Infection Rates in Dogs Vaccinated and Not Vaccinated With an OspA *Borrelia burgdorferi* Vaccine in a Lyme Disease-Endemic Area of Connecticut

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ABSTRACT

A commercially available recombinant OspA vaccine (Recombitek rLyme, Merial Ltd.) was used to immunize dogs against natural infection by *Borrelia burgdorferi* in Connecticut, an area highly endemic for Lyme disease. A C_6 ELISA kit was used to determine infection rates in both vaccinated dogs as well as an unvaccinated control group. Study dogs were patients at a small animal practice in Old Lyme, Connecticut. Vaccinated dogs were immunized exclusively with the recombinant OspA *B burgdorferi* vaccine; controls were not vaccinated against *B burgdorferi* infection. All dogs in the vaccinated group were given 2 doses of the vaccine before 6 months of age and were given boosters each year according to the manufacturer's instructions. Multivariate analysis was performed for both study groups to examine the influence of age, sex, breed, and vaccination on infection rates. A total of 25% (15/60) of vaccinated subjects and 63% (12/19) of non-vaccinated dogs were infected by *B burgdorferi*. Vaccine efficacy was calculated as preventable fraction (PF) by comparing infection rates in unvaccinated and vaccinated dogs. The vaccinated group had a PF of 60.3% (95% CI, 29.3–74.4).

INTRODUCTION

Canine Lyme disease is a common illness of dogs in areas where the vector ticks are active.[1] The vectors of the infectious organism, *Borrelia burgdorferi,* are ticks in the *Ixodes ricinus* complex.[2,3] High rates of infection have been reported in unvaccinated dogs and clinical syndromes involving musculoskeletal, cardiac, and renal pathology have been documented.[1,4] In 1996 a recombinant OspA vaccine (Recombitek rLyme, Merial, Ltd.) was introduced for the prevention of Lyme disease in dogs. A study of its efficacy

$$\text{Preventable Fraction} = \frac{(\text{infection in unvaccinated individuals}) - (\text{infection in vaccinated individuals}) \times 100}{(\text{infection in unvaccinated individuals})}$$

Figure 1. Calculation of Preventable Fraction.

in experimentally exposed laboratory dogs has been published,[5] but studies reporting the field safety and efficacy of the OspA Lyme disease vaccine in naturally exposed dogs have not been published to date.

The goal of this study was to determine the infection rate and preventable fraction (PF) in dogs vaccinated with a recombinant OspA vaccine as well as the rate of infection in non-vaccinated control dogs.

MATERIALS AND METHODS

The study was a non-randomized, retrospective, observational study of pet animals living in Connecticut. All study dogs were presented for heartworm screening between April 1 and August 30, 2001. Selection of dogs was not randomized, but was based on the following criteria: each dog was at least 1 year old at the time of testing and complete immunization and medical histories were available.

A commercially available in-office diagnostic kit (Canine SNAP 3Dx Test, IDEXX Laboratories) was used for the detection of *B burgdorferi* infection. In addition to tests for heartworm and *Ehrlichia canis*, the kit includes the C_6 Enzyme-Linked Immunosorbent Assay (C_6 ELISA) that detects antibodies against the Vmp-like sequence, Expressed (VlsE) gene of the spirochete, *B burgdorferi*. This protein is expressed during active infection, and antibodies directed against it are not cross-reactive with antibodies induced by vaccination. A positive C_6 ELISA for *B burgdorferi* antibodies is an indication of infection by the organism.[6] The test has been demonstrated to be highly sensitive and specific in dogs[7] and was used as the basis for determining the infection status in vaccinated and control (unvaccinated) dogs in this study.

Study Groups

There were two groups of study animals. The first was vaccinated dogs, which received 2 doses of the recombinant OspA vaccine before 6 months of age and received booster doses according to manufacturer's directions in each subsequent year. The second group was non-vaccinated (control) dogs, which had never been immunized with any canine Lyme disease vaccine.

