

Reductions in Human Lyme Disease Risk Due to the Effects of Oral Vaccination on Tick-to-Mouse and Mouse-to-Tick Transmission

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Abstract

Vaccinating wildlife is becoming an increasingly popular method to reduce human disease risks from pathogens such as *Borrelia burgdorferi*, the causative agent of Lyme disease. To successfully limit human disease risk, vaccines targeting the wildlife reservoirs of *B. burgdorferi* must be easily distributable and must effectively reduce pathogen transmission from infected animals, given that many animals in nature will be infected prior to vaccination. We assessed the efficacy of an easily distributable oral bait vaccine based on the immunogenic outer surface protein A (OspA) to protect uninfected mice from infection and to reduce transmission from previously infected white-footed mice, an important reservoir host of *B. burgdorferi*. Oral vaccination of white-footed mice effectively reduces transmission of *B. burgdorferi* at both critical stages of the Lyme disease transmission cycle. First, oral vaccination of uninfected white-footed mice elicits an immune response that protects mice from *B. burgdorferi* infection. Second, oral vaccination of previously infected mice significantly reduces the transmission of *B. burgdorferi* to feeding ticks despite a statistically nonsignificant immune response. We used the estimates of pathogen transmission to and from vaccinated and unvaccinated mice to model the efficacy of an oral vaccination campaign targeting wild white-footed mice. Projection models suggest that the effects of the vaccine on both critical stages of the transmission cycle of *B. burgdorferi* act synergistically in a positive feedback loop to reduce the nymphal infection prevalence, and thus human Lyme disease risk, well below what would be expected from either effect alone. This study suggests that oral immunization of wildlife with an OspA-based vaccine can be a promising long-term strategy to reduce human Lyme disease risk.

Key Words: *Borrelia burgdorferi*—Lyme disease—Oral vaccine—Outer surface protein A—*Peromyscus leucopus*—Tick-borne disease—White-footed mice.

Introduction

INFECTIONOUS DISEASES ARE EMERGING and reemerging at an alarming rate (Daszak et al. 2000, Woolhouse et al. 2005, Jones et al. 2008). The majority of these diseases are zoonoses that are caused by pathogens residing in animal populations that can be transmitted to humans (Jones et al. 2008). An increasingly popular method to reduce human disease risks from zoonotic pathogens is to vaccinate the pathogen's natural reservoir hosts (Cross et al. 2007). However, the efficacy of wildlife vaccination campaigns can be limited by vaccine formulations that are practically difficult to administer to wild animals or by the prevalence of infected animals prior to the

onset of the campaign. Thus, wildlife vaccines must be easily distributable to natural reservoir hosts and protect uninfected animals from infection as well as hinder transmission from infected animals to new hosts (Tompkins et al. 2009). The aim of this work was to test the efficacy of an easily distributable oral vaccine formulation in protecting wildlife from infection with *Borrelia burgdorferi*, the causative agent of Lyme disease, and in preventing transmission from infected animals to the tick vector.

Lyme borreliosis is a tick-borne zoonosis that is of significant public health concern in the Northern Hemisphere, where it ranks 7th among notifiable conditions, just below acquired immunodeficiency syndrome (AIDS) (Prevention

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2011). In the northeastern United States, *B. burgdorferi* is transmitted among wildlife hosts, predominantly small mammals and ground-dwelling birds, by the immature stages (nymphs and larvae) of *Ixodes scapularis* ticks (Anderson 1988, LoGiudice et al. 2003, Brisson and Dykhuizen 2004, Brisson et al. 2008, Ogden et al. 2008, Brinkerhoff et al. 2010, Ogden et al. 2011). The human risk of contracting Lyme disease is strongly correlated with the prevalence of *B. burgdorferi*-infected nymphs (Piesman et al. 1987, Mather et al. 1996, Stafford et al. 1998, Diuk-Wasser et al. 2012). Thus, wildlife vaccination campaigns that reduce the prevalence of infected nymphs can be an effective method to control Lyme disease (Tsao et al. 2004). Because nymphal ticks can only become infected by feeding on an infected wildlife host during their larval blood meal (Magnarelli et al. 1987, Patrican 1997), wildlife vaccination campaigns must interrupt the transmission of *B. burgdorferi* between wildlife hosts and ticks to reduce human Lyme disease risk.

Lyme disease vaccines based on the immunogenic outer surface protein A (OspA) effectively protect uninfected laboratory mice from *B. burgdorferi* when delivered via parenteral (Fikrig et al. 1990, Fikrig et al. 1992b) or oral immunization (Fikrig et al. 1991, Dunne et al. 1995, Luke et al. 1997, Gomes-Solecki et al. 2006, Scheckelhoff et al. 2006, del Rio et al. 2008, Richer et al. 2011). Because the OspA protein is expressed by spirochetes, primarily in the tick midgut (Schwan et al. 1995), OspA-based vaccines work in an unconventional manner. Antibodies elicited by OspA vaccines in the mammalian host are injected by the tick during a blood meal and kill *B. burgdorferi* in the tick midgut and thus prevent transmission of the spirochete to vaccinated hosts (Fikrig et al. 1992b, de Silva et al. 1996). Furthermore, intraperitoneal immunization of previously infected mice can reduce subsequent transmission of *B. burgdorferi* to feeding larval ticks, likely by killing bacteria that migrate to the midgut (Tsao et al. 2001).

The reduction in *B. burgdorferi* transmission from infected and subsequently vaccinated mice was critical to the success of a recent field trial (Tsao et al. 2004). This intraperitoneal vaccination campaign targeted wild white-footed mice (*Peromyscus leucopus*), an important reservoir host of Lyme disease in the northeastern United States (Donahue et al. 1987, Mather et al. 1989), and resulted in significant reductions in the prevalence of infected nymphs and thus human Lyme disease risk (Tsao et al. 2004). However, immunizations by needle inoculation are not practical on scales relevant to public health (Cross et al. 2007). Thus, it is critical to develop an effective oral delivery system that is effective at both protecting wildlife and hindering transmission from infected reservoirs to the tick vector.

We developed an oral reservoir-targeted vaccine based on the immunogenic OspA protein of *B. burgdorferi* aimed at breaking the natural cycle of this spirochete. In the present study, we assessed the efficacy of oral immunization with an OspA protein in protecting uninfected mice from infection and reducing transmission from previously infected mice. Because most white-footed mice are infected prior to vaccination (Bunikis et al. 2004), it is critical to establish whether oral vaccination with an immunogenic protein can reduce *B. burgdorferi* transmission from infected wild mice to uninfected larval ticks. Importantly, we estimated critical pathogen transmission variables (tick-to-mouse and mouse-to-tick) in vaccinated mice and used these estimates to model

the efficacy of an oral vaccination campaign targeting wild *P. leucopus* mice.

Materials and Methods

Mice, ticks, and bacteria

Adult outbred *P. leucopus* mice were obtained from the *Peromyscus* Genetic Stock Center (University of South Carolina, Columbia). Mice were kept at 22°C with a 14:10 light-dark cycle. All mice were approximately 2 months old at the start of the experiments and Institutional Animal Care and Use Committee (IACUC) guidelines were followed. The uninfected larval ticks from 3 different adult female egg masses that were used for xenodiagnosis were purchased from *I. scapularis* colonies at Oklahoma State University. Infected *I. scapularis* nymphs were derived from wild *P. leucopus* mice, as described previously (Gomes-Solecki et al. 2006). Briefly, *I. scapularis* larvae were collected from naturally infected white-footed mice live-trapped in Elverson, Pennsylvania, and were allowed to molt into nymphs. We randomly selected a sample of 80 nymphs and determined that the frequency of infection was 80% by PCR as previously described (Brisson and Dykhuizen 2004).

