (~3×10^8 cells) were thawed and placed in a ball tipped disposable feeding needle (Fisher Scientific, Pittsburgh, PA) for oral gavage inoculation.

2.4. Immunization schedule, breeding and collection of blood

Female, 6-week old, C3H-HeN mice (Charles River) were separated into 2 groups: 10 females were assigned to the oral vaccine group (EcA, labeled as mothers M1 to M10) and 6 females were assigned to placebo (Ec Ctrl, labelled as mothers M11 to M16). A timeline diagram depicting vaccination, breeding and collection of samples is shown in Fig. 1.

Immunization schedule: mice received 1 vaccine dose (~3×10^8 *E. coli* cells) daily by intragastric inoculation for 2 full work-weeks (10 doses), rested for 1 week, received the 1st boost the following week (5 doses), rested for another week, and received a 2nd boost (5 doses) for a total of 20 vaccine doses per mouse over an 8 week period.

Breeding and birth: 2 weeks after receiving the last vaccine dose, pairs of females were co-housed with an age matched male for about 3–4 weeks. After breeding, each female was single housed until parturition, which occurred over the following ~6 weeks due to differences in successful mating between pairs. Females started giving birth to pups ~11–17 weeks after the first vaccine dose. Pups were followed for ~17 weeks.

Collection of blood for serology and neutralization assays: blood was collected from the mothers before immunization on D0, before the first boost on D21, before the second boost on D35, post-vaccination before-breeding on D54, post-vaccination post-birth on D133/154 (when the pups ~7 weeks old) and post-vaccination at termination ~D203/218 (when the pups were ~17 weeks old). The pups were split into two cohorts. The first 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 the second litters born to Mothers 14 and 16) and 32 vaccinated pups (born to the first litter of vaccinated Mothers 6–10). The second cohort (n = 59, Cohort B) was comprised of 33 control pups (born to the first litter of Mothers 12–16) and 27 vaccinated pups (from the first litter of vaccinated Mothers 1–5); blood was collected on a weekly basis starting 2 weeks after birth until the pups reached 17 weeks of age.

2.5. ELISA assay

OspA protein was purified from IPTG induced recombinant *E. coli* by affinity chromatography. ELISA was performed according to modified published protocol [26] as follows. Briefly, purified recombinant OspA quantified by the Lowry protein assay kit (Thermo Fisher Scientific) was used to coat Nunc MaxiSorp flat-bottom ELISA plates (eBioscience, San Diego, CA) at 1 μg/ml, and an indirect ELISA was performed using serum (1:100) from mice to identify OspA-specific IgG antibodies. Goat anti-mouse IgG conjugated to HRP (1:10000, Jackson ImmunoResearch, West Grove, PA) was used as secondary antibody. The OD_{450} was read on a SpectraMax Plus ELISA reader (Molecular Devices). The ELISA cut-off was determined as 3 standard deviations above the average of all timepoints for control mothers (OD_{450} 0.8). We previously determined that vaccinated mice were protected from tick-transmitted *B. burgdorferi* if they had a minimum anti-OspA antibody in serum reading OD_{450} 0.8–1 six weeks after receiving the first dose [17,22].

2.6. Neutralization assay

Presence of neutralizing anti-OspA antibody was determined as described [22]. We used serum from mothers collected after vaccination and before breeding (D54) and the serum from pups was collected on the day of birth (0–24 h), at 2–3 weeks after birth and at 9 weeks after birth. Briefly, 8 μL from a ≤10^7/mL multi-strain culture of *B. burgdorferi* was incubated with 4 μL heat

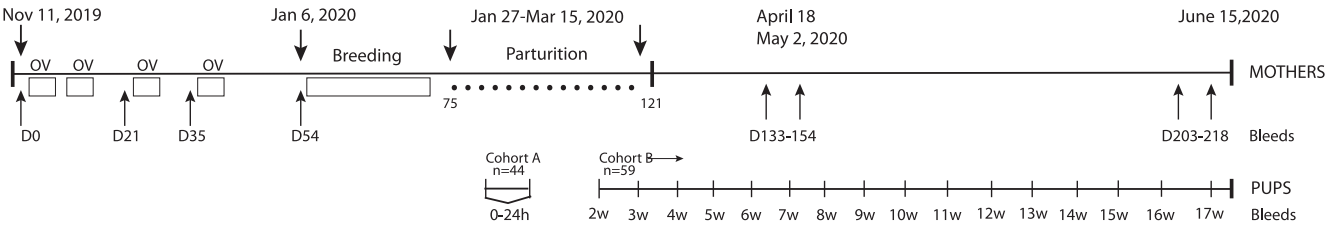

Fig. 1. Diagram of the immunization schedule, breeding, parturition and bleeding of mothers and offspring. OV, oral vaccination; D, day after first vaccine dose; w, weeks; cohort A, pups terminated and bled within 24 h of birth; cohort B, pups bled 2 weeks after birth and then weekly until termination at 17 weeks.

