Canine Lyme Disease: One-Year Duration of Immunity Elicited With a Canine OspA Monovalent Lyme Vaccine

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ABSTRACT

Canine Lyme disease is characterized by various clinical signs ranging from subclinical disease to acute arthritis and arthralgia. Previous reports demonstrate significant protection against Lyme disease is elicited by a vaccine containing the major outer surface protein (OspA) of the causative agent, *Borrelia burgdorferi*. The purpose of the present study was to determine the safety and duration of immunity of protection offered by a recombinant OspA vaccine (RECOMBITEK® Lyme, Merial Limited, Duluth, GA). Twenty beagles, aged 9 to 10 weeks, were vaccinated per label subcutaneously with 2 doses of RECOMBITEK Lyme vaccine 3 weeks apart, and 11 beagles served as unvaccinated controls. The investigators, including those providing daily health assessments post-vaccination, and post-challenge spirochete isolation and clinical signs assessment, were blinded to the vaccination status of the animals. Dogs were challenged 1 year (366 days) after vaccination by exposure to naturally infected ticks. Spirochete reisolation from biopsies was used to assess vaccine efficacy at monthly intervals for 3 months after challenge. All dogs were monitored for clinical signs of Lyme disease. No adverse reactions were noted following vaccination at any time during the study. One-year duration of immunity results demonstrated that the vaccine offered significant protection against Lyme disease as no vaccinated animals showed clinical signs, and no spirochetes were reisolated from vaccinated animals from biopsies performed for 3 months post-challenge. By contrast, 18% of control animals experienced clinical signs, and spirochetes were reisolated from 82% of the non-vaccinated dogs. In conclusion, the canine OspA vaccine is both safe and efficacious with long-lasting protection from clinical signs and spirochete proliferation for at least 1 year (366 days) after vaccination.

INTRODUCTION

Lyme disease, caused by infection with the spirochete *Borrelia burgdorferi* transmitted by ixodid ticks, occurs mostly in dogs and humans and to a lesser extent in horses, cattle, and cats.[1] It remains the most commonly reported vector-born disease in people in North America and Europe. More than 90% of Lyme disease in the United States occurs in the northeastern, mid-Atlantic, and upper Midwest regions, with reported cases in several counties in California.[2]

In dogs, infection with *B. burgdorferi* may cause recurrent arthritis with fever, myalgia, anorexia, and lethargy.[3–6] More rarely, dogs may also experience renal failure, heart block, and neurologic disease. Diagnosis of canine Lyme disease can be challenging due to the variability in clinical signs, nonspecific clinicopathology, and serology using enzyme-linked immunosorbent assay, which may not distinguish between exposure and prior vaccination. The C_6 peptide test (Canine SNAP® 3Dx® Test, IDEXX Laboratories, Inc., Westbrook, Maine) and Western blot assays are frequently used to distinguish post-vaccination titers from those of infection.[3–5] Treatment for Lyme disease includes appropriate antibiotic therapy; however, chronic Lyme disease may be unresponsive. Non-steroidal anti-inflammatory therapy may be indicated but may also obscure the animal's observable response to antibiotic therapy.

Due to the challenges of diagnosis and therapy, vaccination of dogs is recommended in endemic areas.[7–9] However, there is evidence that whole cell bacterins may illicit clinical signs of Lyme disease in dogs showing no evidence of infection with *B. burgdorferi*.[4,8] Therefore, the focus in animal medicine has been the development of a vaccine containing the major outer surface protein (OspA) of *B. burgdorferi* that is also non-adjuvanted, as part of a continual quest for more defined and less aggressive vaccines. The anti-OspA antibody produced by the vaccine enters the tick upon feeding and kills the spirochete in the tick's midgut preventing transmission to the host.[10]

Previously, investigators have conducted several short-term studies to investigate the efficacy of a non-adjuvanted recombinant OspA vaccine (RECOMBITEK® Lyme, Merial Limited, Duluth, GA) in protecting dogs against challenge with *B. burgdorferi*-infected ticks. Results demonstrated vaccinated dogs were protected against both spirochete proliferation and clinical disease. The present study was designed to evaluate the duration of this protective response elicited in dogs vaccinated with the OspA vaccine. Vaccine-induced immunity was evaluated using dogs challenged with a natural infection model using *B. burgdorferi*-infected ticks at 366 days post-vaccination.

