Seroprevalence of *Borrelia burgdorferi*–Specific C₆ Antibody in Dogs Before and After Implementation of a Nonadjuvanted Recombinant Outer Surface Protein A Vaccine in a Rhode Island Small Animal Clinic*

Daniel Hebert, DVMᵃ
Andrew Eschner, DVMᵇ

ᵃVetCor-West Shore Animal Clinic
2500 West Shore Road
Warwick, RI

ᵇField Veterinary Services
Merial Ltd
Gansevoort, NY

**INTRODUCTION**

*Canine Lyme disease* (*borreliosis*) is the term used for the clinical manifestations of *Borrelia burgdorferi* infection in dogs in the United States. The arthropod vector, pathogenesis, and pathophysiology of canine Lyme borreliosis have been previously described.¹⁻³ Veterinarians in Lyme-endemic areas have reported that up to 73% of dogs test serologically positive for *B. burgdorferi* infection.⁴ A recent study⁵ showed that the geographic distribution of *B. burgdorferi* is widespread, with occasional endemic foci (hotspots) within larger areas of relatively low prevalence. In addition to prescribing quality tick-control agents, veterinarians in Lyme-endemic areas often vaccinate to help prevent canine Lyme borreliosis. This clinical study highlights the profile and com-

---

*Study creation, design, and data collection conducted independently by Dr. Hebert without manufacturer input or support. Manuscript review and coauthorship assistance provided by Dr. Eschner.*
position of a canine patient population that tested serologically positive for the C₆ antibody to *B. burgdorferi* after the implementation of a vaccine protocol using a nonadjuvanted recombinant outer surface protein A (rOspA) vaccine (Recombitek, Merial).

Warwick is a coastal city in Kent County, Rhode Island, located approximately 70 miles north of Lyme, Connecticut. It is the second largest city in Rhode Island, and Kent County ranked second in the state for the frequency and rate of confirmed human Lyme disease cases between 2004 and 2008. Large portions of land remain undeveloped, and *Ixodes scapularis* tick activity and *B. burgdorferi* infection rates are high in humans and pets alike, prompting heightened owner concern within my (D.H.) 2.5-doctor small animal clinic. For these reasons and others, the clinic’s veterinarians considered an effective screening and vaccination program for canine Lyme borreliosis essential.

Before January 2006, the clinic did not employ a general screening or vaccine protocol that addressed canine Lyme borreliosis. Serologic testing for evidence of *B. burgdorferi* infection was typically only performed on suspected clinical cases, and vaccination to prevent canine Lyme borreliosis was not routinely recommended.

To determine whether active use of a vaccine to prevent canine Lyme borreliosis made sense for the hospital, the veterinarians performed an analysis to determine the extent to which serologic evidence of *B. burgdorferi* infection was present in the canine patient base. In January 2006, the three-way in-hospital ELISA for C₆ antibodies specific to *B. burgdorferi, Ehrlichia canis*, and *Dirofilaria immitis* (SNAP 3Dx, IDEXX Laboratories) and, subsequently, the four-way ELISA, including *Anaplasma phagocytophilum* (SNAP 4Dx, IDEXX Laboratories), were introduced in place of a stand-alone heartworm test for annual screening of canine patients. At the end of an 18-month screening period, approximately 11% (70 of 615 dogs screened) had tested positive for serologic evidence of *B. burgdorferi* infection. This rate was determined to be high enough to warrant the inclusion of an annual vaccine to prevent Lyme borreliosis in the hospital’s core vaccine protocol. Because the hospital was starting with a pool of client-owned dogs of which most had not previously received a vaccine against Lyme borreliosis, the veterinarians decided to set up an observational study to examine the profile of C₆ antibody–positive dogs after the implementation of a Lyme borreliosis vaccination program.

