

# Efficacy of a Nonadjuvanted, Outer Surface Protein A, Recombinant Vaccine in Dogs After Challenge by Ticks Naturally Infected with *Borrelia burgdorferi*\*

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## ■ ABSTRACT

In a blinded, controlled study, thirty purpose-bred, *Borrelia burgdorferi* negative, mixed-breed dogs 10 to 12 weeks of age were randomly divided into three groups of ten animals each for the purpose of evaluating a recombinant nonadjuvanted *B. burgdorferi* OspA vaccine (Recombitek™ Lyme [Merial Limited]) for efficacy and safety. Two groups received two doses of two different lots of a nonadjuvanted, OspA, recombinant vaccine; the third group served as nonvaccinated controls. All dogs were challenged 3 weeks after the second vaccination with blacklegged deer ticks (*Ixodes scapularis*)

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harvested from a *B. burgdorferi* endemic area in Rhode Island. Clinical signs, antibody titers by ELISA, Western blot assays, and isolation and polymerase chain reaction analyses of spirochetes from biopsy specimens were used to evaluate vaccine efficacy. Direct fluorescent antibody assay was used to evaluate the infection rate in the challenge ticks and in naïve ticks allowed to feed on study dogs after the dogs were infected (xenodiagnosis). Vaccinates responded with high levels of antibodies (determined by ELISA and measured by optical density [OD]), which did not rise after challenge. Vaccinates demonstrated no clinical signs, negative isolation of spirochetes on biopsy, only an OspA antibody pattern on Western blot assay, and neg-

ative isolation of spirochetes on biopsy, confirming that the vaccine blocked infection with *B. burgdorferi* in all vaccinated dogs (20/20). Control dogs demonstrated clinical signs (2/10), antibodies characteristic of infection with *B. burgdorferi* (10/10), isolation of spirochetes (10/10), and polymerase chain reaction (PCR) detection of spirochetes (9/10). The recombinant, nonadjuvanted *B. burgdorferi* vaccine protected 100% of vaccinates against infection after a severe challenge that infected 100% of control dogs. The OspA vaccine proved to be highly safe and effective in this study.

## ■ INTRODUCTION

Lyme disease is caused by the spirochete *Borrelia burgdorferi* and is transmitted by ixodid ticks in the genus *Ixodes*, particularly *Ixodes scapularis*. Lyme disease is the most commonly reported vector-borne disease in the United States, occurring in humans and dogs in endemic areas. The largest number of reported cases is in northeastern, mid-Atlantic, and upper north central regions of the US and in several counties in California. There has been a trend of increasing incidence in endemic areas as well as geographic spread to new areas.<sup>1</sup> The risk of Lyme disease is calculated by the distribution, abundance, and rates of infection with *B. burgdorferi* of the *Ixodes* vector in addition to reported cases and location.<sup>2</sup> Lyme disease can also affect horses, cattle, and cats and occurs in Europe and Asia. In dogs, infection with *B. burgdorferi* causes acute, subacute, and recurrent arthritis with myalgia, fever, anorexia, and lethargy, with rare reports of renal failure, heart block, and neurologic disease.<sup>3-6</sup> In experimentally infected dogs, acute, recurrent lameness has been observed 2 to 5 months after tick exposure. Even in the absence of clinical signs, mild polyarthritis has been detected by histopathology of synovial membranes at least 1 year after exposure.<sup>7</sup>

Infection may be lifelong in untreated animals; the organism has been isolated from dogs as long as 1 year after tick exposure and detected by serology during 17 months of observation.<sup>7-9</sup> Studies have demonstrated that a 30-day regimen of antibiotics is not effective in eliminating *B. burgdorferi* from dogs: Antibody levels dropped shortly after treatment, then began to rise 6 months after treatment, and the spirochete could again be isolated and demonstrated by polymerase chain reaction (PCR) from treated dogs.<sup>8,9</sup> Diagnosis of Lyme disease is difficult: Signs may be vague, a history of exposure 2 to 5 months before onset may be difficult to elicit, clinical pathology is unremarkable, and ELISA serology may indicate nonrelevant exposure or prior vaccination. The Western blot assay is required to differentiate between postvaccination and infectious titers.<sup>3-5</sup>

