

Developing an **integrated approach** for diagnosing, managing tick-borne diseases

Thorough testing aids in determining co-infections in dogs

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Canine granulocytic ehrlichiosis is an emerging tick-borne disease. It has only recently been recognized as a disease of dogs and its full clinical significance is as yet undetermined. *Ehrlichia equi* is a member of the

Ehrlichia phagocytophila genogroup and is the etiologic organism of canine granulocytic ehrlichiosis. The same ticks which vector *Borrelia burgdorferi*, the Lyme disease organism, are responsible for transmission of *E. equi*. The white-footed mouse and likely other rodents are reservoir hosts for *E. equi*. In experimentally infected laboratory mice, strongest response of IgM antibodies and earliest IgG

response occurred 10 days after infection. Morulae of *E. equi* were present in murine neutrophils five days after repletion of feeding ticks.

Dogs infected by *E. equi* may have subclinical infections with no signs of disease observed, while leukopenia, thrombocytopenia, hypoalbuminemia and anemia have been reported in infected canines. Positive titers for antibodies directed against the organism and presence of morulae in granulocytes and monocytes aid in clinical diagnosis. Therapy with tetracycline or doxycycline is indicated in dogs with suspected *E. equi* induced disease or dogs with multiple tick-borne infections responsive to these antimicrobials.

A retrospective study using canine sera collected in 1985 and 1986 from Connecticut and the lower Hudson Valley of New York State (highly Lyme endemic areas) found antibodies to *E. equi* in 10 of 64 (15.6 percent) *Borrelia burgdorferi* positive dogs and none of 42 (0 percent) *B. burgdorferi* negative dogs. In a 2001 study using dogs from Durham, Conn., the author and collaborators detected antibodies to *E. equi* in six of 15 (40 percent) of *Borrelia burgdorferi* positive dogs and 1 of 15 (6.6 percent) of *B. burgdorferi* negative dogs. While this study examined a small number of individuals it suggests that the infection rates for *E. equi* in Lyme endemic areas has in-

Table 1: Durham Veterinary Hospital's Management Protocols for Dogs with positive C₆ ELISA

Dogs with clinical diagnosis of Lyme arthritis:

- Doxycycline therapy- 5mg/lb bid for 28 days given with food.
- (Doxycycline has activity against *Borrelia burgdorferi* and *Ehrlichia equi* both of which are vectored by *Ixodes scapularis* and *I. pacificus*. Further, dogs parasitized by one tick species may have been parasitized by others and spotted fever and canine ehrlichiosis respond to this antibiotic.)
- Recheck dog in 14 days (sooner if signs exacerbate or fail to respond within 3 to 5 days.
- If signs have responded favorably continue therapy for full 28 days in unimmunized dogs first dose of Lyme bacterin is administered on day 14 and the second on day 28 in dogs due for booster immunization give Lyme bacterin on day 14.

Dogs with no signs of Lyme arthritis:

- Doxycycline therapy- 5mg/lb bid for 28 days given with food.
- In unimmunized dogs, first dose of bacterin is administered on day 0 and second on day 14.
- In dogs due for booster immunization give bacterin on day 0.
- Owners of all dogs with positive C₆ ELISA receive tick-control education and a review of early signs of tick-borne diseases.

(NB: This is a working protocol based on practical experience and published data on both experimentally and naturally infected dogs. Utility of this protocol is under investigation in a year-long study.)



creased since the prior study on samples collected 15 and 16 years earlier. These findings make a strong case for consideration of co-infection with multiple tick-borne organisms in dogs which have evidence of infection with any single tick-borne organism or in dogs with evidence of parasitism by any single tick species.

■ Canine Lyme disease

Etiologic organism and tick vectors:

Lyme disease is caused by a spirochete, *Borrelia burgdorferi*, and is vectored by ticks of the genus *Ixodes* on a nearly world-wide basis. The signal outbreak of Lyme disease occurred in Old Lyme, Connecticut in 1975 and was originally thought to be autoimmune arthritis after initial failure to culture microbial organisms from joint effusions of affected humans. An arthropod vector was proposed in 1977 and in 1982 the spirochete was discovered in an *Ixodes scapularis* (formerly *I. dammini*) tick from Long Island, New York. The first canine case of Lyme arthritis was reported in 1984 and since then cardiac and renal syndromes have been well characterized. Lyme disease has been reported in humans in 47 of the lower 48 states and is endemic on the East Coast, West Coast and in the upper Midwest. A clear trend toward a spreading geographic incidence and increasing number of cases is present. Early reports from veterinarians using a new diagnostic test for the detection of infection by *B. burgdorferi* (C₆ ELISA) (SNAP[®]3Dx, IDEXX Laboratories) during routine heartworm screening are indicating the impact of the spread of *Borrelia burgdorferi* infection into new areas.

