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State of the Art Review

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Lyme nephritis

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Objective – To review what is known and highlight knowledge gaps regarding Lyme nephritis (LN).
Data Sources – Publications identified via PubMed using the keywords "*Borrelia burgdorferi*," "Borreliosis," "glomerulonephritis," " protein-losing nephropathy," " autoimmunity," and " retriever," and as generated by investigators working in the fields of Borreliosis and immune-mediated glomerulonephritis.
Human Data Synthesis – Postborrelial immune-mediated glomerulonephritis was described recently in 6 people; 3 responded to antimicrobials/steroids, 1 to antimicrobials/angiotensin-converting enzyme inhibitor/warfarin, 1 required hemodialysis but became hemodialysis independent after 5 months and treatment with antimicrobials, steroids, plasmapheresis, immunoglobulin, and 1 did not respond to steroids and angiotensin-converting enzyme inhibitor and still requires hemodialysis.
Veterinary Data Synthesis – Lyme nephritis is seen in <1–2% of Lyme seropositive dogs, with an average onset at 5–6 years. Labrador and Golden Retrievers are predisposed to this condition. Prior or concurrent lameness is described in 9–28% cases. Historical presentations include acute progressive protein-losing nephropathy with membranoproliferative glomerulonephritis, tubular necrosis/regeneration, and interstitial nephritis, but possibly milder forms exist. Complications include thromboembolic events, hypertension, effusive disease, and oliguric/anuric renal failure. Diagnostic tests help stage disease and rule out other causes. Renal biopsy is advocated early, when intervention may help, and to prove if immune-complex disease exists. Treatment includes standard therapy for protein-losing nephropathy, long-term antimicrobials, and perhaps immunosuppressive therapy.
Conclusions – There is no experimental model of LN to study predisposing factors, pathogenesis, onset, progression, treatment, or prevention. There are no predictive tests to identify the few individuals at highest risk, therefore all seropositive dogs should be screened and monitored for proteinuria. Lyme nephritis mimics other forms of protein-losing nephropathy and sometimes Leptospirosis. Renal biopsy helps show if immune-complex disease exists, but may not prove LN specifically. More studies are warranted on dogs with Lyme-specific immune-complex deposition to evaluate risk factors, understand pathogenesis, variability of expression, and to validate treatment and prevention protocols.
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Introduction

Lyme nephritis (LN), the most serious form of Lyme disease in dogs, is incompletely understood because Koch's postulates have not been satisfied; there is no experimental inducible model to study predisposing factors, pathogenesis, onset, progression, treatment, or prevention.[1,2] Reports of several dogs with renal disease associated with Lyme+ status were first published in 1987–1988,[3,4] soon after Lyme disease was identified as an emerging infectious disease in the United States and serologic testing (eg, indirect fluorescent antibody [IFA], ELISA) became available. Early seroprevalence studies showed

Abbreviations

ACE	angiotensin converting enzyme
Bb	*Borrelia burgdorferi*
CIC	circulating immune-complexes
HUS	hemolytic-uremic syndrome
IHC	immunohistochemistry
IMGN	immune-mediated glomerulonephritis
LN	Lyme nephritis
MPGN	membranoproliferative glomerulonephritis
PLN	protein-losing nephropathy
TE	thromboembolic events
UPC	urine protein/creatinine ratio
VlsE	variable major protein-like sequence, expressed

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that most Lyme+ dogs had no clinical signs of Lyme disease, although exposure was common and high antibody titers persisted for years. In some endemic areas, 70–90% of healthy dogs were Lyme+;[4–6] even after 20 months of observation, < 5% showed signs of fever, anorexia, lameness, the same as in seronegative dogs.[7] Lyme arthritis was self-limiting or quickly responsive to antimicrobials (eg, doxycycline, amoxicillin, or azithromycin).[1,2]

Compared with Lyme arthritis, fewer dogs, perhaps < 1–2% with Lyme+ status,[4,8] developed severe acute progressive and often fatal protein-losing nephropathy (PLN),[1–3,8–11,a,b] and < 30% had a history of lameness.[8,9,a,b] Labrador and Golden Retrievers are predisposed,[8,9,a,b] but proteinuria was rarely found among Lyme+ retrievers.[12] It is unknown if LN is underrecognized, if mild or early-stage disease exists, and if it responds to intervention at early stages. There are no tests to predict which individuals will become affected. Other signs of Lyme disease seen in people (eg, rash, cardiac, or neurologic signs) are not well documented in dogs.

