Lyme Borreliosis In Dogs  
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Introduction
Lyme disease has been recognized in Europe for almost a century [1, 28] but it was not described in humans in the United States until 1975 [36]. The disease occurs also in dogs, horses, cattle, and cats, while many wildlife mammals and birds become infected and serve as reservoirs for tick infection [15, 18, 19, 31]. During the 1980s, the reported disease incidence in both dogs and humans increased dramatically. Lyme borreliosis is now the most common arthropod-borne disease of humans in the United States (Center of Disease Control and Prevention; Division of Vector-Borne Infectious Diseases). Nevertheless, Lyme disease remains predominantly a regional problem (Fig. 1, Fig. 2). Of the human cases reported to the Centers for Disease Control and Prevention, 86.5% came from the northeastern and Atlantic states; 9.2% came from the midwestern focus (Wisconsin, Minnesota, Michigan, Illinois, Missouri, and Iowa), another 2.2% percent from California and Oregon, and the remaining 2.1% were reported from other states.

Epidemiology
Lyme disease is caused by a group of *Borrelia* species, called *Borrelia burgdorferi* sensu lato [5, 20]. Only one species, *B. burgdorferi* sensu stricto, is known to be present in the USA, while at least four pathogenic species, *B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, *B. japonica* have been isolated in Europe and Asia [5, 16, 21]. *B. burgdorferi* sensu lato organisms are corkscrew-shaped, motile, microaerophilic bacteria of the order Spirochaetales (Fig. 3). Among the members of this order, *B. burgdorferi* species are most closely related to *B. hermsii*, which causes tick-borne relapsing fever in the southwestern United States. Better known but more distantly related spirochetes are *Leptospira* spp. and *Treponema pallidum*, the causative agents of leptospirosis and syphilis in man, respectively.

Hard-shelled ticks of the genus *Ixodes*, transmit *B. burgdorferi* by attaching and feeding on various mammalian, avian, and reptilian hosts. In the northeastern states of the US *Ixodes scapularis*, the black-legged
deer tick, is the predominate vector, while at the east coast Lyme borreliosis is maintained by a transmission cycle which involves two tick species, *I. neotomae* and *I. pacificus* [7] (Fig. 4, Fig. 5, Fig. 6) (for more information follow the link to the Iowa State University Entomology Image Gallery).

In Europe and Asia, *I. ricinus* and *I. persulcatus* are the main vectors for *B. burgdorferi* transmission.

Blood-sucking insects may also be involved in the transmission of the organisms, but there is little evidence that they are important vectors [29]. The primary way by which an animal or human becomes infected is by tick bite.

At the time that a tick attaches and begins to feed, spirochetes reside in the midgut of the tick. Stimulated by the blood meal, spirochetes begin to migrate to the tick's salivary glands. From there, they are injected into the skin of the host. The danger of infection increases with the time the tick is allowed to feed on the host. Studies have shown that it takes the organisms at least 24 hours to migrate form the tick midgut to the host's skin [12]. *Ixodes* ticks require three hosts and four different developmental steps to complete their life cycle. The female tick lays about 2000 eggs in the spring. Only a small portion of the larvae that emerge from the eggs carry *B. burgdorferi* [24, 33]. The larvae of *Ixodes* ticks feed mainly on small mammals. In the northeastern states of the US, *I. scapularis* nymphs feed on the white-footed mouse, *Peromyscus leucopus*. Many infected mice harbor *B. burgdorferi* for long periods without developing disease. Tick larvae become infected by ingesting the blood of persistently infected mice or by co-feeding with previously infected ticks on uninfected hosts [32]. After repletion, they drop off and enter a resting stage for the winter. The larvae molt into nymphs the following spring. During spring and early summer, the nymphs feed on new hosts, again small mammals or any of a wide range of animals, including dogs and humans. An infected nymph may infect its new host during its 4-day feeding period. In the fall, nymphs molt again and enter the adult stage. In some areas of the northeastern USA, more than 50% of the adult ticks carry *B. burgdorferi*, and infected adult ticks are the most important source of infection for dogs. Adult ticks can be found on shrubs, where they are high enough off the ground to attach to white-tailed deer and other large animals. Adult ticks mate on the host. Females engorge for 5 to 7 days and then drop off into the leaves, where they live through the winter. The following spring they lay eggs and complete the 2-year cycle. Adult ticks that do not find a host in the fall may survive over the winter and become active again from early spring until about mid-May.