Ixodes scapularis is responsible for vectored the disease throughout the upper-Midwest and eastern states; *Ixodes pacificus* is the vector on the West Coast. These ticks use a 3-host life cycle and parasitize small mammals (usually rodents which serve as reservoirs for *Borrelia burgdorferi* and other microorganisms) in their immature stages and larger mammals as adults (see Table 2). In regions where winters are cold the tick has a 2-year life cycle which is ideal for maintenance of infection when larvae infected by feeding on rodents in the fall molt to nymphs the next spring and transstadially

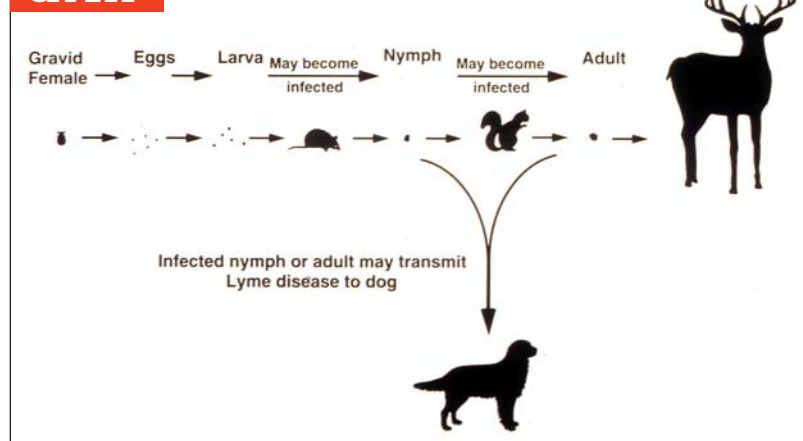
transmitted spirochetes infect the next generation of rodents. On the west coast, immature *I. pacificus* feeding on lizards are not exposed to highly infected rodents and rates of infection in this tick species are limited, yet high infection rates in dogs from California have been reported.

■ Outer surface proteins of *Borrelia burgdorferi*

Borrelia burgdorferi is transmitted via tick saliva after a lag phase of approximately 24 to 48 hours during which the surface structure of the spirochete changes (host-adaptation) in response to cues associated with

In the process of host-adaptation production of Osps A and B is turned off and production of another outer surface protein, OspC, is often turned on. OspA expressing spirochetes are selectively retained in the midgut of the feeding tick and new studies have determined that by the time spirochetes have entered the host they are not expressing Osps A or B but rather some express OspC and others express neither Osp A, or B, or C. These findings help explain the lag phase for transmission and the subsequent absence of antibodies directed against Osps A and B in animals infected by the bite of an infected tick.

Table 2: Tick life cycle and Lyme disease transmission



the host and the blood meal. In mammalian hosts, the spirochete rapidly sequesters in endothelial cells, is difficult to demonstrate in blood or other tissues and is able to evade both the host's immune response and antibiotic therapy. The humoral immune response to *Borrelia burgdorferi* takes three to six weeks to develop. The first antigen recognized in tick-borne infection is often flagellin, which is similar to that in other flagellar organisms. The surface antigens, outer surface proteins A and B (Osps A and B), are prominent on cultured *Borrelia burgdorferi* and on spirochetes in unfed ticks, however, antibodies directed against these antigens are found only rarely in individuals exposed to the spirochete by tick bite. While Osp A was discovered in 1983 it was not until 1994 that it was determined that Osp A (and B) production was down regulated on spirochetes in the midgut of feeding ticks.

■ Serology and antibody testing

One of the most confounding issues in Lyme disease diagnosis is the significance of serologic testing. A positive serology detecting antibodies directed against *Borrelia burgdorferi* is merely an indication of exposure to the organism or to a vaccine which contains an antigen (single subunit vaccine) or multiple antigens (bacterin) of the spirochete. A positive serology by itself does not constitute a diagnosis of Lyme disease. Antibodies induced by natural tick transmission of *Borrelia burgdorferi* detected by IFA, whole-cell ELISA and Western blot persist in infected dogs and do not decrease after antibiotic therapy. Until now, a Western blot was required to examine the full spectrum of the dog's antibody response and thereby determine if the dog was exposed to live *Borrelia burgdorferi* by the bite

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of an infected tick or to antigen(s) in a vaccine. The Western blot is expensive, inherently complex, and evaluation of blots is subject to individual interpretation by the reader.

