

Consensus Statements of the American College of Veterinary Internal Medicine (ACVIM) provide veterinarians with guidelines regarding the pathophysiology, diagnosis, or treatment of animal diseases. The foundation of the Consensus Statement is evidence-based medicine, but if such evidence is conflicting or lacking, the panel provides interpretive recommendations on the basis of their collective expertise. The Consensus Statement is intended to be a guide for veterinarians, but it is not a statement of standard of care or a substitute for clinical judgment. Topics of statements and panel members to draft the statements are selected by the Board of Regents with input from the general membership. A draft prepared and input from Diplomates is solicited at the Forum and via the ACVIM Web site and incorporated in a final version. This Consensus Statement was approved by the Board of Regents of the ACVIM before publication.

ACVIM Small Animal Consensus Statement on Lyme Disease in Dogs: Diagnosis, Treatment, and Prevention

Meryl P. Littman, Richard E. Goldstein, Mary A. Labato, Michael R. Lappin, and George E. Moore

The purpose of this report is to offer a consensus opinion of ACVIM diplomates on the diagnosis, treatment, and prevention of *Borrelia burgdorferi* infections in dogs (canine Lyme disease). Clinical syndromes known to commonly be associated with canine Lyme disease include polyarthritides and glomerulopathy. Serological test results can be used to document exposure to *B. burgdorferi* but not prove illness. Although serum enzyme-linked immunosorbent assay/indirect fluorescent antibody assay titers can stay positive for months to years after treatment, quantitative C6 peptide antibody paired tests need more study. Serological screening of healthy dogs is controversial because it can lead to overdiagnosis or overtreatment of normal dogs, most of which never develop Lyme disease. However, serological screening can provide seroprevalence and sentinel data and stimulate owner education about tick infections and control. Although it is unknown whether treatment of seropositive healthy dogs is beneficial, the consensus is that seropositive dogs should be evaluated for proteinuria and other coinfections and tick control prescribed. Tick control can include a product that repels or protects against tick attachment, thereby helping to prevent transmission of coinfections as well as *Borrelia* spp. Seropositive dogs with clinical abnormalities thought to arise from Lyme disease generally are treated with doxycycline (10 mg/kg q24h for 1 month). Proteinuric dogs might need longer treatment as well as medications and diets for protein-losing nephropathy. The ACVIM diplomates believe the use of Lyme vaccines still is controversial and most do not administer them. It is the consensus opinion that additional research is needed to study predictors of illness, "Lyme nephropathy," and coinfections in Lyme endemic areas.

Key words: Arthritis; *Borrelia burgdorferi*; Glomerulonephritis; Polyarthropathy; Retriever.

Borrelia burgdorferi (*Bb*) is a tickborne organism associated with illness in humans in Lyme, Connecticut in 1975, and the clinical syndrome was termed Lyme disease.^{1–3} The organism is transmitted by *Ixodes* ticks, the life cycle of which has been reviewed extensively.⁴ These field ticks have a 2-year life cycle and mostly quest on vegetation in prime suburban real

estate. Although *Bb* infection of dogs occasionally can be transmitted transplacentally⁵ or by blood,^{6–8} urine,^{7,9–11} or milk,¹² tickborne transmission is considered most common.

Because of its association with a tick vector, the prevalence of Lyme disease varies geographically. The current presumed distribution of *Bb* in humans is shown in Figure 1. Clinical illness from *Bb* was first suggested in dogs in 1984 and 1985.^{13,14} Since those initial reports, hundreds of manuscripts concerning *Bb* in dogs have been written. The combination of this increased knowledge with the introduction and common use of commercially available serological tests and vaccines has resulted in many questions about canine *Bb* infection in veterinary medicine.

Because of the large number of questions and disparity of opinions concerning canine Lyme disease, the need for a Consensus Statement was suggested during the Infectious Disease Study Group (IDSG) meeting at the 21st Annual ACVIM Forum in 2003. During the 22nd Annual ACVIM Forum in 2004 in Minneapolis, the IDSG sponsored an evening Special Interest Group presentation and discussion led by Drs Meryl Littman, Richard Goldstein, and Edward Breitschwerdt. The ACVIM Board then selected the topic for a Small Animal Consensus Statement and chose the chairperson (Littman). During 2004–2005, the

From the Department of Clinical Studies–Philadelphia, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA (Littman); Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY (Goldstein); Department of Clinical Sciences, Tufts University, Cummings School of Veterinary Medicine, N Grafton, MA (Labato); Department of Clinical Sciences, Colorado State University, Ft Collins, CO (Lappin); and Department of Veterinary Pathobiology, School of Veterinary Medicine, Purdue University, West Lafayette, IN (Moore).

Presented in part at the 23rd Annual Veterinary Medical Forum, American College of Veterinary Internal Medicine, Baltimore, MD, June 1–4, 2005.

Reprint requests: Dr Meryl P. Littman, Department of Clinical Studies, University of Pennsylvania School of Veterinary Medicine, 3900 Delancey Street, Philadelphia, PA 19104-6010; e-mail: merylitt@vet.upenn.edu.

Copyright © 2006 by the American College of Veterinary Internal Medicine

0891-6640/06/2002-0032/\$3.00/0

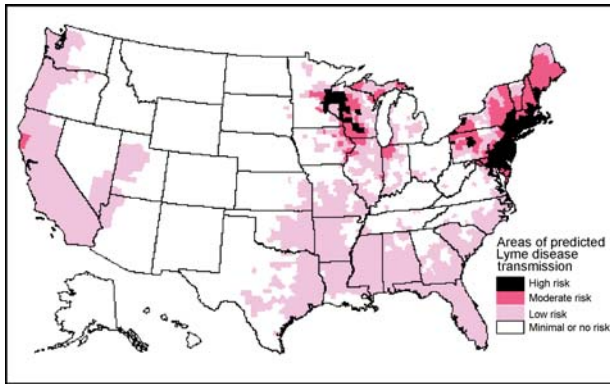


Fig 1. National Lyme disease risk map with 4 categories of risk. (From the CDC website www.cdc.gov/ncidod/dvbid/lyme/index.htm).

other panelists were chosen, reviewed the literature, shared opinions on the structure of the Consensus Statement, and drafted a questionnaire that was distributed to ACVIM small animal diplomates via an e-mail listserve. Of the 45 respondents, 26 worked in endemic areas, 15 worked in nonendemic areas, and 4 worked in “low-risk” areas. Of the respondents, 21 worked in academic practice, 23 in private practice, and 1 in both types of practices. The majority of the respondents had mainly a referral caseload (31) or a well-mixed caseload (11); only 2 saw mostly primary care cases. The responses received were used by the committee to aid in formatting the Consensus Statement draft that was presented orally at the 23rd ACVIM Forum in Baltimore, MD, in June 2005. The written draft then was posted on the ACVIM website for additional comments by the general membership before submission of the revised manuscript to the ACVIM Board of Regents and to the editors of the *Journal of Veterinary Internal Medicine*. The authors are aware that cases seen by specialists in referral centers might not represent the same type of cases seen primarily in the field. The panelists endeavored to use the literature and other information gathered to present evidence-based justification for issues for which there appeared to be a consensus. For issues without clear consensus, pros and cons are presented. The questions addressed primarily the diagnosis, treatment, and prevention of canine Lyme disease.

1. What Clinical Syndromes are Associated with Experimental Infection of Dogs with *Bb*?

Most people exposed to *Bb* show clinical signs, including an acute illness (flulike signs, erythema migrans rash), subsequent arthritis, and possibly cardiac, neurologic, or chronic skin changes; only 10% are asymptomatic.^{1–3} In contrast, 95% of exposed dogs remain asymptomatic.¹⁵ For Lyme disease in dogs, Koch’s postulates have only been satisfied for a syndrome of transient fever, anorexia, and arthritis, which was detected only in puppies. Experimentally, after putting *Ixodes* ticks from New York on Beagles, the

adult dogs seroconverted but remained asymptomatic^{9,16,17}; however, 6–12-week-old puppies showed oligoarthritis in the limb closest to the tick bites with or without lymphadenopathy, about 2–5 months after exposure.^{9,18–24} The illness was self-limiting and resolved in 4 days without treatment. Some puppies developed several similar episodes in the same or different leg a few weeks apart, which also were self-limiting. Tissue migration is more likely than hematogenous spread in the dog; the first leg to show lameness was the limb closest to where the ticks were attached.¹⁸ Older pups (12–26 weeks old) were affected less often and only for 1–2 days.^{25,26} Cytological assessment of synovial fluid collected by arthrocentesis showed neutrophilic inflammation.^{9,13,14} One study suggested that chronic Lyme infection might lead to degenerative joint disease because carrier pups in which postmortem examinations were performed later in life had asymptomatic mild nonsuppurative synovial infiltration⁹; however, the same synovial changes were found in seronegative and vaccinated dogs.²⁷ In small numbers of field cases, renal, cardiac, neurologic, or dermatologic manifestations have been attributed to *Bb* infection of dogs, but these syndromes have not been reproduced in experimental models. At the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania (Ryan VHUP), 55% of retrievers with thrombocytopenia were seropositive for *Bb* antibodies compared with 24% of healthy retrievers, but this association could be because of coinfections (see question 7) or thrombocytopenia might be associated with nephropathy (see question 4) (Littman, personal communication).

2. How Common is Lyme Arthropathy in Dogs in Clinical Practice?

This question is difficult to answer because there are so many seropositive dogs in endemic areas, there is no test result that proves illness from *Bb* infection, and some dogs thought to be subclinically infected could be ill from another cause. For example, there are Lyme endemic areas where 70–90% of all healthy and clinically ill dogs are seropositive,^{28–30} making the diagnosis of Lyme disease in individual dogs problematic. In another study, fewer than 5% of seropositive dogs showed lameness during a 20-month observation period, and the same was true for seronegative dogs.¹⁵ At Ryan VHUP, 37% of the general population and 57% of the polyarthropathy dogs were Lyme-positive.³¹ Breed predisposition also could be involved because 57% of lame retrievers compared with 24% of healthy retrievers were seropositive, but there was no such difference in seropositivity between nonlame and lame German Shepherds at Ryan VHUP (Littman, personal communication).