Lyme disease is caused by *Borrelia burgdorferi* (Bb), a unicellular micro-aerophilic gram-negative motile spirochete. The organism lives extracellularly near collagen and fibroblasts, usually causing very little if any inflammation in most hosts. Unlike Leptospirosis, Bb is not free living in waterways nor mainly transmitted by urine shedding. Transmission is from the bite of a 3-host *Ixodes* tick, in which Bb is transmitted transstadially.[2] Unlike Leptospirosis, LN is not due to renal invasion of spirochetes but due to immune-mediated glomerulonephritis (IMGN) with Lyme-specific antigen-antibody complex deposition.[10,11,13,14] Renal biopsies from Lyme+ dogs with severe proteinuria, hypoalbuminemia, and kidney failure showed membranoproliferative glomerulonephritis (MPGN) with subendothelial C3, IgG, and IgM deposits, diffuse tubular necrosis/regeneration, and lymphocytic-plasmacytic interstitial nephritis.[3,8,14] The histopathologic findings were different, as was the breed predisposition and younger age of onset (5.6 ± 2.6 years) when compared with previously described types of glomerulonephritis (7.1 ± 3.6 years) or amyloidosis (7.8 ± 3.5 years).[8] Since biopsies were obtained from dogs with moderate-to-severe disease, it is unknown if milder forms show glomerular but not tubular/interstitial disease.

Immunohistochemical staining of canine renal cortical biopsies for Lyme-specific antigens is hampered by the subjectivity of observer interpretation of stain patterns for stain localization and intensity. Given these limitations, 84% of renal cortical biopsies from suspected cases showed positive staining with rabbit antibodies directed against Lyme antigens p83, p34 (ospB), p31 (ospA), and lower molecular weight Lyme antigens.[14] Elution studies of immune complexes found involvement of Lyme antigens p39, p41 (flagellin), p22, and small amounts (<10%) p31 (ospA).[11,c] Earlier studies using monoclonal anti-ospA stain showed positive results in suspect kidneys.[3,4] Whether non-Lyme-specific immune-complex deposits also exist in these cases is not known. It is unknown if the Lyme-specific immune-complexes trigger LN or whether they are passively trapped or not cleared properly due to host-pathogen factors, for example Bb strain, genetic podocytopathy, or immunodysregulatory disease.

Dogs with clinical Lyme disease had higher Lyme-specific circulating immune-complexes (CIC) on average than nonclinical dogs,[d] but many asymptomatic dogs have high CIC levels. Levels of CIC decline with therapy.[e]

Immune-Mediated Mechanisms

Type 1 MPGN with subendothelial glomerular immune-complex deposition may occur because of passive entrapment of cationic antigens or circulating complexes in normal or previously altered glomeruli. Complexes then attract inflammatory cells or activate resident glomerular cells to release vasoactive substances, proinflammatory cytokines, activators of platelets and of the injurious classic complement cascade which attacks cell membranes and dissociates the major slit diaphragm protein, nephrin, from the podocyte cytoskeleton.[15–18] Lyme proteins OspE and OspF bind to the soluble complement regulatory protein, factor H, which normally inhibits too much damage from occurring.[19] Genetic deficiencies of factor H have been associated with MPGN and atypical hemolytic-uremic syndrome (HUS).[15]

Secondary tubular changes may be due to hypertension and efferent arteriole vasoconstriction, tubular hypoxia, or toxic proteins in the glomerular filtrate.[8] Very few if any organisms are found by silver stain or PCR in kidneys of dogs with LN.[8,13,14] However, Lyme antigens, DNA, and sometimes organisms have been found in tubular cells and in urine.[3,20–24]

Concerns about immune-mediated disease triggered by Lyme disease are not new. OspA is not only expressed by Bb in the tick and anti-ospA antibodies are found in acute and chronic Lyme disease patients.[25] People with chronic treatment-resistant immune-mediated arthritis triggered by Lyme disease have very high anti-ospA antibodies and ospA-sensitized T cells, correlating with severity of signs.[26–32] Genetic predisposition for a particularly strong T_H1 response to ospA is associated with certain haplotypes (eg, HLA-DRB1).[26] Although amino

acid sequence homology is found between ospA and at least 15 self-proteins[26] and Streptococcal M protein,[25] molecular mimicry is probably less important in causing immune-mediated disease than the proinflammatory properties of ospA.[26] OspA is a polyclonal B cell mitogen and induces upregulation of proinflammatory cytokines IL-6, IL-8, IL-1β, and IFN-γ.[33–35]

Antibodies to other proteins of Bb cross-react with nerve axonal protein, myelin basic protein, heat shock protein, muscle/myosin, cardiolipin, and gangliosides.[33,36] Sonicated fractions of Bb are proinflammatory, inducing upregulation of TNF-α, IL-1α, IL-1β, and viable spirochetes induce IL-8.[37] Lipoproteins, flagellin, and DNA of Bb interact with Toll-like receptors to signal activation of neutrophils, mononuclear cells, and endothelial cells.[38] Spirochetal infections including syphilis, *Leptospira* and *Borrelia* spp. infections are notorious for causing the Jarisch-Herxheimer reaction, due to proinflammatory products released from dying organisms.[39]

It is unknown if there is a particular Lyme antigen that may induce glomerular damage. For example, the dominant nephritogenic antigen associated with poststreptococcal acute glomerulonephritis, another MPGN, is a nephritis-associated plasmin receptor (glyceraldehyde-3-phosphate dehydrogenase) that stabilizes plasmin and activates the alternate pathway of the complement cascade.[40]