A new test has been developed to aid in the diagnosis of canine Lyme disease. The C₆ ELISA is based on an immunodominant conserved region of the *Borrelia burgdorferi*, VlsE (Vmp-like sequence, Expressed). This test uses a synthetic peptide (C₆ peptide) as its diagnostic antigen and detects infections with *B. burgdorferi* with a high degree of sensitivity and specificity. Because C₆ ELISA does not cross-react with antibodies produced by vaccination with either whole-cell bacterin or OspA subunit vaccine it is the new platinum standard in Lyme disease serodiagnosis (see Photo 1).

The specificity of the C₆ ELISA for infection with *Borrelia burgdorferi* allows the canine practitioner to determine if a dog

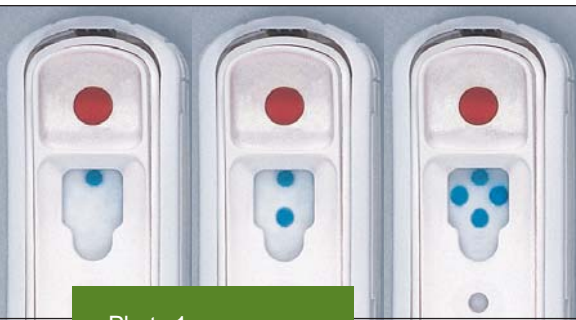


Photo 1:
C6 ELISA test results
Left: negative result
Center: positive result for lyme disease
Right: positive results for heartworm, lyme and E. canis

has anti-*Borrelia burgdorferi* antibodies induced by infection rather than by immunization with either type of vaccine. This distinction is a vital part of the diagnosis

of Lyme disease in dogs that may have been vaccinated against the Lyme organism. Often owners do not know what immunizations their dogs have received and the detection of an antibody produced only by infection eliminates the need for a two-step serologic analysis using first whole-cell ELISA or IFA and then Western blot analysis for dogs positive on initial testing. A positive C₆ ELISA indicates infection, however further steps must be taken to verify a diagnosis of canine Lyme disease.

Table 3: Common ticks parasites of dogs and the organisms they commonly vector

Tick species	Vector organism
• <i>Ixodes scapularis</i>	<i>Borrelia burgdorferi</i> , <i>Ehrlichia equi</i>
• <i>Ixodes pacificus</i>	<i>Borrelia burgdorferi</i> , <i>Ehrlichia equi</i>
• <i>Dermacentor variabilis</i>	<i>Rickettsia rickettsii</i>
• <i>Rhipicephalus sanguenous</i>	<i>Ehrlichia canis</i> , <i>Babesia Canis</i> , <i>Hepatozoon canis</i>
• <i>Amblyomma americanum</i>	Implicated in transmission of <i>Francisella tularensis</i>

■ **Clinical syndromes and diagnosis**

Clinical syndromes of canine Lyme disease include arthritis, carditis, nephritis and neurologic abnormalities. In all cases diagnosis of Lyme disease is made by a consideration of epidemiologic factors, presence of appropriate clinical signs, rule-out of alternate causes of these signs, demonstration of evidence of infection with *Borrelia burgdorferi* (positive C₆ ELISA verifies infection by *Borrelia burgdorferi*), and response to antibiotic therapy. Lyme arthritis is characterized by sudden onset of joint swelling, lameness, fever, and lethargy, often accompanied by prescapular and/or popliteal lymphadenopathy. The incubation period in naturally infected dogs is unknown. In laboratory dogs, clinical signs develop from two to five months after exposure to infected ticks. Abnormal urinalysis characterized by presence of protein, cells and casts has been noted in dogs with naturally acquired Lyme arthritis. Joint tap may reveal WBCs which are mainly neutrophils with smaller numbers of macrophages and monocytes. CBC, serum chemistries and immune profiles are within normal limits. Alternate diagnoses such as trauma, autoimmune arthritis, degenerative joint disease and other tick-borne diseases must be ruled out. Response to therapy with antibiotics such as penicillin, tetracycline or doxycycline may be dramatic in some dogs but about 15 to 25 percent of dogs treated for Lyme arthritis will become chronically affected and have incomplete resolution of signs or recurrent disease. Research in laboratory dogs indicates that, in spite of adequate antibiotic therapy, infected individuals are possibly infected for life. New research, however, has demonstrated decreasing

C₆ ELISA levels in response to antibiotic therapy in experimentally infected laboratory dogs. It remains to be seen if the decrease in C₆ ELISA indicates clearance of infection or rather a sequestration of *Borrelia burgdorferi* in immune privileged sites with the possibility of later recrudescence of active infection. At the Durham Veterinary Hospital, we are currently tracking C₆ ELISA levels in dogs treated twice daily with 5 mg/pound of doxycycline for 28 days to determine if anti-C₆ antibodies diminish with therapy in naturally infected dogs. While we are conducting this year-long study we have developed working protocols to deal with the variety of situations presented by both healthy and ill dogs with positive C₆ ELISA results (see Table 1, p. 14).