inactivated mouse serum in the presence of 4 μL guinea pig complement per reaction and incubated for 6 days at 34 °C. Cultures were checked on days 0, 3 and 6 and the number of motile *B. burgdorferi* were counted in a Petroff-Hausser chamber under a dark field microscope.

2.7. Bayesian model

We introduce a novel Bayesian joint model for maternal and pup antibody levels using coupled, auto-regressive, Gaussian time-series with parameters for oral vaccine timing (mothers) and birth/weaning processes (pups). Parameters were estimated separately between experimental groups; Bayesian techniques allow us to directly compare the posterior distributions of group level parameters, rather than relying on hypothesis tests for interaction terms. For group i, mother j, and time t, we modeled the outcome (antibody levels, denoted Y) as in Eq. (1).

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

The design matrix $\boldsymbol{X}^{(i)}$ contains an intercept, linear-time dose term (up to D54), and change point at D54. For each group, this parameterization allows an average starting mean log-antibody level, after which the average outcome changes continuously up to the end of the feeding period and a separate slope captures the decline in antibody levels. Correlation over time for observations on the same mother is included explicitly with each observation after the first, depending on the previous value via an autocorrelation term. Pups are matched to their respective mothers. For pup j, define (Eq. (2)):

$$\log\left(Z_{ijt}\right) \sim N\left(\zeta_{ijt}, \sigma_2^2\right); \zeta_{ijt} = \boldsymbol{X}^{(i)}_{\boldsymbol{pup}} \boldsymbol{\beta}^{(i)}_{\boldsymbol{pup}} + \boldsymbol{\gamma}^{(i)} \boldsymbol{g}(\boldsymbol{i}, \boldsymbol{j}, \boldsymbol{t}) + \boldsymbol{\rho}_2 \log\left(\boldsymbol{Z}_{\boldsymbol{ij,t-1}}\right) \quad (2)$$

As with mothers, this auto-regressive Gaussian specification allows us to model individual mouse antibody levels over time by group, while accounting for key effects. The pup design matrices contain intercepts and a linear decay term. We further define $g(i,j,t)$ to be zero, except on the first measurement for pups not sacrificed immediately post-parturition, where it is the matched maternal D54 antibody level in blood. This permits appropriate longitudinal characterization of antibody levels, while providing a clear test of post-parturition transfer by coupling the pup anti-OspA level in blood to matched mothers. Vague prior distributions for all parameters were specified:

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

The model was implemented as a reproducible R-markdown document and is referenced in **Supplementary Table S1**. The analysis presents a novel approach to joint modeling of mechanistically-related longitudinal data sets and allows to fully use data from complex experiments and directly measure the effects of interest rather than relying on less powerful methods (e.g., doing separate comparisons at each time point, or for mother and pup groups). The model was fit using the `rstan` R package in R version 4.0.2 [27,28].

3. Results

A total of 105 C3H-HeN pups were born to 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* 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 controls, and as such, those were not included in further analysis.

3.1. Kinetics of αOspA IgG in serum from orally vaccinated mothers over 7 months

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

3.2. OspA-specific IgG was detected in serum from pups born to vaccinated mothers

We measured anti-OspA IgG in serum from offspring born to the vaccinated mothers from two cohorts of pups: one cohort was terminated on the day of birth to assess transplacental transfer of maternal antibodies and a second cohort of pups was bled over a period of 4 months, weekly from week 2–17 after birth, to access transfer of transmammary antibodies through colostrum and milk (Fig. 3).