3. Has “Lyme Nephropathy” Been Definitely Associated with *Bb* Infection of Dogs?

Although there is no experimental model for Lyme nephropathy and Koch’s postulates are not satisfied,

there are multiple reports of dogs with *Bb* antibodies developing a unique renal histopathologic lesion, including immune-mediated glomerulonephritis, diffuse tubular necrosis and regeneration, and lymphocytic-plasmacytic interstitial nephritis.^{11,30,32,33,a} About 30% had a history of arthritis and almost 30% had a history of Lyme vaccination. Labrador Retrievers, Golden Retrievers and Shetland Sheepdogs have been over-represented in some studies (Sanders, personal communication).³² Dogs with Lyme-associated protein-losing nephropathy (PLN) are younger than dogs with PLN from other causes.³² Positive staining for Lyme antigens in canine renal tissue of dogs with this nephropathy were found with monoclonal antibody stains,^{11,30,32} and the agent was isolated in urine,¹¹ but a causal relationship still is not proven.

4. What are the Clinical and Laboratory Abnormalities Associated with Lyme Nephropathy?

Most reported cases^{11,30,32,33,a} of Lyme nephropathy are clinically ill dogs presented mostly in the summer or fall months with an acute or chronic presentation of renal failure with anorexia, vomiting, dehydration, variable polyuria and polydipsia, and wasting. Abnormalities included vasculitis with possible edema or effusions; hypertension with possible blindness or heart murmur; thromboembolic events (eg, pulmonary thrombosis with dyspnea; saddle thrombus with hind limb weakness); and sometimes neurologic signs (eg, seizure, nystagmus, collapse) from vasculitis, hypertension, thromboembolic events, uremic encephalopathy, or meningitis. Laboratory abnormalities in this syndrome included nonregenerative anemia, stress leukogram, thrombocytopenia, hypoalbuminemia, azotemia, hypercholesterolemia, hyperphosphatemia, and sometimes hyperkalemia and hyperbilirubinemia. Urinalysis showed proteinuria and possible decreased concentrating ability with hemoglobinuria, hematuria, glucosuria, bilirubinuria, casts, and an active sediment with negative bacterial culture. Many dogs succumb within days to weeks, some with oliguric or anuric renal failure.

5. Is the Cause of Lyme Nephropathy Known?

Lyme nephropathy probably is an immune-mediated phenomenon^b and might depend on the strain of *Bb*, the genetic predisposition of the patient, and possibly other triggers. The incidence of PLN associated with Lyme disease still is unknown. At Ryan VHUP, presence of *Bb* antibodies was not associated with proteinuria (ie, urine protein-to-creatinine ratio > 1) in the general hospital population (Murray, personal communication); however, the seroprevalence of *Bb* antibodies in healthy retrievers and retrievers with PLN was 24 and 85%, respectively (Littman, personal communication). Occult proteinuria was detected infrequently in young healthy *Bb*-seropositive Labrador and Golden Retrievers at Cornell.^c In addition, proteinuria was not associated with presence of *Bb* antibodies in Bernese Mountain

dogs or control dogs in Europe.^d It currently is unknown why some dogs develop nephropathy and others do not.

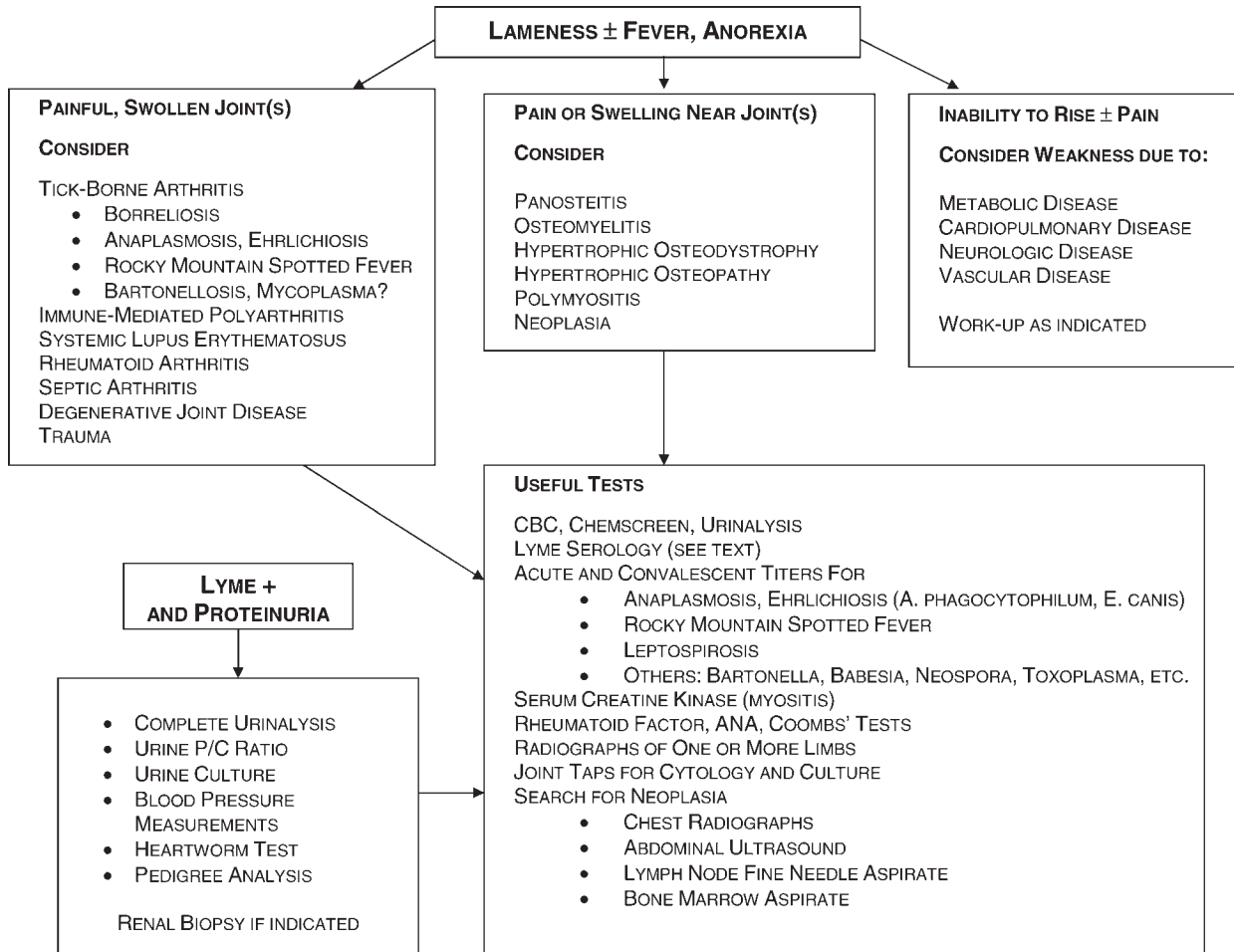
6. Why do Findings from Experimental Studies and Clinical Practice Vary for Lyme Disease?

It is not known why experimental studies fail to replicate what is presumed to occur in naturally occurring cases in veterinary practices. However, differences might relate to breed predispositions, different strains of *Bb* in the field, the likelihood for multiple exposures in pets, and the presence of coinfections.

7. What are the Other Differential Diagnoses or Coinfections in Dogs Suspected to Have Lyme Disease?

Dogs with fever, lameness and anorexia or proteinuria (with or without azotemia) could have many other infectious, immune-mediated, or neoplastic causes, and the clinician should consider all other differential diagnoses (Fig 2). Owners are very willing to accept a popular diagnosis such as “Lyme disease,” but the clinician should be aware that dogs sick with any illness can be seropositive just by coincidence. Because specialists are seeing primarily sick referral cases, there might be bias against Lyme disease as a serious entity causing morbidity. Primary practitioners could be seeing more cases that are mild, self-limiting, or easily treated with a short course of antibiotics that do not require referral. Referred dogs with “nonresponsive Lyme disease” often are found to have other illnesses.

Because *Bb* is tick-associated, coinfections commonly are those that share the same vector or are associated with other ticks, fleas, or environmental exposure including *Anaplasma phagocytophilum*, *Ehrlichia* spp., *Rickettsia rickettsii*, *Neorickettsia risticii*, *Bartonella* spp., *Babesia* spp., *Mycoplasma* spp., and *Leptospira* spp. *Ixodes* ticks can carry many organisms, including *Bb*, *A phagocytophilum*, *Babesia microti*, *Bartonella* spp., tickborne encephalitis virus, and possibly others. In experimental studies with ticks from Westchester County, NY, coinfections with *A phagocytophilum* were found in 32–45% of the dogs.^{19,26} In 2001, 40% of *Bb* seropositive (and 6.6% of seronegative) dogs also were seropositive for antibodies against *A phagocytophilum* (up from 15.6 and 0%, respectively, in 1985).³⁴ *B microti* coinfections with *Bb* also were found in dogs with New York ticks in experimental Lyme studies.⁹ In dogs, a *B microti*-like piroplasm (possibly *Theileria*) can cause PLN and thrombocytopenia.³⁵ Infections and coinfections with *A phagocytophilum*, *B microti*, and *Bartonella* spp. have complicated Lyme disease syndromes in humans.^{36–39} When coinfections were induced in experimentally infected dogs,²⁶ a trend showed an increased frequency of lameness (53%) in dogs coinfecting with *A phagocytophilum* and *Bb*¹⁹ over dogs with *Bb* infection alone (29%). Coinfected dogs might be more likely to have arthritic signs (as seen in mice coinfecting with *A phagocytophilum* and *Bb*⁴⁰ or *B microti* and *Bb*⁴¹) and



Reprinted and adapted from Vet Clin North Am 33(4), Littman MP, Canine Borrelia, pp. 827-862, Copyright 2003 with permission from Elsevier

Fig 2. Flowchart for lameness, with or without fever or anorexia, and Lyme seropositivity and proteinuria.⁵⁶

possibly other manifestations, such as PLN, ocular inflammation, neurologic signs, or thrombocytopenia. Specific diagnostic tests are required for documentation of these infections.

