The Importance of Differentiating Exposure from Infection with *Borrelia burgdorferi* in the Diagnosis and Treatment of Canine Lyme Disease

The C_6 technology used by IDEXX in USDA-licensed kits differentiates Lyme-infected from vaccinated dogs

Introduction

The C_6 peptide used in the IDEXX SNAP® 3Dx®, SNAP® 4Dx® Plus and Lyme Quant C_6® tests has played a foundational role in veterinary infectious disease diagnostics for the last 10 years and has been employed as a method of Lyme disease surveillance in dogs across North America. Recently, the Centers for Disease Control and Prevention (CDC) has recognized that Lyme surveillance data from dogs can be a valuable tool for predicting the emergence of Lyme disease in humans within new geographic regions.[1] This diagnostic update on Lyme C_6 testing will provide information on:

- Why VlsE (C_6) is expressed by the spirochete.
- How the C_6 peptide is used in Lyme diagnostics.
- When quantitative measures of C_6 may help in the management of subclinical cases of *Borrelia burgdorferi* infections.

Background

The C_6 peptide represents one of the constant or invariable regions (IR_6) of the VlsE protein. VlsE is a surface protein of *B. burgdorferi*. It is encoded by the VlsE gene, which contains numerous variable sequences along with the six constant region sequences. The organism selects and expresses different variable sequences over time in order to successfully evade the host immune response and survive within the mammalian host. The VlsE gene is only expressed in the mammalian host. The gene is not expressed when the organism is within the tick or when it is grown in culture to produce the Lyme vaccine. Therefore, antibodies generated as a result of Lyme vaccination do not react with the C_6 peptide.[2] Antibodies to the C_6 peptide are an indication of natural infection with *B. burgdorferi*.

Surface proteins change with different stages of infection

Osp proteins, like VlsE, are also surface proteins of *B. burgdorferi*. OspA is important for localization of the spirochete within the midgut of the tick and is typically not expressed by the spirochete when it is in the mammalian host. OspC begins to be expressed by the spirochete as the tick takes its blood meal. Expression of OspC facilitates movement of the spirochete from the midgut of the tick to tissues of the host. Both OspA and OspC are targets of Lyme vaccines. OspF is generally expressed 4–6 weeks after the spirochete enters the mammalian host with a resulting antibody response that occurs 6–9 weeks postinfection[3] (see figure 1).

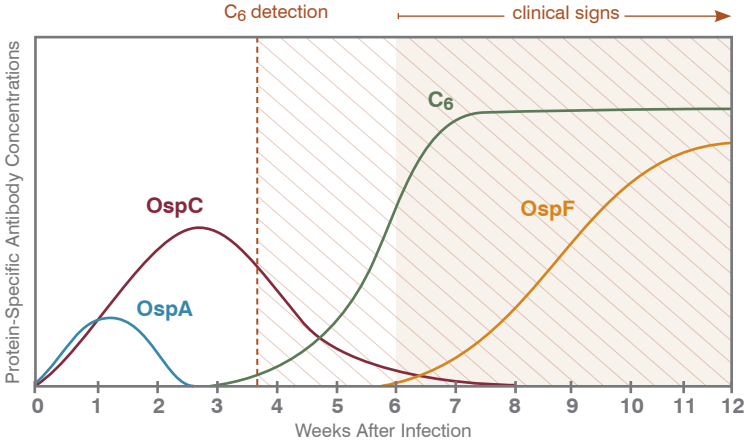

Figure 1. **Schematic representation of antibody concentrations to different outer surface proteins of *B. burgdorferi*. Antibodies to C_6 precede the onset of clinical signs and indicate infection.**

Host immune response to OspC

The early host immune response is primarily directed against OspC. As host antibody titers to this protein increase, the spirochete must turn off expression of OspC or risk elimination from the host. OspC expression is greatly reduced by 10 days postinfection,[4–6] and the OspC antibody response may be variable or begin to wane as early as 49 days postinfection.[3,7]

C_6 antibodies are indicative of infection not exposure

As OspC expression begins to decrease by 10 days postinfection, the VlsE gene is being turned on to allow the organism to employ a novel mechanism of host evasion; it disguises itself with a variable array of different coat proteins. The VlsE protein is only produced after the bacteria has been in the mammalian host for 7–21 days.[4] Antibodies to C_6 indicate infection because the spirochete must infect the dog and be biologically active for at least a week before sufficient amounts of VlsE protein are produced to stimulate the antibody response. Antibodies to C_6 may be detected as early as 3–4 weeks postinfection.[2] Dogs that are protected from infection by vaccination may make more antibodies to OspA and OspC in response to tick–transmitted organisms. However, they do not make antibodies to the C_6 peptide. The organisms appear to be controlled by the immune system prior to the expression of VlsE; this observation provides additional evidence that antibodies to C_6 distinguish infection from exposure.[8]