In the southern United States, *I. scapularis* larvae and nymphs feed primarily on lizards, which do not maintain infection with *B. burgdorferi*. Consequently, nymphal and adult infection rates are low, often less than 1% [23, 30].

Pathogenesis

Spirochete transmission to the host starts approximately 24 to 48 hours after tick attachment. During that time organisms multiply, cross the gut epithelium into the hemolymph, disseminate to the salivary glands, and infect the host through tick saliva [12]. From the site of tick attachment, the organisms then replicate and migrate through tissues. Within weeks, they can spread through many tissues, invading the closest joints. The
interaction of borrelia organisms and host cells leads to the up-regulation and release of immune regulatory factors such as proinflammatory cytokines. The chemokine interleukin (IL)-8, a potent chemotactic factor for polymorphonuclear neutrophils (PMNs), IL-1α, and IL-1β were shown to be up-regulated in inflamed synovial membranes of dogs infected experimentally with *B. burgdorferi* [38, 39]. Locally produced host factors probably accumulate in the joints and body cavities (pericardium, CNS) and, above a certain concentration, may provoke the migration of leukocytes into tissue and body cavities. At the same time, dampening factors such as IL-10 are also up-regulated. Such chemokines are known to inhibit the production and release of proinflammatory factors, and therefore limit the extent of inflammation. Other joints, further removed from the site of tick attachment, may develop arthritis later when *B. burgdorferi* arrive in the synovium, replicate to sufficient numbers, and interact with the host cells. Migration of *B. burgdorferi*, interaction with host cells, and the production of inflammatory and anti-inflammatory factor may be the reasons for the intermittent nature of the arthritis. Not all infected individuals develop clinical signs. The reasons for this phenomenon are not understood. It is speculated, however, that numbers of organisms in tissue differ from individual to individual and large numbers of spirochetes may be essential to induce a clinically apparent response [44]. The genetic background of the host may also be important [35]. Our own studies have shown that the numbers of *B. burgdorferi* in skin biopsy samples decrease (Fig. 7) and are lowest at a time when no clinical signs are apparent. In dogs and humans, *B. burgdorferi* establishes persistent infections. The spirochetes exist extracellularly, but single organisms have been observed intracellularly [9, 10, 17, 22]. At least one mechanism by which extracellular organisms evade the immune system is the production of a variety of surface-exposed proteins that are encoded by variable regions of the genome [25, 42, 45]. After a few weeks of infection, *B. burgdorferi* is difficult to detect or isolate from tissue samples. Western blot analysis has shown that, at this time, specific antibodies are present which, in concert with specific immune cells, probably control the infection more efficiently and keep the spirochete burden at low levels.

**Clinical Signs**

In contrast to the infection in humans, where three different stages are well known [34], the disease in dogs is primarily an acute or subacute arthritis [2, 18] (Fig. 8). In humans, the first stage is characterized by a skin rash called erythema migrans (EM). The rash normally develops within days to weeks at the site of the previous tick bite and expands during the following days (up to 30 cm in diameter). Multiple rashes may develop in approximately 7 to 15% of people with EM lesions. The EM can be accompanied by fatigue, malaise, muscle and joint pain, stiff neck, and fever. The second stage of the disease may occur weeks to months after the infection. It is characterized by acute arthritis, or carditis/pericarditis, or involvement of the central or peripheral nervous system. The third and final stage is characterized by chronic disease. Lesions may develop years after infection and persist for decades. The most prominent changes found in those patients are chronic arthritis, chronic impairment of the CNS, and acrodermatitis chronica atrophicans (ACA). Clinical signs tend to associate with specific species of *B. burgdorferi* sensu lato complex. *B. burgdorferi* sensu stricto is found in annular skin lesions (EM), and in cases with arthritis or meningitis. *B. afzelii* has been isolated from cases with meningopolyneuritis, and *B. garinii* has been isolated with a high frequency from patients with dermatitis and chronic arthritis [4, 43].
Clinical signs associated with the second stage of Lyme borreliosis in humans have also been reported in dogs. Studies with dogs kept as pets in endemic areas have shown that approximately 5% of all infected dogs become ill [27]. However, under experimental conditions, up to 75% of infected animals develop clinically apparent Lyme arthritis [2, 38]. In those experiments, dogs developed mono- or oligoarthritis 2 to 5 months after tick exposure in the joints closest to the tick bites. Joints were painful and had increased volumes of synovial fluids containing mainly PMNs and Type A and B cells derived from the synovial lining. Other clinical signs consist of anorexia and general malaise. Lameness may be intermittent with several episodes of lameness which shifts from one limb to other extremities, lasting for days to weeks. In a few cases heart block [26], fatal kidney failure in certain breeds [11], and neurological changes such as seizures, aggression, and other behavior changes have been reported [3].

