Use of a C₆ ELISA Test to Evaluate the Efficacy of a Whole-Cell Bacterin for the Prevention of Naturally Transmitted Canine Borrelia burgdorferi Infection*

Steven A. Levy, VMD

ABSTRACT
A commercially available C₆ ELISA kit was used to detect antibodies induced by natural infection with Borrelia burgdorferi in dogs that lived in an area endemic for Lyme disease. Rates of infection were determined both for nonvaccinated dogs and those that had been vaccinated with a whole-cell B. burgdorferi bacterin (Lyme Vax, Fort Dodge Animal Health) before 6 months of age and were boostered annually. Vaccinated dogs had an infection rate of 5% (8 of 163), whereas 64% (25 of 39) of the non-vaccinated dogs were positive for B. burgdorferi antibodies. The preventable fraction, determined by comparing infection rates in unvaccinated and vaccinated dogs, was 92.2% (95% confidence interval: 84.3% to 96.3%). In addition, screening of nonvaccinated dogs at six Connecticut clinics (Middletown, Portland, Essex, Old Lyme, Durham, and Marlborough) with the C₆ ELISA test revealed infection rates ranging from 41% to 73%, demonstrating a high level of infected dogs in the area. It was concluded that emphasis should be placed on vaccinating young dogs at risk for Lyme disease before they are exposed to infected ticks. Results of this study support the value of immunization with this whole-cell Lyme disease bacterin for dogs at risk for infection by B. burgdorferi.

INTRODUCTION
Canine Lyme borreliosis is a multisystemic illness caused by the spirochete Borrelia burgdorferi and vectored by ticks in the Ixodes ricinus complex. In endemic areas, 50% or more of unvaccinated dogs have been reported to be infected by the organism, and significant morbidity and mortality involving musculoskeletal, cardiac, and renal systems have been reported. In 1990, a whole-cell bacterin (LymeVax, Fort Dodge Animal Health) was introduced for prevention of Lyme disease in dogs. Studies of its immunogenicity, safety, and efficacy in experimentally exposed laboratory dogs have been published, and in 1993 a field study of safety and efficacy of this bacterin in naturally exposed dogs was reported. The present study was conducted to examine the efficacy of the whole-cell Lyme disease bacterin for the prevention of naturally transmitted B. burgdorferi, using a commercially available in-office kit (SNAP 3Dx, IDEXX Laboratories). The test kit detects antibodies directed against an invariable region (IR₆) of the B. burgdorferi
surface protein VlsE (Vmp-like sequence, Expressed), a surface antigen of the spirochete recognized during active infection. The C₆ ELISA test is not cross-reactive with antibodies induced by vaccination with either recombinant B. burgdorferi outer-surface protein A (OspA) or whole-cell bacterin. In addition to antibodies induced by infection with B. burgdorferi, the test detects antibodies induced by infection with Ehrlichia canis and antigen produced by Dirofilaria immitis. A positive C₆ ELISA result for B. burgdorferi antibodies is an indication of infection by the organism. The test is highly sensitive and specific and was used in this study to determine the infection status of dogs living in an area highly endemic for Lyme disease. The objective was to determine the infection rates and preventable fraction (PF) in dogs vaccinated throughout their lifetime with whole-cell bacterin, as compared with rates in nonvaccinated dogs. Before evaluating the performance of the vaccine, data were collected from several local veterinary practices to determine current rates of infection in the area.

**MATERIALS AND METHODS**

**Prevalence of Lyme Disease**

A total of 345 nonvaccinated dogs were screened with the C₆ ELISA test at six Connecticut clinics (Middletown, Portland, Essex, Old Lyme, Durham, and Marlborough). All dogs tested for B. burgdorferi antibodies were among those presented for heartworm screening between April 1 and August 30, 2001. Examination of hospital records and query of the owners were used to determine the vaccination status of each dog included in the survey.