Vaccination of dogs in endemic areas is recommended because of the inapparent nature of the disease and difficulty in diagnosing and treating it.<sup>10-13</sup> Although whole cell vaccines for the dog have been available for many years, this formulation has been eliminated from consideration in human medicine. There is evidence that Lyme disease pathology may be caused in part by autoimmunity.<sup>14-16</sup> Antibodies to spirochetal antigens cross-react with human nerve, synovial, hepatic, cardiac, and skeletal tissues, raising the specter of immune-mediated chronic inflammatory disease.<sup>17</sup> Appel and Jacobson have reported Lymelike signs in dogs in association with a whole-cell bacterin in the absence of infection with *B. burgdorferi*.<sup>4,11</sup> Severe local reactions are also a potential problem with bacterins.<sup>17</sup>

The focus in human medicine is on developing subunit vaccines, in particular, vaccines containing a lipoprotein in the outer membrane of the spirochete, outer surface protein A (OspA). The OspA lipoprotein is highly conserved between *B. burgdorferi* species and has

induced protection in mice and humans against heterogeneous *B. burgdorferi* from different geographic regions.<sup>17-19</sup> In contrast, the outer surface protein C (OspC) exhibits much greater heterogeneity than OspA, and the outer surface protein B (OspB) has high variability between strains.<sup>18</sup> The Advisory Committee on Immunization Practices of the Centers for Disease Control and Prevention expects OspA vaccines to be protective against most if not all human infections in the United States.<sup>20</sup> The FDA has recently licensed an OspA vaccine for human use, and a second OspA vaccine is under review at this time.<sup>21, 22</sup> Several studies have provided evidence that as tick feeding begins, the expression of OspC is upregulated, and the expression of OspA is downregulated. Research indicates that the anti-OspA antibody kills the spirochete within the gut of the tick before this shift in production and prevents transmission from the tick to the host.<sup>18, 23-25</sup>

To develop and license a Lyme vaccine for dogs, a natural challenge model via tick exposure was developed. This natural challenge model, which allows infected ticks to feed on mammalian hosts, has been demonstrated to be more reliable than syringe inoculation.<sup>18</sup> Naturally infected ticks collected from endemic areas are likely to be infected by two or more strains of *B. burgdorferi* and more closely mimic natural challenge. This study evaluates the efficacy of a nonadjuvanted OspA recombinant vaccine (Recombitek™ Lyme [Merial Limited]) by challenge with naturally infected ticks. Clinical signs, antibody titers by ELISA, Western blot assays, and isolation and PCR analyses of spirochetes from biopsy specimens were used to evaluate vaccine efficacy. Direct fluorescent antibody assay (DFA) was used to evaluate the infection rate in the challenge ticks and in naïve ticks allowed to feed on study dogs after the dogs had become infected (xenodiagnosis).

## ■ MATERIALS AND METHODS

### Vaccines

Two different commercial vaccine lots were used in this efficacy study. One of these vaccine lots (vaccine B) was older than 4 years and had been evaluated previously in a vaccination and challenge study. This vaccine was used in this study to determine the stability of OspA in the vaccine.

### Animals

Thirty purpose-bred, *B. burgdorferi*-negative, mixed-breed dogs aged 10 to 12 weeks and of both sexes were randomly allocated into three groups of ten animals each. One group received two doses (1 mL/dose administered subcutaneously) of vaccine A given on days 0 and 21 of the study. The second vaccinal group received two doses of vaccine B delivered in the same fashion. The third group received no injection and served as the nonvaccinated control group. All animals were initially housed in runs containing two dogs each. During the challenge, the animals were housed singly to prevent dislodging of the tick-containing apparatus.

### Observations

The dogs were observed by the primary investigator and a technician for approximately 30 minutes after each vaccination for any immediate adverse effects. Daily observations were performed and recorded by animal care personnel for the remainder of the study. All animal care personnel were blinded to the randomization of dogs to vaccinal or control groups. Vaccination sites were reexamined at 2 weeks following each vaccination for any evidence of local reaction.