Vaccine construction and formulations

Full-length *ospA*, including the lipidation site, from *B. burgdorferi* strain B31 was cloned into the pET45b plasmid (EMD Millipore) and transformed into *Escherichia coli* strain BL21. Incorporation of the complete recombinant *ospA* (rOspA) sequence into the plasmid was confirmed by Sanger sequencing, and protein expression was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and tandem mass spectrometry. Oral vaccinations used an agar-matrix formulation and an oral gavage formulation (Fikrig et al. 1991). The agar-matrix vaccine was prepared by suspending live OspA-producing *E. coli* at a final density of 2.5 billion cells/mL of Luria-Bertani agar (0.75% agar, 100 µg/mL ampicillin, and 80 mM isopropyl β-D-1-thiogalactopyranoside [IPTG]). OspA production was confirmed via total protein extraction and mass spectrometry. The concentration of rOspA produced by the *E. coli* was determined by purifying a histidine-tagged form of the protein from *E. coli* cells using standard methods (QIAexpressionist handbook). Bradford assays indicated that 1.25×10^{12} rOspA-*E. coli* cells yielded 5 mg of rOspA protein. The purified histidine-tagged rOspA proteins were used in the enzyme-linked immunosorbent assay (ELISA) assays described below.

Enzyme-linked immunosorbent assay

Total immunoglobulin G (IgG) against OspA in mouse blood was determined by ELISA, as previously described (Schwanz et al. 2011). Briefly, Nunc MaxiSorp 96-well plates were coated with purified rOspA protein (10 µg/mL) overnight at 4°C. The rOspA was removed and the plates were blocked using phosphate-buffered saline (PBS) containing 2% bovine serum albumin (BSA) for 2 h at room temperature. Plates were washed three times with PBS+0.1% Tween 20 (PBS-T) and then incubated with 100 µL of 1:100 mouse sera (diluted in PBS) for 45 min. Plates were washed 3 times with PBS-T before adding the secondary antibody for 45 min followed by 3 more washes with PBS-T. Anti-*P. leucopus*

secondary antibody conjugated to horseradish peroxidase was used at a 1:2500 dilution (KPL, Maryland) and the 1-Step Ultra TMB was added to initiate the color reaction. Optical density was read at 652 nm after 20 min. We conducted independent replicate ELISAs for both experiments to determine the precision in measuring the anti-OspA IgG immune response. The efficacy of the vaccine treatment, the effect size, was calculated by dividing the difference in optical density between the vaccine and control mice by the standard deviation of the optical density in the control group.

Xenodiagnosis

The efficacy of the vaccine to protect mice from *B. burgdorferi* challenge as well as to reduce transmission from infected mice was assessed by xenodiagnoses, as previously described (Donahue et al. 1987). Briefly, ~50 uninfected larval ticks per mouse were allowed to feed to repletion and collected after naturally dropping off the host. Each blood-engorged larva was placed in its own Eppendorf tube with a strip of moistened paper towel and allowed to molt to the nymphal stage. Ticks were kept in the same room as the mice, and the temperature and humidity were strictly controlled. A random sample of 10–12 nymphs from each mouse were tested for the presence of *B. burgdorferi* DNA using nested *ospC* PCR (Brisson and Dykhuizen 2004, Gomes-Solecki et al. 2006, Brisson et al. 2008).

Vaccine-protection of uninfected mice

To determine the protective capacity of the vaccine, we challenged uninfected mice (both OspA- and control-vaccinated) with *B. burgdorferi*-infected nymphs. For these nymphal challenge experiments, 13 uninfected *P. leucopus* mice were randomly assigned to either the vaccine ($n=9$) or the control group ($n=4$). Mice were allowed to feed overnight on a 5-gram slice of agar containing $\sim 10^{10}$ cells of OspA-producing *E. coli* (or control *E. coli* strain BL21) 3 nights per week for 5 weeks ($\sim 10^{10}$ cells per dose $\cong 40.5 \mu\text{g}$ of OspA protein per dose). On average, *P. leucopus* mice ate $91.0 \pm 5.7\%$ (mean \pm standard error [SE]; $n=13$) of the agar slice over the 15 immunization nights. To compare the efficacy of the oral agar vaccine with parenteral immunization, 2 mice were immunized via intraperitoneal injection once per week for 5 weeks with 10^8 cells of OspA-producing *E. coli* ($\cong 0.406 \mu\text{g}$ rOspA per dose). Blood was drawn from all mice at 14, 44, and 86 days after the initial vaccine dose to assess the immune response to OspA by ELISA. Mice were subsequently challenged with 10 *B. burgdorferi*-infected nymphal ticks 45 days after the initial vaccine dose. Blood-engorged nymphs were collected after naturally dropping off each mouse and subsequently tested for the presence of *B. burgdorferi*-infection. The infection status of mice was determined by xenodiagnosis 35 days after the nymphal challenge. Generalized linear models with a binomial error function were used to compare the proportion of infected ticks and the proportion of infected mice among treatments.

Vaccine-induced reduction of B. burgdorferi transmission from infected mice to feeding ticks

Infected mice ($n=11$) were subsequently vaccinated to estimate the effect of vaccination on mouse-to-tick transmission. Mice were infected with *B. burgdorferi* by infesting them with

10 *B. burgdorferi*-infected nymphs 30 days before the start of the vaccination schedule. Infected mice were randomly assigned to the vaccine group ($n=6$) or the control group ($n=5$). Mice were subsequently vaccinated using 50 million OspA-producing ($\cong 0.203 \mu\text{g}$ rOspA per dose) or control *E. coli* (strain BL21) via oral gavage once per week for 4 weeks, a vaccination schedule that induced a strong anti-OspA IgG response in uninfected C3H/HeJ mice in a previous study (Fikrig et al. 1991).

The infection status of the mice and the capacity of the vaccine to reduce the transmission from infected mice to feeding larval ticks was assessed by xenodiagnosis 41 days after the initial vaccine dose. The blood-engorged, xenodiagnostic larvae were randomly assigned to be sacrificed on either day 30 ($n=12$ ticks per mouse) or day 150 ($n=10$ ticks per mouse) post blood meal to assess the long-term effect of the vaccine on the reduction of mouse-to-tick transmission. Blood was drawn 14 days prior to the initial vaccine dose and 57 days after the initial vaccine dose to assess the OspA immune response.

Natural vaccination coverage parameter estimates

To estimate the possibility of vaccinating wild mice, we analyzed a mark-recapture survey of wild populations of *P. leucopus* mice in 4 locations in the Crow's Nest Preserve, Elverson, Pennsylvania (April–September, 2009). Each trapping location (225 \times 225 meters each) contained 64 trapping stations spaced every 15 meters with one Sherman live-trap per locality (Sherman Traps, Inc., Tallahassee, FL). Traps were baited (60 total trapping sessions) with rolled oats, set at 17:00, and checked the following morning before 10:00. Each captured mouse was fitted with a unique 4-digit ear tag to facilitate identification and the establishment of recapture histories. From these data, we estimated the probability each resident *P. leucopus* mouse would visit a baiting location given that bait was available as the number of times a mouse was captured divided by the total number of occasions that it was available to be captured.