**MATERIALS AND METHODS**

**Patient Selection for Vaccination**

The three-way and four-way in-hospital ELISAs for the presence (or absence) of *B. burgdorferi* C₆ antibodies were used to screen all dogs entering the hospital for annual or semiannual examinations. Dogs that tested negative at the time of examination were vaccinated immediately. Simultaneous with the decision to implement an active immunization program against canine Lyme borreliosis, a test to quantitatively measure the level of C₆ antibody (Lyme Quant C₆ Test, IDEXX Laboratories) became commercially available. Therefore, any dog testing positive on the in-house C₆ ELISA would receive no vaccinations until a quantitative C₆ antibody test, blood chemistry panel, complete blood count, and urinalysis were performed. Dogs with a quantitative C₆ level <30 U/mL and normal blood and urine parameters were subsequently vaccinated. Dogs with a quantitative C₆ level >30 U/mL were treated for 28 days with doxycycline at 10 mg/kg/d. A recheck physical examination, blood chemistry panel, complete blood count, and urinalysis were performed 30
days after the initiation of antibiotics. If all blood parameters were found to be within normal limits and the dogs remained free of clinical signs, they were eligible to be included in the Lyme borreliosis vaccination program. Quantitative C₆ levels would be checked again 6 months after completion of antibiotic therapy to assess the serologic impact of treatment and establish the individual patient’s baseline C₆ parameters for future screening. All dogs that had a history of being C₆ positive and tested positive on the in-house ELISA at subsequent screening visits would have repeat analysis of the quantitative C₆ antibody level.

**Vaccination Protocol**

At the time of vaccine selection, two *B. burgdorferi* vaccines were readily available for dogs: the rOspA vaccine and an adjuvanted whole-cell bacterin vaccine. The rOspA vaccine for dogs was chosen for its reported short- and long-term efficacy characteristics and anticipated safety owing to its lack of extraneous *Borrelia* proteins and chemical adjuvant. Further reading on the subject indicated that a modification of the initial vaccine series might increase the protective humoral response in some patients. In a pivotal human vaccine study, efficacy improved after the administration of the third vaccine. In light of this information, the hospital administered the second vaccine in the initial series 3 weeks after the initial vaccine, according to the label. However, rather than being delayed a full year, the third booster vaccine in the series was administered 6 months after the second. Yearly vaccination was then recommended. This protocol was initiated in July 2007.

**Data Analysis**

Postvaccination screening for the prevalence of *B. burgdorferi*–specific C₆ antibody in the hospital’s canine population began in the transitional period (July 1, 2008, to January 1, 2009) and continued through the period in which all canine patients would have finished the entire vaccination series. The *transitional period* was defined as the 6-month period wherein some dogs continued to be vaccinated according to protocol and therefore might not have been serologically assessed (screened). Any dog that tested positive for evidence of *B. burgdorferi* infection on the in-clinic C₆ antibody ELISA was identified as belonging to one of the following categories: (1) added after the initiation of the vaccine protocol, (2) did not receive a full complement of vaccines due to compliance failure or preexisting illness, (3) did not initiate a Lyme vaccination series, (4) had a history of *B. burgdorferi*–specific C₆ antibody–positive serology, (5) transitioned from a different brand of vaccine, and (6) considered to have acquired an infection with *B. burgdorferi* (C₆ antibody–positive serology) after a full complement of vaccine had been delivered according to protocol. These numbers were compared with the total number of positive C₆ antibody test results to determine the frequency of positive results in unvaccinated versus vaccinated dogs.

A separate analysis examined the entire pool of dogs that were listed as receiving a 1-year Lyme vaccination, indicating completion of the series. These patients were classified as follows: (1) dogs that had tested positive for *B. burgdorferi*–specific C₆ antibody before initiation of the Lyme vaccination protocol, (2) dogs that tested positive for *B. burgdorferi*–specific C₆ antibody before completing the full vaccine series, (3) new patients to the clinic that had a history of 1-year vaccination from another clinic with unknown vaccine protocols, (4) patients that completed the initial three-vaccine series but failed to return on time for the annual booster, and (5) dogs that tested positive for Lyme C₆ antibody that had completed the vaccination protocol.
RESULTS