### Challenge

Adult ticks (*Ixodes scapularis*) infected with *B. burgdorferi* were obtained for the challenge

from wooded sites in southern Rhode Island. These ticks were collected during fall 1997 from an area endemic for Lyme borreliosis. An infectivity rate of 40% was determined by testing 30 ticks, randomly selected from the challenge cohort, using a standard DFA.<sup>26,27</sup>

All three groups of dogs were challenged with *B. burgdorferi* by exposure to infected ticks via a tick-containing apparatus. Three weeks after the administration of the second vaccination (day 41), a minimum of twelve adult ticks of each sex was placed on each dog. The animals were checked at least once daily for the next 7 days to confirm tick attachment and feeding. A count of ticks feeding was made each day. At the end of the 7-day exposure (day 49), any remaining ticks were carefully detached.

### Serology

Blood was collected from each animal for *B. burgdorferi* antibody titer determination 1 day before both vaccinations (days -1 and 20), 2 weeks after the second vaccination (day 35), before challenge (day 41), and monthly in concurrence with each biopsy (days 76, 104, and 132). Laboratory personnel were blinded to which dogs had received vaccine. Anti-OspA titers were determined by ELISA and measured by optical density (OD). Ninety-six-well microtiter plates were coated with recombinant purified OspA antigen in 0.05M carbonate coating buffer. The plates were coated overnight at 37°C. Following coating, the plates were washed using an excess of phosphate buffered saline (PBS) containing 0.05% Tween 20™ and blocked for 1 hour at 37°C using PBS containing 0.05% Tween 20 and 2% nonfat dried milk. After blocking, the plates were washed with an excess of PBS/Tween 20. All serum samples were diluted 1:25 in blocking buffer and assayed in duplicate at a volume of 100 µL. Duplicate wells of a known positive control serum sample at a 1:25 dilution were

also included on each plate. The plates were incubated for 3 hours at 37°C and then washed with an excess of PBS/Tween 20 solution. A rabbit, antidog, alkaline-phosphatase antibody (Jackson ImmunoResearch) was added to all wells at a dilution of 1:7500 (in blocking buffer). The plates were incubated for 1 hour at 37°C and then washed with an excess of PBS/Tween 20 solution. The plates were then reacted with polynitrophenyl phosphate solution (Pierce Chemical) and reactivity was stopped with 3N NaOH. Antibody concentration (titers) were measured by OD.

To compare results on different plates, each sample was standardized by the following formula:

$$\frac{(\text{Sample Replicate 1} + \text{Sample Replicate 2})}{2} \div \frac{(\text{Positive Control Replicate 1} + \text{Positive Control Replicate 2})}{2}$$

= Adjusted Optical Density (OD) value

The same positive control serum was used on each plate. This technique minimized the effect of plate-to-plate variation.

### Western Blots

The Western blots were completed as outlined.<sup>7</sup> *B. burgdorferi* was cultured in Barbour Stoenner Kelly (BSK II)<sup>28</sup> medium, supplemented with 10% heat-inactivated rabbit serum and antibiotics (0.006 mg/mL kanamycin, 0.045 mg/mL rifampicin, and 240 U/mL nystatin [KRN]), harvested by centrifugation, and subjected to five cycles in a French press (1.26 kg/cm<sup>2</sup>). The lysate was centrifuged to remove particulate matter, and the soluble supernatant was used as antigen. This antigen was subjected to SDS-PAGE (12% acrylamide) at 200 V for 4 to 6 hours. Immunoreactive proteins were detected by Western blot analysis using a miniblotted. Antibody was detected in a 1:10 dilution of dog serum by a

goat, antidog IgG conjugated to horseradish peroxidase.

Three hundred sera were randomized and blinded by sequential numbering for testing in the Western blots. Included were 240 sera from serial bleedings of the experimental dogs. In addition, 60 samples were selected from 100 routine clinical submissions to the Diagnostic Laboratory, College of Veterinary Medicine, Cornell University. These 100 clinical submissions had been run previously in Western blots and were determined to have the following: antibody to vaccination (20 samples), antibody to infection (20 samples), both (40 samples representing infected animals with superimposed vaccinations, thus antibody to both infection and vaccination was present), or no antibody (20 samples). The 100 samples were assembled at Cornell University, sent to the primary investigator's laboratory, transferred to different vials, and coded. From these, 60 sera were selected and included with the 240 samples from the experimental dogs. It was particularly important to include sera from dogs known to have antibody to both infection and vaccination so that bias would be eliminated when reading blots from experimentally vaccinated and challenged dogs.