8. How is Canine Lyme Disease Diagnosed?

No individual test result documents clinical illness from *Bb* infection. Thus, the presumptive diagnosis of Lyme disease should include (1) evidence of exposure to *Bb*, (2) clinical signs consistent with Lyme disease, (3) consideration of other differentials, and hopefully (4) response to treatment. Evidence of exposure to *Bb* generally includes *Ixodes* tick exposure in an endemic area for Lyme or positive test results in tests for *Bb* (see question 9). Endemic areas for Lyme disease in people have been mapped by the Centers for Disease Control (see Fig 1). In 2003, just 12 states (PA > NY > NJ > MA > CN > RI > WI > MD > MN > DE > VA > NH) accounted for 95% of cases, with 89% of the cases occurring in the Northeast and mid-Atlantic region and 6% in the upper Midwest.⁴² Presence of deer is not required for exposure; migratory birds such as robins can carry *Ixodes* ticks to previously naïve areas.^{43,44}

9. What Tests are Available to Evaluate for *Bb* Infection?

Tests for *Bb* can be divided into those that demonstrate presence of the organism and antibody tests. Detection of the organism by culture, cytology, or polymerase chain reaction (PCR) assay can be difficult, expensive, and not readily available in practice. The organism is very rarely found in blood, urine, joint fluid, or CSF⁷; it is more often found in connective tissue, synovia, skin, or fibroblasts. The organism is difficult to culture,⁴⁵ and special stains (eg, silver, acridine orange) or dark-field microscopy are required to visualize *Bb*. Plasmid or chromosomal genomic DNA of *Bb* can be amplified by PCR primers for the outer surface protein A (OspA) gene or 23S RNA gene.²⁶ However, positive PCR test results, especially with target imbalance (OspA versus 23S RNA), do not prove the organism is alive and could be a result of leftover fragments or “blebs,” which are nonviable.⁴⁶ Also, the organism might exist in other forms (eg, L-forms, spheroplasts, cysts).⁴⁷⁻⁵⁵ Because documentation of the organism is problematic, serologic tests for antibodies against the agent usually are performed.

10. What Antibody Tests for *Bb* Currently are Available?

Historically, serum antibodies against *Bb* have been detected by enzyme-linked immunosorbent assays (ELISAs), indirect fluorescent antibody assays (IFAs), and Western blot immunoassays made with a variety of *Bb* antigen preparations. ELISA and IFA antibody responses to antigen preparations could not when first reported differentiate antibodies resulting from natural exposure to *Bb* from those associated with vaccination or infection because of other *Borrelia* spp. In those cases, banding patterns on Western blot immunoassay were used to differentiate the source of the antibody response. The OspA antigen generally is only expressed in the tick and during in vitro culture (and so it is present in Lyme vaccines), but not usually in acute mammalian infections. Thus, recognition of this band (p31) generally suggests that the animal has been vaccinated, not infected. However, the banding patterns that develop after infection are complicated and changes occur as antigenic variation by the organism stimulates new antibody production. It is now known that OspA can be expressed in the mammal during the carrier, subclinical, or chronic phases of *Bb* infection, giving a Western blot pattern that leads to misdiagnosis.^{56–58} Immunoglobulin M (IgM) and G (IgG) ELISA and IFA also are available, but there is no evidence that dogs develop clinical signs of Lyme disease early in the course of infection when only IgM is present. In addition, new IgM antibodies could be produced against new antigens expressed during antigenic variation, so that the appearance of IgM does not always prove recent exposure. The synthetic C6 peptide, derived from the VlsE antigen, is expressed when *Bb* is transmitted to the dog but not expressed in the tick, in tissue culture, or in Lyme vaccines.^{23,59–61} Thus, antibodies against this antigen prove natural exposure. Both qualitative (SNAP-3DX, IDEXX, point-of-care)^e and quantitative (Lyme Quant C6 Test, IDEXX)^f versions of the test are available.⁶² Results of these tests correlate well with those from Western blot immunoassay.^{63,d} Positive results in either C6 antibody assay indicate exposure to *Bb* but do not prove clinical disease.

11. When Does the Qualitative C6 Antibody Assay Become Positive in Dogs Experimentally Infected with *Bb*?

Antibodies against C6 peptide generally can be detected 3–5 weeks after infection, well before signs were noted in the experimental model of canine Lyme disease; the test stayed positive for at least 69 weeks.²³

12. Are Results from Paired Assays Needed to Diagnose Canine Lyme Disease?

Because there were no signs of illness in the experimental Beagle puppy model until 2–5 months after tick exposure (well after seroconversion^{9,18–25}), the necessity of paired titers for the initial diagnosis of Lyme disease is not evident. In addition, titer magnitude is not

associated with the presence or absence of disease.¹⁵ However, some of the common coinfections have a much shorter clinical course of disease, with illness occurring before seroconversion, thus necessitating paired titers to show seroconversion (eg, *Ehrlichia* spp., *A phagocytophilum*, Rocky Mountain spotted fever [RMSF]).

13. What Antibiotics can be Used in the Treatment of Lyme Disease in Dogs?

In humans, doxycycline, amoxicillin, and ceftriaxone are considered drugs of choice.^{64,65} On the basis of extrapolation from results in humans and some research studies (see below), tetracycline derivatives or amoxicillin are recommended most frequently by veterinarians for the treatment of Lyme disease. Because different strains of *Bb* exist in the field and because it is difficult to diagnose Lyme disease in the field or induce clinical disease in experimentally infected dogs, the optimal drugs and duration of therapy are unknown. Most authors and ACVIM diplomates in our survey currently recommend administration of doxycycline at 10 mg/kg PO q24h for a minimum of 1 month (see Fig 3). Doxycycline was recommended most frequently because of the possibility of other coinfections (RMSF, anaplasmosis, ehrlichiosis, leptospirosis) that could respond to doxycycline and because it is inexpensive and has anti-inflammatory properties. In humans, the recommended treatment for acute Lyme disease is only 10 days of doxycycline.⁶⁵ However, experimentally infected dogs do not develop clinical disease during the acute phase,⁹ and the infection is likely to be more widespread than in humans that have acute Lyme disease with flulike signs or erythema migrans rash. In humans, the entity known as “chronic Lyme disease” is debated, and one study showed that long-term antibiotic treatment helped as much as placebo.⁶⁶ Polyarthropathy could be immune-mediated and improve faster with added glucocorticoids. Dogs with presumed Lyme nephropathy might require longer duration of doxycycline therapy and usually are treated with adjunctive therapies such as angiotensin-converting enzyme inhibitors, low-dose aspirin, omega-3 fatty acids, dietary therapy, and if indicated, additional antihypertensives, fluid therapy as needed, and possible immunomodulating drug therapy. However, because there is no model for canine Lyme nephropathy, optimal therapy is unknown.

14. Does Routine Antibiotic Therapy Clear *Bb* From the Tissues?

Results of treatment studies have varied. In studies of experimentally infected dogs (clinically normal), several different antibiotics (doxycycline 10 mg/kg PO q12h; amoxicillin 20 mg/kg PO q8h, azithromycin 25 mg/kg PO q24h, or ceftriaxone 25 mg/kg IV q24h) have been administered for 1 month with failure to clear *Bb* from the tissues in some dogs.^{18,20–22} In those studies, a few (3 of 24) dogs had positive *Bb* culture results and many dogs had positive PCR test results on skin samples from the site of the tick bites, even a year after treatment with