A longitudinal analysis of anti-OspA IgG between individual 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 0–24 h after birth (Cohort A), overall differences in OspA-specific antibody were not significant between pups born to EcA and Ec Ctrl mothers. However, a breakdown of data by birth mother showed there was one litter of pups born to M9 that 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

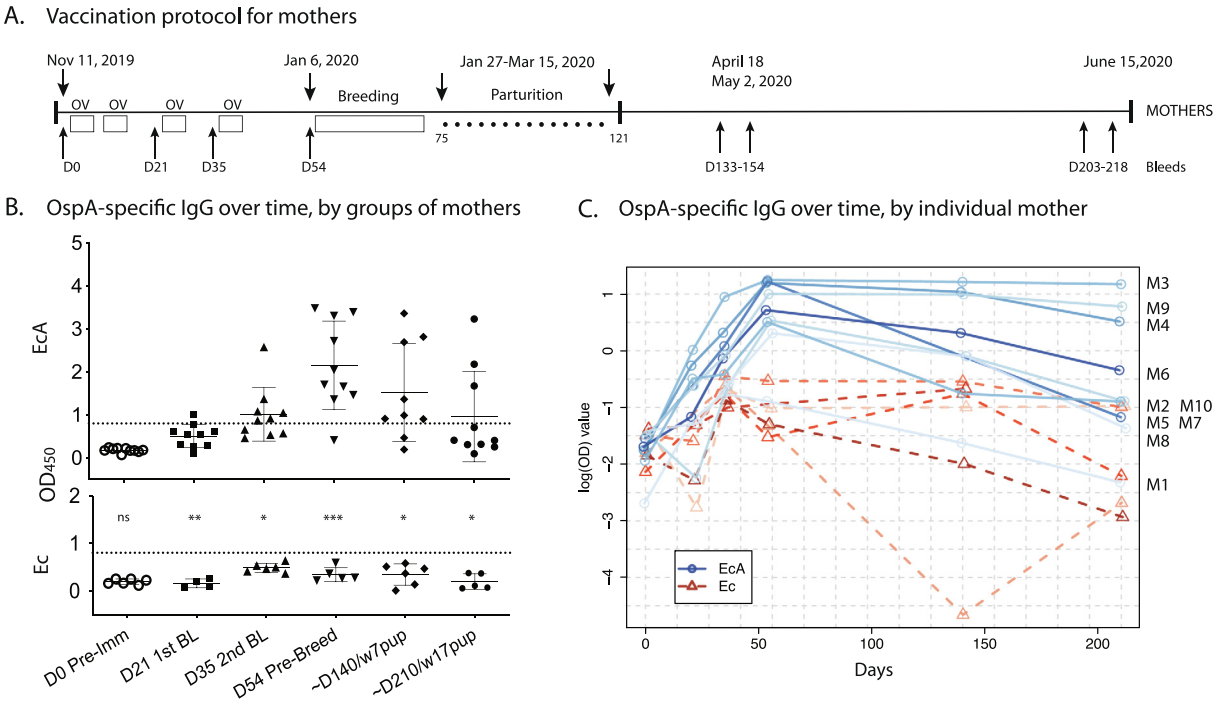

Fig. 2. OspA-specific antibodies in serum from orally vaccinated mothers over ~7 months post first vaccine dose. We measured anti-OspA IgG by ELISA in serum from 10 mothers orally vaccinated with *E. coli* expressing OspA (EcA, M1-M10) and 6 placebo controls (Ec, M11-M16). Mice were bled before (D0) and after immunization (D21, D35), before breeding (D54), after the pups were born at ~D140 (7-week old pups) and at ~D210 (17-week old pups). A, Protocol for vaccination, breeding and blood collection from mothers; B, Comparison of anti-OspA IgG (OD_{450} > 0.8) between EcA and 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. C, Longitudinal log scale trajectory of antibody response over time, each line represents one mother, M5 was not bled on D140.

(OD_{450} = 1.649, 95% CI 0.677–1.832) that steadily decreased until 9 weeks post birth (OD_{450} = 0.382, 95% CI 0.019–0.366). Differences are significant from 2 to 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 in Cohort B we observed significant differences in protective levels of anti-OspA IgG (OD_{450} > 0.8) between litters born to 4 out of 5 mothers (Fig. 3C). Pups born to M1, which never developed anti-OspA antibodies, did not have antibodies in serum. Of the pups born to M2, M3, M4 and M5, 93.3% had protective levels of anti-OspA antibody 2 weeks after birth and 64.7% had protective levels of anti-OspA antibody at weaning, 3 weeks after birth. Interestingly, pups from larger litters had less total antibody to OspA in serum, and pups with the highest levels of antibodies were born to the mothers of this cohort that produced the most robust response to OspA, M3, M4, M5 (Fig. 3C), which suggests a correlation between these two parameters.