Diagnosis
There are no specific clinical, hematological, or biochemical pathognomonic changes that would confirm the diagnosis of Lyme borreliosis. Therefore, additional tests, such as antibody and organism detection, need to be considered in order to produce a specific diagnosis.

Four criteria important in establishing the diagnosis of Lyme disease in dogs:
1. History of exposure to ticks in an endemic area.
2. Typical clinical signs for Lyme borreliosis.
3. Specific antibodies to *B. burgdorferi*.
4. A prompt response to antibiotic therapy.

One or two of these criteria alone are usually not sufficient to confirm a diagnosis. A diagnosis based on clinical signs alone often remains questionable, for there are several other conditions, such as immune-mediated disease and rheumatoid arthritis that cause lameness and pain in dogs.

a) Serologic testing: An enzyme-linked immunosorbent assay (ELISA) or an indirect immunofluorescence assay (FA) with whole cell preparations or single recombinant antigens are useful for detecting antibody responses to infection as well as to vaccination. Antibody titers can first be detected in dogs between 4 and 6 weeks after exposure to infected ticks. In untreated infected dogs, antibody levels increase for several weeks, reaching maximum levels at approximately 90 to 120 days after tick exposure, and then remain constant for at least 22 months in the absence of re-exposure (Fig. 9). Despite high ELISA titers, viable *B. burgdorferi* organisms persist in dogs for more than 600 days, the longest period studied.

![Figure 9](https://www.ivis.org)

It is possible, and likely, that both antibodies and organisms persist in dogs for several years. Several commercial kits are available which allow veterinarians to test for Lyme antibody in dogs without sending samples to diagnostic laboratories. However, well-controlled ELISA test performed in competent diagnostic laboratories are more reliable. Inconsistent results between different laboratories, false-positive results due to cross-reactivity of antibodies with other organisms, and the inability to distinguish between infection and vaccination make it necessary to utilize another serological test, the Western blot.

Immunoblotting or Western blotting improves the specificity of the *B. burgdorferi* antibody assay without loss of sensitivity. This test determines the quality of the antibody response rather than only its quantity (Fig. 10). After natural infection with *B. burgdorferi*, dogs develop antibodies primarily against proteins in the 41-, 39-, and 22-kDa areas. Western blot signals in these areas are indicative of a response to flagellin, p39, and the outer surface protein C (OspC), a borrelia lipoprotein abundantly expressed in mammalian hosts.

![Figure 10](https://www.ivis.org)
However, a reaction to 31-kDa protein (OspA) indicates a response to the currently used subunit vaccine using OspA as an immunogenic protective antigen (Fig. 11; left panel). The vaccinal Western blot banding pattern can be more complex when a bacterin vaccine is used, a formulation that is based on a whole-cell preparation. Here, in addition to OspA, signals to OspB at 34 kDa and many other bands are present (Fig. 11; right panel). 

b) Detection of *B. burgdorferi*. The definitive means for diagnosing infectious agents is the specific detection of the causative organism. In veterinary and human studies, *B. burgdorferi* has been extremely difficult to culture from body fluids and tissues because the organism is very demanding in terms of culture medium and conditions of growth. *B. burgdorferi* can be grown in modified Barbour-Stoenner-Kelly medium (BSK-II) over several weeks and is then detected and identified by dark-field microscopy and indirect FA, respectively. Studies with experimentally tick-infected dogs have shown that skin biopsy samples taken close to the site of tick attachment are a reliable source for organism detection, as are tissue samples from lymph nodes, synovial membranes and the pericardium [8]. However, spirochetal organisms were rarely detected in blood samples [40].

Figure 11. Western blot of sera samples from an OspA-immunized (left panel) and a bacterin-immunized dog (right panel) collected at four-week intervals starting at lane 1 with the day of the first day immunization. - To view this image in full size go to the IVIS website at www.ivis.org.