Screening Period A

During the transitional 6-month period from July 1, 2008, to December 31, 2008, 28 of 372 dogs (7.5%) were positive for C6 antibody to *B. burgdorferi* (Tables 1 and 2). Of these 28 dogs, nine were new patients with an unclear history of vaccination to prevent Lyme borreliosis. Of the 19 established patients, nine had a history of C6 antibody-positive serology and nine had received no vaccination. The remaining dog had received the full complement of vaccine according to clinic protocol. This dog had a quantitative C6 antibody level of <30 U/mL, and the treatment recommendation from the ELISA manufacturer was to monitor. This dog remained clinically asymptomatic for the duration of the study. Of the population tested in this screening period, this dog (0.3% of those tested) was the only patient that completed the vaccine protocol (initiated between 12 and 18 months previously) and tested C6 antibody positive (Tables 1 and 2).

Screening Period B

In this study, the period when all established dogs would have completed the full vaccination series began on or immediately around January 1, 2009. In the 6-month screening period after that date, 55 of 570 (9.6%) dogs tested positive for *B. burgdorferi* C6 antibody. Of these 55 dogs, 28 (51%) were new (unvaccinated) patients to the practice. Of the established patients, 14 dogs had a history of C6 antibody-positive serology, four had not completed the vaccine series, eight had no prior Lyme vaccination, and one had been transitioned from a previously administered whole-cell Lyme borreliosis bacterin vaccine (Tables 1 and 2). Of the population tested in this screening period, no dog that had completed the described vaccine protocol using the nonadjuvanted rOspA vaccine (initiated as early as 24 months prior) tested positive for C6 antibody (Tables 1 and 2).

Screening Period C

The period between July 1, 2009, and De-

---

TABLE 1. Categories of C₆ Antibody–Positive Canine Patients Within Four Defined Screening Periods

<table>
<thead>
<tr>
<th>Screening Period</th>
<th>Total</th>
<th>New C₆ antibody–positive</th>
<th>Incomplete vaccination status</th>
<th>No prior vaccination</th>
<th>Prior bacterin vaccine administration</th>
<th>New C₆ antibody–positive status (protocol compliant)</th>
<th>C₆ antibody–level &gt;30 U/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (7/1/08–12/31/08)</td>
<td>28</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>9</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>B (1/1/09–6/30/09)</td>
<td>55</td>
<td>28</td>
<td>14</td>
<td>4</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>C (7/1/09–12/31/09)</td>
<td>29</td>
<td>10</td>
<td>7</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D (1/1/10–3/31/10)</td>
<td>13</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
December 31, 2009, was examined. During this time, 29 of 422 (6.9%) dogs tested positive for serologic evidence of B. burgdorferi infection. Ten were new patients, seven had a history of C₆ antibody-positive serology, and 12 had received no vaccination against Lyme disease. Of the population tested in this screening period, no dog that had completed the vaccine protocol using the nonadjuvanted rOspA vaccine (initiated as early as 30 months prior) tested positive for C₆ antibody (Tables 1 and 2).

**Screening Period D**

The period between January 1, 2010, and March 31, 2010, was the final period analyzed. During this time, 13 of 227 (5.7%) dogs tested positive for serologic evidence of B. burgdorferi infection. Eight were new patients, and five had received no vaccination against Lyme disease (Tables 1 and 2).

**Composite Analysis**

In total, 1617 B. burgdorferi–specific C₆ antibody tests were conducted on 1220 dogs over a 33-month postvaccination period. Twenty-six of these tests were conducted to test only for heartworm, A. phagocytophilum, or E. canis in dogs that had already been screened for C₆ antibody. These tests were not included in the analysis. Of the 125 C₆ antibody–positive test results, 124 were from a subset of dogs (described above) that were considered either nonvaccinated or suboptimally vaccinated and/or were previously C₆ antibody positive. One dog (<1.0% of total positive tests) that was vaccinated according to protocol tested positive for C₆ antibody. No dog found to be C₆ antibody positive during this observational study was clinically symptomatic. In addition, complete blood counts, blood chemistry results, and urinalyses of all C₆ antibody–positive dogs were within normal limits. Quantitative C₆ antibody levels were maintained below 50% of initial levels in all dogs that tested positive before full vaccination. Therefore, these dogs maintained their postantibiotic treatment.