Experimental Lyme Disease in Dogs

Beagle puppies and adults have been studied extensively in the tick-exposure model,[22,41–51] followed for > 1 year as chronic carriers, and show neither proteinuria nor evidence of renal disease. Therefore, it is difficult to study the risk factors, pathogenesis, onset, progression, treatment, and prevention of LN. After *Ixodes* ticks from Westchester County, New York, fed on adult Beagle dogs, they seroconverted, had high Lyme titers, but remained asymptomatic for >1 year.[22,41,42] Beagle puppies (6–12 weeks) so exposed, had no acute stage of illness, but after a long incubation of 2–5 months, showed a 4-day self-limiting illness of fever, anorexia, and lameness/swelling/lymphadenopathy in the leg closest to where the ticks were applied.[22,43–49] By this time, they had seroconverted to IgG+ status. Some dogs had several shorter episodes several weeks apart in the same or different leg. Beagles exposed at 13–26 weeks had fewer signs than younger puppies.[50,51] Eventually, all dogs became asymptomatic carriers for > 1 year, with positive Bb cultures and PCR+ tests on skin biopsy samples from previous tick attachment sites; dogs treated with antimicrobials for 1 month were not always successfully cleared based on cultures/PCR tests.[43,45–47] Interestingly, 35–45% of the dogs were coinfected with *Anaplasma phagocytophilum*[44,51] and a few with *Babesia microti*,[22] also transmitted by *Ixodes* ticks.

Do People Get Lyme Nephritis?

Glomerulonephritis, recently described in 6 Lyme+ patients,[52–56,f] may be underrecognized.[53] In 4 cases, onset occurred within 1–4 weeks of exposure, often just after acute phase signs of rash and flu-like signs of fever, sore throat, myalgia/arthralgia. Edema, hypertension, PLN and in 2 cases kidney failure requiring hemodialysis ensued. Renal biopsies in 4 cases showed postinfectious type 1 mesangiocapillary MPGN with IgG/C3 deposition. In 1 case, Lyme disease was thought to trigger reactivation of previous IgA nephropathy.[52] One case with membranous GN and IgG/C3 deposition responded to doxycycline, angiotensin converting enzyme (ACE) inhibitor, and warfarin only.[54] Recovery was obtained in 3 cases with antimicrobials and steroids. One case of MPGN received steroids, oral and intravenous antimicrobials, immunoglobulin, plasmapheresis, and became dialysis independent after 5 months.[55] One person had Lyme disease diagnosed 12 years prior with subsequent chronic arthritis before MPGN occurred, which did not respond to antimicrobials, ACE inhibition, and steroids, and requires hemodialysis.[f]

Emergency and Clinical Presentations of Lyme Nephritis

Dogs with Lyme+ PLN may present with dramatic emergent signs, just as dogs with any type of PLN,[57] including thromboembolic events (TE), hypertension, effusions/edema, or signs of kidney failure (eg, vomiting, anorexia, pigmenturia, oliguria/anuria). Hypercoagulability occurs due to hypoalbuminemia, decreased antithrombin activity, vasculitis, and hypertensive damage to endothelium. Pulmonary, arterial, and venous beds may be affected. Signs may include collapse or sudden death (eg, pulmonary, cardiac, or neurologic TE); dyspnea (ie, pulmonary TE); hind end weakness (ie, saddle TE); or pancreatitis/gastrointestinal signs (eg, portal venous TE). Hypertension associated with PLN may present as sudden blindness (eg, retinal hemorrhage/detachment), cerebrovascular accidents, or epistaxis. Hypoalbuminemia from PLN may lead to pleural, pericardial, or ascitic effusions, or dependent edema, which owners may recognize as exercise intolerance, lethargy, dyspnea, bloating, or swollen legs or scrotum.

Anecdotally, LN may present as acute or chronic, stable, or progressive, with mild, moderate, or severe PLN. Cases described with renal biopsies were dramatically ill, with histories ranging from 1 day to 8 weeks

(averaging 2 weeks[a] or 6–8 weeks[8]). Labrador Retrievers (24–32%) and Golden Retrievers (10–20%) were overrepresented.[8,14,a,b] In another study, 49/58 (84%) of Retrievers with PLN compared with 35/146 (24%) of healthy Retrievers were Lyme+.[g] Histories included anorexia, lethargy, vomiting, and sometimes polyuria, with progression and often death within weeks–months of presentation. Acute progressive cases need to be differentiated from Leptospirosis and, if fed raw meat diets and showing diarrhea (± blood), HUS, due to strains of *E. coli* or *Shigella* and Shiga-like toxins that cause thrombotic microangiopathy, PLN, and acute renal shutdown within a week.[58–62] Prior or concurrent lameness was described in 9[b]–28%[a] of cases and some had been treated for Lyme arthritis. Lyme vaccination was administered in 6/46 of cases, 2 weeks to 15 months prior to illness,[8] and in 11–30% of cases,[a,b] but it was unknown if seronegativity existed before vaccination. Dogs that had renal biopsies had azotemia and glomerular changes (eg, proteinuria, hypoalbuminemia, hypercholesterolemia, ascites/effusions, TE, hypertension). Suspect cases also often had mild-moderate thrombocytopenia (79%), possibly due to consumption related to vasculitis, hypercoagulability, or coinfections, and tubular involvement by decreased concentrating ability (72%) or glycosuria (27%).[b]

Diagnostic Tests

Most dogs with high Lyme antibody titers and high CICs are asymptomatic and nonproteinuric. In proteinuric Lyme+ dogs, diagnostic tests may not be able to confirm LN as the cause, but help to indicate therapy and rule out other differential diagnoses. A presumptive diagnosis of LN is based on a history of exposure (ie, positive serology), clinical findings consistent with PLN, and ruling out other causes.[57] Finding evidence of IMGN involving Lyme-specific immune complexes strengthens the association; however, elution studies or specific immunohistochemistry (IHC) stains are not readily available or validated. Response to treatment does not prove LN since dogs may respond to standard PLN therapy and doxycycline treats coinfections and is antiinflammatory.