3.3. Maternal and pup antibody levels are unambiguously connected

To assess evidence connecting anti-OspA maternal antibody levels and antibody transfer to offspring, we developed a Bayesian Hierarchical Model. We found clear evidence of a relationship between antibody in vaccinated mothers and their offspring. We estimate a greater than 99% probability that the effect of maternal vaccination on presence of anti-OspA antibodies in the pups was higher in the EcA group than Ec (Fig. 4). Due to unspecific antibody production to *E. coli*, both maternal groups show positive dose effects (probability $\geq$ 0.97), though temporal trends were very different between EcA and Ec groups. Additionally, EcA exhibited higher autocorrelation (probability 0.88). For pups, we see higher intercepts in the EcA group than the control group, as well as strong evidence of post-parturition transfer. EcA pups exhibited elevated baseline antibody levels relative to controls (probability $\approx$ 1), and a *positive* weaning effect (probabilities $\approx$ 1), capturing the relationship between pup antibody activity and D54 maternal anti-OspA levels. Crucially, Ec pups exhibited strong evidence (probability 0.99) of a small, *negative* weaning effect. Thus, despite presence of unspecific antibody activity in both groups, we observe unambiguous differences between vaccine-induced OspA-specific antibodies and unspecific antibodies in the control group. Autocorrelation was also higher (probability $\approx$ 1) in EcA pups than in control pups, and the drop in antibodies was slower (probability 0.89). Full results in Table 1.

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

The function of neutralizing antibody (nAb) was analyzed in serum from mothers before the females were bred on ~D54, and in the blood of their respective offspring (Fig. 5). nAb was measured over two time points. Due to insufficient sample to test all mothers independently, we pooled serum from the immune mothers used for the analysis of long-term kinetics of antibody to OspA (2–3 weeks and 9 weeks post-birth) and from the mothers used for analysis of antibody transfer at birth (0–24 h). All the mothers included in this analysis (M2 to M10) had levels of OspA-specific antibodies OD_{450} > 0.8 before breeding on D54 (Fig. 2A). We did not include M1 because this mother did not develop antibodies to OspA. We were able to keep one mother from each group used to generate the two cohorts of mice (A and B) that maintained highest levels of antibody to OspA on ~D210 (M3 and M9), to test independently. We ran an immunoblot of a multi-strain culture used for the neutralization assay against an anti-OspA monoclonal antibody and show that OspA is expressed by *B. burgdorferi* (Bb) in culture (**Supplementary Fig S1**).