Model of vaccine efficacy in wildlife

The efficacy of wildlife vaccination in reducing the nymphal infection prevalence (NIP), and thus human Lyme disease risk, was estimated using a deterministic model parameterized with empirical data from this study and from the literature (Fig. 1). The model calculates the expected NIP each year using the sum of the proportion of larval ticks that fed on mice protected due to vaccination (M_P), infected mice that were subsequently vaccinated (M_{IV}), infected mice that were not vaccinated (M_I), and other wildlife hosts (W), as well as the rates at which *B. burgdorferi* is transmitted to feeding ticks from each class of host (C_P , C_{IV} , C_I , and C_W , respectively). The model estimates the proportion of mice that elicit a protective immune response prior to challenge by an infected tick as $M_P = V_C * V_E * (1 - \text{NIP})^D * N$, where V_C is the vaccine coverage, V_E is the vaccine efficacy, NIP is the current nymphal infection prevalence, D is the number of days between the first vaccine dose and full protection, and N is the number of nymphs that attach to each mouse per day. Vaccine coverage is the proportion of mice that successfully complete a vaccination schedule, whereas vaccine efficacy is the proportion of mice that effectively respond to the vaccine. Mice that elicit a protective immune response prior to challenge by

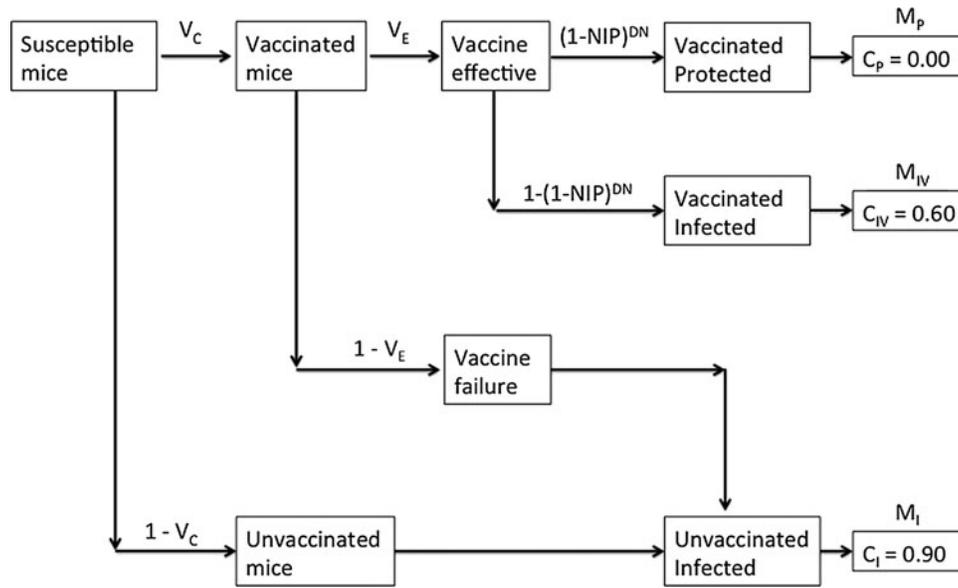


FIG. 1. Diagram of the model parameters that determine 3 classes of *P. leucopus* mice: Vaccinated and protected (M_P), infected and subsequently vaccinated (M_{IV}), and infected and unvaccinated (M_I). Important parameters include vaccination coverage (V_C , the proportion of mice that are vaccinated), vaccination efficacy (V_E , the proportion of mice in which the vaccine is effective), days to complete a vaccination schedule (D), nymphal attachment rate (N), and nymphal infection prevalence (NIP). NIP is determined by the proportions of infected, infected and vaccinated, and vaccine-protected mice and the rates at which *B. burgdorferi* are transmitted to feeding ticks from each mouse class.

an infected tick are not infected and thus do not transmit *B. burgdorferi* to feeding ticks ($C_P=0$). The proportion of mice that are challenged by at least 1 infected tick prior to the vaccine taking full effect is given by $M_{IV}=V_C*V_E*[1-(1-NIP)^D*N]$. These mice are infected and subsequently vaccinated, resulting in a reduced rate of *B. burgdorferi* transmission to feeding ticks as estimated in this study ($C_{IV}=43/71$ or 0.61). The remaining mice ($1-V_C*V_E$) include both unvaccinated mice and vaccinated mice for which the vaccine was not effective. These mice are infected and transmit *B. burgdorferi* to feeding ticks at a standard rate ($C_I=0.90$), as estimated in this and previous studies (Donahue et al. 1987, LoGiudice et al. 2003, Brisson and Dykhuizen 2004, Brunner et al. 2008). Alternative wildlife hosts account for a fixed proportion of tick blood meals ($W=0.70$), as estimated from the literature (LoGiudice et al. 2003, Brisson et al. 2008), but do not transmit the infection to feeding ticks ($C_W=0$). Thus, NIP in the year following a vaccination campaign can be calculated as $M_P * C_P + M_{IV} * C_{IV} + M_I * C_I + W * C_W$. NIP was analyzed with this model by simulating across a wide parameter space for V_C (11 levels: 0.00, 0.10, ..., 1.00), V_E (11 levels: 0.00, 0.10, ..., 1.00), C_{IV} (11 levels: 0.00, 0.10, ..., 1.00), D (4 levels: 14, 28, 42, and 56 days), and N (5 levels: 0.05, 0.10, 0.20, 0.50 1.00 nymphs/day). For each of these 26,620 scenarios, the NIP at equilibrium was calculated.

Results

Oral vaccination protects uninfected mice from *B. burgdorferi* infection

A vaccinated mouse was considered protected if all xenodiagnostic larvae tested negatively for *B. burgdorferi* following nymphal challenge. Vaccination protected 6 of the 9 OspA-vaccinated mice from infection, whereas none of the 4 control-

vaccinated mice were protected (Table 1; Δ deviance=6.49, Δ degrees of freedom [df]=1, $p=0.011$). Protection from infection was likely the result of the anti-OspA IgG immune response of the mice in the vaccine group, which was 6.3 standard deviations (SD) greater than that in the control group by day 42 ($t=18.66$, $df=12$, $p<0.001$; Fig. 2A). The anti-OspA IgG response was similar among the mice in the vaccine group prior to nymphal challenge ($t=1.25$, $df=7$, $p=0.253$; Fig. 2B). However, the anti-OspA IgG levels of the 3 mice that became infected were 1.73 SD lower than the 6 protected mice 41 days after nymphal challenge ($t=4.66$, $df=7$, $p=0.002$; Fig. 2B).

The anti-OspA IgG levels at day 42 were similar in the orally ($n=9$) and parentally ($n=2$) vaccinated mice (Fig. 2). Replicate measures of the anti-OspA IgG levels (absorbance at 652 nm at 20 min for 1:100 serum dilution) were highly correlated between independent ELISA assays ($r=0.98$, $p<0.001$), indicating a very low measurement error.

Oral vaccination reduces *B. burgdorferi* from feeding nymphs

At least 1 blood-engorged *B. burgdorferi*-infected nymph was recovered from all of the protected mice, indicating that all mice were exposed to at least 1 *B. burgdorferi*-infected nymph. However, the proportion of nymphs that remained infected after feeding on mice in the vaccine group (36.4%=20 infected/55 nymphs) was significantly lower than those nymphs that fed on mice in the control group (71.4%=20 infected/28 nymphs; Δ deviance=9.35, $\Delta df=1$, $p=0.002$).