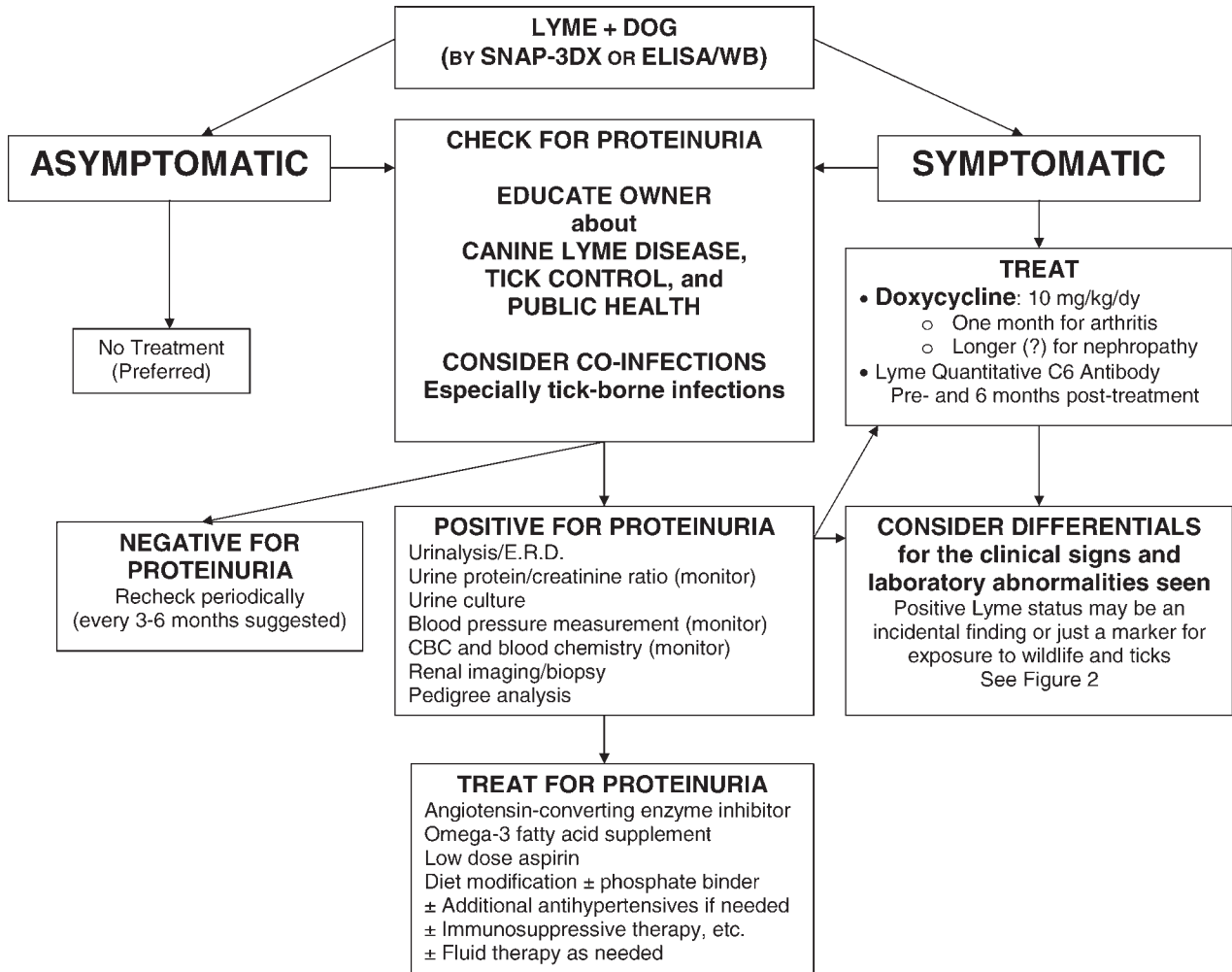


Fig 3. Flowchart for the Lyme-positive dog.

antibiotics. But these studies only used PCR probes for plasmid DNA and not genomic DNA^{21,22} or did not state which probes were used^{18,20}; consequently, PCR results could have been a result of nonviable remnant spirochetes or blebs. These could remain immunologically active and result in maintenance of positive antibody titers but not indicate presence of live *Bb*. In another study, after treatment was administered and titers waned, a new rise in titers after 6 months in isolation was seen,²⁰ presumably because of proliferation of the surviving pool of spirochetes. However, in other studies, titers dropped and stayed low for 35 weeks⁶⁷ or more than a year posttreatment.^{21,22} In addition, some antibiotic-treated experimentally infected dogs with persistently positive PCR assay or skin culture results did not have recurrent clinical signs, even if treated with glucocorticoids.^{18,26} Evidence for and against persistence of infection despite treatment is further reviewed elsewhere.⁵⁶ There is debate about whether “chronic Lyme disease” is caused by cystic forms (see question 15) that are difficult to culture, persistence of viable *Bb* in immunoprivileged sites, immune-mediated disease triggered by Lyme antigens, or occult coinfection or other diseases, in which case the positive Lyme test could be a coincidence.

15. Is There Evidence for Antimicrobial-Resistant Strains of *Bb* in Dogs?

It has been proposed that there are nonmotile forms of the spirochete that are resistant to antibiotics generally used. In a hostile in vitro environment (during antibiotic administration), *Bb* could transform into a spheroplast (L-form or cystic form),^{47–55,68–70} which might be resistant to antibiotics. This transformation is more likely to occur with penicillins and ceftriaxone than with tetracyclines or macrolides.⁷¹ When the antibiotic is stopped, the spheroplast or cyst releases new motile spirochetes. The spheroplast form might be sensitive to metronidazole⁵⁴ and hydroxychloroquine.⁵³

16. What is the Expected Response to Treatment of Lyme Disease in Dogs?

Response to treatment is expected within 1–2 days for acute Lyme arthropathy, although titers can remain positive for many months to years. The response could be the result of the self-limiting nature of the disease, inadvertent treatment of another doxycycline-responsive infection, reactive arthritis,⁷² or the anti-inflammatory

and antiarthritic properties of doxycycline.^{73,74} However, PLN associated with Lyme disease tends to respond slowly, possibly because chronic disease changes exist before diagnosis and initiation of treatment.

17. How Should Dogs Treated for Lyme Disease be Monitored?

Clinical signs of disease should be monitored routinely. Although a consensus opinion was not reached, most ACVIM diplomates recommend following proteinuria in all dogs with Lyme disease (complete urinalysis, in-house E.R.D.–HealthScreen Urine Test,⁸ or a urine protein/creatinine ratio).⁷⁵ However, the optimal recheck interval or duration is unknown. In a limited number of experimentally infected dogs, quantitative C6 antibody concentrations decreased after treatment, so evaluating pre- and 6-month post treatment quantitative C6 concentrations has been recommended by some.^{62,67,76} However, information concerning quantitative C6 kinetics in untreated field cases has not been published, and the predictive value of the test for subsequent illness is unknown. In addition, a low positive titer might not disappear with treatment, perhaps because of immune memory.⁷⁷ Thus, it is the consensus opinion that this protocol cannot be routinely recommended until published data from a large number of field cases is made available.

18. Should Healthy Dogs be Screened for *Bb* Antibodies?

Whether healthy dogs should be screened for antibodies against *Bb* proved controversial, and a consensus could not be reached. The points that follow are those raised in the literature and in our ACVIM diplomate survey that we think should be considered when clinicians are making a decision about whether to screen healthy dogs for evidence of *Bb* exposure. Similar discussion was presented for screening healthy dogs for *Ehrlichia canis* antibodies in a previous ACVIM Consensus Statement.⁷⁸

Potential benefits cited most frequently for serologically screening healthy dogs include:

1. We might detect a potentially dangerous disease (Lyme-associated nephropathy) before clinical illness develops by screening and monitoring seropositive dogs for proteinuria. Although checking for proteinuria is part of an annual wellness examination, if a dog is known to be seropositive, one might want to check for proteinuria more frequently than annually, especially in retrievers and Shelties. (see Fig 3).
2. We can track seroprevalence data in the practice area in both healthy and sick dogs.
3. Our vaccination protocol can be individualized because there is no evidence that vaccine is helpful for seropositive dogs.
4. We can provide information about the environment because dogs are sentinels.⁷⁹
5. We can inform owners about landscaping changes and the importance of checking daily for ticks on

themselves as well as their pets, and we can emphasize the importance of using tick control.

6. We can inform owners that if they remove an engorged *Ixodes* tick from a person, they should call a physician who could prescribe 1 dose of doxycycline (200 mg) to be taken within 72 hours, which has been shown to prevent Lyme disease in people.⁸⁰ Owners are very thankful for this public health information. No such study has been done on dogs.

Arguments against serologically screening healthy dogs (or treating them) that were mentioned most frequently include:

1. Routine testing often results in overdiagnosis and overtreatment of dogs on the basis of a test that does not diagnose Lyme disease nor predict whether Lyme disease will ever occur; most seropositive dogs will never become ill with Lyme disease and do not need to be treated.
2. Overtreatment with incomplete clearance of the organisms potentially can induce resistant strains.
3. Overuse of antibiotics generally increases other microbial resistance in the environment.
4. Not all dogs are cleared of infection even after 1 month of antibiotics.
5. Immunity is not permanent, and treated dogs could be reinfected.
6. Subclinically infected seropositive dogs might be in a premunitive state that could be protective.
7. Drugs used for treatment of Lyme disease have potential adverse effects.
8. Detection of positive test results could cause unnecessary owner distress.
9. False positive test results involve expense for the owner, unnecessary owner distress, and potential induction of drug reactions in animals that do not need to be treated.
10. Lyme endemic areas are already well-known, and tick control and public health information should be recommended in all Lyme endemic areas regardless of results of blood tests.
11. Screening for proteinuria should be part of a routine wellness examination and not done merely because of a seropositive test result.

19. Should all Dogs with Proteinuria be Screened for *Bb* Antibodies?

The consensus opinion is that dogs with proteinuria in endemic areas should be screened for exposure to *Bb* with antibody tests and that *Bb* seropositive dogs should be screened for proteinuria (see Fig 3). However, proteinuria has many causes. Thus, instead of screening all dogs for *Bb* antibodies and then screening the seropositive ones for proteinuria, it is the general recommendation that all veterinarians screen dogs for proteinuria as part of a wellness visit and then recommend a complete workup to identify the cause.⁷⁵

20. How Should a Subclinically Infected Seropositive Dog be Managed?

For reasons discussed previously, the clinical course of a seropositive but apparently healthy dog cannot be predicted. The pros and cons of treatment and monitoring (see question 18) should be discussed with the owner, and a case-by-case decision should be made. In one study, seropositivity and proteinuria was not associated in young (mean age, 34 months) apparently healthy Labradors.^d In another study, neither seropositivity nor titer magnitude were correlated with whether a dog would show clinical signs of arthritis over the 20-month period of observation.¹⁵ There is no evidence that vaccination of seropositive dogs (asymptomatic or symptomatic) is helpful, with either bacterin or recombinant OspA subunit (rOspA) vaccine,^{81–83} and there is some potential for exacerbation of immune-mediated consequences (see question 25). Further epidemiologic studies will be needed before a consensus concerning management of healthy seropositive dogs can be made.