MATERIALS AND METHODS

Dogs

Thirty one male and female Beagle puppies, 9 to 12 weeks of age, were obtained commercially (Harlan Sprague Dawley, Indianapolis, IN). These puppies were tested negative for Lyme and Leptospira vaccination as well as Lyme disease. The puppies were randomly split into 2 groups: 20 dogs were vaccinated subcutaneously with the OspA vaccine, and 11 dogs served as unvaccinated controls.

Vaccine Preparation and Monitoring

The OspA monovalent vaccine used in this study was prepared utilizing a stock concentration of the OspA vaccine and diluting the vaccine with sterile diluent to a concentration exactly equal to the minimum release dose allowed for the commercially available product. The preparation was placed into single-use vials under sterile conditions (1 mL each) and was deemed satisfactory by quality control.

Vaccination Protocol

Two doses of vaccine (1 mL/dose) were administered subcutaneously to dogs at a 3-week vaccination interval. Concurrently, the control dogs received a placebo injection. All assessments were performed and recorded by trained individuals who were blinded to vaccination status. Dogs were monitored for

signs of anaphylaxis, including labored breathing, pruritis, and edema, for 15 minutes following each injection. Animal technicians observed dogs continuously for 1 hour following vaccination and then at regular daily intervals during the 14 days after each injection for clinical signs, including swelling, pain, tenderness, and scratching at the injection site. The injection site was palpated for signs of tenderness and swelling prior to administration of the second injection.

Challenge

All dogs were challenged with 50 *Ixodes scapularis* nymphs infected with *B. burgdorferi* at 366 days after vaccination according to a previously demonstrated procedure.[1] Nymphs were used due to the scarcity of adult ticks as a result of an unseasonably dry summer. Dr. Thomas Mather (Director, Center for Vector Borne Diseases, University of Rhode Island) determined the nymph infection rate to be 100%.

Skin Biopsy and Spirochete Reisolation

Upon challenge, tick attachment sites were marked with indelible ink to allow identification and future biopsy. At 1, 2, and 3 months postchallenge, site-specific skin biopsies were performed. Tick attachment sites were shaved, prepped with surgical scrub, anesthetized with 2% lidocaine injected intradermally, and biopsied using a Baker skin punch. Skin samples were placed in tubes containing Barbour-Stoenner-Kelly culture medium (BSK media) with heat-inactivated rabbit serum and antibiotics and transported to the laboratory. Samples were supplemented with additional BSK media, placed in a candle jar, and incubated for 6 weeks. The investigator was blinded to the dog's vaccination status for the samples submitted. The tubes were examined weekly for spirochetes using dark field microscopy. When 10 or more fields were examined under the 40× objective with no evidence of spirochetes, the sample was considered negative.

Clinical Signs

All dogs were monitored daily for any signs of reaction or illness, including those consistent with Lyme disease, such as fever, lameness, ataxia, depression, anorexia, and pain and tenderness at the infection site. Again, all health assessments were performed and recorded by trained individuals who were blinded to vaccination status. Due to the variable manifestation of Lyme disease, the absence of clinical signs was not considered to be an indication of protection; however, the presence of clinical signs would lend support to a positive diagnosis, and be confirmed by spirochete reisolation.

RESULTS

Vaccine Safety

No general adverse reactions to the vaccine were noted at any time during the study. This included a lack of any detectable pain, swelling, tenderness, or itching at the injection site, which was examined daily for 2 weeks following vaccination.

Spirochete Reisolation From Skin Biopsies

No vaccinated dogs were positive for spirochetes at any time during the study (Table 1). However, 6 of the 11 control animals were biopsy positive at 1 month post-challenge, 8 of the 11 control animals were positive at 2 months post-challenge, and by the third biopsy date, spirochetes were reisolated from biopsy samples from 9 of the 11 control animals (82%).

Clinical Signs of Lyme Disease

None of the 20 vaccinated dogs experienced clinical signs of Lyme disease. Two of the 11 unvaccinated controls (18%) experienced lameness attributable to Lyme disease.

DISCUSSION

Lyme disease is the most commonly reported tick-borne disease in humans in the United States. High infection rates are reported to occur in unvaccinated dogs, but the disease remains difficult to diagnose and treat. Research has shown that the OspA of *B. burgdorferi* is a strong immunogen that provides protection against *B. burgdorferi* in a variety of animals.[11,12]

Table 1. Results of Spirochete Isolation and Clinical Signs.