Oral vaccination reduces *B. burgdorferi* transmission from infected mice to feeding ticks

The anti-OspA IgG levels in the vaccinated mice were 1.4 SD greater than the control mice, a statistically nonsignificant

TABLE 1. EFFICACY OF VACCINATION SCHEDULES IN UNINFECTED AND *B. BURGDORFERI*-INFECTED MICE

Experiment	Infection status ^a	Vaccine	Schedule ^b	N	Effect size ^c	Protected ^d	Transmission ^e	Statistical significance ^g
Vaccine protection	Uninfected	OspA	15 doses* 10 × 10 ⁹ cells	9	6.3 (<i>p</i> < 0.001)	6/9	0.80–0.90 ^f	Tick-to-mouse transmission (<i>p</i> = 0.011)
	Uninfected	Control	15 doses* 10 × 10 ⁹ cells	4		0/4		
Transmission reduction	Infected	OspA	4 doses* 0.5 × 10 ⁸ cells	6	1.4 (<i>p</i> = 0.574)	NA	0.33–0.91	Mouse-to-tick Transmission (<i>p</i> < 0.001)
	Infected	Control	4 doses* 0.5 × 10 ⁸ cells	5		NA	0.75–1.00	

^aThe infection status of mice prior to the first vaccine treatment.
^bThe number of doses and the number of cells per dose.
^cThe effect size of the vaccination treatment on anti-OspA immune response.
^dThe proportion of mice protected (tick-to-mouse transmission).
^eThe minimum and maximum proportions of infected larvae (mouse-to-tick transmission).
^fFor 3 nonprotected mice only.
^gStatistical significance of vaccination treatment on transmission
 NA, not applicable.

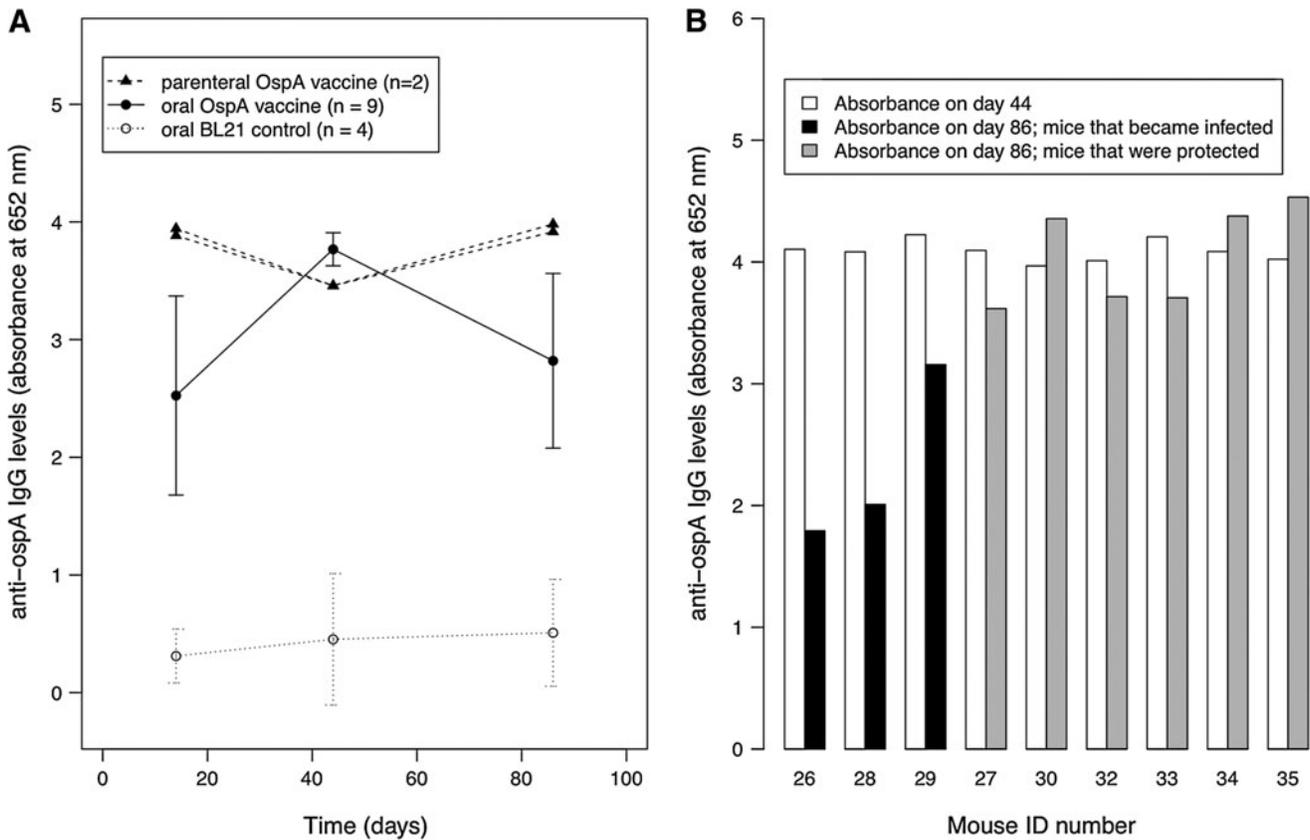


FIG. 2. Oral vaccination with *Escherichia coli*-expressing OspA suspended in an agar matrix results in a strong and rapid anti-OspA immunoglobulin G (IgG) immune response in uninfected *P. leucopus* mice. The anti-OspA IgG immune response was measured as the absorbance reading at 652 nm after 20 min using a 1:100 dilution of mouse serum. **(A)** The anti-OspA IgG immune response on day 42 in vaccinated, uninfected mice (solid circles; *n* = 9 mice) was 6.3 standard deviations (SD) greater than the control mice (open circles; *n* = 4 mice; *t* = 18.66, [df] = 12, *p* < 0.001). Orally vaccinated mice were immunized 15 times with 10 billion live cells per dose starting on day 0. Parenterally vaccinated mice (*n* = 2) were immunized 5 times with 100 million cells per dose starting on day 0. Shown are the means and the 95% confidence limits. There are no confidence limits for the parenterally vaccinated mice because there were only 2 individuals. **(B)** *B. burgdorferi* infection reduced anti-OspA IgG levels in mice following the nymphal challenge. On day 86, the anti-OspA IgG response in rOspA-vaccinated mice that became infected following nymphal challenge (black bars; *n* = 3) was 1.73 SD lower than the mice that remained uninfected following nymphal challenge (grey bars; *n* = 6; *t* = 4.66, df = 7, *p* = 0.002).

