Research Review

Assessing Response to Antibiotic Treatment: Quantitative Measurement of C₆ Antibody in Dogs

Despite years of research and the availability of safe and efficacious canine vaccines, Lyme disease remains the most common vector-borne disease in the United States, with dogs in many regions of the country susceptible. Whether to vaccinate, whether to treat subclinical cases, and how to best diagnose the disease represent only a few areas of controversy surrounding Lyme disease. These questions leave today’s veterinarian in a complicated position—dealing with a disease that seems to be increasing in prevalence and complexity while striving to incorporate the latest research findings into daily clinical practices that will benefit patients and pet owners.

Diagnosing Lyme disease presents clinicians with several challenges. Currently, no test can document clinical illness resulting from *Borrelia burgdorferi* infection. If a dog in a Lyme-endemic area presents with fever and joint pain, tests cannot definitively prove that Lyme disease is the cause of the clinical signs. Clinical suspicion increases if the patient has a history of exposure to *Ixodes* spp ticks and responds to treatment, but these determinations are subjective. Documenting the presence of the causative organism should be another important piece of the diagnostic puzzle; however, although the *B. burgdorferi* organism can be detected in joint fluid or tissues by polymerase chain reaction or culture, these procedures can be costly and impractical in a clinical setting. Instead, clinicians tend to rely on serologic tests for antibodies against *B. burgdorferi* to diagnose Lyme disease in dogs. This method must also be approached carefully, because some serologic tests, such as traditional kinetic ELISA (KELA) titers, cannot distinguish vaccine-induced antibodies from those resulting from natural exposure, limiting their diagnostic usefulness in practice.

*B. burgdorferi* expresses several outer membrane proteins, some of which have become important in the diagnosis and prevention of Lyme disease. For example, the outer surface protein A (OspA) antigen is a component of all approved canine Lyme disease vaccines, and anti–OspA antibodies have an important role in preventing transmission of the *B. burgdorferi* organism when a dog is bitten by an infected tick. Another outer membrane protein, VMP-like sequence, expressed (VlsE), has become important in the diagnosis of Lyme disease in dogs. VlsE contains antigenically variable and invariant regions. Detection of antibody to the sixth invariant region of the VlsE protein (a peptide known as C₆) has evolved as a reliable serologic test for Lyme disease. The C₆ peptide is expressed when the *B. burgdorferi* organism infects a dog but is not contained in Lyme vaccines. Therefore, detection of C₆ antibodies indicates infection, regardless of vaccine history.
Assessing Response to Antibiotic Treatment: Quantitative Measurement of C\textsubscript{6} Antibody in Dogs

detection of antibody to C\textsubscript{6} and quantitative measurement of serum C\textsubscript{6} antibody levels were the basis for three recent studies that examined Lyme disease diagnosis and the influence of antimicrobial therapy on detectable measures of Lyme disease in naturally infected dogs.\textsuperscript{7–9}

Study Overview

A study by Levy and colleagues compared changes in quantitative C\textsubscript{6} antibody levels between Lyme-positive dogs that were treated with antibiotics and those that were untreated.\textsuperscript{7} This study involved 132 client-owned dogs that presented to veterinary practices for routine care. At study initiation, none of the dogs had clinical signs consistent with Lyme disease. Sixty-four of the dogs tested negative for Lyme disease and 68 tested positive using the in-house qualitative C\textsubscript{6} ELISA (SNAP 3Dx or SNAP 4Dx, IDEXX Laboratories, Inc.). Of the 68 positive dogs, 53 were treated with either doxycycline (low dose of 5 mg/kg PO bid or high dose of 10 mg/kg PO bid) or amoxicillin (5 mg/kg PO bid) for 28 days. All of the treated dogs also received two doses of a whole-cell B. burgdorferi vaccine. The remaining 15 positive dogs served as untreated controls.