Underdiagnosis of LN, especially if mild or early, may occur in Lyme+ asymptomatic or sick dogs that are not screened for proteinuria. Overdiagnosis of LN occurs since Lyme+ status in proteinuric dogs may be coincidental, with proteinuria due to lower urinary tract disease, pyelonephritis, Leptospirosis, or PLN due to other causes (eg, infectious diseases, genetic nephropathies, lupus, neoplasia, HUS). In one study, 16% of suspected LN cases did not show positive IHC staining for Lyme immune complexes.[14]

Tests for Lyme Exposure

Ixodes ticks are at higher risk of carrying Bb in certain endemic areas. In 2010 in the United States, 94% of human Lyme disease was reported in just 12 states (NJ > PA > WI > NY > MA > CT > MN > MD > VA > NH > DE > ME).[h] Awareness of neighborhood seroprevalence[i] in healthy dogs helps avoid overdiagnosing Lyme disease; for example, if 40% of healthy dogs are Lyme+, perhaps 40% of sick dogs with any illness will be Lyme+ coincidentally. Since Lyme+ status is a marker for tick and wildlife exposure, the clinician needs an open mind for differentials.

The search for Bb antigens is not helpful in dogs because very few organisms are found in tissues or fluids. In the experimental model, organisms migrated interstitially, causing lameness in the leg closest to tick attachment.[22,43–49] It is unknown if Bb strain or host factors might allow for hematogenous spread in some dogs and whether that could predispose for LN.

Exposure to Bb is best documented by finding natural exposure antibodies, by qualitative or quantitative tests. The height of any titer does not predict illness since high titers are seen in asymptomatic dogs in field and experimental cases.[46,48,63] Its unknown what the factors are to initiate LN or why some dogs with high titers (which correlate with high CIC) have proteinuria but most do not, even among breeds at risk.[12]

While in the tick, Bb expresses a number of antigens including outer surface protein A (ospA), which functions to attach organisms to the tick's midgut. During tick feeding, ospA expression is downregulated so that Bb detaches from the midgut and is able to be transmitted to the host. This occurs after 2–4 days of tick feeding, beginning at 52–53 hours in the mouse.[64] A few organisms may still be expressing some ospA as they enter the host, but new antigens are expressed such as ospC, and at least 116 new antigens are upregulated during early infection in the host, during the first 10 days, waning between days 17 and 30.[65] Antibodies directed against OspC rise 2–3 weeks after infection and wane after 3–5 months.[j] Possibly OspA is expressed in the host during the carrier phase;[25,66] nonvaccinated humans with nonresponsive arthritis triggered by Lyme disease have very high anti-ospA titers.[26–32] Antibodies against OspF rise 6–8 weeks after infection and remain increased.[j] Whether ospF antibodies wane with treatment needs more study.

There are many tests for antibodies directed against either whole cell antigens (eg, IFA, ELISA, KELA, IgM, IgG) or individual Lyme antigens (eg, Western blot, C6 peptide, ospA, ospC, OspF). The tests for antibodies directed against whole cell antigens are less specific for natural exposure antibodies, since they cross-react with vaccinal antibodies as well as antibodies to other

spirochetes.[1,2] Western blot testing shows an array of antibody bands against many Lyme antigens (eg, p31 (ospA), p34 (ospB), p22-23 (ospC), p28-29 (ospF), p41 (flagellin)); the banding pattern will change over time during infection because new antigens are expressed by the organism via antigenic variation, which helps Bb survive host immunity. We do not know if there are specific antigens that induce LN and whether they are likely to be expressed early or late after exposure. Western blot banding patterns need to be studied in well-documented cases of LN.

Lyme bacterin vaccines are made from killed whole Bb bacteria grown in culture in vitro, where Bb express antigens as when in the tick, including ospA, ospB, many others, and sometimes ospC. Subunit recombinant ospA vaccines only induce ospA antibodies; protective titer levels are unknown. The Western blot utilizes cultured whole cell Bb antigens to capture antibodies in a banding pattern according to molecular weight. The pattern differentiates between vaccinal and natural exposure antibodies since ospA antibodies are usually vaccinal antibodies; however, 17% of nonvaccinated dogs showed ospA bands in one study.[66] The banding pattern changes over time with antigenic variation.