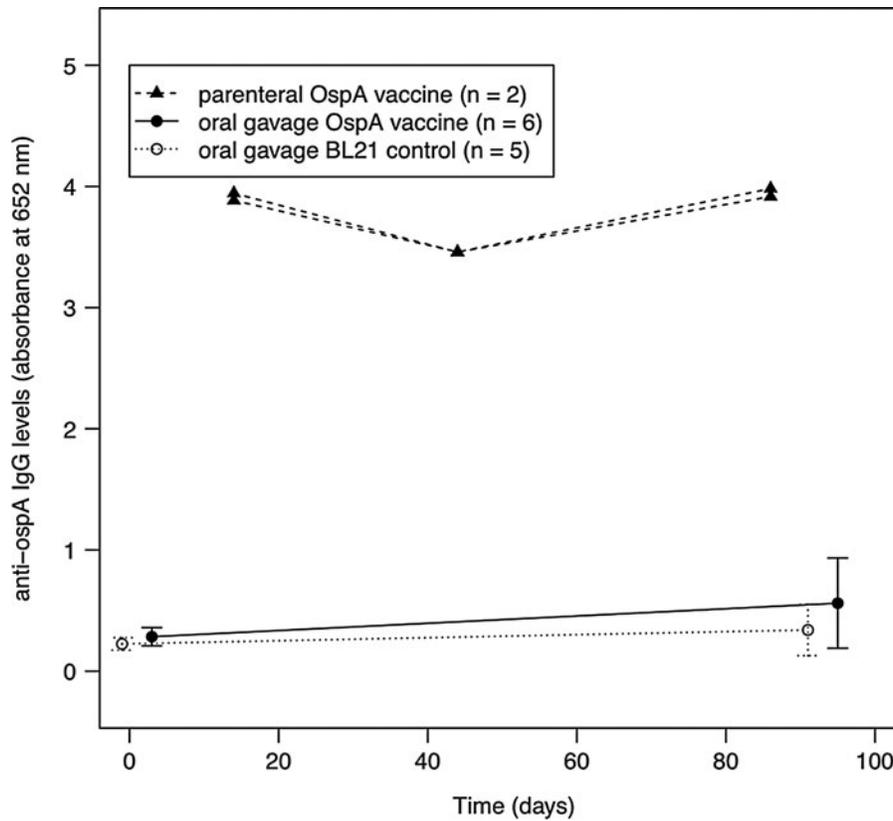


FIG. 3. Oral vaccination with *E. coli*-expressing OspA did not result in a strong anti-OspA immunoglobulin G (IgG) immune response in previously infected *P. leucopus* mice. The anti-OspA IgG levels were similar among the groups of infected mice prior to vaccination. Post vaccination, the anti-OspA IgG immune response in infected and subsequently vaccinated mice ($n=6$) was 1.4 standard deviations (SD) greater than in infected control mice ($n=5$), but this difference was not statistically significant ($t=0.58$, [df]=9, $p=0.574$). Mice were first infected with *B. burgdorferi* 31 days prior to the first of 4 vaccine doses of 50 million live cells per dose. For comparison, the anti-OspA IgG immune responses of the parenterally vaccinated mice ($n=2$) were included from the previous experiment. These mice were immunized 5 times with 100 million cells per dose via intraperitoneal injection. Shown are the means and the 95% confidence limits.

difference ($t=0.58$, $df=9$, $p=0.574$). Replicate measures of the anti-OspA IgG levels (absorbance at 652 nm at 20 min for 1:100 serum dilution) were highly correlated between independent ELISA assays ($r=0.74$, $p=0.005$), indicating low measurement error. Comparison with the parenterally immunized mice from the previous experiment revealed that the oral gavage treatment induced a weak anti-OspA IgG immune response (Fig. 3). Despite this weak immune response, mouse-to-tick transmission of *B. burgdorferi* in infected and subsequently OspA-vaccinated mice (43 infected/71 ticks=60.6%; $n=6$ mice) was one-third lower than that of infected and control-vaccinated mice (54 infected/60 ticks=90%; $n=5$ mice), and this overall difference in the percentage of infected ticks was highly statistically significant ($\Delta df=1$, $\Delta dev=20.48$, $p<0.001$; Fig. 4). Among infected and OspA-vaccinated mice, there was considerable variation in the mouse-to-tick transmission rate (33–91% in Table 1). Despite this variation, the conservative independent 2-sample t -test (using mice as the units of replication) confirmed that the difference in the mean transmission rate between the vaccinated and control mice was statistically significant ($t=2.98$, $df=9$, p value=0.015). To test whether there was a relationship between mouse anti-OspA IgG antibody levels and the mouse-to-tick transmission rate, we used a logistic regression with a quasi-binomial error func-

tion. There was a negative, but not statistically significant, relationship between anti-OspA IgG levels and the transmission rate (slope on the logit scale = -0.97 ± 0.426 , $F=1.88$, $p=0.203$). When the analysis was restricted to the 6 vaccinated mice, the slope remained negative (-0.58 ± 0.480) and not statistically significant.

Interestingly, for both OspA-vaccinated and control mice, the prevalence of *B. burgdorferi* infection was significantly lower ($df=1$, $\Delta dev=95.95$, $p<0.001$; Fig. 4) in the nymphs sacrificed on day 150 (17 infected/110 ticks=15.5%; $n=11$ mice) than the nymphs sacrificed on day 30 (97 infected/131 ticks=74.0%; $n=11$ mice). Because all nymphs (those sacrificed at day 30 and at day 150) were fed as larvae on the same 11 mice at the same time, the results suggest that infection prevalence in nymphal ticks can decrease over time. Importantly, the relative difference in the infection prevalence of nymphs from vaccinated and unvaccinated mice remained similar across time, indicating that the vaccine effect remains constant regardless of other biological factors.

Vaccine coverage

A total of 296 unique *P. leucopus* individuals were captured a total of 2089 times with an average daily capture probability of 0.70/day. Assuming that the capture rate is equivalent to

