

# News

August • 1999

**ANTECH**  
D I A G N O S T I C S

## Lab Locations

Atlanta  
Georgia

Chicago  
Illinois

Dallas/  
Fort Worth  
Texas

Denver  
Colorado

Honolulu  
Hawaii

Houston  
Texas

Los Angeles  
California

Memphis  
Tennessee

New York  
New York

Phoenix  
Arizona

Portland  
Oregon

San Francisco  
California

Tampa  
Florida

## BORRELIOSIS UPDATE

*Borrelia burgdorferi* is the causative agent of borreliosis (Lyme disease). This spirochete was first identified in 1995 to cause an infectious arthritis, in the region of Old Lyme, Connecticut. *B. burgdorferi* produces disease in humans and dogs, with less clearly defined syndromes in cattle, horses and cats. Avian and mammalian wildlife in endemic areas harbor the organism, which is transmitted primarily by ticks of the genus *Ixodes*. These ticks have 3 hosts during a 2-year life cycle, and the nymph and adult phases may both be sources of infection for dogs. Adult ticks in the northeast commonly feed on white-tailed deer and are frequently referred to as 'deer ticks'.

Other *Borrelia* spp. have been reported to cause spirochetemia associated with relapsing fevers and hematologic changes that respond to antibiotics, but their prevalence is unknown and no specific diagnostic serologic tests are available.

### EPIDEMIOLOGY

The prevalence of *B. burgdorferi* in *Ixodes* spp. varies regionally. Areas in the United States recognized as endemic include the northeastern and mid-Atlantic states, midwest, and northwest. Of reported cases, 85% are from the northeast, where 50% of ticks in this area may harbor *Borrelia* spp.

Transmission occurs solely by the tick vector in dogs; other blood-sucking arthropods have not been proven to transmit the disease. There is no direct transmission between dogs, and no vertical transmission has been documented with experimental models that mimic patterns of natural exposure. During prolonged feeding of infected ticks on the host, the organism migrates from the tick's midgut to the salivary gland, and infection occurs. Ticks removed relatively soon (12-24 hours) after attachment are unlikely to cause infection. Dogs are not considered to be a reservoir for human infection, based on the fact that neither the nymph nor adults stages of *Ixodes* spp. feed alternately on different hosts. Thus, dogs are unlikely to be a source of ticks which will later attach to people. Although spirochete antigen can be found in the urine and saliva of infected dogs, it is labile, virtually impossible to

culture, and does not appear to be a source of infection for other dogs or people.

### CLINICAL SYNDROMES

Once an animal has been infected, the spirochetes migrate to target tissues, such as the joints, lymph nodes and connective tissue. The incubation period is long (2-5 months) from the time of infection to the onset of clinical signs.

Serologic studies in dogs from endemic areas suggest that infection with *B. burgdorferi* most commonly produces asymptomatic seropositive dogs. Seropositive status has been documented for up to 50% of dogs in some areas.

Approximately 5-10% of infected dogs will develop clinical signs. Typically, these dogs are lame, and exhibit varying degrees of joint swelling and myalgia. The lameness typically involves just one leg. Fever, malaise and inappetance can occur but are less common. Untreated, this arthropathy can be self-limiting, but may recur at roughly monthly intervals.

Renal disease, characterized by a membranoproliferative glomerulonephritis has been associated with Lyme disease. These dogs are azotemic, hypoalbuminemic, and proteinuric. The glomerular lesions are progressive, and the long-term prognosis for these patients is poor. A breed predilection for Labrador and Golden retrievers has been reported.

Heart block and neurologic disease have been reported in association with *B. burgdorferi*, but are believed to be rare.

### DIAGNOSIS

The following criteria have been proposed for establishing a diagnosis of Lyme disease in dogs: a history of tick exposure in an endemic area, clinical signs, positive serology, and prompt response to antibiotics. With the exception of cases where glomerular disease has developed, results of complete blood counts, serum chemistries and urinalysis on these patients are generally normal. The presence of hematologic abnormalities, such as an elevated white blood count or thrombocytopenia should prompt a search for concurrent disease.

# BORRELIOSIS UPDATE (cont'd.)

Dual infections with rickettsial organisms may occur in some endemic areas. Joint taps reveal elevations in cell counts of up to 50,000 cells/mm<sup>3</sup>, with neutrophils as the predominant cell type. Differential diagnoses include immune-mediated arthropathies, degenerative joint disease, and other infectious causes of arthritis, such as Rocky Mountain spotted fever and ehrlichiosis.

Serology remains the most practical tool in veterinary medicine for the diagnosis of Lyme disease. Antech offers an ELISA test for IgG, and an IFA test for IgM. Clinical signs lag behind infection for 2-5 months, so that clear serologic evidence of infection is almost always present. IgM titers may remain elevated for prolonged periods after infection, and are not definitive of recent infection. IgG antibodies are detectable 4 weeks after infection and levels peak at 3 months. Antibodies in untreated dogs may persist for 12-18 months. Titers in treated animals may drop transiently, but because the organism is not completely cleared from the dog by treatment, titers may persist. Treatment will reduce the number of spirochetes and alleviate clinical signs, but small numbers of the organism remain and elicit a persistent antibody response. Dogs who have been vaccinated for Lyme disease can have positive IgM and IgG titers as early as 7-10 days after vaccination (earlier than in natural infection).