		Spirochete Isolation (post-challenge)			Clinical Signs Consistent With
Dog #	Vaccine	1 Month	2 Months	3 Months	Lyme Disease
4D499	Control	-	+	+	+
4D513	Control	+	+	+	-
4D520	Control	-	-	+	-
4D557	Control	-	-	-	-
4D561	Control	-	-	-	-
4E410	Control	+	+	+	+
4E420	Control	+	+	+	-
4E441	Control	-	+	+	-
4E478	Control	+	+	+	-
4E469	Control	+	+	+	-
4E583	Control	+	+	+	-
4E482	Vaccinate	-	-	-	-
4D516	Vaccinate	-	-	-	-
4D563	Vaccinate	-	-	-	-
4D597	Vaccinate	-	-	-	-
4E408	Vaccinate	-	-	-	-
4E421	Vaccinate	-	-	-	-
4E430	Vaccinate	-	-	-	-
4E436	Vaccinate	-	-	-	-
4E442	Vaccinate	-	-	-	-
4E450	Vaccinate	-	-	-	-
4D526	Vaccinate	-	-	-	-
4E411	Vaccinate	-	-	-	-
4E417	Vaccinate	-	-	-	-
4E425	Vaccinate	-	-	-	-
4E434	Vaccinate	-	-	-	-
4E437	Vaccinate	-	-	-	-
4E440	Vaccinate	-	-	-	-
4D444	Vaccinate	-	-	-	-
4D454	Vaccinate	-	-	-	-
4D458	Vaccinate	-	-	-	-

In 1996, a recombinant non-adjuvanted OspA vaccine (RECOMBITEK Lyme) was introduced for the prevention of Lyme disease in dogs. The current study demonstrates that vaccination with RECOMBITEK Lyme is safe. Moreover, RECOMBITEK Lyme is highly efficacious and provides at least 1-year duration of immunity.

Safety of the OspA vaccine was assessed via monitoring of animals for acute and subacute clinical signs following vaccination. No local or systemic reaction was detected in any vaccinated animal. This vaccine contains no adjuvant thus eliminating even mild or transient granulomatous response characteristic of vaccination with most adjuvanted preparations. This study supports previous study results demonstrating the vaccine's high level of safety.

Efficacy of the vaccine was established at a 1-year period following administration of the initial vaccination protocol. Evaluation of clinical signs, as well as determination of the vaccine's ability to prevent spirochete dissemination, was used to

assess protection elicited in this long-term duration of immunity trial. Dogs were monitored daily for clinical signs associated with Lyme disease, including fever, lameness, ataxia, depression, and anorexia. No clinical signs of Lyme disease were observed in any vaccinated dogs; however, 2 unvaccinated controls (18%) demonstrated lameness associated with spirochete infection.

Clinical signs of Lyme disease in dogs are variable, with the majority of infections being subclinical, so this study primarily focused on spirochete reisolation from skin biopsy samples as the most accurate measure of the vaccine's effectiveness. In this study, with a significant challenge at 366 days, 100% of the vaccinated dogs were protected from infection. These results were verified by negative spirochete reisolation from multiple biopsies performed on 3 separate occasions. In contrast, spirochetes were reisolated from 82% of the unvaccinated controls.

These results confirm protection against infection seen in several short-term duration of immunity studies.[13–15] Most notably, Conlon and colleagues[13] purpose-bred mixed breed dogs 10 to 12 weeks of age that were sequentially vaccinated with 2 doses of the same OspA vaccine. Dogs were challenged with *I. scapularis* ticks 3 weeks after the second vaccination, and this study demonstrated that the OspA vaccine blocked proliferation of *B. burgdorferi* in all vaccinated dogs (20/20). Control dogs seroconverted following challenge, and 2 of the dogs experienced clinical signs associated with Lyme disease (2/10). Spirochetes were reisolated from all of the controls (10/10). Results from this short-term study demonstrated that 100% of vaccinates were protected against infection after challenge,[13] results similar to those reported in the present study.

CONCLUSION

The recombinant OspA vaccine administered subcutaneously to puppies 9 weeks of age and older is shown to be both safe and efficacious. The vaccine induced an immune response that protected vaccinates against spirochete infection and clinical signs for a full 12 months after vaccination.

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