**Evaluation of Vaccinated Versus Nonvaccinated Dogs**

At the Durham Veterinary Hospital, 39 nonvaccinated and 163 vaccinated dogs were selected for testing by the C₆ ELISA test to examine the efficacy of the whole-cell B. burgdorferi bacterin. Selection of dogs was based on the dog being at least 1 year of age at the time of inclusion in the study, having been a patient at the practice from the time of its first Lyme disease vaccination until the time of testing, and having complete immunization and medical histories available. Vaccinated dogs had received two doses of the whole-cell bacterin before 6 months of age, and booster doses had been given according to manufacturer’s directions each subsequent year. Nonvaccinated (control) dogs had never been immunized with any canine Lyme disease vaccine.

All dogs were tested using the in-office C₆ ELISA diagnostic kit. Because the C₆ ELISA test does not cross-react with vaccine-induced antibodies and is highly sensitive, dogs found positive by the C₆ ELISA test for B. burgdorferi antibodies were considered infected with the organism.

**Vaccine Efficacy**

Preventable fraction is the proportion of infection prevented by the vaccine relative to the infection rate in nonvaccinated dogs and is calculated by subtracting the rate of infection in vaccinated animals from the rate in unvaccinated animals and expressing the difference as a percentage of the incidence in unvaccinated individuals. Vaccine efficacy, PF, and the 95% confidence interval were calculated. All statistical analysis was performed using EpiInfo, Version 6.04d (Centers for Disease Control and Prevention).

**RESULTS**

Results of testing 345 nonvaccinated dogs at the six clinics are presented in Table 1. The infection rates at the clinics ranged from 41% to 73%, demonstrating a high level of infection among dogs in the geographic area of the study. At the Durham Veterinary Clinic, 163 dogs vaccinated annually with the whole-cell bacterin
and 39 nonvaccinated dogs qualified for evaluation. Nonvaccinated controls ranged from 1 to 16 years of age, and vaccinated dogs were 1 to 11 years of age. When the two groups were combined, the average age at the time of testing was 8.5 years. From the time of first vaccination until \( C_6 \) testing, all dogs lived in the Connecticut River Valley, 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 inclusion criteria when they presented for routine heartworm screening.

### Comparison of Infection Rates

Sixty-four percent (25 of 39) of nonvaccinated dogs were infected, as determined by the \( C_6 \) ELISA test, compared with 5% (8 of 163) of dogs that had been vaccinated annually with whole-cell bacterin. The PF for prevention of Lyme disease by the whole-cell bacterin in dogs tested at this clinic was 92.2% (95% confidence interval: 84.3% to 96.3%).

### DISCUSSION

Canine Lyme disease is a significant clinical entity in certain areas of the United States.\(^1\)\(^{–}\)\(^{5}\)\(^{,}\)\(^{7}\)\(^{–}\)\(^{11}\)\(^{,}\)\(^{16}\) Syndromes involving limb or joint abnormalities are common.\(^3\)\(^{,}\)\(^{5}\)\(^{,}\)\(^{7}\)\(^{–}\)\(^{11}\)\(^{,}\)\(^{17}\) Renal disease associated with *B. burgdorferi* infection is difficult to treat and generally results in either death or euthanasia of the dog.\(^3\)\(^{,}\)\(^{5}\) Cardiac Lyme disease has been reported, although it is a rare presentation.\(^3\)\(^{–}\)\(^{5}\) Diagnosis of canine Lyme disease is performed by a process of rule-in and rule-out of alternative diagnoses, evaluation of epidemiologic risk, and consideration of laboratory data, including urinalysis, electrocardiogram, serum chemistries, and serologic analysis for evidence of infection by the etiologic organism.\(^2\)\(^{–}\)\(^{5}\)\(^{,}\)\(^{7}\)\(^{–}\)\(^{11}\)\(^{,}\)\(^{17}\) Serologic evaluation for diagnosis of Lyme disease has been confounded by several factors, including high prevalence of seropositive, clinically normal dogs; high cost and subjective nature of Western blot analysis; and the introduction of vaccines that will induce antibodies detected by immunofluorescent assay, ELISA, and Western blot.\(^3\)\(^{,}\)\(^{5}\)\(^{,}\)\(^{10}\)\(^{,}\)\(^{16}\)\(^{,}\)\(^{17}\)\(^{,}\)\(^{26}\)

