

# Diagnosis of Lyme Disease in Dogs

**IVMA CE Self-Study Offering**  
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## AIM OF THIS ARTICLE

Canine Lyme disease is typically diagnosed on the basis of a combination of features, including: a history of exposure to tick bites, clinical signs consistent with Lyme disease, serological testing, elimination of other differential diagnoses, and response to therapy.

This article—the second in a 2-part series—aims to review some key aspects of the diagnosis of Lyme disease in dogs.

## LEARNING OBJECTIVES

Following completion of this continuing education article, you will be able to:

- Understand the difficulties associated with reaching a definitive diagnosis of Lyme disease
- List key criteria to consider when making a clinical diagnosis of Lyme disease
- Identify the most frequently used laboratory method used in practice to detect *B. burgdorferi* itself
- Identify the different serological assays used in practice to detect antibodies to *B. burgdorferi*
- Identify different stages of infection with *B. burgdorferi* based on host antibody responses

- Identify other diagnostic test-related findings that may occur in dogs with Lyme disease, including from evaluation of hematological and serum biochemistry profiles, urine samples, and synovial fluid
- List the characteristic histological features seen in the kidneys of dogs with Lyme nephritis

## **Diagnosis**

Reaching a definitive diagnosis of Lyme disease in dogs is a difficult and often long process, because of the lack of a specific and all-encompassing test for the disease. Lyme disease is therefore typically a clinical diagnosis that should be based on five key criteria:

- Clinical presentation consistent with Lyme disease
- History of tick exposure and/or Lyme disease risk
- Positive serological test results
- Elimination of differential diagnoses
- Response to treatment for Lyme disease

### ***Laboratory testing for B. burgdorferi infection***

Tests for *B. burgdorferi* infection detect the presence of either the organism itself, or the presence of antibodies to it.

#### Detecting the organism

Although culture of *B. burgdorferi* from tissue or blood is the gold standard in laboratory diagnosis of *Lyme disease*, this test has low sensitivity, requires incubation periods up to six to eight weeks, and is typically used only in research settings. Similarly, detection of the organism by polymerase chain reaction (PCR) or cytology can also be difficult.

Although expensive, the PCR method is frequently used in practice, however. This technique amplifies borrelial DNA and can produce results quickly, within just a few hours. Nevertheless, for various reasons, this method should not be used as a single diagnostic tool when clinical Lyme disease is suspected: it cannot differentiate between viable and dead spirochetes, and false-negative results are common because it is difficult to detect *Borrelia* organisms in naturally-infected dogs due to low spirochetal burdens. Samples of tissue are recommended for PCR testing, because spirochetes are rarely detected in body fluids such as blood, urine, synovial fluid, or cerebrospinal fluid—they are more commonly found in the tissues of persistently-infected animals, including the skin, connective tissue, and joint capsule.

Overall, however, because of the associated difficulties, in dogs that are naturally infected with *B. burgdorferi*, it is uncommon to make a diagnosis of Lyme disease by demonstrating the presence of the organism within the host.

#### Detecting antibodies to *B. burgdorferi*

The changes in host antibody responses that reflect switches in antigenic protein expression by *B. burgdorferi*—to protect itself from the host's immune response—can serve as markers of different stages of infection, as well as treatment outcomes and vaccination status in dogs.

Following *B. burgdorferi* infection, the initial host immune response is primarily directed against OspC, an antigenic protein that facilitates transmission of the spirochete from the tick into the host. With rising OspC antibody titers, the organism reduces expression of OspC to evade the host's immune response; consequently, OspC expression begins to decrease by about 10 days post-infection. At this point, the VlsE gene (VlsE is the spirochete's variable surface antigen) begins to switch its antigenic protein expression to allow the spirochete to continue to evade the host's defense mechanisms.

OspF protein is expressed about 4 to 6 weeks after the spirochete enters the host. Some studies suggest this protein can bind complement inhibitor, helping the spirochete to avoid host-mediated serum killing.

C6 is a conserved peptide that is a component of VlsE. Its expression begins when the spirochete is transmitted to the host. Antibodies to C6 are detectable by about 3 to 5 weeks after infection. Their presence indicates active infection with *B. burgdorferi*. C6 antibody levels also fall after treatment in dogs with clinical Lyme disease.

In general, detection of only OspC antibodies is indicative of very early infection (within the first few weeks; levels peak at about 3-weeks post-infection). The combined presence of antibodies to OspC, OspF, and C6 is indicative of an intermediate stage of infection (2 to 5 months). And antibodies to both OspF and C6 (with antibodies against OspC either absent or at a very low level) are indicative of chronic infection (approximately 5 months or longer duration).

Dogs that are vaccinated against *B. burgdorferi* do not appear to make antibodies to C6 peptide. They do, however, appear to generate more antibodies to OspA and OspC in response to tick-transmitted organisms.

*Serological tests:* Serological assays for detection of *B. burgdorferi* antibodies are highly sensitive and specific, and are used in clinical practice to identify *B. burgdorferi* infection—detection of specific antibodies to *B. burgdorferi* in serum is the most commonly used test.

- Confirmation of a high titer of *B. burgdorferi*-specific antibodies has traditionally involved a 2-step procedure that begins with a quantitative, nonspecific, enzyme-linked immunosorbent assay (ELISA) or indirect fluorescent antibody (IFA) test. Because these tests are nonspecific, they measure antibodies to the whole organism and cannot differentiate between those from natural exposure to the

organism and vaccination, and produce positive results in both cases. The second step therefore involves the use of a qualitative, specific, Western blot test to confirm infection.