21. What are the Pros and Cons for the Use of Vaccines Against *Bb* in Dogs?

The many arguments for and against vaccination against *Bb* in dogs are summarized in Table 1. The only consensus reached is that immunization of dogs in nonendemic states is unneeded (Fig 1). The majority of respondents do not recommend *Bb* vaccination in endemic states either. However, which vaccine antigens are to be administered to individual animals is a medical decision that should be made between each owner and their veterinarian on an individual case basis.^{87,89}

22. What Vaccines Currently are Available for *Bb* Infections of Dogs?

In the United States, 4 vaccines are currently available: (1) monovalent bacterin,^h available since 1990; (2) bivalent bacterin,ⁱ available since 1994; (3) nonadjuvanted rOspA vaccine,^j available since 1996; and (4) adjuvanted rOspA vaccine,^k available since 1999.

23. How Effective are Lyme Vaccines?

Response to Lyme vaccination can be measured by determining how many vaccinated dogs become ill (preventative fraction for disease) or by determining how many vaccinated dogs seroconvert (preventative fraction for seroconversion). In one study,⁸⁴ use of the monovalent bacterin resulted in an average preventative fraction for illness of 78% (58% in seropositive and 86% in seronegative dogs). In a separate study, preventative fraction for seroconversion was roughly 90% for the monovalent bacterin and 60% for the rOspA vaccine.⁸⁵ Unfortunately, in these studies,^{84,85} dogs were not evaluated for *Bb* exposure before vaccination, complicating the interpretation of the data presented. Studies evaluating preventative fraction for disease are probably of most value but are marred by the difficulties

associated with making an accurate diagnosis of Lyme disease. OspA antigen is present in both bacterins and the rOspA vaccine. The anti-OspA antibodies generated by vaccination kill *Bb* within the tick. Bacterins have been purported to stimulate anti-OspC antibodies as well as anti-OspA antibody titers; thus, the preventative efficacy of a bacterin might be greater than for the subunit vaccine. However, anti-OspC antibody bands are rarely seen on Western blot tests from dogs vaccinated with bacterins (Goldstein, personal communication). Because bacterin contains more types of antigens, there might be more risk for immune-mediated reactions and adverse effects.

24. If a Lyme Vaccine is Going to be Administered, What is a Common Protocol?

Because an enhanced benefit is possible in animals immunized before exposure, some recommend administration of the vaccines before exposure occurs. In this scenario, puppies initially would be vaccinated at 9 or 12 weeks of age with a 2nd dose administered 2–4 weeks later, followed by annual boosters, preferably in the spring before tick exposure. Duration of immunity against *Bb* is short, and annual boosters would be needed.⁹⁰

25. What Adverse Effects are Associated with Administration of Lyme Vaccines?

It has been estimated that administration of Lyme vaccine is associated with <2% adverse effects.⁸⁴ Because Lyme arthropathy and Lyme nephropathy arise at least in part from immune responses against the organism, there are concerns that vaccination might contribute to the immunopathogenesis of disease in some dogs. This sensitization has been demonstrated for other organisms, including feline infectious peritonitis virus, caprine encephalitis virus, and ovine visna-maedi virus.⁹¹ In humans, antibodies against several *Bb* antigens react with several autoantigens (ie, molecular mimicry), including lymphocyte function-associated antigen 1 (LFA-1), myelin, myosin, cardiolipin, and thyroid,^{92–95} which has complicated the use of Lyme vaccines in people. The development of a Lyme bacterin for humans was considered too risky and was discontinued. The human subunit OspA vaccine was launched in December 1998 but was taken off the market in February 2002 because of poor sales. There were concerns about possible immune-mediated sequelae in people with HLA-DR4 haplotype, who are predisposed to chronic nonresponsive Lyme disease, which might be triggered by OspA mimicry of LFA-1.^{92,96–99} A new, potentially safer 2nd-generation vaccine for humans that excludes the cross-reactive epitope is being studied.¹⁰⁰ OspA has been shown to be proinflammatory and sensitizing in rat and hamster models of Lyme arthritis, causing them to have more severe signs upon challenge or re-exposure.^{92,101–107} Nonviable spirochetes trigger the production of inflammatory cytokines in dogs.^{24,27} Almost 30% of dogs with putative Lyme nephropathy had been

Table 1. Pros and cons of Lyme vaccination.

Proponents Say ...	Naysayers Say...
Private practitioners have given many doses of <i>Bb</i> vaccines since 1990 without side effects.	39 diplomates do not recommend vaccine, only 2 recommend vaccine, and 4 use it rarely.
Because you might not always clear the infection and some dogs get serious illness from <i>Bb</i> , it is best to attempt to prevent infection with vaccination.	Most <i>Bb</i> infections are subclinical or respond rapidly to cheap/safe/oral antibiotics. The dogs that develop serious illness from <i>Bb</i> are probably genetically predisposed to having immune-mediated disease triggered by Lyme antigens and vaccine might not be best for them.
Owners might not want to use tick control products and prefer a vaccine. Owners are worried about Lyme disease in their area.	We need to use good tick control in Lyme endemic areas anyway because of <i>Anaplasma</i> , RMSF, <i>Ehrlichia</i> , <i>Babesia</i> , <i>Bartonella</i> spp., etc.
Private practitioners using vaccine say they are seeing fewer Lyme disease cases now.	Practitioners not using vaccine are also seeing fewer Lyme disease cases.
Efficacy	Efficacy
<ul style="list-style-type: none"> • Bacterin preventive fraction for illness = 78%⁸⁴ • Bacterin preventive fraction for seroconversion = 90%⁸⁵ or 92%⁶¹ • rOspA 100% efficacious⁸⁶ • Need annual boosters (economic incentive) 	<ul style="list-style-type: none"> • Variable, 50–100% • Not that good for preventing signs • Preventive fraction for illness is more important than preventing seroconversion • rOspA only 50% efficacious²⁷ • rOspA only 60% efficacious⁸⁵ • Need annual boosters
Safety	Safety (see text for more references)
<ul style="list-style-type: none"> • <2% adverse effects⁸⁴ • “Post-vaccinal Lyme-like syndrome” might be a result of misinterpretation of Western blots (carrier dogs’ patterns can appear as those vaccinated with bacterin), <i>Anaplasma</i> infection, or other causes. 	<ul style="list-style-type: none"> • Possible anaphylaxis; adverse effects are “moderate”⁸⁷ • “Post-vaccinal Lyme-like syndrome”⁸⁸ needs more study • Many respondents associated adverse effects (PLN, IMPA) with Lyme vaccines • Molecular mimicry <ul style="list-style-type: none"> ◦ OspA/LFA-1 (HLA-DR4 predisposed) ◦ Myelin, myosin, cardiolipin, thyroid • OspA is in all vaccine types <ul style="list-style-type: none"> ◦ OspA is proinflammatory ◦ OspA sensitizes ◦ OspA in human chronic Lyme arthritis ◦ OspA in Lyme nephropathy ◦ Canine Lyme nephropathy is an immune complex disease ◦ 30% of Lyme PLN dogs were vaccinated ◦ Postvaccinal Lyme nephropathy (Fincham, personal communication)
Treat all positive dogs with doxycycline for 1 month and vaccinate with bacterin at 0, 14 days. ³⁴	No evidence that vaccinating seropositive dogs is a good idea; it could even be harmful to some.

IMPA, immune-mediated polyarthropathy; LFA-1, lymphocyte function-associated antigen-I; Osp, outer surface protein; PLN, protein-losing nephropathy; RMSF, Rocky Mountain spotted fever.

vaccinated.³² OspA was found in the renal cortex of dogs with Lyme nephropathy with the use of a mouse monoclonal anti-OspA stain.^{11,30} OspA is not yet proven to be the antigen involved in immune complex deposition in glomeruli but Lyme-associated nephropathy is an immune complex disease.^b At Ryan VHUP, a dog with Lyme nephropathy that became very ill a few days after its 2nd rOspA vaccine was described (Fincham, personal communication). The Western blot and SNAP tests showed no evidence of natural exposure, but a very high anti-OspA antibody concentration was detected. Because OspA is in both bacterins and rOspA vaccines, there is concern about whether it could trigger, sensitize, or add antigens to antigen-antibody immune complex

deposition in target tissues such as synovia or glomeruli in genetically predisposed individuals. Additional data will be required to determine the incidence of *Bb* vaccine-associated disease.

26. Can Lyme Vaccination be Used as an Immunotherapy in Seropositive Dogs?

It has been recommended that all seropositive dogs be treated with a month of doxycycline and vaccinated with Lymevox^c at 0 and 14 days.³⁴ Currently, no published data from a controlled study supports this recommendation. Some data suggest that this approach might be harmful (see question 25), and overuse of antibiotics for

all asymptomatic seropositive dogs might not be warranted.