Blots were examined for vaccinal antibody (bands at p31 and p34) and/or antibody to infection. At least three bands from among p39, p29-30, p25-26, p22, and p19 were required to consider the serum positive. Data were recorded systematically on a scoring sheet to ensure that each relevant band observed was recorded. The blinded data were submitted to the primary investigator, at which point the test was unblinded and submitted for evaluation. The samples were separated into the seven serial bleedings of each experimental dog representing a prevaccination bleed and six postvaccinal bleeds drawn over 5 months. They were rerun side by side in Western blots to evaluate change in antibody patterns.

### **Skin Biopsies and Culture for Spirochete Isolation**

A skin biopsy was taken from each dog at 1, 2, and 3 months after challenge. The biopsy site of each dog was shaved with clippers, the area disinfected, and local anesthetic instilled. A biopsy sample was taken using a 5- or 6-mm biopsy punch. The sample was placed in a sterile tube containing 2 mL of BSK II + KRN medium.

The samples were returned to the lab and aseptically transferred to fresh sterile medium tubes containing 5 mL of the above-mentioned medium formulation. Tubes were then incubated at  $33^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . Antibiotics were replenished during the incubation period using the following schedule: nystatin stock every 3 days and rifampicin/kanamycin stock every 5 days. According to the manufacturer, the toxicity of these antibiotics on *B. burgdorferi* has been shown to have no effect on the viability of the organism.<sup>d</sup> The cultures were examined approximately every 2 weeks. A minimum of 15 fields of a wet-mount slide preparation was examined, using the 40 $\times$  objective and dark-field condenser, and scored as to the presence or absence of spirochetes. All scoring of biopsies was performed without knowledge of the allocation of dogs to vaccinal groups.

### **DNA Extraction and PCR Amplification**

To confirm the culture results, DNA from biopsy cultures taken at 1 and 2 months after challenge was extracted using DNA STAT-60 (Tel-Test, Inc., Friendswood, TX). An equal volume (500  $\mu\text{L}$ ) of each culture was mixed with DNA-STAT. After adding 60  $\mu\text{L}$  of chloroform, the mixture was centrifuged at 12,000 $\times$  g for 10 minutes. DNA was precipitated from the aqueous layer with isopropanol for 10 minutes at room temperature. The precipitated DNA was pelleted by centrifugation at 12,000 $\times$  g for 10

<sup>d</sup>Data on file with Merial Limited.

minutes. The pellet was washed with 500  $\mu$ L 75% ethanol, air-dried, and resuspended in 50  $\mu$ L TE (10 mM Tris-HCl, pH 8.0; 1 mM EDTA). PCRs were performed in an MJ Research PTC-100 thermal cycler (MJ Research, Inc., Watertown, MA) and reagents were from Bioline USA Inc. (Reno, Nevada). Five  $\mu$ L of purified DNA was used as template in a total volume of 50  $\mu$ L. The reaction mixtures contained 125  $\mu$ M each deoxynucleoside triphosphate, 1 U of *Taq* DNA polymerase, 3 mM  $MgCl_2$ , and 50 pmol of each primer. Primers were OSP-A4 (5'-CTGCAGCTTGOAATTCAGGCACCTTC-3') and OSP-A2 (5'-G TTTTGTAATTTCAACTGCTGACC-3'). According to the manufacturer, PCR with these primers is expected to amplify a 158 base pair region of the *B. burgdorferi* OspA gene.<sup>c</sup> Cycling conditions included an initial denaturation step at 4°C for 5 minutes; then 40 cycles each of 94°C for 45 seconds, 52°C for 45 seconds, and 72°C for 45 seconds; followed by a 7-minute extension step at 72°C. A 5  $\mu$ L volume of the reaction mixture was electrophoresed through a 1% agarose gel containing ethidium bromide and viewed under ultraviolet light.