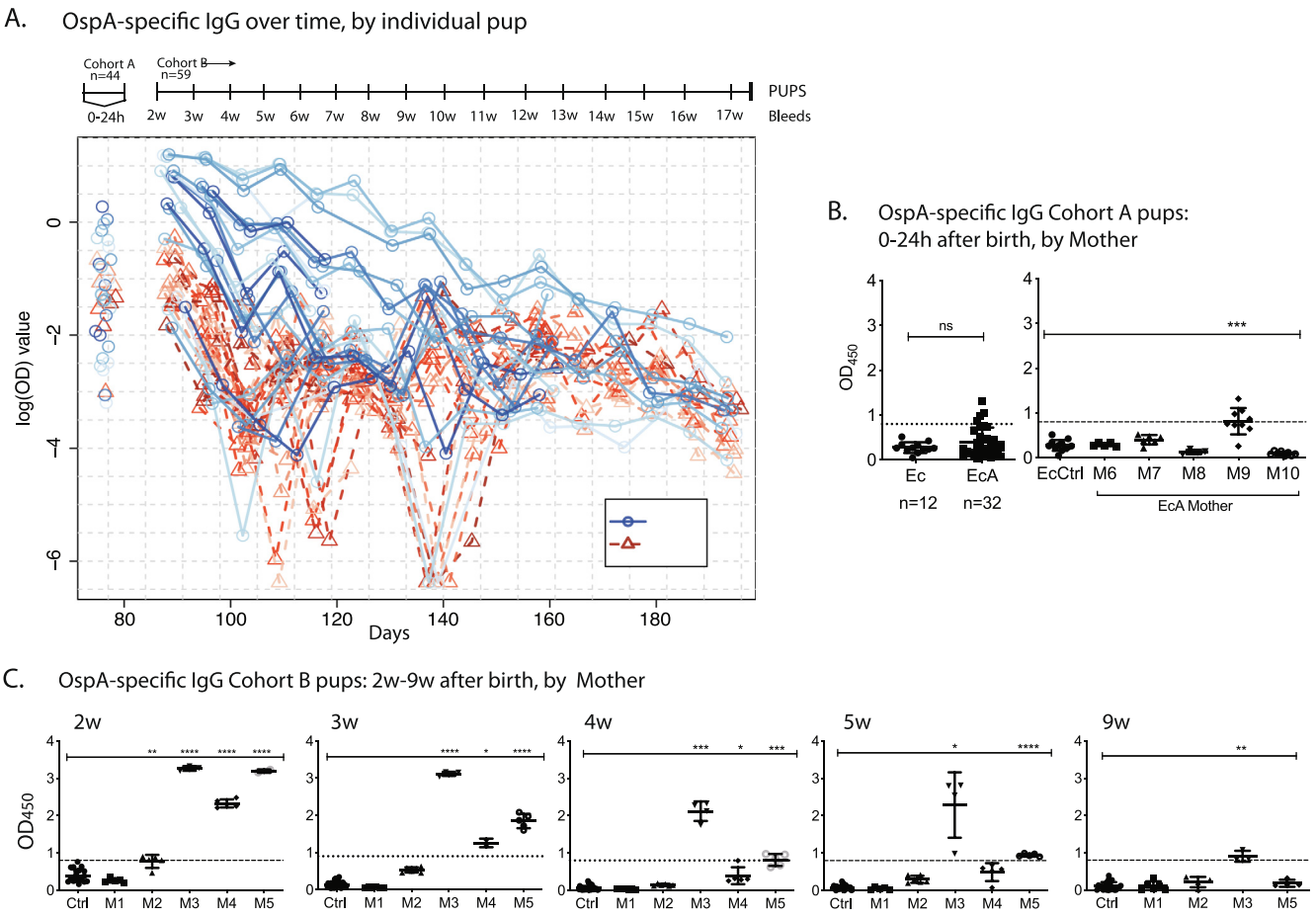

Fig. 3. OspA-specific antibodies in serum from offspring born to orally vaccinated mothers within 24 h of birth and over 4 months post birth. A, Longitudinal log scale trajectory of anti-OspA IgG measured in serum from both cohorts of pups, by individual pup: Cohort A pups were analyzed on the day of birth, and Cohort B pups were analyzed 2 to 9 weeks after birth. B and C, Anti-OspA IgG in both cohorts of pups, grouped by mother. Statistics by Unpaired *t* test with Welch's correction between EcA and Ec, * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$ and **** $p < 0.0001$. EcA, oral vaccination with *E. coli* expressing OspA; Ec Ctrl, placebo control; M1-M10 EcA vaccinated mothers; w, weeks.

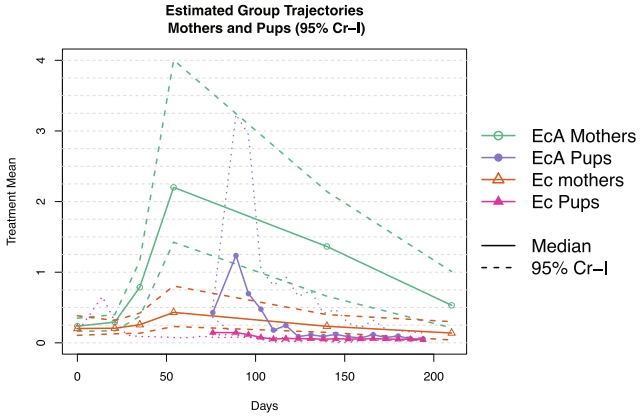

Fig. 4. Bayesian hierarchical model showing median anti-OspA antibody levels in EcA vaccinated and control mothers, and pups from each group. Each group median is given along with 95% posterior credible intervals (Cr-I). Differences in antibody curves between vaccinated and control mothers and their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

We found that serum from mothers M6-M10, that produced Cohort A pups used to evaluate transplacental transfer, had sufficient anti-OspA antibody to reduce the number of motile Bb in culture by about 1Log_{10} (Fig. 5A); serum from the mothers that produced Cohort B pups, M2-M5 used to evaluate transmammary transfer of antibody, had sufficient anti-OspA IgG to reduce the number of motile Bb in culture by about 7Log_{10} (Fig. 5A). Serum from M9 pups collected on the day of birth did not have enough anti-OspA antibody to neutralize motile Bb in culture (Fig. 5B). Serum from 2 to 3 week old pups born to M2-M5 had enough anti-OspA IgG to neutralize motile Bb in culture by 1–2 Log_{10} (M2, M3 and M5) and by 7 Log_{10} (M4) (Fig. 5C). Serum from pups born to M3 still had enough anti-OspA antibody to neutralize motile Bb in culture by 1 Log_{10} 9 weeks after birth (Fig. 5D).

