LYME DISEASE

Lyme disease is caused by the spirochete *Borrelia burgdorferi*. This pathogen is transmitted by hard ticks of *Ixodes* spp, such as *Ixodes scapularis*, the blacklegged tick or deer tick common to the northeastern US. The Centers for Disease Control and Prevention (CDC) in annual maps of human case locations show slow but consistent spread of Lyme disease, and therein expanding exposure risk. Tick vectors, more common in northern climates, appear to be spreading slowly in a southern and perhaps westward pattern. This tick expansion is undoubtedly influenced by movement of birds and mammals that can carry pathogen-laden ticks. Largest geographical jumps are probably made by birds transporting infected ticks, but local spread is most influenced by the presence of white-tailed deer - a preferred host for the adult stage of this tick. Further complicating this picture is the fact that many (an estimated 20-30%) ticks carry multiple pathogens of the same or different genus. The enzootic life cycle is a 2-year, 3-host cycle. Exposure of dogs and humans is most common in mid-late spring to early summer due to questing nymphs. The nymphs molt into adults which feed in fall, repeating exposure risk, before breeding, laying eggs and dying.

The spirochete is maintained in the midgut of the tick but, after ingestion of a blood meal from a warm-blooded mammal, changes outer surface proteins (Osps) and moves to the tick’s salivary glands so it can enter the mammalian host. Based on research in other (non-canine) species, a minimum of 24 hours is required after tick attachment before *Borrelia* is transmitted.

OspA is the predominant surface protein for *Borrelia* in the tick midgut but it then declines, giving way to OspC (necessary for mammalian infection) in the tick salivary gland. Once in the mammalian host, however, OspC evokes a strong immune response so the Osp change again to evade the immune response. In order to create a persistent infection, OspC is down-regulated in the mammalian host and variable surface antigen (VlsE) and OspF are up-regulated. The C6 peptide, detected in some antibody diagnostic tests, is a component of VlsE.

Clinical signs have been difficult to consistently produce in dogs in experimental settings, but transient fever and/or polyarthritis were most commonly reported— albeit 2 to 5 months post infection. Thus, clinical signs subsequent to a spring exposure will not occur until summer or fall. Nevertheless, most experimentally infected dogs demonstrate immune-related pathologic lesions histologically around many joints, e.g. plasmacytic synovitis. Clinical signs related to *Borrelia* infection may be primarily due to immunological sequelae, such as immune damage to synovium. The consistency and high prevalence of these lesions in infected, untreated dogs prompted the USDA Center for Veterinary Biologics to issue a Memorandum in 2007 which allows the presence/absence of these lesions to a marker of Lyme vaccine efficacy.

Protein-losing glomerulopathy (termed ‘Lyme nephritis’) has been described in a few naturally infected dogs, but a cause-and-effect mechanism remains unclear. Lesions are described as an immune-mediated, antibody-complement deposition glomerulopathy in *Borrelia*-positive dogs, and unvaccinated dogs positive for *Borrelia* antibodies should be routinely tested for proteinuria. Although originally described in terminal cases, there is limited evidence that the antibody-related disease is responsive to antibiotic treatment for the *Borrelia* infection. Thus, reductions in circulating *Borrelia* antibodies were correlated with reductions in proteinuria, yet measurable reductions in circulating antibodies may not occur for 4-6 months after antibiotic therapy. Patients may receive other therapy for a protein-losing nephropathy, such as an ACE inhibitor and lower protein diet, but immunosuppressive therapy is not...
currently recommended unless proteinuria increases in spite of the aforementioned interventions. There is no evidence that vaccination and antibody formation related to vaccination initiate Lyme nephritis.

Other manifestations such as neuroborreliosis and Lyme carditis occur in people and may infrequently occur in dogs, but diagnosis is hindered in that clinical dogs would likely be antibody negative!

