Confirmation of Presence of *Borrelia burgdorferi* Outer Surface Protein C Antigen and Production of Antibodies to *Borrelia burgdorferi* Outer Surface Protein C in Dogs Vaccinated with a Whole-cell *Borrelia burgdorferi* Bacterin.

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ABSTRACT

The findings in this study demonstrate the presence of *Borrelia burgdorferi* outer surface protein C (OspC) antigen in a whole-cell bacterin[a] (BBB) and the production of antibodies directed against *Borrelia burgdorferi* OspC in dogs immunized with this bacterin. Client owned dogs were determined to have negative test results for infection with *Borrelia burgdorferi* prior to initial or booster immunization. Monoclonal OspC antibody Western blot demonstrated presence of *Borrelia burgdorferi* OspC antigen in the whole-cell bacterin. Serum samples obtained post-immunization and evaluated with an in vitro qualitative assay for the detection of IgG antibodies in canine serum that react with *Borrelia burgdorferi* antigens, including OspC, revealed that dogs under field and laboratory conditions had Western blot evidence of production of antibodies directed against *Borrelia burgdorferi* OspC.

INTRODUCTION

Lyme disease is caused by the bite of an *Ixodes* spp tick infected with *Borrelia burgdorferi* .[1] Dogs infected with *Borrelia burgdorferi* may develop Canine Lyme disease. [2 3] Affected dogs may display a well character-

[a]LymeVax® and Duramune® Max 5-Cvk + LymeVax® Fort Dodge Animal Health, Overland Park, KS.

ized syndrome with shifting leg lameness, joint swelling, fever, lethargy, inappetence, and often lymphadenomegaly.[4,5] A common Lyme disease-associated syndrome characterized by protein-losing nephropathy, azotemia, hyperphosphatemia, hypoglobulinemia, and peripheral edema generally leads to death or euthanasia.[5,6,7] A rare syndrome characterized by myocardial disease and associated arrhythmia also occurs in dogs.[8] In 1990, a whole-cell *Borrelia Burgdorferi* bacterin was introduced for immunization of dogs as an aid in the prevention of the disease caused by *Borrelia burgdorferi*. Subsequent studies of field-exposed dogs determined that immunization with this bacterin not only prevented disease[9] but also prevented infection[10] in dogs in highly Lyme disease-endemic areas.

Expression of surface proteins of *Borrelia burgdorferi* is highly variable[11] and dependent upon the environment in which the spirochete is living and the stage at which the organism is examined in the transmission cycle from reservoir to vector to host.[12] Outer surface proteins and their expression play a major role in the host-adaptation process[11] that supports the survival of the organism in the various environments, and thus the ability of the organism to persist in and to infect susceptible hosts. Outer surface protein A (OspA) has been determined to play a role in protecting the organism from the innate immune response in the invertebrate tick vector. Changing of the organism's surface proteins to OspC, conversely, helps protect it from the innate immune response of the vertebrate hosts and promotes invasion and survival in those hosts.[12]

Antibodies directed against both OspA[13] or OspC[14] have been demonstrated to protect against infection in laboratory animals. However, in natural exposure challenge, vaccines limited to the production of OspA antibodies have been demonstrated to produce only moderate protection against field acquired *Borrelia burgdorferi* in both humans [15] and dogs. [16] Conversely, dogs vaccinated with BBB have been demonstrated to have high levels of protection against clinical disease[9,17] and infection.[10,17] Although the data is from two separate studies in the same geographic area, we have hypothesized that the lower infection rate in dogs immunized with BBB as compared to dogs immunized with OspA-only vaccine[b] is, at least in-part, attributable to the production of antibodies directed against OspC in dogs immunized with the whole-cell BBB. This study verified the presence of OspC antigen in BBB and demonstrated the repeated and reliable production of anti-OspC antibodies in both field and laboratory dogs immunized with BBB.

MATERIALS AND METHODS

Dogs

Client-owned dogs: Twenty-five adult dogs that had been immunized previously against *Borrelia burgdorferi* infection with BBB and presented for annual booster vaccination and seven young dogs presenting for their initial puppy series of immunizations with BBB. Laboratory dogs - Twenty-four (24) 9 week old purpose bred dogs.

Vaccine for animal administration

Borrelia Burgdorferi Bacterin (BBB), multiple serials of commercially available product, was administered subcutaneously in a single dose to 25 client-owned adult dogs presenting for annual booster vaccination. Products used for vaccine administration were: LymeVax® and Duramune® Max 5-Cvk + LymeVax®. A 2-dose vaccine series was administered according to label instructions to seven client-owned young dogs and 12 laboratory dogs.