Cardiac Lyme disease is characterized by high degree of heart block and therapy must include pacemaker implantation as well as antibiotics. Renal Lyme disease is generally fatal, often occurs in young adult dogs and is characterized by uremia, hyperphosphatemia, hypoalbuminemia, peripheral edema and severe protein losing nephropathy. Spirochetes have been demonstrated in the cardiac and renal tissues (respectively) of dogs with each syndrome. Central nervous system infection with *Borrelia burgdorferi* has been associated with seizure disorder.

■ **Integrated management of tick-borne diseases of dogs**

An integrated approach to the management of tick-borne diseases is required to clinically manage the potential of co-infection by multiple tick-borne organisms. Individual dogs may be bitten by individual ticks containing single or multiple pathogens or by many ticks of different

species which may each contain individual pathogens. The net result is the potential for the dog to be infected by multiple organisms (See Table 3, p. 17).

■ Lyme disease prevention

Lyme disease prevention for humans has been centered on tick avoidance and early removal of attached ticks. The effort to develop and license an effective vaccine for human use had initially focused on Osp A vaccines but the discovery that Osp A down regulates (host-adapts) in feeding ticks has led to the investigation of vaccines made up of multiple antigen "cocktails". In 1998, a human OspA, recombinant vaccine (LymeRix[®], Smith Kline Beecham Pharmaceuticals) was licensed by the Food and Drug Administration as being both safe and effective. However, an examination of the published data in the field study evaluating this vaccine raises some questions about efficacy, according to a 1998 article in *The New England Journal of Medicine*. In the first year, efficacy was only 49 people in people receiving two doses. In the second year (after a booster dose in month 13) efficacy was listed as 76 percent. When overall results are examined for the full two-year study period, efficacy was only 65 per-

cent. These results clearly highlight the shortfalls of a single subunit strategy for protection against this very complex organism. In March of 2002, the human single subunit OspA Lyme vaccine was removed from production and distribution by its manufacturer.

Attempts to limit numbers of *Ixodes* ticks through habitat modification are often limited to the immediate area of the modification (cutting brush, destroying wood piles, etc). Host reduction has been attempted but the wide variety of small mammals which serve as reservoirs for *Borrelia burgdorferi* and the large variety of animals on which adult *Ixodes scapularis* will feed make this strategy problematic. Biological control has been less than adequate and while repeated area treatment with chemicals can effectively control tick populations, environmental considerations make wide treatment problematic. New, host-directed products for dogs (amitraz-impregnated collars and fipronal spot-ons) have produced good results in tick control but do not necessarily result in control of tick-borne diseases. The amitraz-impregnated collar (Preventic[®], Virbac Inc.,) has been demonstrated to prevent tick bites and a recently published study demonstrated that

this collar could interrupt the transmission of *Borrelia burgdorferi* to dogs exposed to *Ixodes scapularis* in a model approximating natural tick parasitism. Proof of interruption of pathogen transmission is required to make a claim for interruption of disease transmission.

■ Canine vaccination

There are currently two types of Lyme disease vaccine available for dogs: whole-cell, killed bacterin and recombinant Osp A vaccine. The original bacterin (LymeVax[®], Fort Dodge Animal Health) has been available since 1990 and the original Osp A vaccine (Recombitek[®] Lyme, Merial Ltd.,) since 1996. Peer reviewed, published data on the safety and efficacy in dogs naturally exposed to *Borrelia burgdorferi* is available only for the original whole-cell bacterin (Levy, Lissman, and Ficke, *JAVMA* 1993;202:1834-1838).

Post-vaccination signs of Lyme disease may indicate vaccine failure, however, the long incubation for canine Lyme disease may allow for vaccination of healthy, infected dogs which will develop signs of disease some time after immunization. In endemic areas, any dog older than 6 months may reasonably be considered to be at risk for prior exposure. Use of the in-office C₆ ELISA can provide immediate information on the infection status of an adult dog.

The author and collaborators at two nearby small animal practices are currently in a multi-hospital study using the C₆ ELISA to compare efficacy of the original whole-cell bacterin and the original recombinant OspA vaccine. The study is currently in the review process. To learn more about this study, contact Dr. Levy. **DVM**



Dr. Levy, VMD is a 1977 graduate of the University of Pennsylvania School of Veterinary Medicine and is in full-time private small animal practice in Durham, CT. Dr. Levy is a nationally recognized authority on Lyme disease and ticks, has published scientific papers in major veterinary and science journals, and has presented continuing education programs widely. He was awarded the AVMA practitioner Research Award and the AAHA Outstanding Regional Practitioner Award and is a Past-President of the Connecticut Veterinary Medical Association.

Suggested reading

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