Evaluation of the efficacy of Lyme disease vaccines has been accomplished through laboratory models that attempt to reproduce natural exposure to tick-borne *B. burgdorferi*\(^28\)\(^{,}\)\(^{29}\) and one field study in naturally exposed dogs immunized with whole-cell bacterin.\(^6\) Each method has its limitations. Laboratory models use a limited number of infected ticks and challenge may take place a short time after immunization.\(^29\) The field study was complicated by the inherent difficulty of diagnosing canine Lyme disease and the long lag time from infection to demonstration of clinical signs.\(^30\) Use of the \( C_6 \) ELISA to evaluate vaccine efficacy in natural exposure studies overcomes some of the limitation imposed by diagnosis of clinical disease as a criterion for vaccine efficacy. Prevention of infection is a more sensitive criterion because it eliminates both of the limitations previously encountered. \( C_6 \) ELISA has been demonstrated to be 100% specific and 94.4% to 100% sensitive for detection of antibodies induced by infection with the
The lag phase for the development of antibodies detected by the C6 ELISA is 4 to 6 weeks after infected tick bite. The limitation of a one-time challenge of laboratory dogs by a specified number of ticks was eliminated in the present study by natural challenge in a highly endemic environment with tick exposure spanning the full period of activity by the vector Ixodes scapularis. Tick challenge for dogs in this study ranged from 1 to 16 years.

The prevaccination infection status of dogs in this retrospective study was not available. Risk of infection was high in the study area, ranging from 41% to 73% among the six clinics surveyed.

A PF of 92.1% is higher than that previously reported for the bacterin. In that study, PF was 58% for dogs known to be infected and 86% for uninfected dogs or of unknown status at the time of immunization. In the earlier study, an infection rate of 40% was determined in 1,252 dogs that were initially immunized at any age. In the present study, only dogs immunized by 6 months of age were included in the vaccinated group. The risk of infection increases with age for dogs living in endemic areas. In the study reported here, it is possible that immunizing young dogs with this whole-cell bacterin before they have an opportunity to be exposed to infected ticks produced a much higher PF than in that earlier study. A high PF for a bacterin may also be related to the dynamics of surface antigens on B. burgdorferi during the life cycle of vector ticks. In infected, questing (unfed) ticks, spirochetes express OspA as a dominant surface antigen. Within the early period of tick attachment on a mammalian host, cues associated with the host temperature and blood meal induce host adaptation, the down-regulation of OspA, and often the up-regulation of a new surface antigen, OspC, on spirochetes. Examination of populations of B. burgdorferi transmitted to hosts reveals that spirochetes expressing OspA are absent, whereas those expressing OspC are often present. The whole-cell bacterin induces antibodies not only to OspA but also to a wide variety of other B. burgdorferi antigens, including OspC. Animal studies and in vitro experiments have demonstrated that many of these antibodies have borreliacidal properties and can prevent infection in immunized animals. The whole-cell bacterin is adjuvanted and produces high antibody titers.

CONCLUSION

The finding of high efficacy for the whole-cell Lyme disease bacterin in this study in preventing infection supports results of a previous field study in which prevention of episodes of clinical Lyme disease was the criterion for efficacy. Clinically, emphasis should be placed on vaccinating dogs at risk before they are exposed to infected ticks. Vaccination against canine Lyme disease should be included as a routine component of immunization protocols for puppies living in areas having a high risk of infection. Results of this study support the value of immunization with this whole-cell Lyme disease bacterin for dogs at risk for infection by B. burgdorferi.

ACKNOWLEDGMENTS

Serum samples and patient data for some control dogs were contributed by Dr. Kathy Clark, Clark Veterinary Hospital, Old Lyme, CT, and Dr. Robert Olson, Pieper-Olson Veterinary Hospital, Middletown, CT.

REFERENCES

4. Levy SA, Duray PH: Complete heart block in a dog.