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BRIEF REPORT

Deployment of a Reservoir-Targeted Vaccine Against *Borrelia burgdorferi* Reduces the Prevalence of *Babesia microti* Coinfection in *Ixodes scapularis* Ticks

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In the Northeast and upper Midwest of the United States, *Babesia microti* and *Borrelia burgdorferi* use *Ixodes scapularis* ticks as vector and *Peromyscus leucopus* mice as major reservoir host. We previously established, in a 5-year field trial, that a reservoir-targeted outer surface protein A vaccine reduces the prevalence of *B. burgdorferi*-infected ticks. We accessed ticks and mouse blood samples collected during the trial, extracted total DNA, and amplified the *B. microti 18S rRNA* gene. Vaccine deployment reduced the prevalence of ticks coinfected with *B. microti* and that of mice infected with *B. microti*. Breaking the enzootic cycle of *B. burgdorferi* may reduce the incidence of babesiosis.

Keywords. *Babesia microti*; *babesiosis*; *Borrelia burgdorferi*; *coinfection*; *Lyme disease*; *Peromyscus leucopus*; tick; vaccine.

In the United States, nearly all cases of human babesiosis are caused by the hemoparasite *Babesia microti* and develop following the bite of an infected *Ixodes scapularis* tick [1]. Common symptoms include fatigue, fever, chills, sweats, headache, myalgia, and anorexia. In the elderly and the immunocompromised, the illness can be severe. Complications include severe anemia, acute respiratory distress syndrome, and renal insufficiency or failure. Despite antimicrobial therapy, babesiosis can be fatal. No vaccine is available. Current prophylaxis, which is limited to endemic area avoidance and tick exposure reduction, has been ineffective in containing the emergence of babesiosis [2].

Babesia microti is maintained in its enzootic cycle by alternate transmission between *I. scapularis* ticks and reservoir hosts such as white-footed mice (*Peromyscus leucopus*), short-tailed shrews, and eastern chipmunks [3, 4]. Larval ticks, which are free of *B. microti* because the parasite does not colonize the ovaries of adult female ticks, may acquire *B. microti* when taking a blood meal from reservoir hosts. Nymphs that result from the molt of infected larvae harbor *B. microti* and infect reservoir hosts upon which they feed. If nymphs become infected when feeding, adult ticks that result from the molt are infected. Thus, the enzootic cycle of *B. microti* resembles that of *Borrelia burgdorferi*, the causative agent of Lyme disease [4].

Ixodes scapularis nymphs are the primary vector for transmission of *B. microti* to humans [1], which are incidental hosts. Adult female ticks also transmit *B. microti* to humans. Given that *I. scapularis* nymphs and adults can transmit *B. microti* and *B. burgdorferi* simultaneously [4], a diagnosis of babesiosis should prompt testing for Lyme disease [5]. Conversely, babesiosis should be considered in Lyme disease patients for whom symptoms are severe, not typical of Lyme disease, or poorly responsive to standard antibiotic therapy [5].

Babesiosis is reported from areas that have long been endemic for Lyme disease but not from those in which *B. burgdorferi* recently spread [4]. The delayed spread of babesiosis has been attributed to the poor transmission of *B. microti* from reservoir host to tick and to the facilitation of this transmission by *B. burgdorferi* [6]. To assess the importance of *B. burgdorferi* in maintaining *B. microti* in their shared enzootic cycle, we accessed *I. scapularis* ticks and *P. leucopus* blood samples collected during a field trial of an oral reservoir-targeted vaccine against *B. burgdorferi* [7].

METHODS

Ethics Statement

Experiments were in compliance with the regulations of the Institutional Biosafety Committees (IBC) and Institutional Animal Care and Use Committees (IACUC) of the Cary Institute of Ecosystem Studies (IACUC 07-021, 10-01III), the University of Tennessee Health Science Center (IBC 08-313/12-416; IACUC 1741/13-010/13-012/14-007), and Tufts Medical Center (IBC 2013-TIA61; IACUC B2014-56).

Vaccine Deployment

The field study was conducted at the Cary Institute of Ecosystem Studies and has been described elsewhere [7].