C\textsubscript{6} antibody levels were quantified and evaluated throughout the study using blood samples from B. burgdorferi-positive dogs (Lyme Quant\textsuperscript{a} C\textsubscript{6} Test [QC\textsubscript{6}], IDEXX Laboratories). The diagnostic range for this test is 10 to >400 U/ml. At study initiation, 41 dogs (30 treated dogs and 11 controls) had QC\textsubscript{6} levels \geq 29 U/ml and 27 dogs (23 treated dogs and 4 controls) had QC\textsubscript{6} levels <29 U/ml. Dogs with initial QC\textsubscript{6} levels \geq 29 U/ml demonstrated significant decreases in QC\textsubscript{6} antibody levels 6 months after treatment with antibiotics, whereas dogs with initial QC\textsubscript{6} levels <29 U/ml demonstrated smaller, less consistent changes in QC\textsubscript{6} levels after treatment. QC\textsubscript{6} levels decreased an average (median) of 68% at 6 months and 83% at 12 months after antibiotic therapy in dogs with initial QC\textsubscript{6} levels \geq 29 U/ml. In contrast, there was little or no change in QC\textsubscript{6} levels in positive dogs with initial levels <29 U/ml. Interestingly, QC\textsubscript{6} levels in untreated dogs had actually increased by the end of the study.

Two other recent studies examined the relationship between serologic tests for Lyme disease (including measurement of QC\textsubscript{6} levels) and circulating B. burgdorferi immune complexes in Lyme-positive dogs.\textsuperscript{8,9} As in the first study, initial Lyme disease status was based on results of the SNAP 3Dx in-house qualitative ELISA, but these studies included both clinical and nonclinical dogs.

In the first of these studies, serum samples from 156 Lyme-positive, client-owned dogs were evaluated to determine QC\textsubscript{6} antibody levels, KELA results, and Western blot scores and to identify the relationship between these parameters and concentrations of B. burgdorferi–induced circulating immune complexes (CIC).\textsuperscript{8} QC\textsubscript{6} levels correlated well with KELA and Western blot scores in Lyme-positive dogs. Investigators also found that QC\textsubscript{6} concentrations correlated well with CIC concentrations and that CIC concentrations were increased in dogs with clinical signs of Lyme disease compared with nonclinical dogs. These findings suggest that increased CIC concentrations may be a consistent finding in dogs with clinical signs of Lyme disease.

A related study evaluated the effect of antimicrobial therapy on selected serologic tests for Lyme disease.\textsuperscript{9} Blood samples from 156 Lyme-positive, client-owned dogs were analyzed at baseline and 5 months after a month-long course of doxycycline (10–20 mg/kg/day PO) in an effort to compare CIC concentrations with serum parameters (QC\textsubscript{6} antibody levels, KELA, and Western blot assay) for dogs with and without clinical signs. Clinical status was based on initial veterinary evaluation. Ninety-six dogs were reported as nonclinical, and 35 had clinical signs consistent with Lyme disease. The remaining 25 dogs were reported to have exhibited clinical signs that were less likely induced by Lyme disease, such as vomiting, seizures, and diarrhea. The data from these dogs were excluded when comparing clinical versus nonclinical dogs. Investigators found that QC\textsubscript{6} antibody levels, KELA results, and Western blot scores were all decreased in posttreatment samples compared with pretreatment samples (\(P < .001\)). Concentration of CICs also decreased following doxycycline therapy (\(P < .001\)). However, no changes in any of these parameters were associated with
the clinical status of the dog. Additionally, the magnitude of reduction in CIC concentrations was positively correlated with decreases in QC6 and KELA results.

Summary of Results

Collectively, these results highlight the utility of the qualitative and quantitative C6 ELISA tests for the diagnosis and management of canine Lyme disease. In the first study,7 dogs with moderate to high positive QC6 scores at study initiation demonstrated significant decreases in QC6 antibody levels after antimicrobial therapy. In contrast, QC6 levels for untreated dogs increased during the 12-month study, which supports the contention that although antibody levels do not necessarily correlate with clinical signs of disease, nonclinical dogs may benefit on some level from antimicrobial therapy. This study also demonstrates that the magnitude of the QC6 value does not necessarily correlate with clinical signs of Lyme disease or predict which dogs will develop clinical signs.