### TABLE 2. Percentage of C₆ Antibody–Positive Canine Patients by Category

<table>
<thead>
<tr>
<th>Screening Period</th>
<th>Total number of dogs tested</th>
<th>Total (%)</th>
<th>Existing clients (%)</th>
<th>Prior C₆ antibody--positive status (%)</th>
<th>Incomplete vaccination status (%)</th>
<th>No prior vaccination (%)</th>
<th>Prior bacterin administration (%)</th>
<th>New C₆ antibody--positive status (protocol compliant; %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (7/1/08–12/31/08)</td>
<td>372</td>
<td>7.5</td>
<td>5.1</td>
<td>2.4</td>
<td>0</td>
<td>2.4</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>B (1/1/09–6/30/09)</td>
<td>570</td>
<td>9.6</td>
<td>4.7</td>
<td>2.5</td>
<td>0.7</td>
<td>1.4</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>C (7/1/09–12/31/09)</td>
<td>422</td>
<td>6.9</td>
<td>4.5</td>
<td>1.7</td>
<td>0</td>
<td>2.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D (1/1/10–3/31/10)</td>
<td>227</td>
<td>5.7</td>
<td>2.2</td>
<td>0</td>
<td>0</td>
<td>2.2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
C₆ antibody baseline, which likely indicates that B. burgdorferi was not retransmitted within the subsequent revaccination interval.

Analysis of All Vaccine Protocol–Compliant Dogs

To assess the effectiveness of the vaccine on the full canine population, an analysis was conducted between July 1, 2008, and March 31, 2010, on all dogs that received all three initial vaccines as described (Table 3). Of 840 dogs that received the prescribed vaccine series, 60 were positive for serologic evidence of B. burgdorferi infection using the in-house C₆ antibody test within the defined test period. Of these dogs, 48 tested positive before initiation of the Lyme vaccination series, nine had not completed the prescribed vaccine series before testing positive (i.e., they did not receive the full series until after a positive result for Lyme C₆ antibody), one had previously received a “1-year” Lyme vaccination of unknown source, one failed to return on time for its annual booster, and one had completed the full vaccination series. This last dog is the same patient reported in the Period A analysis.

### DISCUSSION

The described study period captures a snapshot of the composition and profile of Lyme-specific C₆ antibody–positive dogs under field conditions in one clinic during a 33-month period after the clinic began using a nonadjuvanted rOspA vaccine. The data show that the implementation of a slightly modified three-vaccine protocol may have contributed to what the clinic considered a highly effective Lyme borreliosis management program. A commercially available in-clinic test kit for the detection of B. burgdorferi–specific C₆ antibody was used for 1617 individual serologic screenings, of which 125 results were positive. Only one dog that had completed the defined vaccine protocol had a positive result. This dog had a low quantitative C₆ antibody titer (<30 U/mL), did not require treatment, and remained clinically asymptomatic. All other C₆ antibody–positive dogs were not vaccinated (new to the clinic), vaccine protocol violators (noncompliant), vaccinated with another product, or previously C₆ antibody positive. Thus, when the nonadjuvanted rOspA vaccine was used in this hospital during this defined study period, less than 1% (0.99%) of all dogs testing C₆ antibody positive had completed the recommended vaccine protocol. Described differently, 99.0% of the C₆ antibody–positive test results were in dogs that were either previously positive or not optimally vaccinated. In summary, the hospital staff observed that the distribution of C₆ antibody–positive test results in their canine patients was skewed almost completely toward the unprotected, poorly compliant, or previously C₆ antibody–positive population.
These observations may cast an interesting light on the protection afforded by a Lyme borreliosis vaccine program when comparing seroprevalence before and after implementation. During the 18-month prevaccination screening period, 11% of the tested canine patient population was serologically positive for *B. burgdorferi*-specific C₆ antibody. However, during the 33-month postvaccination period, 4.1% of established patients tested positive. As the study period progresses, the data show a steady decline in the overall percentage of dogs testing positive for C₆ antibody (2.4%, 2.5%, 1.7%, 0%; Table 2). Further studies and analysis would be necessary to determine the relationship between the observed decrease in Lyme seropositivity after implementation of a vaccine program. Nonetheless, the observed drop in the background C₆ antibody positivity may be a reflection of the benefit of vaccine performance given that other methods of prevention (tick control) remained constant across the two periods. Since approximately 30% (38 of 125) of the dogs that were positive for C₆ antibody in the period after initiation of the Lyme vaccination protocol either received no vaccines or were not vaccine protocol compliant, additional emphasis on compliance and client education may go a long way toward further reducing the prevalence of canine Lyme borreliosis seropositivity in this clinic.