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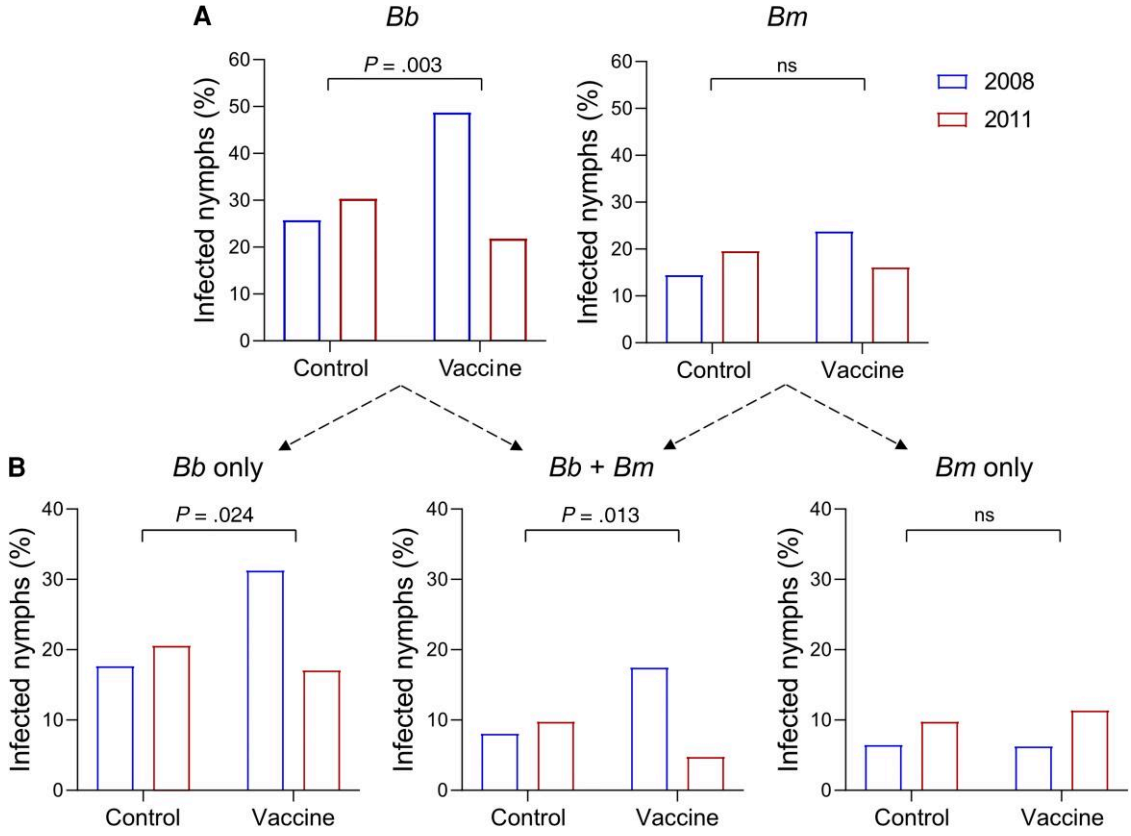

Figure 1. A reservoir-targeted outer surface protein A (OspA) vaccine reduces the prevalence of *Ixodes scapularis* ticks coinfected with *Borrelia burgdorferi* (*Bb*) and *Babesia microti* (*Bm*). From mid-May to mid-September, crimped oat baits were placed at control sites (labeled as "control") and vaccination sites (labeled as "vaccine") [7]. Baits for vaccination sites contained lyophilized OspA-expressing *Escherichia coli*, whereas baits for control sites contained no *E. coli*. From May to June, ticks were collected by drag sampling. Ticks available for the present study (see Supplementary Table 1) were those collected in 2008 and 2011 at 2 control sites (Ctrl1, Ctrl2) and 2 vaccination sites (NY1, NY2). Ticks were tested for *Bb* and *Bm* by amplifying the *flaB* gene and the *18S rRNA* gene, respectively. (*A*) Graphs depict the proportions (%) of ticks infected with *Bb* or *Bm* among those collected at the control sites (n = 62 in 2008, n = 102 in 2011) and the vaccination sites (n = 80 in 2008, n = 105 in 2011). Whether deployment of the OspA vaccine had an effect on either pathogen was tested by use of binomial logistic regression. The term "site [vaccine] * time [2011]" differed from the term "site [control] * time [2008]" for *B. burgdorferi* ($P = .003$) but not for *B. microti* ($P = .145$). (*B*) Graphs depict the proportions (%) of ticks infected with a single pathogen or both pathogens among those collected at the control and vaccination sites (see A). Whether deployment of the OspA vaccine had an effect on single infection and/or coinfection was tested by use of multinomial logistic regression (see Supplementary Table 2). The term "site [vaccine] * time [2011]" differed from the term "site [control] * time [2008]" for singe infection with *B. burgdorferi* ($P = .024$) and for coinfection ($P = .013$) but not for single infection with *B. microti* ($P = .69$). ns, not significant ($P > .05$).