The Western Blot test separates antibodies for *Borrelia* spp. based on the antigen they target. Flagellar antibodies from natural exposure can be distinguished from the other surface protein antibodies induced by vaccination. With this technique, when sufficient antibody is present, a band forms on the nitrocellulose test strip. The intensity of this banding will vary with the amount of antibody, although the test is only subjectively quantitative. This test is indicated when evaluating vaccinated dogs to determine whether natural exposure has occurred. Results may indicate the presence of vaccine-induced antibodies, antibodies from natural exposure, or a combination of both.

Isolation of the organism is difficult and expensive, and so it is rarely performed. The paucity of organisms in blood and body fluids render both culture and PCR techniques unrewarding. Use of collagen-rich tissue samples, such as skin, fascia, peritoneum and synovium may be more productive, but the poor sensitivity and high cost involved remain as deterrents.

## TREATMENT

Tetracycline and  $\beta$ -lactam antibiotics are both effective in the treatment of Lyme disease. Due to the slow growth of the organism, 21-28 day courses of therapy are recommended. Doxycycline at 10 mg/kg SID, or amoxicillin at 22 mg/kg BID are both appropriate choices. The clinical response is usually prompt, with improvement evident in 1-2 days. Dogs that experience relapses usually respond equally well to therapy. In the event of a poor clinical response, the diagnosis should be reevaluated.

A common clinical dilemma is posed by the question of using antibiotics in asymptomatic seropositive dogs. Only a small percentage of seropositive dogs ever develop clinical signs, and many dogs remain seropositive despite successful treatment of their symptoms. In the absence of symptoms, therefore, it can be argued that the use of antibiotics is unjustified, except in those dogs receiving ongoing immunosuppressive therapy. Also, as dogs in endemic areas are at risk for repeated exposure, continual re-treatment is impractical. It is not known whether treatment of such dogs would prevent or lessen the rare, more serious complications of Lyme disease in dogs (e.g., chronic arthritis or glomerulonephritis).

## PREVENTION

The use of tick repellents and the prompt removal of ticks are important practices to decrease exposure to *B. burgdorferi* in endemic areas. Two commercial vaccines are currently available to veterinarians; a bacterin, and a single protein recombinant vaccine containing outer surface protein A. While a decreased incidence of clinical illness has been demonstrated in vaccinated populations of dogs, the low rate of disease despite the high rate of infection in endemic areas warrants careful evaluation of the risk:benefit ratio of vaccination for each patient. It is unclear at this time whether vaccinating seropositive dogs is beneficial, and some experts believe it presents the increased risk of adverse reactions (immune-complex arthritis).

## TEST CODES

	EAST	WEST
Lyme IgG	545	16530
Lyme IgM	546	16537
IgG & IgM	1546	16531
Western Blot	2545	16836
PCR Testing	7001	7001

## LAB TIPS

### COMPARISON OF PLATELET NUMBERS AND SIZE IN CITRATE VS EDTA ANTICOAGULATED BLOOD

Platelet numbers and size can vary when blood is collected in either EDTA or trisodium citrate tubes. The platelet count also can vary between freshly collected and stored blood samples. To assess these variables, blood was drawn daily from 10 dogs into EDTA (LTT) and trisodium citrate (BTT) tubes. The fresh blood was analyzed immediately by automated and slide techniques, and after storing the blood at 4-8° C for 24 hours.

Platelet counts (repeated 5 times on each sample) from BTT, were lower than those of the LTT tubes. These differences were striking when results of the fresh and stored blood samples were compared. Platelet counts from stored BTT blood averaged 30% lower than those of the freshly drawn samples. BTT slide smears from stored blood showed platelet clumping to variable degrees. However, only 1 of the 10 slide smears showed platelet clumping in the fresh BTT, or fresh or stored LTT tubes.

This study confirms that platelet counts are unreliable in blood stored 24 hours (or longer), especially when it is collected in citrate anticoagulant (BTT). Based on these findings, platelet counts should be verified on LTT rather than BTT tubes. For urgent situations, if an EDTA (LTT) tube is unavailable, a stored BTT tube can be used to repeat the platelet count. However, the final count will be about 30% lower (i.e. multiply count obtained by 1.6 to obtain the *estimated* original number).

**References:** Appel and Jacobson, Current Vet Ther XII (Kirk and Bonagura, eds). WB Saunders, Philadelphia, 1995, pp 303-314; Breitschwerdt et al, Am J Vet Res 57: 505-512, 1996; Dambach et al, Vet Pathol 34(2): 85-88, 1997; Appel et al, 10th Ann Int Sci Conf on Lyme Disease, April 28-30, 1997.

Join us at our Web Site @ [antechdiagnostics.com](http://antechdiagnostics.com)

**ANTECH**  
D I A G N O S T I C S