4. Discussion

In this study we demonstrate that IgG antibodies from mothers immunized with *E. coli* overexpressing OspA were transferred to the respective offspring. Immunoglobulin G specific to OspA was passed mostly through ingestion of colostrum and milk during the lactation period shortly after birth. We were unable to conclude with all confidence that antibodies are not transferred via the placenta. We found a strong statistical correlation of antibody transfer between mothers that produced the most robust IgG response to OspA and their respective pups. Presence of protective levels of antibodies lasted until 4–5 weeks of age in the majority of pups but persisted in some mice until 9 weeks. We also show that serum obtained from vaccinated mothers and from the respective

Table 1
Parameter Estimates and Comparisons.

Parameter Estimates				
Parameter	Cohort	Mean (95% CrI)	P(x > 0)	P(x < 0)
EcA Intercept - baseline antibody activity	Mothers	−1.44 (-1.81, −1.05)	0	1
EcA Linear Dose Effect - change from baseline during dosing	Mothers	2.38 (1.82, 2.93)	1	0
EcA Decay Term – decline/change in antibody levels after dosing stops	Mothers	−3.9 (-5.21, −2.58)	0	1
Control Intercept – baseline antibody activity	Mothers	−1.6 (-2.25, −0.96)	0	1
Control Linear Dose Effect – change from baseline during dosing	Mothers	0.94 (-0.08, 1.96)	0.97	0.03
Control Decay Term – decline/change in antibody levels after dosing stops	Mothers	−2.48 (-4.88, −0.07)	0.02	0.98
EcA Autocorrelation – temporal dependence within mouse (persistence)	Mothers	0.4 (0.2, 0.6)	0	1
Control Autocorrelation – temporal dependence within mouse (persistence)	Mothers	0.2 (0.01, 0.5)	0.56	0.44
EcA Intercept – baseline antibody activity	Pups	−0.85 (-1.02, −0.67)	0	1
EcA Decay Term – decline/change over time	Pups	0.03 (-0.4, 0.46)	0.06	0.94
Control Intercept – baseline antibody activity	Pups	−1.94 (-2.17, −1.7)	1	0
Control Decay Term – decline/change over time	Pups	−0.34 (-0.78, 0.09)	1	0
EcA Autocorrelation – temporal dependence within mouse (persistence)	Pups	0.69 (0.61, 0.77)	1	0
Control Autocorrelation – temporal dependence within mouse (persistence)	Pups	0.27 (0.19, 0.36)	1	0
EcA Pup Maternal Exposure Term – effect of D56 maternal antibodies on pup	Mothers/Pups	1.39 (1.08, 1.71)	1	0
Control Pup Maternal Exposure Term – effect of D56 maternal antibodies on pup	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 – overall mean difference	Mothers	0.16 (-0.59, 0.91)	0.67	0.33
Linear Dose Effect – difference during dosing	Mothers	1.44 (0.26, 2.6)	0.99	0.01
Decay – rate of decline after dosing	Mothers	−1.42 (-4.16, 1.35)	0.15	0.85
Autocorrelation – difference in persistence	Mothers	0.2 (-0.14, 0.48)	1	0
Intercept – overall mean difference	Pups	1.09 (0.79, 1.39)	0.89	0.11
Decay – difference in rate of decline	Pups	0.37 (-0.24, 0.98)	0.88	0.12
Autocorrelation – difference in persistence	Pups	0.42 (0.3, 0.54)	1	0
Maternal Exposure Term – difference in dependence on maternal antibody levels	Mothers/Pups	1.41 (1.09, 1.72)	1	0