Briefly, vaccination plots and control plots had comparable basic vegetative structure and small mammal community. Each plot consisted of an 8-by-8 array of Sherman live traps, which were distributed every 15 m. Plots were separated by at least 500 m, ensuring biological and statistical independence. From mid-May to mid-September, in the late afternoon of each workday, every trap was set with 1 single bait. Baits for vaccination plots consisted of crimped oats admixed with 200 mg of lyophilized, outer surface protein A (OspA)-expressing *Escherichia coli*. Baits for control plots consisted of crimped oats only.

Sample Collection

In May and June, on at least 2 occasions, host-seeking nymphs were collected from each plot by drag sampling along a series of linear transects [7]. In August and September, blood was drawn at the submandibular veins of *P. leucopus* mice randomly selected at the Sherman traps (see section above). Blood specimens (50–100 µL) were stored at −20°C.

Pathogen Detection

Individual ticks were crushed using single-use sterile pestles. Total DNA was extracted from tick homogenates and mouse blood samples using DNeasy Blood & Tissue kits (QIAGEN, Valencia, CA). *Borrelia burgdorferi* was detected by amplifying the *flaB* gene, as previously described [7]. *Babesia microti* was detected by amplifying the *18S rRNA* gene (see Supplementary Material). A tick or a mouse was declared infected with *B. microti* when ≥10 copies of the *18S rRNA* gene were detected per reaction.

Statistical Analysis

Differences in the proportion of infected ticks or infected mice were tested for significance by use of logistic regression. Differences in pathogen burden (log-transformed data) were tested for significance by use of two-way analysis of variance (ANOVA) followed by Fisher's LSD test or by unpaired *t* test with Welch's correction. A difference was declared significant when $P \leq .05$. Multinomial logistic regression was run using SPSS v.28.0.1.1; all other analyses were run using GraphPad Prism v.9.3.1.

RESULTS

Most nymphs that had been tested for *B. burgdorferi* during our field trial [7] were available for *B. microti* testing (see Supplementary Table 1). As expected, OspA vaccination reduced the prevalence of *B. burgdorferi*-infected nymphs (Figure 1*A*; binomial logistic regression, $P = .003$ for site*time) but failed to reduce the prevalence of *B. microti*-infected nymphs ($P = .145$). We next classified nymphs as infected with *B. burgdorferi*, *B. microti*, or both (Figure 1*B*). OspA vaccination reduced the prevalence of nymphs infected with only *B. burgdorferi* (multinomial logistic regression, $P = .024$) as well as the prevalence of coinfected nymphs ($P = .013$) but had no effect on the prevalence of nymphs infected with only *B. microti* ($P = .69$). At the end of the 4-year period, when compared with the start of this period (see Supplementary Material), the odds of finding a nymph infected with only *B. burgdorferi* were 3.5 times lower at a vaccination site than at a control site (odds ratio [OR] = 0.28) (Supplementary Table 2). The odds of finding a coinfected nymph were 7.5 times lower at a vaccination site than at a control site (OR = 0.13) (Supplementary Table 2). We conclude that our reservoir-targeted vaccine is effective at clearing *B. microti* from coinfected nymphs and that this effect does not come at the cost of a greater prevalence of nymphs infected with only *B. microti*.

Coinfection of *P. leucopus* mice with *B. burgdorferi* and *B. microti* facilitates the transmission of *B. microti* to feeding larvae as revealed by the greater prevalence of *B. microti* in nymphs generated by the molt of these larvae [6]. To test whether *B. microti* burden is greater in nymphs that are coinfected with *B. burgdorferi*, we turned to nymphs collected at the control sites in 2008 and 2011. *Babesia microti* burden, as assessed by the mean *18S rRNA* gene copy number, did not differ between coinfected ticks and ticks infected with only *B. microti* (Supplementary Figure 1*A*). Likewise, *B. burgdorferi* burden, as assessed by the mean *flaB* gene copy number, did not differ between coinfected ticks and ticks infected with only *B. burgdorferi* (Supplementary Figure 1*C*). To test whether OspA vaccination lowers the pathogen burden of ticks, we analyzed ticks collected at the control and vaccination sites. Having established that *B. burgdorferi* coinfection has no impact on *B. microti* burden and vice versa, we classified ticks as infected with *B. microti* (Supplementary Figure 1*B*) or