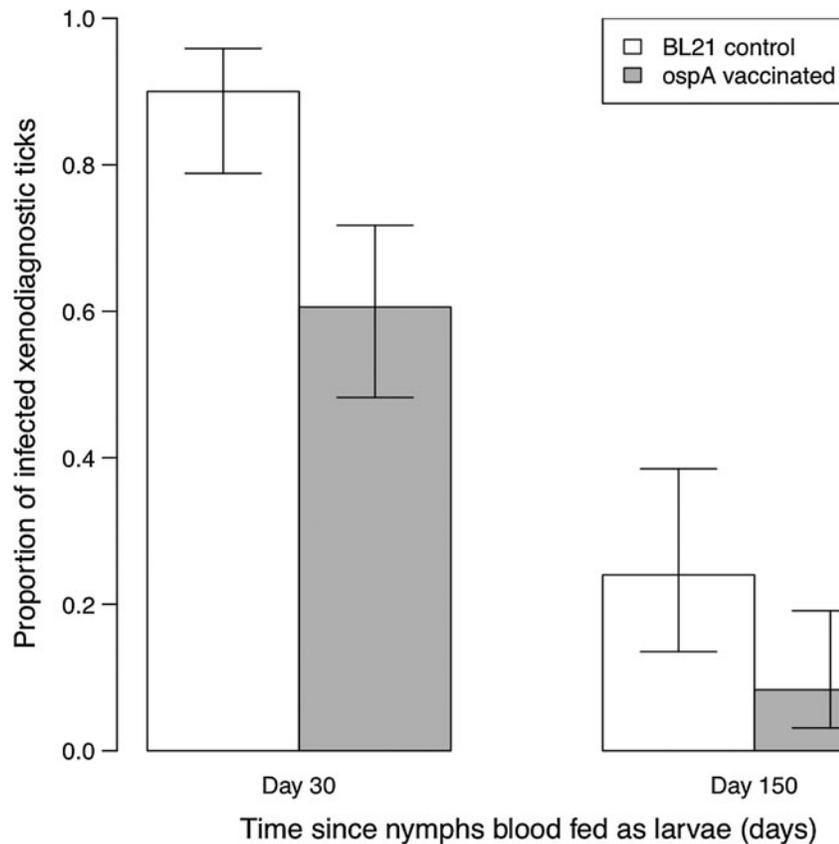


FIG. 4. OspA vaccination reduces transmission of *B. burgdorferi* from previously infected *P. leucopus* mice to feeding larval ticks. For each of the 11 mice, xenodiagnostic larvae were collected and randomly assigned to be sacrificed on day 30 or day 150 after the blood meal. For each of the 11 mice, the prevalence of *B. burgdorferi* infection was estimated for 12 ticks sacrificed on day 30 and 10 ticks sacrificed on day 150. Nearly all of the day-30 nymphs that took their larval blood meal from control mice were infected (54 infected/60 ticks=90%; $n=5$ mice), whereas fewer day-30 nymphs from vaccinated mice were infected (43 infected/71 ticks=60.6%; $n=6$ mice; [df]=1, Δ dev=20.48, $p<0.001$). Interestingly, the prevalence of *B. burgdorferi* in ticks that were sacrificed 150 days after the transmission event (17 infected/110 ticks=15.5%; $n=11$ mice) was 4.8 times lower than ticks that were sacrificed 30 days after the transmission event (97 infected/131 ticks=74.0%; $n=11$ mice; df=1, Δ dev=95.95, $p<0.001$). However, the decrease in prevalence occurs at the same rate in the ticks that fed on vaccinated and unvaccinated mice such that the effect of the vaccine remains unchanged due to this phenomenon. The bars represent the means and the whiskers represent the 95% confidence intervals.

the daily probability of a mouse encountering the vaccine, we can calculate the proportion of mice that will be vaccinated a specified number of times within a specified time period. For example, our data indicate that 47% of all *P. leucopus* mice would receive a complete vaccination schedule (15 doses) within 19 days and over 90% of mice would be completely vaccinated within 24 days. Thus, high vaccination coverage is readily achievable within a short period of time.

Reducing NIP through wildlife vaccination

Oral vaccination of wild mouse populations can reduce the NIP both by preventing infection in uninfected mice and reducing the transmission from infected mice (Figs. 5 and 6). Importantly, reductions in current NIP reinforce lower future NIP by increasing the proportion of mice that can be vaccinated prior to exposure to a *B. burgdorferi*-infected tick, which in turn reinforces lower future NIP in this positive feedback loop. In the absence of vaccination, the equilibrium NIP is 0.270, which is similar to the NIP observed in natural systems (LoGiudice et al. 2003, Brisson and Dykhuizen 2004, Tsao et al.

2004, Hoen et al. 2009). Using the estimates of vaccination efficacy ($V_E=6/9=0.667$) and the rate of *B. burgdorferi* transmission from infected and vaccinated mice ($C_{IV}=43/71=0.606$) from this study, and optimistic vaccination parameters (100% vaccination coverage, 15 days to full vaccination, daily nymphal attachment rate=0.05 nymphs/day), our model predicts an equilibrium NIP of 0.097 (Fig. 5). The estimates of equilibrium NIP were highly sensitive to vaccine coverage and the daily nymphal attachment rate. More conservative estimates of these vaccination parameters led to greater equilibrium NIP.

The importance of the vaccine's ability to reduce *B. burgdorferi* transmission from infected mice to feeding ticks (C_{IV}) is readily apparent when the time to complete vaccination is long ($D=56$ days) and the nymphal attachment rate is high (1.00 nymphs/day) (Fig. 6). Under these circumstances, virtually all mice become infected prior to becoming vaccinated. Despite these challenging conditions, high vaccination coverage ($V_C=0.90$) with an effective oral vaccine ($V_E>0.80$) that reduces *B. burgdorferi* transmission from infected mice to feeding ticks ($C_{IV}<0.10$) has the potential to reduce the

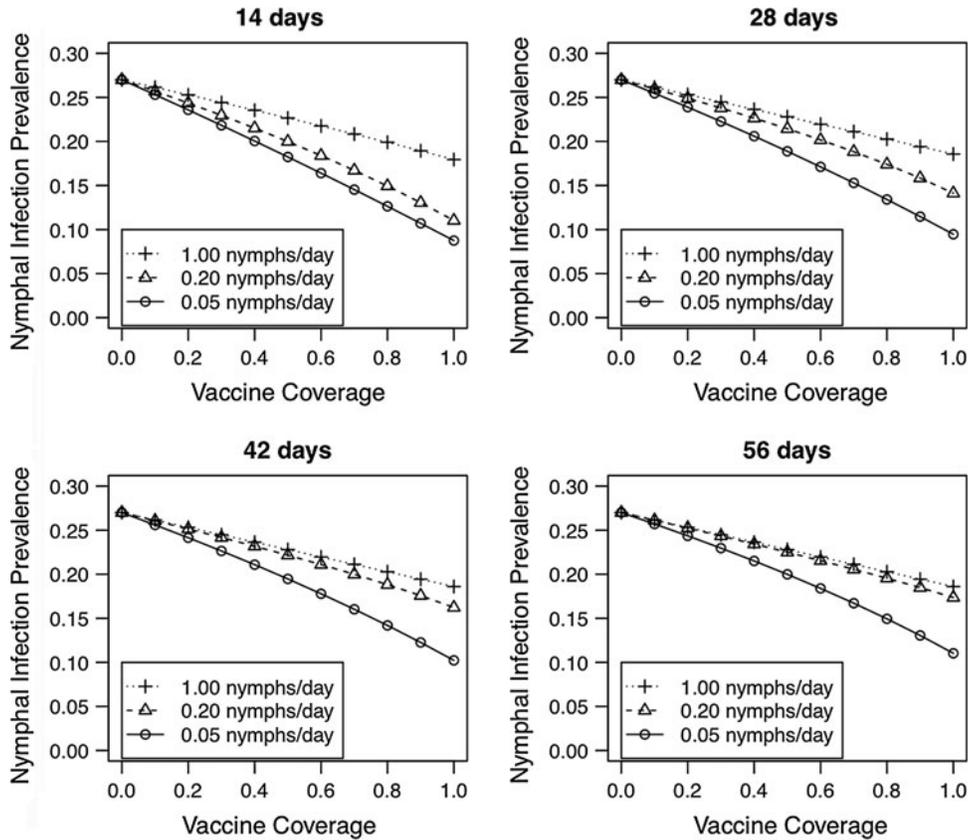


FIG. 5. Wildlife vaccination can result in dramatic reductions in the nymphal infection prevalence (NIP) by preventing infection in uninfected mice and reducing the transmission from infected mice. The equilibrium NIP decreases with vaccination coverage and increases with the nymphal attachment rate ($N=0.05$, 0.20 , and 1.00 nymphs/day) and the time to complete vaccination ($D=14$, 28 , 42 , and 56 days). The vaccination efficacy parameter ($V_E=6/9=0.67$) and the *B. burgdorferi* transmission rate from infected and vaccinated mice ($C_{IV}=43/71=0.60$) were estimated from the data in the present study.

equilibrium NIP to below 10%, even under otherwise challenging vaccination conditions.