A limitation of this study is the fact that it was not a randomized, blinded, placebo-controlled prospective study that would have allowed for the comparison of two defined groups—one vaccinated and one not—followed over time. Therefore, a precise efficacy value for the vaccine used cannot be definitively ascertained. Despite this, and for all intents and purposes, this study was of a size (1220 dogs) and duration (33 months) that allowed for a more precise characterization of the patient profile of a Lyme-specific C₆ antibody–positive dog in this practice after immunization. The veterinarians essentially wanted to answer two questions to help rationalize the cost and time spent recommending a vaccine against canine Lyme borreliosis in their hospital: (1) would serologic testing for Lyme borreliosis reveal that vaccinated dogs are benefited (e.g., remain serologically C₆ antibody negative), and (2) might they see a decline in serologically positive dogs in the general canine population after implementation of an active vaccination program? At this 2.5-doctor practice in a Lyme-endemic area of Rhode Island, the veterinarians believe strongly that they achieved both ends.

**CONCLUSION**

Veterinary Lyme disease vaccines have been available since the 1990s in the United States and currently come in two general presentations: whole-cell bacterin formulations using inactivated *B. burgdorferi* spirochetes and formulations using purified rOspA. Each vaccine type is licensed as safe and effective by the US Department of Agriculture, and vaccine manufacturers consistently highlight and use OspA as a key protective immunogen.⁹,¹⁰,¹⁴,¹⁵ Recombinant vaccine technology allows the use of specific proteins involved in a functional immune response without the simultaneous delivery of nonessential and potentially inflammatory proteins associated with whole-cell bacterin preparations. The recombinant vaccine induces OspA-specific borrelicidal antibodies and has been shown to be an immunogenic and effective vaccine antigen for the prevention of Lyme disease in a variety of species, including dogs, horses, mice, nonhuman primates, and humans.⁹,¹⁰,¹²,¹⁶–¹⁸

The most effective canine Lyme borreliosis prevention program relies on three pillars: education, effective tick control, and a dog's ability to effectively mount an effective vaccine re-
sponse. Given the 11% seroprevalence rate for B. burgdorferi in its canine patients during the screening period, this hospital decided to implement an active immunization strategy against canine Lyme borreliosis. Based on their observations during this 33-month study, the veterinarians are highly satisfied with the decision to incorporate a Lyme borreliosis vaccine into their core canine vaccine protocol. They remain strongly convinced and encouraged by the observation that dogs newly screened as C₆ Lyme antibody positive are not likely to have been previously vaccinated according to their protocol. Furthermore, they seem to have markedly altered the seroprevalence of B. burgdorferi (C₆) antibody in their canine patients compared with the prevaccination population.

REFERENCES


*RECOMBITEK is a registered trademark of Merial Limited, Duluth, GA 2010. All rights reserved.