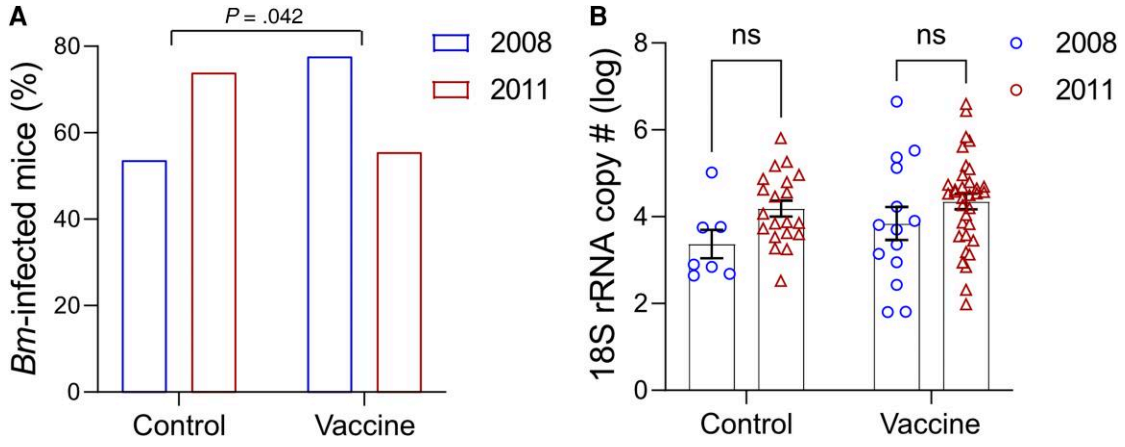

Figure 2. A reservoir-targeted outer surface protein A (OspA) vaccine reduces the prevalence of *Peromyscus leucopus* mice infected with *Babesia microti*. Vaccination baits and control baits were deployed as reported in Figure 1 legend. From August to September, *P. leucopus* mice were trapped at control (labeled as "control") and vaccination sites (labeled as "vaccine"). Mice were randomly selected for a blood draw. Blood specimens available for the present study were those collected in 2008 and 2011 at 1 control site (Ctrl2) and 2 vaccination sites (NY1, NY2). Total DNA was extracted and the *B. microti 18S rRNA* gene amplified. (*A*) The graph depicts the proportions (%) of *B. microti*-infected *P. leucopus* mice among those trapped at the control (n = 13 in 2008, n = 27 in 2011) and vaccination sites (n = 18 in 2008, n = 61 in 2011). Whether deployment of the OspA vaccine had an effect on *B. microti* infection was tested by use of binomial logistic regression (see Supplementary Table 3). The term "site [vaccine] * time [2011]" differed from the term "site [control] * time [2008]" ($P = .042$). (*B*) The graph depicts *18S rRNA* gene copy numbers detected in blood from *P. leucopus* mice trapped at the control (n = 7 in 2008, n = 20 in 2011) and vaccination sites (n = 14 in 2008, n = 34 in 2011). Log copy numbers are reported individually and as mean ± standard error of the mean. Whether deployment of the OspA vaccine had an effect on *B. microti* burden was tested by use of two-way analysis of variance. No such effect was observed ($P = .60$ for site*time). An overall time effect was noted ($P = .026$), but increases in *B. microti* burden at the control ($P = .09$) and vaccination ($P = .14$) sites did not reach statistical significance (post-hoc analysis using Fisher's LSD test). ns, not significant ($P > .05$).

B. burgdorferi (Supplementary Figure 1*D*). In either tick population, OspA vaccination had no effect on pathogen burden (either pathogen; two-way ANOVA, $P > .05$ for site*time). We conclude that *B. microti* burden in questing nymphs is neither increased by coinfection with *B. burgdorferi* nor reduced by the deployment of our reservoir-targeted OspA-based vaccine.

Unlike *B. burgdorferi* spirochetes, which reside transiently in the bloodstream, *B. microti* parasites are easily detected in blood because they are obligate pathogens of red blood cells [1, 4]. Blood samples obtained from *P. leucopus* mice at the control and vaccination sites were tested for the *B. microti 18S rRNA* gene. Deployment of our OspA-based vaccine reduced the prevalence of *P. leucopus* mice infected with *B. microti* (Figure 2*A*; binomial logistic regression, $P = .042$ for site*time). At the end of the 4-year period, when compared with the start of the experiment, the odds of finding a mouse infected with *B. microti* were 6.8 times lower at a vaccination site than at a control site (OR = 0.15) (Supplementary Table 3). Among mice that remained infected with *B. microti*, OspA vaccination had no effect on their burden (Figure 2*B*; two-way ANOVA, $P > .05$ for site*time). We conclude that our reservoir-targeted vaccine reduces the prevalence of *B. microti*-infected *P. leucopus* mice without reducing their pathogen burden.