Testing for *Borrelia burgdorferi* Infection

All dogs were tested using the in-office C_6 ELISA diagnostic kit, which detects antibodies induced by infection with *B burgdorferi*, antigen produced by *Dirofilaria immitis,* and antibodies induced by infection with *Ehrlichia canis*. Dogs with positive C_6 ELISA for *B burgdorferi* antibody were considered to be infected with the organism.

Determination of Vaccine Efficacy

Infection rates were calculated for each group by dividing the number of infected dogs by the total number of dogs in each group. Preventable fraction (PF) was calculated to examine efficacy of vaccination. Vaccine efficacy is the proportion of infection prevented by vaccine in vaccinated individuals and may be calculated by subtracting the rate of infection in vaccinated animals from the rate in non-vaccinated animals and expressing the difference as a percentage of the incidence in non-vaccinated individuals (Figure 1). A negative C_6 ELISA for *B burgdorferi* antibodies was interpreted to indicate absence of infection and positive C_6 ELISA was interpreted to indicate evidence of infection by *B burgdorferi*.

Data Analysis

Multivariate logistic regression analysis was conducted in which the outcome variable was

a positive or negative Lyme test. The predictor variables in the model were age in years at the time of test, sex, breed, and number of Lyme disease vaccinations. Infection rates and vaccine efficacy (PF) in each group was calculated. All statistical analysis was performed using EpiInfo Version 6.04d (US Centers for Disease Control and Prevention).

RESULTS

Dogs Studied

A total of 79 dogs qualified for evaluation in this retrospective study. There were 60 dogs immunized with the OspA vaccine and 19 non-vaccinated control dogs. At the time of testing the average age of the immunized dogs was 2.96 years and 3.16 years for control dogs. The vaccinated group contained 25 males (20 castrated and 5 intact) and 35 females (all spayed). The control group contained 13 males (10 castrated and 3 intact) and 6 females (5 spayed and 1 intact). Fifteen of the vaccinated dogs were mixed breed and 45 were various purebred dogs. Five of the control dogs were mixed breed and 14 were various purebred dogs. All dogs lived in Connecticut, an area highly endemic for Lyme disease. All were pet dogs from a rural area and were admitted to the study based on meeting the criteria when they presented for routine heartworm screening.

Infection Rates

Twenty-five percent (15/60) of vaccinated dogs and 63% (12/19) of control dogs were infected with *B burgdorferi* (Figure 2).

Preventable Fraction

PF in vaccinated dogs was 60.3% (95% CI, 29.3–74.4) (Figure 3).

Statistical Analysis

Predictor variables were age in years at the time of test, male versus female sex, breed (mixed versus pure), and number of Lyme disease vaccinations administered.

DISCUSSION

Canine Lyme disease has been associated with syndromes involving musculoskeletal, renal, and cardiac abnormalities. Diagnosis is made by a process of clinical evaluation and consideration of alternative diagnoses and of epidemiological factors that may affect risk of exposure.[1] Evaluation of antibody status as part of Lyme disease diagnosis is complicated by the large numbers of seropositive dogs that have no signs of Lyme disease, the inherent complexity of antibody patterns in and expense of western immunoblot (WB) testing, and the common use of vaccines that produce antibodies detected by indirect fluorescent antibody assay, ELISA, and WB.[7–11]

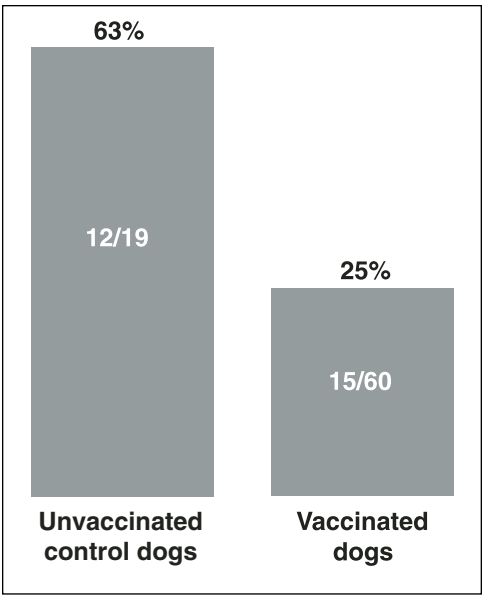

Figure 2. Infection rates in unvaccinated controls and vaccinated dogs.

