IS IT LYME NEPHRITIS OR LEPTOSPIROSIS?

Meryl P. Littman, VMD, DACVIM (SAIM)
Professor Emerita of Medicine, Clinician-Educator
University of Pennsylvania School of Veterinary Medicine
Email: merylitt@vet.upenn.edu
Keystone Veterinary Conference, August 17, 2018

INTRODUCTION

Just because the dog is a Lyme-positive retriever with proteinuria and hypoalbuminemia does not mean it has Lyme nephritis. It could have another cause for protein-losing nephropathy (PLN) or even have a tubular disease such as leptospirosis, which can mimic PLN in many ways and is also seen in Lyme endemic regions. Misdiagnosis may occur because so many healthy (and sick) dogs are coincidentally Lyme-seropositive, and because Lyme nephritis is difficult to study because there is no experimental model for it. Lyme nephritis is difficult to prove because even if a renal biopsy shows immune-complex glomerulonephritis (ICGN), there are no validated stains to prove the immune-complexes are Lyme-specific antigen-antibody complexes. Here we will discuss the many ways that Lyme nephritis and leptospirosis may mimic one another, and how we might try to sort things out. We will also discuss if (and when) recent vaccinations for these spirochetal infections may make a diagnosis more difficult, by with some diagnostic test results. In addition, inconsistent duration of immunity means that being up-to-date on vaccinations may not preclude infection. While doxycycline is the antibiotic of choice for both of these spirochetal infections, differentiating Lyme nephritis and leptospirosis is important in order to properly isolate leptospirosis cases, to initiate other treatments (e.g., standard protocols for PLN and possibly immunosuppressives for Lyme nephritis, which would be contraindicated for leptospirosis), and to educate owners and staff regarding the public health implications of each disease.

THE ORGANISMS[1-3]

Lyme *Borrelia* and *Leptospira* are gram-negative spirochetes, related to *Treponema* (the cause of syphilis). A double cell membrane with a flagellum (axial filament) running lengthwise between the inner and outer cell membranes gives the bacteria a corkscrew motility. They do not grow in the lab easily, so although *Leptospira* are shed in urine, the bacteria are not found when we do a urine culture, and they are not seen in the urine sediment without dark-field microscopy or special stains (e.g., silver stain or acridine orange). *Leptospira* are not tickborne. There are more than 200 species, including pathogenic, saprophytic, and opportunistic species. The pathogenic ones are shed in urine of carriers and are free-living in moist environments, in waterways, and can survive in biofilms on rocks in even pristine looking creeks. In the host they are extracellular, with a short spirochetemic phase for about a week, and then localize in the medulla of the kidney near the tubules. *Leptospira* outside the host are easily killed with soap, disinfectants, and drying out in sunshine.

Borrelia species are not shed in urine; they are vector-borne. At least 52 species of tickborne *Borrelia* have been identified including 21 in the Lyme group, the major one being *Borrelia burgdorferi*, which is transmitted within *Ixodes* ticks trans-stadially and to the host after about 2 days of tick attachment. Lyme *Borrelia* spp. migrate interstitially in the host (*B. mayonii* also travels hematogenously). There are at least 30 strains of *B. burgdorferi*, based on their OspC genotypes. Lyme endemic areas are still mostly in the Northeast, MidAtlantic, and upper Midwestern USA, extending northward into Canada and westward from the coast. There are 29 known species so far in the tickborne relapsing fever (TBRF) Borreliosis group, which migrate hematogenously in the host. *B. miyamotoi* in Lyme endemic areas is transmitted transovarially in *Ixodes* ticks, so even larvae can be infective, and transmission time is less than 24 hours. Others in the TBRF group such as *B. turicatae* (in the South) and *B. hermsii* (in the Northwest) are transmitted by soft *Ornithodorus* ticks, which only feed on hosts for 15-90 minutes.