Of the 68 Lyme-positive, nonclinical dogs in this study, 41 had QC6 levels ≥29 U/ml—a range considered moderate to high. Investigators reported that the QC6 value of 29 U/ml may be used as a guideline to predict expected changes in antibody levels after appropriate antimicrobial therapy and that determining QC6 levels at baseline and 6 months after treatment may be an effective way to monitor Lyme-positive dogs after therapy.

Circulating Lyme antigen–associated immune complexes have been implicated in the pathogenesis of Lyme-associated nephropathies in dogs and may also have clinical implications for Lyme disease in humans.10–12 Two studies described here8,9 investigated possible correlations between QC6 antibody levels, CIC concentrations, and other serologic measures of Lyme disease in dogs.

In the first study,8 CIC concentrations correlated well with QC6. In addition, CIC concentrations increased in dogs with clinical signs of Lyme disease, suggesting potential utility as a measure of clinical disease.

The final study described here9 identified repeated measurements of QC6 as a possible method of predicting decreases in CIC concentrations following treatment of infected dogs. Although CIC levels may increase in dogs with clinical signs, investigators caution that decreases in levels of CIC concentrations, QC6, and other parameters may not necessarily correlate with the clinical status of the dog. This area deserves further investigation.

Clinical Implications

In 2006, the American College of Veterinary Internal Medicine (ACVIM) published a consensus statement on diagnosis, treatment, and prevention of Lyme disease in dogs.2 The statement addressed several areas of clinical interest, including routine screening of asymptomatic dogs and antimicrobial therapy for symptomatic and asymptomatic dogs.

Although the ACVIM consensus statement does not directly endorse routine screening of asymptomatic dogs, the benefits of the practice are described; these include early detection of potential complications (e.g., Lyme-associated nephropathy), promoting owner education about the risks of tick-borne diseases, and obtaining regional seroprevalence data on Lyme disease. Results of the studies described here highlight the benefits of serologic tests using the C6 peptide. The in-house qualitative C6 ELISA tests (SNAP 3Dx and SNAP 4Dx) have been shown to be effective screening tests for Lyme disease in clinical practice as a result of their high sensitivity (96.2%), high specificity (100%), and lack of false-positive results in vaccinated dogs.6,13 Quantifying C6 antibody levels does not yield false-positive results in Lyme-vaccinated dogs.14 The QC6 can provide baseline antibody levels and can be a useful tool for assessing response to treatment, even in previously vaccinated dogs and dogs without clinical signs of Lyme disease.

Treatment of asymptomatic dogs remains an area of controversy because as many as 95% of *B. burgdorferi*–exposed dogs may not exhibit any clinical signs.2,15 While the decision to treat is at the discretion of the veterinarian, diagnostic methods are available for monitoring a patient’s response to antimicrobial therapy. For example, levels of *B. burgdorferi* isolated from culture and polymerase chain reaction testing of skin biopsy samples have been shown to decrease significantly after antimicrobial therapy in dogs concomitant with a significant decline in C6 antibody levels.16–18 Similar findings have been reported in humans, in whom antimicrobial therapy was associated with significant declines in C6 antibody levels for patients with early Lyme disease.19 Preventive strategies,
including vaccination and tick prevention, must also be considered when managing Lyme disease in dogs.

In the current studies, QC<sub>6</sub> levels, CIC concentrations, and Western blot scores all decreased following antimicrobial therapy. These decreases were not dependent on the clinical status of the dog; however, the magnitude of decrease in QC<sub>6</sub> correlated with decreases in CIC concentrations. This suggests that QC<sub>6</sub> may be an efficient method for estimating CIC concentrations in the patient and may therefore be valuable in both diagnosis and monitoring response to therapy. Currently, there is no objective way to assess resolution of Lyme-associated clinical signs. Developing markers for clinical disease has been a focus of human research and may also benefit canine patients at risk for exposure to this disease.

References