The antigenic repertoire of Bb includes families of antigens that change over time during infection because of promiscuous recombination of a gene locus with many silent cassettes nearby on its chromosome to produce new antigen variants that can adapt to host immunity. One such site is the VlsE (variable major protein-like sequence, expressed), with variable and conserved regions, which is only expressed in the host (not in the tick or in culture, and therefore not present in vaccines). Since this antigen is not expressed by cultured Bb, there is no corresponding band for antibodies to it on Western blot. A recombinant protein, C6, mimics the 6th conserved invariable region (IR6) of the VlsE family, and antibodies against the C6 peptide are a sensitive and specific confirmation for natural exposure antibodies.[48] In-house test kits for C6 antibodies include the SNAP-3Dx, SNAP-4Dx, and SNAP-4DxPlus tests (IDEXX[k,l,m]). These antibodies rise 3–5 weeks after infection, well before lameness signs were seen experimentally, stay high in carriers for at least 69 weeks,[48] and were shown to wane by the Lyme Quant C6 test (IDEXX[n]) 6 months after treatment,[63,67] whereas whole cell ELISA or KELA antibodies did not wane as much.[46]

New tests are available for Lyme antibodies and for some other infections that can cause PLN. The SNAP-4DxPlus (IDEXX[n]) tests for heartworm antigen and antibodies against Lyme C6 peptide, *Anaplasma phagocytophilum*, *A. platys*, *Ehrlichia canis*, *E. chaffeensis*, *and E. ewingii*. The Multiplex[j] test (Cornell University) includes Western blot, and quantitation of antibodies against OspA, OspC, and OspF. The AccuPlex4[o] test (Antech Diagnostics) tests for heartworm, Lyme, Anaplasmosis, and Ehrlichiosis and may show evidence of infection 1–2 weeks earlier than other tests (although Lyme disease may not cause signs then). It uses an algorithm comparing the relationship and magnitude of 5 markers that differentiate vaccinal and natural exposure antibodies, and may help differentiate early versus late infection. The results are given in qualitative terms since it is debatable if the specific height of titers is clinically useful. Abaxis is developing new quantitative and in-house qualitative tests intending to differentiate vaccinal from natural exposure antibodies that wane after treatment.

Quantitation of natural exposure antibodies that wane after treatment may be useful clinically. Dogs with high titers are likely to have high CICs. Even though few of these dogs will show proteinuria, decisions about further treatment may be made based on how high the quantitative titers are, especially in retrievers. After treatment, qualitative tests may remain positive for years, but having a new quantitative baseline level 6 months after starting treatment may be useful by suggesting decreased antigenic load, decreased CICs, and possible clearance (if there is $\geq$ 50% decline). If signs recur, comparisons of future quantitative test results with the new baseline may indicate reexposure or relapse, and show if retreatment is warranted.

Tests to differentiate early and late infection are based on the experimental model of one time exposure. In field cases, multiple exposures are likely, and the finding of early indicators may not be that helpful. Since the canine experimental model did not show lameness until 2–5 months after exposure, we do not expect to see Lyme arthritis signs before seroconversion for C6 antibody or during IgM+/IgG– or OspC+/OspF– status. IgM antibodies rise first after infection, and rise in chronic carriers as new antigens are expressed. OspC antibodies may be present due to this season's tick exposure, but by the time dogs are ill, ospF antibodies are probably present as well. OspF antibodies could be due to this past season or from exposures years ago. We may suspect when the dog was last exposed, but we may not be able to know when it was first exposed. We suspect that LN occurs when antibody titers and CICs are high, well after conversion, since arthritis signs were seen before LN in many cases. But human MPGN occurred earlier, and without an experimental canine model, we do not know when onset may occur.

Tests for PLN and Differential Diagnoses

Diagnostic tests to stage PLN and rule out other differential diagnoses[57] include CBC, biochemical profile, urinalysis, urine protein/creatinine ratio (UPC), urine

culture, blood pressure measurements, imaging (eg, thoracic radiographs, abdominal ultrasound), tests for coinfections,[68] DNA tests (if available), and if the dog is a candidate, renal cortical biopsy to document IMGN to prove immunosuppressive therapy is warranted. If dogs present with arthropathy, joint tap cytology may indicate septic changes or *Anaplasma/Ehrlichia* spp. morulae.

Dogs with LN often showed mild-moderate nonregenerative anemia (92%), thrombocytopenia (79%), urine-specific gravity (USG) < 1.022 (72%), proteinuria (100%), hemoglobinuria/hematuria, glucosuria (27%), cylinduria, azotemia (96%), hypoalbuminuria (90%), hypercholesterolemia (29%); and/or hyperphosphatemia.[b] Hematuria/hemoglobulinuria, an active sediment, and negative urinary culture were also common.[a]

All Lyme+ dogs should be screened and monitored for proteinuria even if they do not present with PLN, since early identification and intervention may ward off progression. Persistent moderate or high microalbuminuria, +2 dipstick proteinuria (or if USG ≤ 1.012, +1 dipstick proteinuria)[69] warrants follow up UPC determination.[57] Daily variation of proteinuria can be averaged by pooling samples.[70,71] When proteinuria (UPC > 0.4) is found, localization to the kidney versus the lower urogenital tract is important.[72] For renal proteinuria, a urine protein electrophoresis test (SDS-PAGE, available at the Texas Veterinary Renal Pathology Service, TVRPS) identifies the source as likely glomerular (high molecular weight) versus tubular (low molecular weight), and LN may have both.