Increased levels of C_6 antibodies reflect active infection

Biological activity on the part of the organism is reflected by continuous antigenic variation in response to immune pressure from the host. In other words, an active spirochete must continue to disguise itself with new coat proteins to evade the antibody response of the host. Each new coat (VlsE) protein that is expressed also contains C_6. So, as the dog makes antibodies to the new coat proteins, it also makes more antibodies that react with the C_6 peptide. Experimental infection studies have demonstrated that the higher the C_6 antibody levels, the greater the number of organisms that could be recovered from the skin or tissues of infected dogs. Furthermore, these studies have shown that these organisms are more likely to survive in culture when removed from the dog.[2,9,10] Concentrations of C_6 antibody decline rapidly in response to antibiotic therapy and so do the numbers of organisms that can be recovered from the dog.[2,9,10] As the organisms are eliminated or driven to a state of dormancy, they are no longer changing their coat proteins in an attempt to evade the immune response. There is no further stimulation of the C_6 antibody response and as a result, C_6 antibody concentrations decline (see figure 2).

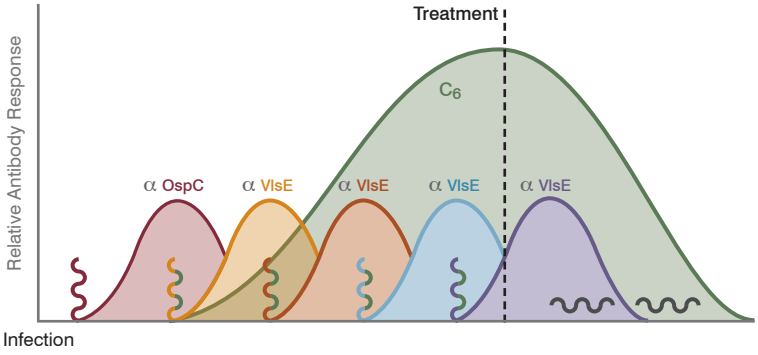

Figure 2. **Active *B. burgdorferi* infections stimulate the C_6 antibody response.** At initial infection, a spirochete (ʃ) expresses OspC on its surface (red). With time, it must change the coat protein to evade the immune response (α OspC, red line). So by 10 days postinfection, the spirochete no longer expresses OspC and instead begins to express different VlsE coat proteins over time, each of which includes C_6. This drives C_6 antibody concentrations higher. Following treatment, the organism becomes dormant, no longer expresses VlsE and C_6 antibody concentrations decline.

Quantitative concentrations of C_6 antibodies help to identify and monitor dogs that may benefit from treatment.

Because quantitative C_6 antibody concentrations correlate with organism load and viability, the Lyme Quant C_6® Test can help to identify *B. burgdorferi*-infected dogs that would benefit from antibiotic therapy, including those that lack the more recognizable clinical signs or laboratory abnormalities of Lyme disease. In general, *B. burgdorferi*-infected dogs, humans and nonhuman primates with high concentrations of C_6 antibody respond to antibiotic therapy with a marked reduction in C_6 antibody concentrations.[9,11,12] Thus, even in dogs that show no clinical signs, treatment response can be monitored by measuring a reduction in the concentrations of C_6 antibody. Failure of the test to show a reduction in the concentration of C_6 antibody following treatment may indicate treatment failure, recrudescence, noncompliance with administering medication or reexposure to *B. burgdorferi*. C_6 antibody concentrations do not correlate with disease or predict which dogs will become sick with Lyme disease or Lyme nephritis. Dogs with lower concentrations of C_6 antibodies (<30 U/mL) may or may not benefit from antibiotic treatment; a means to monitor response to therapy in this population is currently not available.

Clinical uses and advantages of C_6 testing

Testing for antibodies to C_6 as part of the vector-borne disease screening protocol is easy with the IDEXX SNAP® 3Dx®, SNAP® 4Dx® Plus and Lyme Quant C_6® tests. It provides a number of unique benefits for veterinarians:

- Most important, antibodies to C_6 are specific for infection, which minimizes the risk of false positives and avoids confusion with Lyme-vaccinated patients.
- C_6 also distinguishes between infection and exposure, so practitioners can be confident in their diagnostic conclusions and effectively recommend the appropriate diagnostic tests and treatment for each patient.
- Furthermore, with regard to therapy, C_6 provides an effective means to monitor treatment, as declining concentrations of C_6 antibody indicate a reduction in organism load and viability.

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