- Other confirmatory tests have also emerged recently, especially tests that are based on C6 peptide detection. C6 peptide is associated with the IR6 region only in live spirochetes, and is immunodominant in dogs infected with *B. burgdorferi*; it is expressed when the organism is transmitted to the dog, but is not expressed in the tick, in tissue culture, or in Lyme disease vaccines. Antibodies against C6 are therefore highly specific for *B. burgdorferi* and indicate natural exposure because the spirochete must infect the dog and be biologically active for at least one week before enough VlsE protein is produced to stimulate an antibody response; however, although the presence of C6 antibodies is indicative of active infection with the organism, it does not prove clinical Lyme disease. C6 antibody responses to *B. burgdorferi* occur 3 to 5 weeks after infection, and decline following treatment in dogs with clinical Lyme disease. Both qualitative (point-of-care tests for use in clinics) and quantitative (performed in reference laboratories) versions of the C6 test are available.

The qualitative in-clinic test can be performed rapidly in-house, and can therefore be useful for screening dogs for *B. burgdorferi* infection, either as part of a screening program for dogs without clinical signs or when Lyme disease is suspected. Because dogs that are vaccinated against *B. burgdorferi* do not appear to make antibodies to C6, vaccination with a commercial Lyme disease vaccine does not interfere with this test to produce a false positive result.

In contrast, because most dogs infected with *B. burgdorferi* have no clinical signs at the time of testing, the quantitative C6 test can be effective in measuring response to antibiotic therapy in dogs with Lyme disease, as decreasing levels of C6 antibody indicates infection control.

- Fluorescent bead-based, multiplex assay of antibodies to *B. burgdorferi* is a novel and highly sensitive approach that simultaneously evaluates antibodies to several *B. burgdorferi* antigens as indicators of acute or chronic infection. These antibodies include the different Osp types that are differentially expressed either in ticks (OspA) during transmission to the host (OspC), or later in the host (OspF). This test is reported to be helpful to differentiate between natural exposure and vaccination, as well as between early and chronic infection. However, it has several limitations, including the inability to differentiate between dogs with chronic infection and those that have been treated and re-infected. In addition, it cannot reliably differentiate between OspA antibodies that are a result of vaccination and those that may be generated because of infection.

*Seroprevalence of B. burgdorferi antibodies:* It is important to remember that serological test results can be used to document exposure to or infection with *B. burgdorferi*, but not diagnose Lyme disease. For example, data from some studies in Lyme endemic areas in the United States have demonstrated that 70% to 90% of all healthy and clinically ill dogs are seropositive for *B. burgdorferi*. Serological screening of healthy dogs for exposure to *B. burgdorferi* therefore remains controversial because it can lead to overdiagnosis or overtreatment of Lyme disease, even though most of these dogs will never develop clinical disease. The benefit of treatment for seropositive healthy dogs also remains unknown.

However, dogs that live in—or have travelled to—endemic areas and develop proteinuria should be screened for *B. burgdorferi* exposure and, if necessary, for co-infection with other tick-borne pathogens that are known to occur in the area. Dogs that are seropositive for *B. burgdorferi* should be screened for proteinuria, and tick control strategies initiated. Guidelines recommend that, in endemic areas, seropositive dogs without proteinuria should be re-tested for proteinuria every 3 to 6 months.

### ***Ancillary laboratory tests***

In dogs that either present with clinical signs suggestive of Lyme disease or are seropositive for *B. burgdorferi*, additional laboratory testing will inevitably be performed as part of the diagnostic workup; however, findings tend to be variable and nonspecific:

- **Hematology and biochemistry:** Hematological and/or biochemical abnormalities are unlikely in most cases. However, seropositive dogs are at risk for co-infection with other tick-borne pathogens, especially those dogs that have travelled and potentially been exposed to other tick-borne pathogens such as *Rickettsia rickettsii* and *Neorickettsia risticii*. These dogs may have abnormalities such as anemia (non-regenerative), thrombocytopenia, and/or hypoalbuminemia; azotemia is another nonspecific finding.

In the cases of Lyme nephritis that have been documented, progressive renal failure has been associated with positive Lyme borreliosis serology. However, all dogs with *B. burgdorferi* antibodies do not necessarily go on to develop proteinuria. Findings in affected dogs may include non-regenerative anemia, stress leukogram, thrombocytopenia, hypoalbuminemia, azotemia, hypercholesterolemia, hyperphosphatemia, and sometimes hyperkalemia and hyperbilirubinemia.

- **Urinalysis:** Urinalysis—in particular, determination of the urine protein to creatinine (UPC) ratio—is recommended to monitor for protein-losing nephropathy in the case of renal involvement. In addition to proteinuria (UPC ratio >1), findings in dogs with Lyme nephritis may also include oliguria, decreased concentrating ability (urine specific gravity often below 1.022), hemoglobinuria, hematuria, glucosuria, bilirubinuria, casts, and an active sediment with no bacterial growth on culture.
- **Evaluation of synovial fluid and synovial membranes:** In acute cases, synovial fluid will have increased protein content, will contain large numbers of cells—predominantly neutrophils, without any bacterial growth on culture;

suppurative inflammation (predominantly neutrophils and fibrin) is also seen on cytological and/or histopathological evaluation of synovial membranes. In more chronic cases, non-suppurative inflammation (predominantly lymphocytes and plasma cells) is seen on histopathological evaluation of synovial membranes.

A unique histopathological lesion has been described in the kidneys of dogs with Lyme nephropathy: this involves glomerulonephritis with diffuse tubular necrosis and regeneration, and lymphoplasmacytic interstitial nephritis. This lesion is considered immune-mediated, not a direct consequence of renal invasion by spirochetes. A causal relationship between *B. burgdorferi* and the renal changes remains unproven.

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