27. Should Tick Control be Maintained?

Tick control in Lyme endemic areas is not only important to prevent Lyme disease but also to prevent RMSF, ehrlichiosis, anaplasmosis, babesiosis, bartonelosis, and other infections (see question 7). Tick control begins with avoidance of tick habitats, careful landscaping, and daily checking for ticks. *Ixodes* ticks are field ticks that quest for hosts, especially from leaf litter; low-lying vegetation; overhanging branches; and wooded, brushy, or overgrown lawns. State and other public health websites help owners analyze their property and create relatively safe “tick-free” zones.¹⁰⁸⁻¹¹⁰

Tick control products often recommended include fipronil,¹ amitraz collar,^m permethrin/imidacloprid,ⁿ and other permethrin-containing products.^o In some situations, combinations of products might be beneficial (eg, the amitraz collar is frequently combined with fipronil by ACVIM diplomates). Any of these products can be effective in reducing transmission of *Bb* to dogs. However, products that prevent tick attachment (eg, amitraz collar) or repel ticks (eg, those containing permethrin) are needed to decrease transmission of other tickborne infections. For example, although *Bb* and *B microti* require 2–3 days of tick attachment for effective transmission, *Rickettsia* and *A phagocytophilum* potentially can be transmitted during the 1st day of attachment.^{111,112} Theoretically, transmission times might be shorter if a tick is detached and refeeds on another host, but after a tick cements itself to begin feeding, it is unlikely to become detached with intact mouthparts. The amitraz collar works very well to help prevent transmission of *Bb*.¹¹³ It needs to be applied tightly enough to have skin contact (not just hair), it is only active against ticks (not fleas), the chemical is washed away from the skin if swimming or bathing occurs, and it should not be used on dogs receiving tricyclic antidepressant medications for behavioral disorders. It is very toxic if eaten, and the veterinarian should have the antidote yohimbine in the office. Fipronil has been shown to decrease transmission of *Bb* and *E canis*.¹¹² It is not washed away by swimming or bathing, kills fleas as well as ticks (but not until the 2nd day of attachment), and can be used safely on cats. Permethrin/imidacloprid has been proven to help prevent transmission of *A phagocytophilum* and *Bb*.¹¹⁴ It is not washed away by swimming or bathing with mild shampoo, repels and kills ticks as well as fleas and mosquitoes, but is toxic to cats.

Summary and Future Questions

Many questions about Lyme disease in dogs and humans remain. Studies for the future that are recommended include the following:

1. Elution studies to find the antigen(s) that are bound in the immune complexes associated with immune-mediated Lyme-associated nephropathy or chronic synovitis.

2. Experimental studies that use predisposed breeds (or certain lines) such as Labradors or Golden Retrievers instead of Beagles. If we can produce a model of Lyme nephropathy, we can study treatment protocols and whether vaccination protects, sensitizes, or aggravates it.
3. Studies that use capillary-fed ticks artificially infected with *Bb*¹¹⁵ to identify the relative contributions of infections with *Bb* or other pathogens because field ticks used in previous studies were collected in New England and often were coinfecting, especially with *A phagocytophilum*, possibly *B microti*, *Bartonella* spp., and very rarely tickborne encephalitis virus.
4. Studies of possible predictive tests in Lyme-positive dogs that will help indicate which dogs are more likely to become sick, such as evaluating circulating immune complexes,¹¹⁶ sedimentation rate, inflammatory mediators, or certain types of antibodies. A prospective study could be done on the relationship of the concentration of Lyme Quantitative C6 antibody in asymptomatic seropositive dogs and the development of future signs of illness.

Veterinarians should keep their minds open and re-evaluate these questions in the years to come.

Footnotes

^a Sanders NA, Dambach DM, Littman MP. Clinical characterization of a rapidly progressive and fatal glomerulonephritis associated with *Borrelia burgdorferi* infection in the dog (“Lyme nephritis”). *J Vet Intern Med* 1997;11:127 (abstract)

^b Shanies TA, Goldstein RE, Njaa BL, et al. The search for intact *Borrelia burgdorferi* bacteria in kidneys from dogs suspected of suffering from “Lyme nephritis.” *J Vet Intern Med* 2005;19:471 (abstract)

^c Goldstein RE, Sandler JL, Bellohusin BA, et al. Microalbuminuria testing in asymptomatic Labrador Retrievers naturally exposed to *Borrelia burgdorferi*. *J Vet Intern Med* 2005;19:464 (abstract)

^d Gerber B, Eichenberger S, Wittenbrink MM, et al. Urine protein excretion of healthy Bernese Mountain Dogs and other dogs with and without antibodies against *Borrelia burgdorferi*. *J Vet Intern Med* 2005;19:431 (abstract)

^e SNAP-3Dx heartworm, *Ehrlichia canis*, and *Borrelia burgdorferi* test, IDEXX Laboratories, Westbrook, Maine

^f Lyme Quant C6 test, IDEXX Laboratories, Westbrook, Maine

^g ERD trademark for a laboratory test for microalbuminuria, HESKA Corporation, Fribourg, Switzerland

^h Monovalent bacterin, Lymeavax, Fort Dodge Animal Health, Fort Dodge, IA

ⁱ Bivalent bacterin, Galaxy Lyme, Schering-Plough Animal Health, Union, NJ

^j Nonadjuvanted rOspA vaccine, Recombitek, Merial, Duluth, GA

^k Adjuvanted rOspA vaccine, ProLyme, Intervet, Millsborough, DE

^l Fipronil, Frontline Top Spot, Merial, Duluth, GA

^m Amitraz collar, Preventic collar, Virbac, Fort Worth, TX

ⁿ Permethrin/imidacloprid, Advantix, Bayer, Shawnee Mission, KS

^o Defend EXspot or Proticall, Schering-Plough Animal Health, Union, NJ

Acknowledgment

Supported by the American College of Veterinary Internal Medicine.

References

1. Steere AC. Lyme disease. *N Engl J Med* 2001;345:115–125.
2. Sigal LH. Misconceptions about Lyme disease: Confusions hiding behind ill-chosen terminology. *Ann Intern Med* 2002;136:413–419.
3. Nadelman RB, Wormser GP. Lyme borreliosis. *Lancet* 1998;352(9127):557–565.
4. Keirans JE, Hutcheson HJ, Durden LA, Klompen JS. *Ixodes (Ixodes) scapularis* (Acari:Ixodidae): Redescription of all active stages, distribution, hosts, geographical variation, and medical and veterinary importance. *J Med Entomol* 1996;33:297–318.
5. Greene CE, Appel MJG, Straubinger RK. Lyme borreliosis. In: Greene CE, ed. *Infectious Diseases of the Dog and Cat*. Philadelphia, PA: WB Saunders; 1998:282–293. or Greene CE, Straubinger RK. Borreliosis. In: Greene CE, ed. *Infectious Diseases of the Dog and Cat*, 3rd ed. Philadelphia, PA: Elsevier Saunders; 2006:417–435.
6. Badon SJ, Fister RD, Cable RG. Survival of *Borrelia burgdorferi* in blood products. *Transfusion* 1989;29:581–583.
7. Chang YF, Straubinger RK, Jacobson RH, et al. Dissemination of *Borrelia burgdorferi* after experimental infection in dogs. *J Spirochetal Tick-Borne Dis* 1996;3:80–86.
8. Burgess EC. Natural exposure of Wisconsin dogs to the Lyme disease spirochete (Bb). *Lab Anim Sci* 1986;36:288–290.
9. Appel MJG, Allen S, Jacobson RH, et al. Experimental Lyme disease in dogs produces arthritis and persistent infection. *J Infect Dis* 1993;167:651–664.
10. Bushmich SL. Lyme disease: Comparative aspects. *Proc 18th ACVIM Forum* 2000;203–205.
11. Grauer GF, Burgess EC, Cooley AJ, Hagee JH. Renal lesions associated with *Borrelia burgdorferi* infection in the dog. *JAVMA* 1988;193:237–239.
12. Post JE, Shaw EE, Wright SD. Suspected borreliosis in cattle. *Ann NY Acad Sci* 1988;539:488.
13. Lissman BA, Bosler EM, Camay H, et al. Spirochete-associated arthritis (Lyme disease) in a dog. *JAVMA* 1984;185:219–220.
14. Kornblatt AN, Urband PH, Steere AC. Arthritis caused by *Borrelia burgdorferi* in dogs. *JAVMA* 1985;186:960–964.
15. Levy SA, Magnarelli LA. Relationship between development of antibodies to *Borrelia burgdorferi* in dogs and the subsequent development of limb/joint borreliosis. *JAVMA* 1992;200:344–347.
16. Greene RT, Levine JF, Breitschwerdt EB, et al. Clinical and serologic evaluations of induced *Borrelia burgdorferi* infection in dogs. *Am J Vet Res* 1988;49:752–757.
17. Cerri D, Farina R, Andreani E, et al. Experimental infection of dogs with *Borrelia burgdorferi*. *Res Vet Sci* 1994;57:256–258.
18. Straubinger RK, Straubinger AF, Summers BA, et al. Clinical manifestations, pathogenesis, and effect of antibiotic treatment on Lyme borreliosis in dogs. *Wien Klin Wochenschr* 1998;110:874–881.
19. Straubinger RK, Straubinger AF, Härter L, et al. *Borrelia burgdorferi* migrates into joint capsules and causes an up-regulation of interleukin-8 in synovial membranes of dogs experimentally infected with ticks. *Infect Immun* 1997;65:1273–1285.
20. Straubinger RK, Summers BA, Chang YF, Appel MJG. Persistence of *Borrelia burgdorferi* in experimentally infected dogs after antibiotic treatment. *J Clin Microbiol* 1997;35:111–116.
21. Straubinger RK, Straubinger AF, Summers BA, Jacobson RH. Status of *Borrelia burgdorferi* infection after antibiotic treatment and the effects of corticosteroids: An experimental study. *J Infect Dis* 2000;181:1069–1081.
22. Straubinger RK. PCR-based quantification of *Borrelia burgdorferi* organisms in canine tissues over a 500-day postinfection period. *J Clin Microbiol* 2000;38:2191–2199.
23. Liang FT, Jacobson RH, Straubinger RK, et al. Characterization of a *Borrelia burgdorferi* VlsE invariable region useful in canine Lyme disease serodiagnosis by enzyme-linked immunosorbent assay. *J Clin Microbiol* 2000;38:4160–4166.
24. Harter L, Straubinger RK, Summers BA, et al. Up-regulation of inducible nitric oxide synthase mRNA in dogs experimentally infected with *Borrelia burgdorferi*. *Vet Immunol Immunopathol* 1999;67:271–284.
25. Callister SM, Jobe DA, Schell RF, et al. Detection of borreliacidal antibodies in dogs after challenge with *Borrelia burgdorferi*-infected *Ixodes scapularis* ticks. *J Clin Microbiol* 2000;38:3670–3674.
26. Chang YF, Novosel V, Chang CF, et al. Experimental induction of chronic borreliosis in adult dogs exposed to *Borrelia burgdorferi*-infected ticks and treated with dexamethasone. *Am J Vet Res* 2001;62:1104–1112.
27. Straubinger RK, Rao TD, Davidson E, et al. Protection against tick-transmitted Lyme disease in dogs vaccinated with a multiantigenic vaccine. *Vaccine* 2002;20:181–193.
28. Magnarelli LA, Anderson JF, Schreier AB. Persistence of antibodies to *Borrelia burgdorferi* in dogs on New York and Connecticut. *JAVMA* 1990;196:1064–1068.
29. Frank JC. Taking a hard look at *Borrelia burgdorferi*. *JAVMA* 1989;194:1521.
30. Magnarelli LA, Anderson JF, Schrier AB, Ficke CM. Clinical and serologic studies of canine borreliosis. *JAVMA* 1987;191:1089–1094.
31. Rondeau MP, Walton RM, Bissett S, et al. Suppurative, nonseptic polyarthropathy in dogs. *J Vet Intern Med* 2005;19:654–662.
32. Dambach DM, Smith CA, Lewis RM, Van Winkle TJ. Morphologic, immunohistochemical, and ultrastructural characterization of a distinctive renal lesion in dogs putatively associated with *Borrelia burgdorferi* infection: 49 cases (1987–1992). *Vet Pathol* 1997;34:85–96.
33. Sanders NA. Canine “Lyme nephritis.” *Proc. 18th ACVIM Forum* 2000;627–628.
34. Levy S. Developing an integrated approach for diagnosing, managing tick-borne diseases. *DVM Magazine* 2002;June:14–18.
35. Camacho AT, Guitian FJ, Pallas E, et al. Azotemia and mortality among *Babesia microti*-like infected dogs. *J Vet Intern Med* 2004;18:141–146.
36. Belongia EA. Epidemiology and impact of coinfections acquired from *Ixodes* ticks. *Vector Borne Zoonotic Dis* 2002;2:265–273.
37. Adelson ME, Rao RV, Tilton RC, et al. Prevalence of *Borrelia burgdorferi*, *Bartonella* spp, *Babesia microti*, and *Anaplasma phagocytophila* in *Ixodes scapularis* ticks collected in northern New Jersey. *J Clin Microbiol* 2004;42:2799–2801.
38. Mitchell PD, Reed KD, Hofkes JM. Immunoserologic evidence of coinfection with *Borrelia burgdorferi*, *Babesia microti*, and human granulocytic *Ehrlichia* species in residents of Wisconsin and Minnesota. *J Clin Microbiol* 1996;34:724–727.
39. Eskow E, Rao RVS, Mordechai E. Concurrent infection of the central nervous system by *Borrelia burgdorferi* and *Bartonella henselae*: Evidence for a novel tick-borne disease complex. *Arch Neurol* 2001;58:1357–1363.
40. Thomas V, Anguita J, Barthold SW, Fikrig E. Coinfection with *Borrelia burgdorferi* and the agent of human granulocytic