Vaccine for Western Blot Analysis

Borrelia Burgdorferi Bacterin (BBB) sourced directly from the Fort Dodge Animal Health manufacturing facility, lot # 06321B, was used for the Western blot test to confirm the presence of OspC in BBB.

Vaccine Control

A true placebo (antigen free diluent blended

[b] *rLyme®, Merial, Athens, GA*

with adjuvants) was administered to12 laboratory dogs according to label instructions for BBB.

Serum Samples

All samples were collected as whole blood and allowed to clot. Serum was harvested and frozen at -20 C. Whole blood was collected from adult client-owned dogs prior to annual booster vaccination and approximately 4-6 weeks later. Whole blood was collected from young client-owned dogs prior to initial immunization and approximately 4-6 weeks after the second dose was administered. Whole blood was collected from the laboratory dogs 4-6 weeks after the second dose of BBB or placebo was administered.

Serology

C6 antibodies: Serum samples were evaluated using a patient-side test kit[c] for the detection of *Borrelia burgdorferi* infection. The test includes the C6 Enzyme-Linked Immunosorbent Assay (C6 ELISA) that detects antibodies against the Vmp-like sequence, Expressed (VlsE) gene of the spirochete, *Borrelia burgdorferi*. Canine vaccines do not induce antibodies detected by this test. 18 A positive C6 ELISA is an indication of infection with *Borrelia burgdorferi*.19 This test also detects presence of antibodies to Ehrlichia canis, Anaplasma phagocytophilum, and antigen of Dirofilaria immitis.

Western Blot

A Western blot using a commercial test kit[d] was performed on BBB to determine the presence of OspC antigen. The vaccine was loaded at 15ug/lane on a 15% SDS-PAGE and electrophoresed. Transfer was performed to nitrocellulose following the manufacturer's instructions (Biorad, Hercules, CA). The blot was probed with a commercially sourced monoclonal antibody against OspC diluted 1/1000 and an anti-mouse IgG (H + L) secondary antibody (KPL Lot # 0300782).

OspC Antibodies

A commercially available in vitro qualitative assay[e] was used for the detection of IgG antibodies in canine serum that react with *Borrelia burgdorferi* antigens. The kit comes with pre-identified antigens on the immunomembrane including OspC. Post-immunization serum samples from each animal were tested for the presence of OspC antibodies on the Qualicode Canine Lyme test following the manufacturer's directions.

RESULTS

The serology test described previously determined that no field samples from either adult or young dogs had antibodies indicating infection with *Borrelia burgdorferi* prior to vaccination. (Table 1.) In contrast, 20% of the adult client-owned dogs were shown to test positive for antibodies to Anaplasma phagocytophilum. Samples from all dogs were negative for Dirofilaria immitis and negative to Ehrlichia canis antibodies.

The Western blot performed on BBB showed a single band in the 20k Da region at the location consistent with the commercially available OspC monoclonal antibody used as a reference (Figure 1).

Serum samples collected as described above were tested for the presence of antibodies to OspC at 4-6 weeks after the booster, second vaccination, or second dose of placebo using the commercially available Immunetics test kit (Figure 2). Twenty-five of 25 client-owned adult dogs devel-

***Table 1**: IDEXX 4Dx test results prior to vaccination of the client-owned dogs*

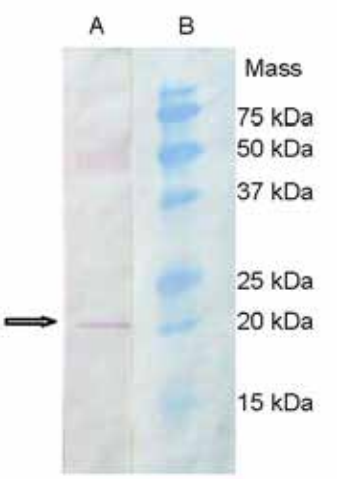
	A. Phagocytophilum	*E. Canis*	*B. burgdorferi*	*D. immitis*
Adults	5/25	0/25	0/25	0/25
Puppies	0/7	0/7	0/7	0/7

[c] SNAP®4DX, IDEXX Laboratories, Westbrook, ME. (

[d] *Biodesign International, OEM Concepts, Meridian Life Sciences, Saco. ME, USA*

[e] *Qualicode Canine Lyme disease kit, Immunetics, Boston, MA*

Figure 1 *– Western blot of BBB antigen probed with an OspC monoclonal antibody. Reaction of monoclonal OspC antibody with the vaccine antigen indicates presence of OspC in the vaccine.*



A = Monoclonal antibody to OspC run against BBB vaccine antigen.
B = Molecular mass markers