Diagnosis of Lyme borreliosis in people is aided by the characteristic bull’s eye skin lesion (erythema migrans) developing in a circular pattern around the bite wound as an immune response to the migrating spirochete. Regrettably dogs do not develop this overt acute pathognomonic sign. The organism migrates and hides in tissues such that routine antigen testing, e.g. PCR testing of blood or urine, is unproductive in dogs. Diagnosis therefore remains confined to antibody testing – which is really more a test for antigen exposure.

Administration of doxycycline, >10 mg/kg once a day for 4 to 6 weeks, is considered effective although there are no published large studies in dogs to confirm this. Effective treatment should be followed by a decline (>50% reduction) in C6 antibody concentrations within 6 months. Efficacy of other antibiotics for canine infections remains undetermined, and doxycycline often has efficacy against potentially co-infecting tick-borne pathogens. Published recommended dosage for an alternative to doxycycline, namely minocycline at 12.5 mg/kg/day, are generally higher than those for doxycycline. A recently (2015) published small pilot study has also shown that 2 cefovecin injections two weeks apart would be as efficacious as the recommended treatment of doxycycline.

Clients should be clearly informed that tick prevention is the first line-of-defense against Lyme disease. A positive Lyme test in a dogs means tick control has failed! Lyme vaccines are available which invoke canine antibodies to *Borrelia* OspA. These antibodies, ingested in the tick’s blood meal, will bind the pathogen before it can be transmitted to the dog. Inclusion of OspC in the vaccine would theoretically and practically provide additional protection by binding *Borrelia* in the tick (or dog) not expressing (or minimally expressing) OspA. C6-antibody positive dogs should be treated before vaccination, but the impact of vaccination (against presumably down-regulated Osps) in infected dogs is unclear. C6-antibody positive dogs should not be vaccinated, however, if they are proteinuric, as the impact of antibodies on a potentially damaged glomerular basement membrane is a concern.

Problematic to veterinarians is the interpretation of in-house diagnostic tests, expanding prevalence of tick exposure, the dilemma of how to manage asymptomatic, C6 antibody-test-positive dogs (e.g., treat them all vs. ignore them all), and the decision of if or when to consider Lyme vaccine in vaccination protocols. In essence, what does “first do no harm” mean in canine Lyme disease? Some steps however are indicated as a Lyme+ dog clearly is not adequately protected from ticks and is exposed to the *Borrelia* organism!

Evidence of periarticular inflammation as previously described provides very strong support for antibiotic treatment of asymptomatic, test-positive dogs. With/after antibiotic treatment, further protection is necessary (tick control +/- vaccination) for these dogs. These patients should also be monitored periodically for proteinuria (UPC >0.05) for early detection of Lyme nephritis.

Vaccines against *Borrelia burgdorferi* have been available for more than a decade with continuing improvement in vaccine quality. Initial vaccines were directed against OspA alone, with plasma antibodies taken in by the tick with its blood meal and binding OspA in the tick midgut. Subsequent recognition that some ticks also had OspC present led to OspA and C combinations. More recently (2013) it was reported that OspC had several genotypes which may be present in a population and infective of dogs in North America. This has led to new (2016) production of a chimeric recombinant vaccine incorporating epitopes of 7 OspC genotypes in addition to an OspA component. Each of these progressive steps in vaccine development have been designed to minimize any vaccine failures. All Lyme vaccines are killed bacterins, necessitating 2 parental injections for protection with an annual booster to maintain protective immunity.
Lyme disease, specifically chronic Lyme disease, has become a controversial topic in human medicine. Strongly divergent opinions by infectious disease physicians exist regarding the actual occurrence, incidence, and symptoms related to chronic Lyme disease, but CDC has in 2015 acknowledged the existence of chronic Lyme disease on its website. Complicating this scenario and its interpretation is the potential impact of co-infection(s) caused by other tick-borne pathogens—pathogens that may or may not be detected/diagnosed. Of recent concern is the recognition that all *B. burgdorferi* may not be the same, with cell wall–deficient forms of the spirochete possibly explaining some chronic Lyme disease cases in people.