*B. burgdorferi* can also be detected by the polymerase chain reaction (PCR). This technique is based on the amplification and detection of a *B. burgdorferi*-specific DNA fragment with the help of specific synthetic DNA sequences called primers. Total DNA (host and bacterial DNA) is recovered from tissues or biological fluids and then subjected to several cycles of DNA denaturation, primer annealing, and DNA extension. The duplication of the specific target DNA during each cycle results in an exponential amplification of DNA throughout the procedure, yielding enough of the specific DNA fragment, that it can be detected by conventional electrophoresis and staining techniques (Fig. 12). PCR has the advantage that it is extremely sensitive and specific. However, unless additional modifications are implemented into the detection protocol, the technique does not allow the differentiation between life and dead organisms. Furthermore, negative PCR results do not exclude an infection with *B. burgdorferi*, and positive results need to be interpreted cautiously, since this technique is sensitive to carry-over contamination and may produce false-positive results. For diagnostic purposes, it is therefore advisable to send test samples to experienced laboratories.

Figure 12. Detection of *B. burgdorferi*-specific DNA (ospA gene) by conventional qualitative PCR and agarose gel electrophoresis. DNA is stained with ethidium bromide and visualized over a ultraviolet light source. Skin biopsy samples were takes near the site of tick exposure in four-week intervals. - To view this image in full size go to the IVIS website at www.ivis.org.

**Treatment**

Antibiotics are the treatment of choice for Lyme disease. Tetracyclines (doxycycline), penicillins, (amoxicillin and ceftriaxone), and macrolides (azithromycin) are very effective in improving the clinical status of the patient but fail to eliminate the infection [40, 41]. Antibiotics should be given for 3 or 4 weeks, even though a beneficial effect can be seen after a few days of treatment. The long duration of therapy is warranted because of the very slow multiplication rate of the organism, which takes 12 hours or more to double in number, in contrast to the much shorter times for most other bacteria. Antibiotic therapy reduces the number of organisms in the host, and due to the decreased antigen load, antibody titers drop off. However, positive moderate antibody responses can be expected for years, especially when treatment has been initiated long time after the infection had occurred [40].

Corticosteroids and other anti-inflammatory drugs are sometimes used for treatment of Lyme disease in dogs. These drugs should be applied cautiously and in combination with antibiotics. Our studies have shown that persistent, subclinical infection with *B. burgdorferi* can be reactivated to clinical Lyme arthritis by a two-week course of prednisone [40].
Transmission From Dogs To Humans
It has been speculated that *B. burgdorferi* in the saliva or urine of infected dogs might be transmissible to humans. Experiments to test this hypothesis, in which infected and uninfected dogs have been kept in close contact for extended periods, have failed to provide any evidence of urine or saliva transmission and infection [2]. So far, there is no evidence of human infection resulting from contact with dogs. It is possible that dogs carry home loosely attached infected ticks, which may then transfer to humans and induce infection. In such a case, dogs are not be the source of infection but function merely as tick carriers.

Prevention
There are several approaches to prevent infection in dogs. Tick exposure can be reduced by either modifying the tick habitat (trimming trees, mowing lawns, removing bushes, reducing deer traffic) or by limiting tick engorgement on dogs by using tick repellents and/or grooming daily. If this is not feasible, vaccination against *B. burgdorferi* may be considered.

Several vaccines against *B. burgdorferi* are now available for the use in dogs in the USA. Vaccines are either based on a single antigen with or without adjuvants (OspA subunit vaccine) or on a whole-cell bacterin, which contains all antigens of culture-grown and chemically inactivated *B. burgdorferi* organisms complemented with adjuvant. In a limited field study it was concluded that the incidence of disease (4.7% in infected, non-vaccinated dogs) was reduced to about one percent [27]. Both vaccine types noted above prevent the transmission of *B. burgdorferi* to the host. Vaccinated dogs produce borreliacidal antibodies, which are present in the blood and tissues. After a blood meal, ticks acquire these protective antibodies. *B. burgdorferi* organisms expresses a different set of antigens in the tick than they do in the mammalian host. OspA is normally up-regulated and expressed in the midgut of the tick, while OspC is down-regulated. However, in response to the blood meal, the spirochetes begin to down-regulate OspA and up-regulate OspC. In the tick's midgut, 'neutralizing' (protective) antibodies bind to the expressed OspA and kill, or immobilize, the bacteria [13, 14]. Consequently, no infectious organisms are transmitted into the host's skin. Since no organisms and no immunogenic booster by natural exposure are encountered by the host's immune system, yearly re-vaccination is required to sustain antibody titers at a protective levels [37].

No information is available on the performance of the vaccine in individuals with an infection that had occurred prior to immunization. It is known that immunization with OspA does not eliminate the infection with *B. burgdorferi* [6]. No data are available on whether the simultaneous presence of both borreliacidal antibodies and organisms in the host pose a risk to the health of vaccinated dogs; however, immune complexes may form and induce inflammation in predisposed tissues such as joints, blood vessels, and kidneys.

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