ehrlichiosis alters murine immune responses, pathogen burden, and severity of Lyme arthritis. *Infect Immun* 2001;69:3359–3371.

41. Moro MH, Zegarra-Moro OL, Bjornsson J, et al. Increased arthritis severity in mice coinfecting with *Borrelia burgdorferi* and *Babesia microti*. *J Infect Dis* 2002;186:428–431.

42. Hopkins RS, Jajosky RA, Hall PA, et al. Summary of notifiable diseases, United States, 2003. *MMWR* 2005;52(54):1–85. (www.cdc.gov/mmwr/)

43. Richter D, Spielman A, Komar N, Matuschka FR. Competence of American robins as reservoir hosts for Lyme disease spirochetes. *Emerg Infect Dis* 2000;6:133–138.

44. Gylfe A, Bergstrom S, Lundstrom J, Olsen B. Reactivation of *Borrelia* infection in birds. *Nature* 2000;403(6771):724–725.

45. Burgdorfer W, Barbour A, Hayes S, et al. Lyme Disease—A tick-borne spirochetosis? *Science* 1982;216(18Jun):1317–1319.

46. Persing DH, Rutledge BJ, Rys PN, et al. Target imbalance: Disparity of *Borrelia burgdorferi* genetic material in synovial fluid from Lyme arthritis patients. *J Infect Dis* 1994;169:668–672.

47. Kersten A, Poitschek C, Rauch S, Aberer E. Effects of penicillin, ceftriaxone, and doxycycline on morphology of *Borrelia burgdorferi*. *Antimicrob Agents Chemother* 1995;39:1127–1133.

48. Gruntar I, Malovrh T, Murgia R, Cinco M. Conversion of *Borrelia garinii* cystic forms to motile spirochetes in vivo. *APMIS* 2001;109:383–388.

49. Brorson O, Brorson SH. A rapid method for generating cystic forms of *Borrelia burgdorferi*, and their reversal to mobile spirochetes. *APMIS* 1998;106:1131–1141.

50. Brorson O, Brorson SH. In vitro conversion of *Borrelia burgdorferi* to cystic forms in spinal fluid, and transformation to mobile spirochetes by incubation in BSK-H medium. *Infection* 1998;26:144–150.

51. Brorson O, Brorson SH. Transformation of cystic forms of *Borrelia burgdorferi* to normal, mobile spirochetes. *Infection* 1997;25:240–246.

52. Murgia R, Piazzetta C, Cinco M. Cystic forms of *Borrelia burgdorferi* sensu lato: Induction, development, and the role of RpoS. *Wien Klin Wochenschr* 2002;114:574–579.

53. Brorson O, Brorson SH. An in vitro study of the susceptibility of mobile and cystic forms of *Borrelia burgdorferi* to hydroxychloroquine. *Int Microbiol* 2002 Mar;5:25–31.

54. Brorson O, Brorson SH. An in vitro study of the susceptibility of mobile and cystic forms of *Borrelia burgdorferi* to metronidazole. *APMIS* 1999;107:566–576.

55. Mursic VP, Wanner G, Reinhardt S, et al. Formation and cultivation of *Borrelia burgdorferi* spheroplast L-form variants. *Infection* 1996;24:218–226.

56. Littman MP. Canine borreliosis. In: Macintire DK, Breitschwerdt EB, eds. *Emerging and Re-emerging Infectious Diseases*. *Vet Clin North Am Small Anim Pract* 2003;33:827–862.

57. Greene RT, Walker RL, Burgess EC, Levine JF. Heterogeneity in immunoblot patterns obtained by using four strains of *Borrelia burgdorferi* and sera from naturally exposed dogs. *J Clin Microbiol* 1988;26:2287–2291.

58. Guerra MA, Walker ED, Kitron U. Quantitative approach for the serodiagnosis of canine Lyme disease by the immunoblot procedure. *J Clin Microbiol* 2000;38:2628–2632.

59. Bacon RF, Biggerstaff BJ, Schriefer ME, et al. Serodiagnosis of Lyme disease by kinetic enzyme-linked immunosorbent assay using recombinant VlsE1 or peptide antigens of *Borrelia burgdorferi* compared with 2-tiered testing using whole-cell lysates. *J Infect Dis* 2003;187:1187–1199.

60. Levy S, O'Connor TP, Hanscom JL, Shields P. Utility of an in-office C6 ELISA test kit for determination of infection status of dogs naturally exposed to *Borrelia burgdorferi*. *Vet Ther* 2002;3:308–315.

61. Levy S. Use of a C6 ELISA test to evaluate the efficacy of a whole-cell bacterin for the prevention of naturally transmitted canine *Borrelia burgdorferi* infection. *Vet Ther* 2002;3:420–424.

62. IDEXX Laboratories, Lyme Quant C6 Test. Available at: www.idexx.com/animalhealth/laboratory/c6/index.jsp. Accessed February 27, 2006.

63. Mogilyansky E, Loa CC, Adelson ME, et al. Comparison of Western immunoblotting and the C6 Lyme antibody test for laboratory detection of Lyme disease. *Clin Diagn Lab Immunol* 2004;11:924–929.

64. Steere AC. Duration of antibiotic therapy for Lyme disease. *Ann Int Med* 2003;138:761–762.

65. Wormser GP, Ramanathan R, Nowakowski J, et al. Duration of antibiotic therapy for early Lyme disease. *Ann Int Med* 2003;138:697–704.

66. Klemmner MS, Hu LT, Evans J, et al. Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. *N Engl J Med* 2001;345:85–92.

67. Philipp MT, Bowers LC, Fawcett PT, et al. Antibody response to IR6, a conserved immunodominant region of the VlsE lipoprotein, wanes rapidly after antibiotic treatment of *Borrelia burgdorferi* infection in experimental animals and in humans. *J Infect Dis* 2001;184:870–878.

68. Murgia R, Cinco M. Induction of cystic forms by different stress conditions in *Borrelia burgdorferi*. *APMIS* 2004;112:57–62.

69. Alban PS, Johnson PW, Nelson DR. Serum-starvation-induced changes in protein synthesis and morphology of *Borrelia burgdorferi*. *Microbiology* 2000;146:119–127.

70. Brorson O, Brorson SH. An in vitro study of the susceptibility of mobile and cystic forms of *Borrelia burgdorferi* to tinidazole. *Int Microbiol* 2004;7:139–142.

71. Murgia R, Piazzetta C, Cinco M. Cystic forms of *Borrelia burgdorferi* sensu lato: Induction, development, and the role of RpoS. *Wien Klin Wochenschr* 2002;114:574–579.