All dogs with suspected LN should be tested for additional infectious diseases that cause PLN in the geographic area the dog lives and travels in. The presentation of LN mimics PLN due to other causes. If the Labrador retriever with PLN due to *Babesia gibsoni*[73] had coincidentally been Lyme+ and Babesiosis not discovered, its demise would have wrongly been blamed on LN. That dog made a full recovery after treatment for Babesiosis, so it is definitely worth checking for other causes. Lyme+ status is a marker for tick and wildlife exposure. Coinfection may increase risk for illness.[74] Other infectious agents are found in *Ixodes* ticks (eg, *Anaplasma*, *Bartonella*, *Babesia microti*), lameness history may be due to other diseases, and Leptospirosis may cause proteinuria, glycosuria, and active urinary sediment with negative culture. In our endemic area, we test for Leptospirosis (titers, PCR), Anaplasmosis (titers), Ehrlichiosis (titers, PCR), Bartonellosis (Western blot ± titers, PCR/cultures), Babesiosis (PCR, titers), and for acute presentations, follow-up convalescent titers for these as well as Rocky Mountain-Spotted Fever titers. In certain circumstances, other infectious agents may be considered, for example Mycoplasmosis, Brucellosis, Leishmaniasis, fungal agents.[57,68] Tests for immune-mediated disease (eg, ANA test) may be positive in cases of Ehrlichiosis or Bartonellosis.[75]

Another differential for PLN progressing rapidly to anuria is HUS (with diarrhea ± blood, or atypical HUS without diarrhea). Genetic glomerulopathies may be the cause of PLN. Bernese Mountain dogs in Europe were first thought to have LN; however, a genetic MPGN was found, mostly affecting females. The breed happens to have a high natural exposure rate, but neither lameness nor proteinuria was associated with Lyme+ status when dogs were followed for 2.5–3 years.[76,77] There are DNA tests for a few breeds with genetic glomerular basement membrane defects, for example Samoyeds (VetGen) and English Cocker Spaniels (OptiGen),[57] and a new test for genetic podocytopathy in Soft-coated Wheaten terriers at risk for PLN.[p,78,79] If Lyme+, these cases should also be treated for LN, since high CIC may trigger or aggravate disease expression.

Renal cortical biopsy is best done before chronic changes of end-stage disease mask the initiating cause and while intervention might still have an impact. Hypertension must be regulated, platelet count must be adequate, and antithrombotic therapy stopped for at least several days. Coordination with the TVRPS is recommended for full evaluation of samples by transmission electron microscopy, immunofluorescence, and thin section light microscopy, for recognition of IMGN versus glomerulosclerosis, amyloidosis, or another cause for which immunosuppressive therapy would not be helpful. Elution studies and specific IHC stains for Lyme antigens are not readily available or validated.

Treatment

Management protocols are not validated because diagnostic confirmation is difficult without elution or specific immunohistochemistry studies and because of the lack of an inducible model. Treatment of nonproteinuric, asymptomatic Lyme+ dogs is controversial,[80] but all agree that all dogs that have renal proteinuria and Lyme+ status should be treated for presumed LN. Doxycycline (10–20 mg/kg/d) is usually chosen because susceptible coinfections may exist. In the experimental tick-exposure model, treatment for 30 days with high-dose antimicrobials such as oral doxycycline (10 mg/kg q12 h), amoxicillin (20 mg/kg q8 h), azithromycin (25 mg/kg q24 h), or intravenous ceftriaxone (25 mg/kg q24 h) did not clear all dogs, based on positive PCR or cultures from skin biopsies at the tick-bite sites,[43,45–47] so long-term antimicrobial therapy appears warranted. If the Lyme Quant C6 level taken 6 months after the start of treatment has substantially declined ≥50% compared with the pretreatment level, antimicrobials can probably be stopped. The new baseline Quant C6 can then

be used for comparison with future levels should reexposure or relapse be suspected. The qualitative Lyme antibody tests are likely to be positive for many years, even with treatment. Other quantitated tests such as IFA, ELISA, KELA, IgG, IgM, and OspF Lyme antibody tests have not been shown to wane as much as Quant C6 posttreatment.

All dogs with LN should also get standard treatment for PLN,[57] that is an inhibitor of the renin-angiotensin-aldosterone system such as an ACE inhibitor (eg, enalapril or benazepril, 0.5–2 mg/kg/d) to reduce proteinuria by decreasing glomerular filtration pressure due to dilatation of both the efferent and afferent arterioles; an antithrombotic (eg, aspirin 1–2 mg/kg/d, clopidogrel 1–3 mg/kg/d, and/or dipyridamole 1 mg/kg q12 h); a modified medium-low protein/phosphorus diet; and an omega-3 fatty acid supplement. Dogs with hypertension despite ACE inhibition receive anti-hypertensive calcium channel blocker (amlodipine, 0.1–0.75 mg/kg/d), and dogs with renal failure may need other medications (eg, phosphate binders, anti-emetics, antacids, appetite stimulants).