C_6 ELISA is 100% specific and sensitive for detection of antibodies induced by infection with *B burgdorferi*.[7] C_6 ELISA determines infection status and eliminates the confounding factors introduced by nonspecific serologic tests. Use of prevention of infection as the criterion for vaccine efficacy eliminated the diagnostic challenge involved in utilizing prevention of signs of disease as the measure of efficacy. Vaccinated versus non-vaccinated dogs from the same practice were used to determine infection rates and PF, which is defined as the portion of infection prevented by administration of the vac-

$$\text{Preventable Fraction} = \frac{(\text{infection in unvaccinated dogs}) - (\text{infection in vaccinated dogs}) \times 100}{(\text{infection in unvaccinated dogs})}$$

$$\frac{63-25}{63} \times 100 \quad \textbf{PF = 60.3\%} \quad (95\%\ \text{CI}, 29.3 - 74.4)$$

Figure 3. Preventable Fraction (PF) for dogs vaccinated by 6 months of age.

cine in vaccinated dogs. In this study, only dogs immunized by 6 months of age were included in the vaccinated group. While the prevaccination infection status was unknown, the risk of infection increases with age[12] and dogs in both groups had no statistical difference between average age. *B burgdorferi* infection was found in 25% (15/60) of the vaccinated dogs and 63% (12/19) of the unvaccinated dogs. Dogs immunized with the recombinant OspA vaccine had a PF of 60.3% (95% CI, 29.3–74.4 upper) (Figure 3).

Signalment for the 2 study groups was statistically similar. Multivariate analysis revealed a decreased risk of 3% per year of age. Females had a 43% increased risk of infection versus males, and mixed-breed dogs had a 26% lower risk versus pure-bred dogs. Differences associated with age, sex, or breeds were not statistically significant. The infection rates were statistically different between the vaccinated and non-vaccinated dogs, with each vaccination decreasing the risk of infection by 28% ($P < 0.05$).

Confounding factors in this study include the unknown infection status at the time of initial vaccination and the basic design of a non-randomized observational study. Conducting studies in the practice environment creates certain limitations that are not encountered in laboratory studies. Pet dogs may not be subjected to forced exposure and infection and the number of participants is limited by the number of dogs meeting the criteria and presented during the study period. Yet age, sex, and breed presented no statistical difference in the 2 groups in this study.

Infection status before vaccination is nearly impossible to assess in a naturally exposed, owned population of pet dogs because there is a 4- to 5-week lag time from exposure to *B burgdorferi* by the bite of an infected tick and the development of antibodies detected by a C_6 ELISA.[6] Purpose-bred laboratory dogs may be determined to be uninfected by testing before immunization and later maintenance in a tick-free environment. Pet dogs in Connecticut are maintained in an environment with risk of tick-bite at nearly all times throughout the year. In this study, testing dogs on the day of initial immunization would only have provided data on the infection status up to a time 5 weeks prior to the test. Repeat testing of dogs at intervals up to 5 weeks after immunization would still produce results confounded by the question of whether positive tests represented infection prior to the initial test and vaccine administration or infection due to failure of immunization to protect against infection. The fact that dogs in both groups were maintained under the same exposure conditions in a uniformly high-risk environment justifies the creation of 2 groups with equal risk of infection. Analysis of other factors reveals that the only significant variant was immunization.

CONCLUSION

Immunization with an OspA recombinant *B burgdorferi* vaccine can reduce the risk of infection of naturally exposed dogs when compared with non-vaccinated dogs.

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