72. Fendler C, Laitko S, Sorensen H, et al. Frequency of triggering bacteria in patients with reactive arthritis and undifferentiated oligoarthritis and the relative importance of the tests used for diagnosis. *Ann Rheum Dis* 2001;60:337–343.

73. Yu LP, Smith GN, Brandt KD, et al. Reduction of the severity of canine osteoarthritis by prophylactic treatment with oral doxycycline. *Arthritis Rheum* 1992;35:1150–1159.

74. Yu LP, Burr DB, Brandt KD, et al. Effects of oral doxycycline administration on histomorphometry and dynamics of subchondral bone in a canine model of osteoarthritis. *J Rheum* 1996;23:137–142.

75. Lees GE, Brown SA, Elliott J, et al. Assessment and management of proteinuria in dogs and cats: 2004 ACVIM Forum consensus statement (small animal). *J Vet Intern Med* 2005;19:377–385.

76. Straubinger RK, Straubinger AF, Summers BA, et al. Status of *Borrelia burgdorferi* infection after antibiotic treatment and the effects of corticosteroids: An experimental study. *J Infect Dis* 2000;181:1069–1081.

77. Peltomaa M, McHugh G, Steere AC. Persistence of the antibody response to the VlsE sixth invariant region (IR6) peptide of *Borrelia burgdorferi* after successful antibiotic treatment of Lyme disease. *J Infect Dis* 2003;187:1178–1186.

78. Neer TM, Breitschwerdt EB, Greene RT, Lappin MR. Consensus statement of ehrlichial disease of small animals from the infectious disease study group of the ACVIM. *J Vet Intern Med* 2002;16:309–315.

79. Duncan AW, Correa MT, Levine JF, Breitschwerdt EB. The dog as a sentinel for human infection: Prevalence of *Borrelia burgdorferi* C6 antibodies in dogs from southeastern and mid-Atlantic states. *Vector Borne Zoonotic Dis* 2004;4:221–229.

80. Nadelman RB, Nowakowski J, Fish D, et al. Prophylaxis with single-dose doxycycline for the prevention of Lyme

disease after an *Ixodes scapularis* tick bite. *N Engl J Med* 2001;345:79–84.

81. Wallich R, Siebers A, Jahraus O, et al. DNA vaccines expressing a fusion product of outer surface proteins A and C from *Borrelia burgdorferi* induce protective antibodies suitable for prophylaxis but not for resolution of Lyme disease. *Infect Immun* 2001;69:2130–2136.

82. Zhong W, Gern L, Stehle T, et al. Resolution of experimental and tick-borne *Borrelia burgdorferi* infection in mice by passive, but not active immunization using recombinant OspC. *Eur J Immunol* 1999;29:946–957.

83. Appel MJG. Lyme disease in dogs. *Suppl Comp Cont Ed* 2002;24(1A):19–23.

84. Levy SA, Lissman BA, Ficke CM. Performance of a *Borrelia burgdorferi* bacterin in borreliosis-endemic areas. *JAVMA* 1993;202:1834–1838.

85. Levy SA, Clark KK, Glickman LT. Infection rates in dogs vaccinated and not vaccinated with an OspA *Borrelia burgdorferi* vaccine in a Lyme disease–endemic area of Connecticut. *Int J Appl Res Vet Med* 2005;3:1–5.

86. Conlon JAR, Mather TN, Tanner P, et al. Efficacy of a non-adjuvanted, outer surface protein A, recombinant vaccine in dogs after challenge with ticks naturally infected with *Borrelia burgdorferi*. *Vet Ther* 2000;1:96–107.

87. Klingborg DJ, Husted DR, Curry-Galvin EA, et al. AVMA council on biologic and therapeutic agents' report on cat and dog vaccines. *JAVMA* 2002;221:1401–1407.

88. Jacobson RH, Chang YF, Shin SJ. Lyme disease: Laboratory diagnosis of infected and vaccinated symptomatic dogs. *Semin Vet Med Surg (Small Anim)* 1996;11:172–182.

89. Paul MA, Appel M, Barrett R, et al. Report of the AAHA Canine Vaccine Task Force: Executive summary and 2003 canine vaccine guidelines and recommendations. *JAAHA* 2003;39:119–131.

90. Straubinger RK, Chang YF, Jacobson RH, Appel MJG. Sera from OspA-vaccinated dogs, but not those from tick-infected dogs, inhibit in vitro growth of *Borrelia burgdorferi*. *J Clin Microbiol* 1995;33:2745–2751.

91. Littman MP. Why I don't use Lyme disease vaccines. *Comp Cont Ed* 1997;19:1269, 1272, 1274–1275.

92. Littman MP. Immune-mediated reactions against Lyme vaccine. *Proc 18th Ann Vet Med Forum, ACVIM* 2000;629–630.

93. Guerau-de-Arellano M, Huber BT. Development of autoimmunity in Lyme arthritis. *Curr Opin Rheum* 2002;14:388–393.

94. Birner P, Gatterbauer B, Drobna D, et al. Molecular mimicry in infectious encephalitis and neuritis: Binding of antibodies against infectious agents on Western blots of human nervous tissue. *J Infect* 2000;41:32–38.

95. Benvenga S, Guarneri F, Vaccaro M, et al. Homologies between proteins of *Borrelia burgdorferi* and thyroid autoantigens. *Thyroid* 2004;14:964–966.

96. Steere AC, Gross D, Meyer AL, Huber BT. Autoimmune mechanisms in antibiotic treatment-resistant Lyme arthritis. *J Autoimmun* 2001;16:263–268.

97. Gross DM, Fortshubar T, Tary-Lehmann M, et al. Identification of LFA-1 as a candidate autoantigen in treatment-resistant Lyme arthritis. *Science* 1998;281:703–706.

98. Sigal LH, Williams S, Soltys B, Gupta R. H9724, a monoclonal antibody to *Borrelia burgdorferi*'s flagellin, binds to heat shock protein 60 (HSP60) within live neuroblastoma cells: A potential role for HSP60 in peptide hormone signaling and in an autoimmune pathogenesis of the neuropathy of Lyme disease. *Cell Mol Neurobiol* 2001;21:477–495.

99. Trollmo C, Meyer AL, Steere AC, et al. Molecular mimicry in Lyme arthritis demonstrated at the single cell level: LFA-1 α_L is a partial agonist for outer surface protein A–reactive T cells. *J Immunol* 2001;166:5286–5291.

100. Willett TA, Meyer AL, Brown EL, et al. An effective second-generation outer surface protein A–derived Lyme vaccine that eliminates a potentially autoreactive T cell epitope. *Proc Natl Acad Sci U S A* 2004;101:1303–1308.

101. Lim LC, England DM, DuChateau BK, et al. Development of destructive arthritis in vaccinated hamsters challenged with *Borrelia burgdorferi*. *Infect Immun* 1994;62:2825–2833.

102. Du Chateau BK, England DM, Callister SM, et al. Macrophages exposed to *Borrelia burgdorferi* induce Lyme arthritis in hamsters. *Infect Immun* 1996;64:2540–2547.

103. Hu LT, Klempner MS. Host-pathogen interactions in the immunopathogenesis of Lyme disease. *J Clin Immunol* 1997;17:354–365.

104. Philipp MT. Studies on OspA: A source of new paradigms in Lyme disease research. *Trends Microbiol* 1998;6:44–47.

105. Sigal LH. Lyme disease: A review of aspects of its immunology and immunopathogenesis. *Ann Rev Immunol* 1997;15:63–92.

106. Croke CL, Munson EL, Lovrich SD, et al. Occurrence of severe destructive Lyme arthritis in hamsters vaccinated with outer surface protein A and challenged with *Borrelia burgdorferi*. *Infect Immun* 2000;68:658–663.

107. Gondolf KB, Mihatsch M, Curschellas E, et al. Induction of experimental allergic arthritis with outer surface proteins of *Borrelia burgdorferi*. *Arthritis Rheum* 1994;37:1070–1077.

108. Westport Weston Health District, Analyze your property. Available at: www.wvhd.org/TLD_CD/anaprop2.htm. Accessed February 27, 2006.

109. Westport Weston Health District, Tick safe zones. Available at: www.wvhd.org/TLD_CD/zones.htm. Accessed February 27, 2006.

110. Stafford III, KC. Tick management handbook. Available at: www.cdc.gov/ncidod/dvbid/lyme/resources/handbook.pdf. Accessed February 27, 2006.

111. desVignes F, Piesman J, Heffernan R, et al. Effect of tick removal on transmission of *Borrelia burgdorferi* and *Ehrlichia phagocytophila* by *Ixodes scapularis* nymphs. *J Infect Dis* 2001;183:773–778.

112. Kidd L, Breitschwerdt EB. Transmission times and prevention of tick-borne diseases in dogs. *Comp Cont Ed* 2003;25:742–750.

113. Elfassy OJ, Goodman FW, Levy SA, Carter LL. Efficacy of an amitraz-impregnated collar in preventing transmission of *Borrelia burgdorferi* by adult *Ixodes scapularis* to dogs. *JAVMA* 2001;219:185–189.

114. Blagburn BL, Spencer JA, Billeter SA, et al. Use of imidacloprid-permethrin to prevent transmission of *Anaplasma phagocytophilum* from naturally infected *Ixodes scapularis* ticks to dogs. *Vet Ther* 2004;5:212–217.

115. Korshus JB, Munderloh UG, Bey RF, et al. Experimental infection of dogs with *Borrelia burgdorferi* sensu stricto using *Ixodes scapularis* ticks artificially infected by capillary feeding. *Med Microbiol Immunol (Berl)* 2004;193:27–34.

116. Zhong W, Oschmann P, Wellensiek HJ. Detection and preliminary characterization of circulating immune complexes in patients with Lyme disease. *Med Microbiol Immunol (Berl)* 1997;186:153–158.