Discussion

Oral vaccination of *P. leucopus*, an important wildlife host of *B. burgdorferi*, elicits an immune response that reduces transmission of *B. burgdorferi* at the two critical stages of the Lyme disease life cycle. Consistent with previous studies (Fikrig et al. 1991, Gomes-Solecki et al. 2006, Scheckelhoff et al. 2006, del Rio et al. 2008, Richer et al. 2011), oral vaccination of uninfected mice prevents transmission of *B. burgdorferi* from infected ticks to uninfected mice due to a strong immune response. Here we demonstrate that oral vaccination of previously infected mice with an OspA protein significantly reduces the transmission of *B. burgdorferi* to feeding larval ticks. Projection modeling suggests that the two effects of the vaccine on the transmission cycle of *B. burgdorferi* will act synergistically in a positive feedback loop to reduce the nymphal infection prevalence in wildlife communities well below what would be expected from either effect alone. This study suggests that oral immunization of wildlife with an OspA-based vaccine is a promising long-term strategy to reduce human Lyme disease risk.

Uninfected *P. leucopus* mice immunized with an easily distributable OspA vaccine were protected from *B. burgdorferi*

infection when challenged with infected ticks (Table 1). As shown previously (Fikrig et al. 1992b), the protective feature of the vaccine was caused by the immune response of the vaccinated mice, which also cleared *B. burgdorferi* from a substantial proportion of the challenge nymphs. However, it is important to note that the “nymphal clearance” aspect of the vaccine is unlikely to affect its capacity to break the transmission cycle of Lyme disease in nature (Tsao 2009, Tsao et al. 2001, 2004). This is because nymphs molt to adults, which tend to feed on large hosts such as deer, which are not competent for transmitting *B. burgdorferi* (Bosler et al. 1984, Magnarelli et al. 1984, Telford et al. 1988, Jaenson and Talleklint 1992).

With respect to the capacity of the agar vaccine to protect mice, our results are directly relevant to field conditions because the experimental mice were challenged with ticks that were infected with a variety of natural strains. Furthermore, the number of infected ticks that fed on each mouse (mean = 5.9 nymphs; range = 1–8 nymphs), and thus the infectious dose of *B. burgdorferi* and the associated immunomodulatory tick salivary proteins (Randolph 2009), were greater in this study than what *P. leucopus* mice experience in nature (~ 0.2 nymphs/day) (Mannelli et al. 1994, Daniels and Fish 1995, Lyon et al. 1996, Brisson and Dykhuizen 2004). Thus, wild mice may be better protected from *B. burgdorferi* than suggested by the present data if the inoculating dose of

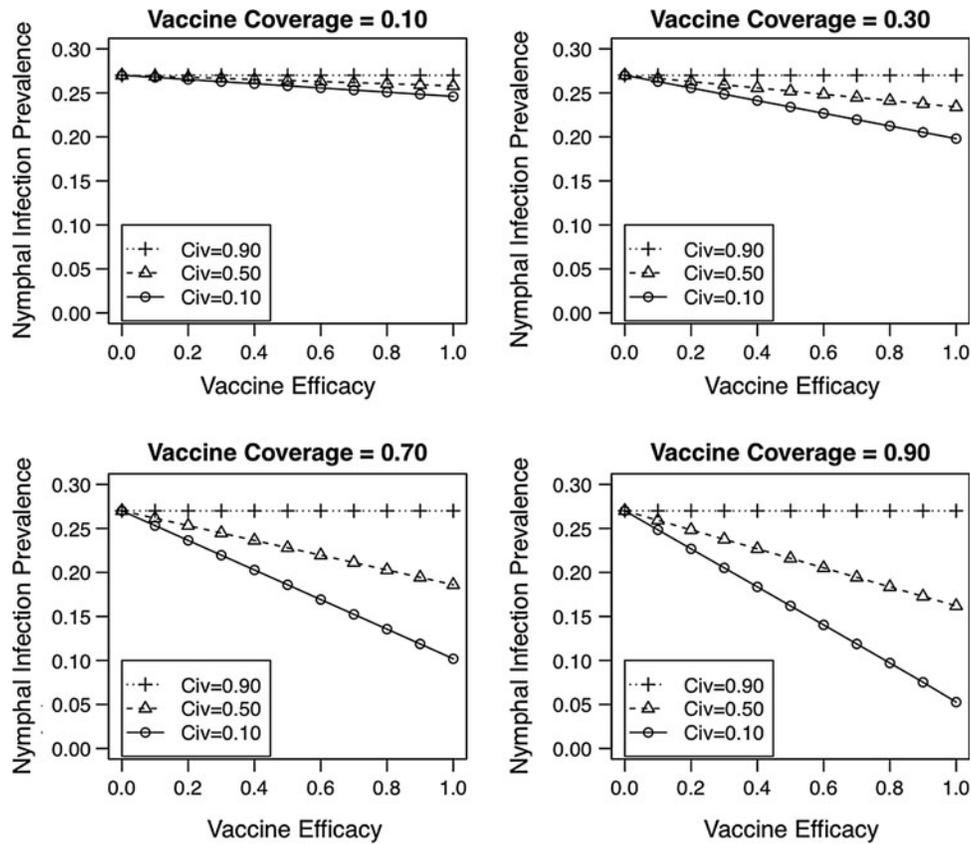


FIG. 6. The nymphal infection prevalence depends strongly on the capacity of the vaccine to reduce *B. burgdorferi* transmission (C_{IV}) from infected mice to feeding ticks. The equilibrium NIP decreases with higher vaccination coverage and vaccine efficacy (V_E) and decreases at lower values of C_{IV} . These simulations assumed the most conservative vaccination conditions with respect to the time to complete vaccination ($D=56$ days) and nymphal attachment rate ($N=1.00$ nymphs/day).

B. burgdorferi or the concentration of tick salivary proteins affects the probability of successful infection (Ribeiro et al. 1990, Nuttall and Labuda 2004). Further data are needed to determine if the infection of mice in this study, despite prior vaccination, resulted from the extreme dose of *B. burgdorferi* or salivary proteins that overwhelmed the vaccine protection, or if some strains of *B. burgdorferi* are less affected by the OspA immunogen.

Vaccinating previously infected mice effectively reduced mouse-to-tick transmission of *B. burgdorferi* from 90.0% to 60.6% (Fig. 4) despite a statistically nonsignificant immune response caused by the vaccine. Interestingly, the mouse-to-tick transmission efficiency varied substantially among infected, vaccinated mice, with 1 mouse showing no effect while others showed a 63% reduction (Table 1). Such variation can have important consequences for field vaccination strategies if ticks aggregate on rodent hosts that are the least responsive to the oral vaccination treatment. This scenario is plausible because ticks are often aggregated on their hosts (Randolph et al. 1996, Randolph et al. 1999, Brunner and Ostfeld 2008, Devey and Brisson 2012) and tick saliva is known to inhibit the host immune response (Wikel 1999, Randolph 2009). Although there was a negative relationship between anti-OspA IgG levels and the transmission rate to feeding ticks, this relationship was not statistically significant, likely due to the very limited range of the immune responses among these mice. Thus, we cannot definitively say that the anti-OspA IgG

levels are the mechanism that caused the transmission rate reduction in the vaccinated mice. One possibility is that the anti-OspA IgG levels were higher in the experimental mice at the time of the nymphal challenge (day 41) and then declined so that they were no longer different from the control mice after xenodiagnosis (day 90).