### Tick Xenodiagnosis

Three weeks following the adult tick challenge, about 200 laboratory-reared larval *I. scapularis* were placed into the tick apparatus attached to each dog. Larvae engorging on vaccinated and nonvaccinated dogs were collected as they detached and were held at 95% relative humidity at 23.5°C until they molted to the nymphal stage. Nymphs were tested for spirochetes about 3 weeks after molting by dissecting and examining their midgut tissues by DFA.<sup>26,27</sup>

## RESULTS

### Vaccine Safety

All vaccinated dogs were monitored for acute adverse reactions during the first 30 min-  
<sup>c</sup>Report on file with the USDA and Merial Limited.

utes following vaccination. Injection sites were reexamined at 2 weeks following each vaccination for any swelling, pain, tenderness, or itching at the injection site. No adverse reactions were observed at any time following the injection of either Lyme vaccine.

### Challenge Effects

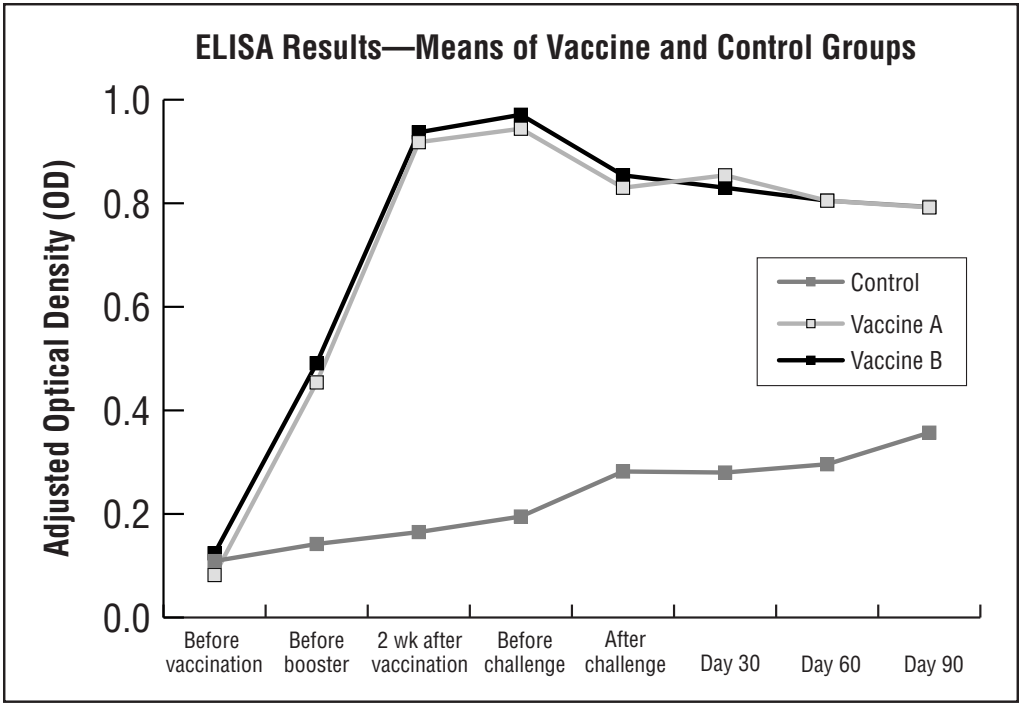
Animal caretakers were blinded to which dogs had received vaccinations. Clinical signs characteristic of Lyme disease were detected in two of the test animals. Two nonvaccinated control animals were observed to have slightly stiff gaits and movement between 31 and 41 days after challenge. These signs abated quickly without treatment. No other clinical signs were observed.

### Tick Challenge

Prolonged tick feeding of infected ticks is believed to be necessary for transmission of *B. burgdorferi*. In this study, the challenge ticks demonstrated a 40% infectivity rate; therefore at least three ticks feeding for a prolonged period would be necessary to ensure an adequate challenge. On the day of challenge (designated day 0 of challenge), 12 adult ticks of each sex were applied to each dog. On the following day, additional ticks were added to replace any unattached ticks thereby ensuring adequate infestations. At least three of the female ticks were seen attached to and engorging on all dogs for 3 days following infestation. On subsequent days (days 4 to 6), an increasing number of dogs were observed with fewer than three female ticks attached as the feeding cycle was completed. By day 6, most dogs had three or fewer ticks still attached. All remaining ticks were removed on either days 6 or 7.