DISCUSSION

Nearly all cases of babesiosis are reported from the Northeast and the upper Midwest of the United States, particularly from areas that have long been endemic for Lyme disease [2, 4]. In these areas, Lyme disease has greater incidence than babesiosis, a difference attributed to the greater prevalence of *B. burgdorferi*-infected *I. scapularis* nymphs [8]. Some of the nymphs that harbor *B. burgdorferi* also harbor *B. microti*. The prevalence of coinfected nymphs is greater than predicted by independent assortment of the 2 pathogens [4, 9], stressing the importance of coinfected nymphs to the rising incidence of babesiosis. In the past 2 decades, considerable efforts have been invested in developing a reservoir-targeted vaccine that would reduce the incidence of Lyme disease [7, 10, 11]. We previously reported that deployment of one such vaccine reduces the prevalence of *B. burgdorferi*-infected nymphs [7]. We now report that this OspA-based vaccine reduces the prevalence of nymphs coinfected with *B. microti*.

OspA antibodies interrupt the enzootic cycle of *B. burgdorferi* by preventing transmission of *B. burgdorferi* from nymph to host and from host to larva [12, 13]. How can OspA antibodies interfere with *B. microti*? OspA antibodies are unlikely to be detrimental to *B. microti* in feeding nymphs because OspA antibodies ingested with a blood meal are confined to the midgut, whereas *B. microti* sporogony is confined to the salivary glands [1]. Accordingly, OspA vaccination of humans will not protect them from *B. microti* present in feeding nymphs. Keeping in mind that we studied host-seeking (unfed) nymphs, we postulate that OspA antibodies interfere with *B. microti* in feeding larvae. As larvae feed on a coinfected host, *B. burgdorferi* spirochetes and *B. microti*-infected red blood cells accumulate in their midgut. If the host has been immunized with OspA, OspA antibodies contained in the blood meal block the acquisition of spirochetes by larvae in a host complement-dependent manner [14]. We speculate that complement activation in the gut lumen of larvae that feed on OspA-immunized coinfected hosts leads to the lysis of red blood cells, including those containing *B. microti*, thereby explaining why our vaccine is effective against coinfected nymphs but spares nymphs that are infected with only *B. microti*. Red blood cell lysis would occur early because spirochetes begin expressing OspA in the larva midgut within 24 hours postattachment [13]. Early red blood cell lysis would halt the metamorphosis of *B. microti* gametocytes and the ensuing generation of gametes, processes that are initiated in red blood cells while in the tick midgut and are completed only when ticks are replete [15]. Experiments are needed to establish that complement-mediated red blood cell lysis can halt sexual reproduction of *B. microti*.

Reservoir hosts that ingest an OspA-based vaccine are not protected from *B. burgdorferi* infection because spirochetes express OspA in the tick midgut but not in their salivary glands [12, 13]. Accordingly, reduction in the prevalence of *B. microti*-infected mice by our vaccine is best explained by a reduction in the prevalence of coinfected larvae before their molt to coinfected nymphs. By reducing the prevalence of nymphs that are infected with only *B. burgdorferi* [7], our vaccine may also hinder the maintenance of *B. microti* in its enzootic cycle because (1) white-footed mice that are chronically infected with *B. microti* may become infected with *B. burgdorferi* and (2) such coinfection may create an immunological conflict that favors *B. microti* in mice [4].

As the geographic range of *B. microti* continues to expand into areas already endemic for *B. burgdorferi*, the incidence of coinfections in ticks and humans will rise further. In this study, we provide evidence that a reservoir-targeted OspA vaccine is effective at reducing the prevalence of coinfection with *B. burgdorferi* and *B. microti* in their shared tick vector and the prevalence of *B. microti* infection in their major reservoir host. Initially designed to reduce the risk of Lyme disease, our reservoir-targeted vaccine may also reduce the risk of concurrent babesiosis.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. MGS has stocks and patents that relate to the use of a reservoir-targeted OspA-based vaccine.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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