Immunosuppressant therapy may be warranted if renal biopsy confirms that there is active immune-complex disease and if the disease is progressive, but the best protocol is unknown. If the dog is not a candidate for renal biopsy due to thrombocytopenia, unregulated hypertension, or owner constraints, immunosuppressives may be attempted in cases that are deteriorating despite antimicrobials and standard therapy for PLN.

Dogs that present with dehydration, for example, due to azotemia/vomiting, are challenging and have a poorer prognosis. If dehydration is mild and conservative management suffices, subcutaneous fluid administration is preferred. If more aggressive hydration therapy is required, catheters may provoke thrombogenesis, intravenous crystalloid therapy may induce/aggravate effusive disease, and intravenous colloid therapy is then needed. These treatments may be accompanied by increased hypertension, coagulopathy, or volume overload. Urine output must be monitored; progression to oliguric/anuric acute kidney failure may require therapies such as diuretics, and even hemodialysis. Dogs with TE events may require thrombolytic therapy. Antithrombotic medications should be stopped at least several days before renal biopsy is performed.

In people, MPGN is primarily treated by addressing the underlying causative infection if known, and secondly, with steroids at 1 mg/kg q24–48 h (up to 60–80 mg) for many months before tapering.[16] Other protocols sometimes include cyclophosphamide, mycophenolate, cyclosporine, anti-platelet therapy, immunoglobulin, or plasmapheresis. New therapies will inhibit specific inflammatory mediators (eg, NFκB, TGFβ, PDGF) or target mesangial cells with immunoliposomes.[17,81]

Indiscriminant use of steroids in stable dogs is not recommended because of possible side effects, for example increased risk for TE, gastric ulceration, and hypertension, for which PLN cases are already predisposed. However, if renal biopsy confirms IMGN, or in cases without biopsy that are rapidly deteriorating, steroids may be used alone or in combination with other immunosuppressives. The best protocol is unknown but agents tried include mycophenolate (10–20 mg/kg q12 h IV or PO long term), pulse steroids (eg, methylprednisolone sodium succinate, 5 mg/kg q24 h IV for 2 days or prednisolone 1–2 mg/kg/d PO, pulse or with taper), cyclophosphamide (200–250 mg/m^2 q 2–3 wks IV or 50 mg/m^2 4 d/wk PO q 2–3 wks), azathioprine (2 mg/kg q24 h PO for 2 wks, then 2 mg/kg q48 h PO), or chlorambucil (0.2 mg/kg q24–48 h PO). In one study of dogs with unspecified PLN, the cyclosporine-treated group showed worse outcome than placebo; however, the characterization of the cause of PLN was limited to light microscopy, which does not differentiate PLN subtypes completely, thus perhaps one or more PLN subtypes may respond to cyclosporine but the numbers were too small to gain statistical significance for efficacy in this first study.[82] When studies with biopsy-confirmed IMGN show validation of treatment protocols, the subset of LN may still require identification.

If discharged from the hospital, moderately severe cases are monitored every 1–2 weeks for blood pressure measurement, packed cell volume, serum albumin, creatinine, BUN, phosphorus, Na/K, and bicarbonate concentration. Milder cases are monitored monthly at first. The trend of stability or progression is used to reassess and modify recommendations of treatments and frequency of rechecks. Proteinuria is monitored by UPC measurements. Since daily variation can alter UPC by 35% near a UPC of 12.0 and 80% at UPC of 0.5,[70] owners may save 3 urine samples taken over several days in their refrigerator until bringing these to their veterinarian; 1 mL is taken from each sample and mixed so this pooled sample is submitted as an average representative UPC determination, which is within 20% of the average of 3 separate samples.[71] If more RAA inhibition is sought and if the ACE inhibitor dose is maximal, an angiotensin-receptor blocker may be added (losartan 0.125–0.25 mg/kg/d in azotemic dogs, 0.5–1 mg/kg/d in nonazotemic dogs). Spironolactone, an aldosterone receptor antagonist, may be helpful as a diuretic to decrease effusive disease (1–2 mg/kg q12 h). Care must be taken with inhibition of the RAA so that serum potassium concentrations do not exceed 6.5 mmol/L (6.5 mEq/L). Nutrition consultations may offer low potassium diets as required.

In general the prognosis for LN is guarded, especially if no other cause is found. Dogs that present dehydrated with moderate-severe azotemia, requiring intravenous fluids, have a poor prognosis, often progressing rapidly within a week or 2, similar to dogs with thrombotic microangiopathic and hypertensive injury to glomeruli, as in HUS. But proteinuric, hypoalbuminemic dogs that are active, eating, and with serum creatinine < 221–265 μmol/L [<2.5–3.0 mg/dL] appear to have a better chance at stabilization. A few dogs have shown normalized creatinine, albumin, and UPC, but whether those dogs actually had LN is difficult to know.

Prevention

Tick control is paramount since there are many tick-borne diseases in Lyme endemic areas. Some may be transmitted earlier than the 2–4 days it takes for Bb transmission, so when possible prevention of tick attachment with amitraz or permethrin is preferred rather than products that only kill ticks after they have been attached for hours-days.