### Serology

Serum samples taken at regular intervals during the study were evaluated by ELISA for



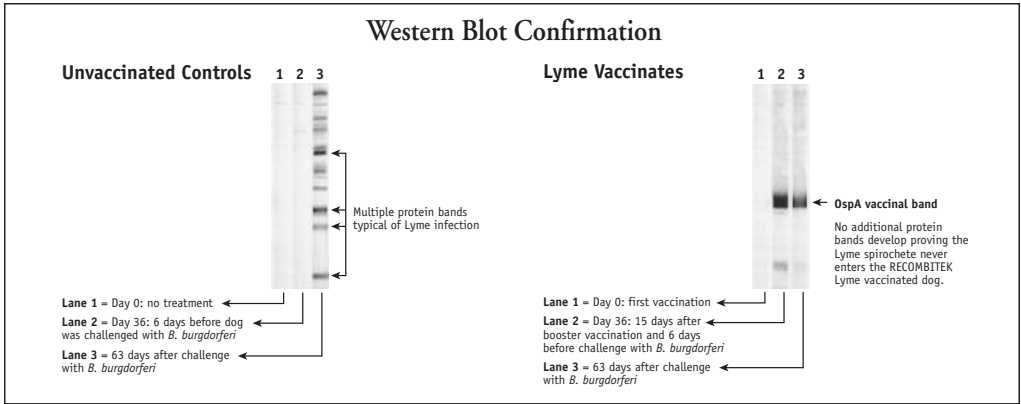
**Figure 1.** Anti-OspA titers were determined by ELISA measured by optical density. The two vaccinate groups developed high antibody levels, which they maintained through the end of the study (after challenge day 90). There was no booster effect after challenge, demonstrating solid immunity. The nonvaccinated control group had low titers that increased somewhat in response to challenge.

the presence of anti-OspA antibody. An analysis of variance was used to evaluate the effect of group, day, and group by day interaction on the OspA antibody levels using day -1 as a covariate. The covariate was not significant. The results of the “by day” analysis demonstrated that there was significant difference between vaccinate groups and the control group on each day tested ( $P < .0001$ ). That is, there was a significant difference on each day between each vaccine group compared with control animals; however, there were no significant differences between vaccine groups. Postvaccinal antibody levels remained elevated throughout the study with no increase in antibody level occurring after challenge in the vaccinated dogs.

Sera from nonvaccinated control animals demonstrated a significant rise in antibody level after challenge that continued throughout the postchallenge sampling period ( $P = .0165$ ). Means of vaccinal and control group ELISA results are shown in Figure 1.

**Western Blot**

All of the 20 vaccinated dogs developed antibodies to p31. Following tick challenge, none of these dogs developed antibodies to antigens that are specific for infection to *B. burgdorferi*. All of the 10 nonvaccinated control dogs developed antibodies characteristic of infection with *B. burgdorferi* that remained detectable through the end of the experiment. The most common-



**Figure 2.** An example of a Western blot pattern for the nonvaccinated challenge control dogs demonstrates antibodies characteristic of infection with *Borrelia burgdorferi*. The most commonly observed “infection bands” were at p28, p29, p30, and p39. A typical Western blot pattern for vaccinated dogs demonstrates antibodies to p31. Following tick challenge, only the p31 band is present, demonstrating that no antibodies developed to antigens that are specific for infection to *B. burgdorferi*.

ly observed “infection bands” were at p39, p29-30, and p28 (Figure 2).

In addition to the experimental samples, the serologist correctly identified the antibody status of 20 infected dogs, 9 vaccinated dogs, 20 vaccinated and infected dogs, and 11 uninfected dogs. These sera from clinical submissions to Cornell University had been included and randomized with the 240 experimental dog samples to remove bias in reading of blots.