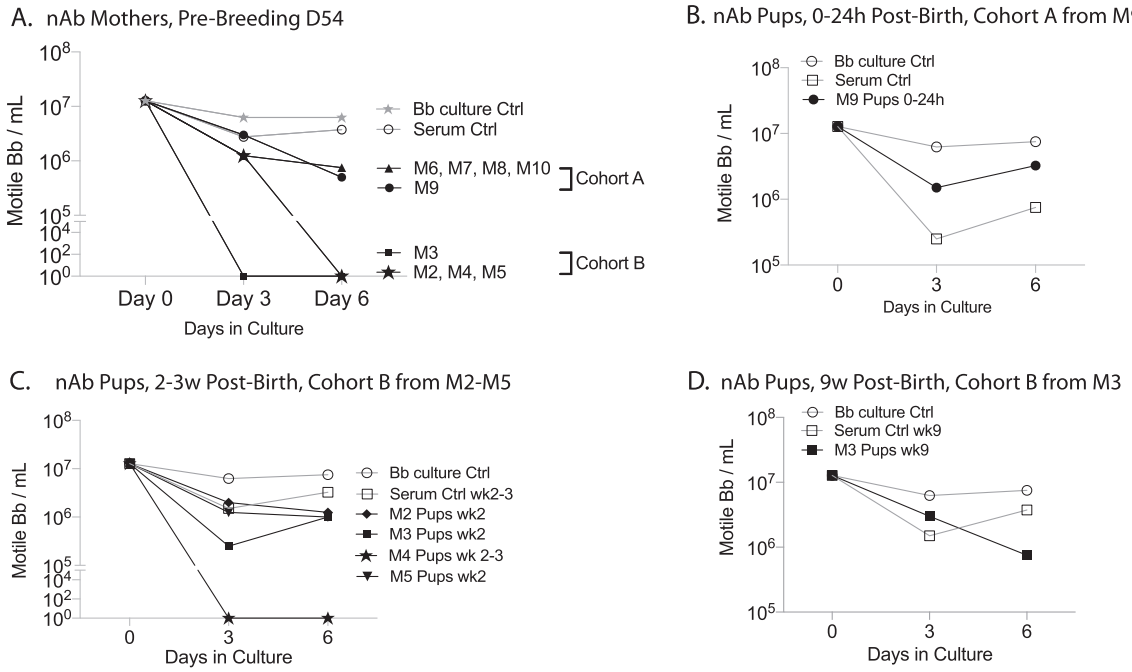

Fig. 5. Anti-OspA neutralizing antibody (nAb) function against *B. burgdorferi*. Number of motile *B. burgdorferi* (Bb) after treatment of a multi-strain culture with A) pooled serum from mothers M6 M7 M8 M10, pooled serum from mothers M2 M4 M5 and individual serum samples from mothers M3 and M9; B, 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 (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.

offspring neutralized motility of cultured *B. burgdorferi* containing several strains, demonstrating broad antibody function.

B. burgdorferi is maintained in the environment by tick-mediated infection of reservoir host species [29], mostly rodents, rather than through transovarial transmission between tick cohorts [30] and between mammalian hosts [31]. As a logical follow up to our previous findings we hypothesized that maternal transfer of vaccine-induced OspA-specific IgG to offspring may help explain how the enzootic cycle of *B. burgdorferi* can be disrupted by oral bait immunization of field mice with OspA vaccines [32,17]. We vaccinated C3H-HeN mothers using a standard intermittent 8-week immunization schedule for oral gavage of an *E. coli* culture overexpressing OspA and, due to COVID-19 social distancing restrictions, we extended the schedule of blood

collection until day 203–218 post first vaccine dose. We found that the mean of anti-OspA IgG remained above OD_{450} ~0.8 for about ~5.5 months after the 1st boost at D35 (Fig. 2B). In our previous studies [17,22] we determined that, unlike levels of LA-2 equivalent antibodies, an OspA ELISA 2 < OD_{450} > 0.8–1 can be used to empirically establish the minimum levels of protective/neutralizing antibodies in serum from adult mice orally vaccinated with *E. coli* expressing OspA. We found that the number of mice with protective levels [22] of anti-OspA IgG remained above 60% for about ~3.5 months (between D35 until D154), and it remained above 30% for two additional months. This is important given that 30% oral vaccine antibody prevalence in immunized foxes resulted in disrupted transmission of Rabies virus [33]. In addition, the potency of anti-OspA immunity differed considerably between the mothers (Fig. 2C). This is also consistent with our previous findings using the same vaccine formulation and schedule [19].