ANAPLASMOSIS

Due to the common *Ixodes scapularis* tick vector, dogs at risk for exposure to Lyme disease are also at risk for exposure to the rickettsia *Anaplasma phagocytophilum*—causative agent of granulocytic anaplasmosis. In contrast however to Lyme borreliosis which typically requires several (>4-8) weeks for clinical signs and detectable antibodies to develop, clinical illness of anaplasmosis can occur within 1-2 weeks post-exposure. Thus, signs and symptoms related to infection are more likely to appear early in the tick season. These clinical signs include fever, anorexia, lethargy, arthropathy, stiffness, and lymphadenopathy. Much less common are petechiae or epistaxis, signs associated with a related thrombocytopenia.

In the acute phase of the *A. phagocytophilum* infection, morulae may be detected within neutrophils of a blood smear although these morulae are morphologically indistinguishable from *Ehrlichia ewingii* inclusions. The reported likelihood of morulae detection is quite varied, with estimates ranging from 10-90% of acutely-ill canine patients. The most common clinicopathologic abnormality is a mild, or even severe, thrombocytopenia. Mild hypoalbuminemia, lymphopenia, eosinopenia, monocytosis, neutropenia or mild neutrophilia, and/or mild normochromic normocytic anemia are also seen in some patients.

Clinical signs may appear before ELISA-based semi-quantitative antibody tests register a positive result. These patients should be positive however on more sensitive PCR testing of whole blood. Although false-positives and false-negatives can occur on PCR tests, PCR tests are generally considered less cross-reactive to closely related rickettsiae.

Fortunately doxycycline/minocycline are considered effective treatments for all *Anaplasma* and *Ehrlichia* infections, and differentiation between infections is not always required. Just as clinical signs secondary to *A. phagocytophilum* appear much quicker than possible signs of Lyme disease, response to treatment and duration of treatment is also quicker than with Lyme disease. Treatment recommendations may be as short as 10-14 days of therapy, but recommendations of 28 days of antibiotic therapy are sometimes made in light of possible co-infections.

EHRLICHIOSIS

Much less commonly, but occasionally, seen in areas of *Ixodes* ticks are the rickettsiae *Ehrlichia ewingii* and *Ehrlichia canis*. These *Ehrlichia* are not carried by *Ixodes* ticks, but are transmitted by *Amblyomma americanum* and *Rhipicephalus sanguineus*, respectively. Infection should nevertheless be considered in light of documented northward expansion of southern tick ranges and/or documented travel history to southern at-risk areas.

ELISA-based SNAP tests can give positive results for *Ehrlichia* spp. antibodies, without distinguishing between *E. canis* and *E. ewingii* infection. Assumptions of infective organism based on geographical location, i.e. what’s most likely, may be erroneous. IFA serology tests or PCR tests of whole blood are required for identification of the infective pathogen.

As noted, the morulae of *Ehrlichia ewingii* detected within neutrophils of a blood smear are morphologically indistinguishable from *A. phagocytophilum* inclusions. Nevertheless, morulae in
neutrophils due to *E. ewingii* (canine granulocytotropic ehrlichiosis) should be distinguishable from those in monocytes due to *E. canis* (canine monocytotropic ehrlichiosis).

Clinical signs due to *E. ewingii* infection are poorly described but include lethargy, anorexia, lameness, and joint swelling, thus resembling other mild rickettsial disease. Fever may or may not be present. CNS signs of head tilt and anisocoria have also been reported. Mild thrombocytopenia may be noted as a clinical laboratory finding, but platelet counts may be within the normal reference range.

As already noted, doxycycline/minocycline are considered effective treatments for all *Anaplasma* and *Ehrlichia* infections, and differentiation between infections is not always required. Treatment recommendations for *E. ewingii* may be as short as 10-14 days of therapy, but 28 days of antibiotic therapy are commonly recommended for *E. canis* infection.