### Biopsy Culture Results

Each skin biopsy culture was examined for growth of spirochetes. Biopsy cultures from all of the control dogs (10/10) were positive for spirochetes at all three sampling times, whereas all of the biopsy cultures from the vaccinated dogs (20/20) were negative for spirochetes at all three sampling times. Isolation was consistent throughout the study, with no inconclusive samples. Contaminants were detected in approximately 5% of the observations; however, this did not interfere with detection of spirochetes (Table 1).

### Biopsy PCR Results

PCR analyses on biopsy cultures taken at 1 and 2 months after challenge were negative in all dogs in the vaccinated groups at both sampling times. In the control group, four dogs had positive biopsy samples by PCR at both sampling times, four dogs were negative at the first sampling but positive at the second sampling, one dog was positive at the first sampling and negative at the second sampling, and one dog was PCR negative at both sample time periods (Table 1).

### Tick Xenodiagnosis

Five or more ticks were recovered from 13 of the 30 dogs, while less than five ticks were recovered from 11 dogs. No ticks were recovered from the six remaining dogs (three in vaccine group A and three in vaccine group B). In the two vaccinal groups, ticks were recovered from 14 dogs (a total of 62 ticks), and all were negative by DFA. In cases of *Ixodes dammini* ticks and *B. burgdorferi* (the vector and bacterium of interest), DFA can be a more sensitive test than



**TABLE 1. Results of Biopsy Culture, Biopsy Polymerase Chain Reaction (PCR), and Xenodiagnosis (isolation of *Borrelia burgdorferi* from ticks fed on dogs) in Vaccinated and Control Dogs\***

Group or Dog	Biopsy Culture	PCR at Day 30	PCR at Day 60	Xenodiagnosis Tick Reisolation = Number Positive (number recovered)
Vaccinate A-1	Neg	Neg	Neg	0 (6)
Vaccinate A-2	Neg	Neg	Neg	0 (10)
Vaccinate A-3	Neg	Neg	Neg	None recovered
Vaccinate A-4	Neg	Neg	Neg	0 (1)
Vaccinate A-5	Neg	Neg	Neg	0 (5)
Vaccinate A-6	Neg	Neg	Neg	None recovered
Vaccinate A-7	Neg	Neg	Neg	0 (3)
Vaccinate A-8	Neg	Neg	Neg	0 (3)
Vaccinate A-9	Neg	Neg	Neg	0 (2)
Vaccinate A-10	Neg	Neg	Neg	None recovered
Vaccinate B-1	Neg	Neg	Neg	None recovered
Vaccinate B-2	Neg	Neg	Neg	0 (4)
Vaccinate B-3	Neg	Neg	Neg	None recovered
Vaccinate B-4	Neg	Neg	Neg	0 (4)
Vaccinate B-5	Neg	Neg	Neg	0 (10)
Vaccinate B-6	Neg	Neg	Neg	None recovered
Vaccinate B-7	Neg	Neg	Neg	0 (2)
Vaccinate B-8	Neg	Neg	Neg	0 (8)
Vaccinate B-9	Neg	Neg	Neg	0 (3)
Vaccinate B-10	Neg	Neg	Neg	0 (1)
Control 1	Pos	Pos	Pos	2 (8)
Control 2	Pos	Pos	Pos	0 (10)
Control 3	Pos	Pos	Pos	3 (8)
Control 4	Pos	Neg	Neg	0 (10)
Control 5	Pos	Neg	Pos	1 (7)
Control 6	Pos	Neg	Pos	1 (4)
Control 7	Pos	Neg	Pos	2 (7)
Control 8	Pos	Neg	Pos	5 (10)
Control 9	Pos	Pos	Neg	4 (10)
Control 10	Pos	Pos	Pos	4 (4)

\*Cultures on skin biopsies at 30, 60, and 90 days after challenge were negative at each sampling for all vaccinates and positive at each sampling for all control dogs. PCR on skin biopsies taken at 30 and 60 days after challenge were negative in all vaccinated dogs and positive in 9 of 10 control dogs on one or both samplings. Xenodiagnosis was performed by placing ticks on study dogs 3 weeks after challenge. Ticks were recovered from 14 vaccinated dogs, and all were negative. Ticks were recovered from all control dogs; 8 out of 10 were positive for spirochetes.