Arrow indicates the OspC band of interest

Figure 2. *Immunetics Qualicode Western blot of serum sample from client-owned LyneVax immunized dog demonstratting banding in OspC region*

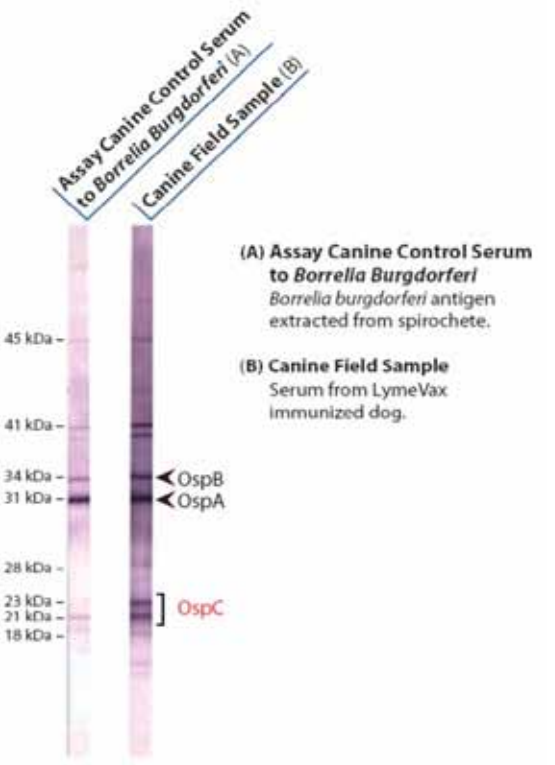


***Table 2**: The presence of OspC antibodies in dogs after immunization*

	Total	Antibodies present	Antibodies absent
Field samples			
Puppies	7	7/7	0/7
Adults	25	25/25	0/25
Laboratory samples			
Negative controls	12	0	12
Vaccinated	12	11	1

oped antibodies to OspC following booster vaccination. All client-owned young dogs possessed antibodies to the OspC protein following a 2-dose vaccination series. Similar results were observed in laboratory vaccinated dogs, in which 11 of 12 vaccinated had OspC antibodies. Conversely, 0 of 12 of the negative control laboratory dogs demonstrated production of OspC antibodies (Table 2).

DISCUSSION

The demonstration of OspC in BBB and antibodies to OspC in vaccinated dogs may explain the high level of efficacy of BBB in dogs.

Vaccination with the commercially available canine BBB has been demonstrated to prevent both signs associated with infection with *Borrelia burgdorferi* [9] as well as to prevent infection with the organism [10] under natural exposure challenge in a highly Lyme disease-endemic area. The vaccine is a multiantigenic, whole-cell bacterin produced by harvesting and inactivating twostrainsf strains of *Borrelia burgdorferi* grown in bioreactors. The vaccine is adjuvanted and produces a robust and long-lasting antibody response to multiple *Borrelia burgdorferi* antigens [20].

Variable expression of *Borrelia burgdorferi* outer surface proteins is dependent on the differing environments in the invertebrate arthropod vector and the mammalian host. In the tick vector, the organism expresses abundant amounts of OspA where this antigen serves to protect the organism from the innate immune surveillance of the tick and to anchor the organism in the midgut of the tick. Upon attachment of the tick to a mammalian host, cues in the blood meal and higher body temperature induce down regulation of OspA and up regulation of OspC. OspC plays a role in virulence and invasion. In the host-adaptation process the shift from OspA to OspC expression prepares the spirochetes to survive the early, innate immune surveillance of the mammal.[11,12]

The original multiantigenic BBB has been demonstrated to be highly effective in naturally exposed dogs (9) (10) as well as dogs in controlled laboratory experiments. [17] Greater preventable fraction in client-owned dogs vaccinated with BBB (92.2%) [10] as compared to the recombinant subunit OspA vaccine (60%) [16] has been demonstrated. Although the data is from two separate studies in the same geographic area, this difference may, in-part, be attributed to the presence of adjuvant and production of higher antibody titers in the bacterin.[20] Additionally, the multiantigenic bacterin produces borreliacidal antibodies that may kill *Borrelia burgdorferi* spirochetes at various stages of surface protein expression. first, in the tick's midgut prior to OspA down regulation; second in the tick's salivary glands after OspC up regulation; and finally, in the dog after transmission of OspC-expressing, host-adapted spirochetes.[17,21]

CONCLUSION

The multiantigenic BBB contains OspC antigen and is capable of producing antibodies to OspC in client owned adult and young dogs as well as in laboratory dogs.

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