Vaccination for LN, a disease with an immune-mediated pathogenesis, may be problematic and is still controversial even in endemic areas. Tick control is needed to prevent other diseases; 95% of Lyme+ dogs remain asymptomatic; Lyme arthritis in the <5% responds quickly to safe, inexpensive antimicrobials; the duration of immunity requires frequent boostering and is not 100%;[83] Lyme bacterin has been associated with more postvaccinal adverse events than other vaccines we use;[84] and there are concerns about possible immune-mediated sequellae in genetically predisposed dogs. Since this most serious form of Lyme disease affecting <2% of Lyme+ dogs is caused by immune-complex disease, and since high CIC occur for weeks to months after vaccination (longer in boostered or Lyme+ dogs, and longer with bacterin than subunit ospA vaccine), it is theoretically possible that vaccinal immune-complexes could be deposited in glomeruli of a genetically predisposed individual. Potentially, this could happen over time and not appear temporally related to vaccination. Without an inducible experimental model, it is difficult to determine the host-pathogen factors that are associated with LN and whether vaccine antigens protect against it, or if they could sensitize or aggravate the condition. Early reports showed up to 30% of LN suspects had been vaccinated; 6 dogs had been vaccinated with Lyme bacterin 2 weeks to 15 months prior to presentation with LN.[8]

OspA is in all currently available vaccines and is proinflammatory enough that it does not require an adjuvant. OspA has been associated with immune-mediated disease in other species including people with nonresponsive Lyme arthritis.[26] Monoclonal anti-ospA stains showed positive staining in kidneys of dogs with LN.[3,4] In hamsters, Lyme bacterin or subunit ospA vaccine caused more severe arthritis in vaccinates than in nonvaccinates after challenge.[85,86] OspA alone is arthritogenic in rats; sensitization occurs so that even more intense arthritis is seen with boosters.[87] Many doses of Lyme vaccines have been used and most dogs do not get sick, but most dogs do not get sick with Lyme disease either.

Recommendations for Future Studies and Changes in Current Practices

Banking of DNA samples from dogs with well-characterized phenotypes is recommended in preparation for genome-wide association studies to search for genetic markers associated with LN, especially in retrievers. Affected Lyme + PLN dogs should have serologic tests ruling out other causes and renal biopsies proving IMGN. Control dogs with matched signalment would have Lyme+ status but without proteinuria. Pedigree analysis may be helpful. If the genome-wide association studies showed a statistically significant difference for a region, it would narrow the field and point to specific candidate genes that could be sequenced and validated in a larger group of affected versus control dogs. Possible candidate genes might include but not be limited to genes encoding podocyte proteins associated with PLN such as NPHS-1 (nephrin), NPHS-2 (podocin), NEPH1-3 complex, ACTN4, TRPC6, CD2AP, LAMB2, etc.[88] or immunoregulatory genes that might cause an aberrant host-pathogen response (eg, MHC, DLA, DQA, complement).

Studies are needed to document that LN is indeed the cause of PLN in an individual. Renal biopsies need to be sampled earlier in the course of illness, classifying the PLN subtype as much as possible. Therapy protocols can then be validated. Documentation of LN requires better study and validation of specific IHC stains to prove specific Bb antigens exist in glomerular immune complexes in renal biopsies, and to identify if there are particular nephritogenic Lyme antigens involved. Serum complement levels should be tested to see if they are low in cases of LN. Western blot and quantitation tests for a wide variety of antibodies need to be done on well-documented cases of LN to see if there are associated patterns of antigen expression associated with LN. The question of whether to do paired titers (acute/convalescent) should be reassessed. More dogs need to be tested for coinfections, especially those found in *Ixodes* ticks (*Bartonella*, *Anaplasma phagocytophilum*, *Babesia microti*) to see if coinfections are more likely to cause disease and to be sure we are treating for them.

Notes

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[h] http://www.cdc.gov/lyme/stats/chartstables/reportedcases_statelocality.html, last accessed 2-19-12.

[i] http://www.dogsandticks.com/diseases_in_your_area.php, last accessed 2-27-12.

[j] http://ahdc.vet.cornell.edu/docs/Lyme_Disease_Multiplex_Testing_for_Dogs.pdf, last accessed 2-27-12.

[k] http://www.idexx.com/view/xhtml/en_us/smallanimal/inhouse/snap/3dx.jsf, last accessed 2-27-12.

[l] http://www.idexx.com/view/xhtml/en_us/smallanimal/inhouse/snap/4dx.jsf, last accessed 2-27-12.

[m] http://www.idexx.com/view/xhtml/en_us/smallanimal/inhouse/snap/snap-4dx-plus.jsf, last accessed 2-27-12.

[n] http://www.idexx.com/view/xhtml/en_us/smallanimal/reference-laboratories/testmenu/innovative-tests/quant-c6.jsf, last accessed 2-27-12.

[o] http://www.antechdiagnostics.com/Main/AccuPlex4.aspx, last accessed 2-27-12.

[p] http://www.scwtca.org/health/dnatest.htm, last accessed 2-8-13.

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