PCR.<sup>29</sup> In the control group, ticks were recovered from all ten dogs (a total of 78 ticks), and ticks derived from eight of them tested positive for spirochetes. Results of DFA on tick midgut tissues are presented in Table 1. The mean infectivity of xeno-positive dogs was 37.9% (22 ticks infected of 58 recovered).

## DISCUSSION

The safety of the two test vaccines was very high, with no local or systemic reactions noted in any vaccinee. This is to be expected because this vaccine is a highly purified, nonadjuvanted product known to have lower incidences of local and systemic reactions than adjuvanted products.

The tick challenge model used in this study mimicked the natural exposure of dogs to *B. burgdorferi*. All control dogs (100%) were culture positive at all three biopsy times, validating the strength of this challenge. Even in the face of this strong challenge, 100% of vaccinated dogs were protected, as evidenced by negative culture results from all three biopsies.

Infection with *B. burgdorferi* does not always produce objective clinical signs in dogs. In this study, two of the ten nonvaccinated control dogs exhibited symptoms consistent with Lyme disease. No definitive clinical signs were observed among dogs receiving vaccine. Thus, in order to assess vaccine efficacy, we relied on a combination of serology and recovery of the infectious agent.

Antibody levels after challenge in vaccinated dogs did not increase, whereas control dogs demonstrated a significant increase in serologic titer throughout the postchallenge sampling period. This lack of increase in antibody in vaccinees is evidence of blocking immunity in the host. The protection conferred by the two test vaccines and the ELISA titers generated by these vaccines were not significantly different. These data support both the efficacy of the

OspA antigen and its long-term stability (4 years). This vaccine has previously demonstrated protection of all vaccinees in a 1-year duration of immunity study.<sup>c</sup>

The Western blot portion of the study confirmed that no infection occurred in the vaccinated dogs following challenge with ticks infected with *B. burgdorferi*. The OspA vaccine prevented infection in the face of a challenge that infected all of the control dogs, as determined by the presence of antibody characteristic of infection and positive biopsy cultures.<sup>7</sup> The extra precaution of including sera of known antibody status was essential to ensure that the reader of the blots would not be biased if infections did become established in the vaccinees. By providing 100 additional sera to the primary investigator, 60 of which were randomized along with the 240 experimental samples, the serologist could not determine a priori which of these samples were from infected, vaccinated, infected-vaccinated, or uninfected dogs. This eliminated potential bias and ensured that the Western blot testing would detect vaccinated dogs that became infected after challenge.

Infection, or a lack thereof, was confirmed by recovering live spirochetes after challenge using biopsy culture and tick xenodiagnosis. Spirochetes were readily isolated in culture from 100% (10/10) of the nonvaccinated dog biopsy samples but not from any of the 20 vaccinated animals. PCR testing confirmed microscopic observations and contributed to the stringency of the assay. Tick xenodiagnosis has been used effectively to detect the presence of infection with *B. burgdorferi* in a variety of wildlife hosts and dogs<sup>30, 31</sup> and to determine canine Lyme disease vaccine efficacy.<sup>32</sup> Infected ticks were recovered from eight of the ten control dogs, and the mean infectivity of xeno-positive ticks was nearly 40%. In contrast, no xeno-positive ticks were detected from the 14

vaccinated dogs in which ticks were recovered. The lack of any detectable infection in the 62 ticks that were derived from vaccinated dogs provides good evidence for vaccine protection from challenge.

## CONCLUSION

The challenge with ticks infected with *B. burgdorferi* was sufficient potentially to infect all dogs as indicated by the prolonged attachment of female ticks and isolation of spirochetes from all nonvaccinated control dogs. In this blinded study, both test vaccines demonstrated highly protective efficacy as demonstrated by 0% isolation of *B. burgdorferi* from vaccinated dogs and 100% isolation from the controls at multiple sample times and confirmed by PCR. This was additionally confirmed by ELISA antibody to *B. burgdorferi* after challenge, showing no increase in any vaccinee and typical response to infection in all controls. Western blot analysis confirmed that spirochete transmission to vaccinees was completely blocked at challenge 42 days after vaccination. This study demonstrated complete protection by the nonadjuvanted, recombinant, OspA vaccine that contains no extraneous antigens or adjuvants.

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