Transfer of immune elements from mother to offspring via placenta and milk is a classical mechanism by which neonates are protected from infections, acknowledged for over 120 years [23,34,35]. Recently it was found that maternal antibodies to *Borrelia afzelii* infection provided strain-specific protection against tick-transmitted *B. afzelii* in bank voles [36]. When we tested for presence of anti-OspA antibodies in pups born to vaccinated mothers we found a clear difference in antibody levels at birth versus 2+ weeks after birth (Fig. 3). Antibodies to OspA remained detectable in some pups from birth to about 2 months. In addition, the robustness of the maternal immune response to OspA correlated with the amount of antibody transferred to offspring. We observed a positive association between high levels of antibodies in the birth mother and the length of time these antibodies persisted in the offspring serum; for example, the mother with the highest level of anti-OspA antibodies produced offspring that had persistent antibodies 9 weeks after birth, indicating a more durable antibody response. Another interesting finding was that pups from larger litters had less total antibody to OspA in serum which strongly suggests a dilution effect between mother and offspring. In other words, the mother produces a fixed amount of antibody to OspA at the peak of her immune response and this is split between the number of offspring and the time of suckling. At birth, the pups born to 4/5 mothers did not have antibody to OspA. However, one mother (M9) produced pups that had antibodies to OspA on the day of birth. Since most of the pups were born overnight, M9 may have been the first mother to give birth and her pups may have begun nursing much sooner than the pups born to the other mothers. Although the data is suggestive of absence of transplacental transfer, we cannot reach an unequivocal conclusion, given that the mothers that produced the Cohort A pups also developed less overall antibody to OspA after vaccination. This difference may be explained by the longer time these mothers took to breed and produce offspring.

Through dual modelling of mothers and their corresponding pups we observed systematic differences in antibody profiles. In addition to the unambiguous detection of immunization effects in mothers and the presence of maternal transfer of antibodies to pups (Fig. 4), maintenance of antibody activity was substantially different between vaccine and placebo groups. In addition, these comparisons remained clear in the presence of substantial effect heterogeneity from both mothers and pups, arising from factors such as the timing of birth of some litters, variability in maternal antibody levels and litter size variation. In addition to providing an efficient use of all the data available for these paired cohorts, this approach provides a template for how similar studies might make use of Bayesian hierarchical models. These techniques allow researchers to directly explore mechanisms under study and can be of use in a wide variety of problems where complex dependence among data sets is expected.

The protective mechanism of action of OspA based vaccines relies on the OspA antibody ability to neutralize motile spirochetes in the nymphal tick midgut, thus preventing migration to the tick salivary gland and subsequent transmission to the next mammalian host [6]. We and others have developed transmission blocking vaccines based in OspA [19,32,37]. Our reservoir targeted vaccine reduced prevalence of infected nymphal ticks by 76% after vaccination of the primary reservoir host species (*P. leucopus*) over 5 years [17]. In the study reported here we show that 90% of vaccinated mothers produced neutralizing anti-OspA antibodies for 4–5 months after receiving the last vaccine dose. Transfer of these antibodies from mother to offspring happened mostly through consumption of colostrum and milk, and neutralizing antibodies persisted in some pups through two months of age (Fig. 5). We speculate that in a field setting where adult female mice are vaccinated in April and May, transfer of neutralizing anti-OspA antibodies from mother to offspring over a 4–5 month period through late Spring and Summer may lead to a population of reservoir offspring with significant levels of neutralizing anti-OspA antibodies in blood through July and August when the uninfected larva start to feed. This factor may impact the enzootic cycle of *B. burgdorferi*. This novel observation may explain how vaccinating field mice through the summer with baits containing OspA led 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 spring [17]. Further experimentation is needed to sort out transplacental transfer of anti-OspA antibodies and provide unambiguous protection results in pups after tick challenge, which was not possible to do in 2020 for this study.

CRediT authorship contribution statement

Kathryn Phillip, Nisha Nair: Experimental investigation, Data analysis, Editing manuscript; **Kamalika Samanta, Jose F Azevedo:** Experimental investigation, Data analysis; **Grant Brown:** Bayesian model development, Implementation and analysis, Writing original draft and editing; **Christine Petersen:** Funding acquisition and editing manuscript; **Maria Gomes-Solecki:** Funding acquisition, Conceptualization, Supervision, Writing original draft and editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: MGS (grants from federal agencies, employment, patents and consultant), CAP (grants from federal agencies, employment, and consultant). All other authors declare no conflicts.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2021.06.025.

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