The effects of the OspA vaccine on mouse-to-tick transmission described here are conservative as the experimental mice received a very weak vaccination schedule (4 doses * 0.203 μ g rOspA per dose) and had a correspondingly weak anti-OspA immune response (Fig. 3). More intense vaccination schedules, which are feasible in natural conditions, may further reduce mouse-to-tick transmission, although more experimental data are needed to confirm this hypothesis. Two sets of data suggest that more intense vaccination schedules are feasible under natural conditions. First, *P. leucopus* in this study ate >90% of the agar containing 10^{10} cells of live OspA-expressing *E. coli* over the 15 immunization sessions. Second, in an observational pilot study, field-captured *P. leucopus* mice willingly ate agar containing *E. coli* even in the presence of other food resources (pellet food or apple). In addition, PVC tubes containing agar that were distributed at our field site had evidence of rodent activity, including substantial reductions in agar, teeth marks in the agar, and rodent droppings in the PVC tube. Although observational, these data suggest wild *P. leucopus* willingly consume agar-based vaccines. Finally, our capture rate estimates suggest that it is possible to

vaccinate a large proportion of the population (>90%) in a short period of time (24 days).

Previous studies demonstrate that parenteral immunization of previously infected *P. leucopus* mice with purified rOspA can reduce *B. burgdorferi* transmission to as little as 1% after just 3 immunizations. A recent experiment similarly demonstrated that vaccinating previously infected *P. leucopus* mice with a vaccinia virus-based OspA vaccine also significantly reduced transmission to feeding ticks, although not as effectively as parenteral immunizations (Bhattacharya et al. 2011). This vaccinia-based vaccine has the added advantage of requiring only a single immunization for inducing a strong immune response (Scheckelhoff et al. 2006, Bhattacharya et al. 2011). However, the mode of action of vaccinia-based vaccines differs considerably from the data shown here because the virus does not express the immunogen directly but infects host cells, which then express the immunogen (Scheckelhoff et al. 2006). This methodology has proven highly effective but has several potential regulatory hurdles because vaccinia-based vaccines are infectious to people and can be difficult to control in nature (McGuill et al. 1998, Rupprecht et al. 2001, Sepkowitz 2003). Thus, it is important to understand if oral immunization with a protein antigen is effective at reducing mouse-to-tick transmission despite promising data from needle- and virus-based delivery vehicles.

The reduction in the proportion of ticks testing positively for *B. burgdorferi* caused by the OspA immunization was apparent in both newly molted and 150-day-old nymphal ticks (Fig. 4). Interestingly, the proportion of *B. burgdorferi*-infected ticks was significantly lower in the 150-day-old ticks from both the control mice and the vaccinated mice (Fig. 4). These data may suggest that the duration of *B. burgdorferi*-survival in *I. scapularis* nymph midguts is limited. However, the experimental ticks were maintained at room temperature for 6 months, which is not analogous to the natural conditions that *B. burgdorferi* experiences in overwintering nymphs in the northeastern United States. Furthermore, the reduction in spirochete load in all ticks was not a product of vaccination and may not be biologically relevant. There are several experimental factors that may influence the survivorship of spirochetes in ticks, none of which were explored here. Future investigations into the effects of factors such as temperature, humidity, and tick activity-levels on the survivorship of *B. burgdorferi* in tick midguts are necessary to understand the relevance of these data. Future experiments should keep replicate samples of ticks in different incubators.

The anti-OspA IgG levels from the mice that were not protected by OspA vaccination decreased significantly after these mice became infected with *B. burgdorferi*. Although there was no difference in the anti-OspA IgG levels among vaccinated mice prior to nymphal challenge, the anti-OspA IgG levels were significantly reduced in the mice that became infected, suggesting that infection with *B. burgdorferi* may have caused a reduction in the standing anti-OspA IgG antibody levels relative to the mice that were successfully protected (Fig. 2). The transmission rate of *B. burgdorferi* to feeding larval ticks from these infected mice was also very high and similar to the transmission rate from control mice (Table 1). These results may suggest that, despite a strong immune response and apparent success of the vaccination, there was neither a protective effect nor an effect on transmission suggesting vaccination failure. Additionally, these

data may suggest that infection with *B. burgdorferi* may hinder an immune response against an oral OspA-based vaccine, a topic that should be investigated explicitly in future studies. Regardless, vaccinating previously infected mice significantly reduces mouse-to-tick transmission and thus could have a substantial impact on the prevalence of infected ticks in natural systems (Figs. 4–6).

Our model assumes that vaccinated mice remain protected for the duration of their lifetimes. This assumption was validated by a previous study that found that uninfected mice gained yearlong protection following oral OspA immunization (Richer et al. 2011). Further studies are necessary to determine the duration of transmission reduction following oral OspA immunization of *B. burgdorferi*-infected mice. However, the agar-matrix-based oral vaccine described in this study could be easily distributed to wildlife throughout the nymphal activity season and thus provide a continual booster to increase vaccine efficacy. Agar has little digestible material and thus adding agar-matrix-based baits to a natural ecosystem is unlikely to affect *P. leucopus* population demography dramatically. In addition, our bait vaccine formulations are readily eaten by wildlife and remain immunogenic for several days under environmental conditions representative of the summers in the northeastern United States (Brisson, unpublished data). Restricted distribution of oral vaccines to the primary natural reservoirs of *B. burgdorferi* (small mammals) can be achieved via bait palatability or by bait repositories that exclude larger animals, as shown in our preliminary field trials. Hence, any shortcomings in the duration of vaccine efficacy could be overcome using an extended vaccination schedule with an easily distributable bait vaccine.

The inhibitory effects of OspA vaccination on both tick-to-mouse and mouse-to-tick transmission will benefit wildlife vaccination campaigns aimed at reducing the prevalence of infected nymphal ticks and thus human Lyme disease risk (Figs. 5 and 6). The reductions in mouse-to-tick transmission are imperative in practice because a majority of mice in Lyme disease endemic areas cannot be vaccinated prior to becoming infected (Bunikis et al. 2004). Our projection models suggest that the reductions in NIP achieved by vaccinating infected mice will increase the probability of successfully vaccinating uninfected mice prior to exposure with infected ticks, which further reduces NIP. Thus, the inhibition of mouse-to-tick and tick-to-mouse transmission works synergistically to reduce NIP below levels that would be expected from either effect alone. The model results suggest that the equilibrium NIP achieved through a vaccination campaign is sensitive to the proportion of animals vaccinated, the efficacy of the vaccine in protecting animals from infection, and the ability of the vaccine in reducing transmission from infected mice.

Although vaccine coverage in *P. leucopus* mice is relatively easy to achieve, future studies should investigate whether a more aggressive oral vaccination regime could improve the protection efficacy and reduce the transmission rates from infected animals to levels similar to those reported from needle vaccination studies (Tsao et al. 2001). Combining *B. burgdorferi*-targeted and *I. scapularis*-targeted oral vaccines will also likely amplify these synergistic effects by further reducing the number of ticks that feed on mice (Maritz-Olivier et al. 2007). Although the data and models presented have focused on *P. leucopus* mice, a major wildlife reservoir of

B. burgdorferi, these results can be extended to include other wildlife reservoirs as data on the effect of the vaccine on other species become available. These results suggest that wildlife vaccination can be an effective and long-term strategy to reduce human Lyme disease risk.

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Author Disclosure Statement

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