GLOMERULAR VERSUS TUBULAR DISEASES CAUSING PROTEINURIA

There is no experimental model for Lyme nephritis so it is difficult to study. When infected *Ixodes* ticks are experimentally placed on adult dogs, the dogs serconvert but show no signs of illness even after a year of observation. They remain carriers with positive antibody titers and high Lyme Quant C6 (IDEXX) results. Spirochetes can be found by culture or PCR testing of skin biopsy samples from tick bite sites more than a year later. If treated with antibiotics, 85-90% of the dogs can be cleared.[1,2] When infected ticks are experimentally placed on very young puppies (6-12 weeks old), a model of Lyme arthritis occurs, i.e., after 2-5 months incubation they may show 2-4 days of a self-limiting illness including fever, anorexia, and lameness in the leg closest to where the ticks fed, sometimes followed by a few similar episodes every 2 weeks, also self-limiting. **There is no experimental model of Lyme nephritis** but in the field it is usually

seen in young to middle-aged retrievers. Incubation, progression, best treatment, and best prevention are still unknown. Only around 30% of these cases have any history of lameness, and so they often present to their veterinarians for the first time as sick dogs, anorexic, vomiting, and azotemic, and by then they have a guarded prognosis. In the original series of cases[1,2] described in the 1990's, about 30% of the cases had been vaccinated, and it was unclear whether vaccine just failed to prevent Lyme nephritis or whether vaccine could sensitize or aggravate it, since Lyme nephritis was found to be an immune-mediated glomerular disease, without spirochetal invasion of the kidney or urinary shedding, as occurs in leptospirosis. In elution studies of Lyme nephritis kidneys after death, Lyme-specific antigen-antibody complexes are found (Goldstein RE, unpublished), but there are no validated stains to prove it in the tiny samples we get when we do renal biopsies in living dogs. Other diseases, e.g., Babesiosis and heartworm, can also cause ICGN.[4-7]

Leptospirosis on the other hand is a tubular disease. Clinically you may not be expecting to see proteinuria and hypoalbuminemia, which are the hallmarks of glomerular disease, in leptospirosis cases. But in two papers, each with more than 50 cases of leptospirosis seen at PennVet[8] and Cornell,[9] azotemia was seen in 75-93%, hypoalbuminemia in 27-35%, and proteinuria in 66-76% of those tested. Other findings included thrombocytopenia (30-51%) and glucosuria (18-30%), changes also described in field cases of suspected Lyme nephritis. These retrospective studies show that tubular disease can mimic glomerular disease and PLN. At PennVet we found that 45% of leptospirosis diagnoses would have been missed if we had not done convalescent titers.[8] In areas with high Lyme exposure rates and with the availability of rapid in-house test results, we may be tempted to quickly diagnose Lyme nephritis and thus miss leptospirosis or other causes of proteinuria. If we miss diagnosing leptospirosis cases, proper isolation and treatment may not occur, and we put other dogs, staff, owners and their families at risk for exposure in our clinic and community.

HINTS TO DIFFERENTIATE LYME NEPHRITIS AND LEPTOSPIROSIS

I. HISTORY:

A. Signalment: Dogs of any age, breed, and sex can get these infectious diseases but most are young to middle-aged, and often active outdoor breeds, for instance, retrievers. Retrievers appear to be predisposed to Lyme nephritis; it may be that they have a genetic predisposition for glomerular disease possibly associated with a number of environmental triggers. It is unknown why perhaps 1-2% of seropositive dogs show Lyme nephritis, but the vast majority of seropositive dogs, even retrievers, do not get it.[1,2] Active middle-aged dogs are also at risk for exposure to leptospirosis, but even small breeds housed in the city are potentially exposed, from rat urine in the basement or raccoons visiting balconies.

B. Risk of exposure:

1. **Does the dog live or travel to endemic areas?** Helpful seroprevalence maps are available at www.capcvet.org which show seroprevalence data based on IDEXX (SNAP 4Dx Plus) or Antech (AccuPlex4) test results reported from USA and Canada, down to the county, state, or province level. Information is also shown there regarding seroprevalence of heartworm antigen and antibodies against *Anaplasma* and *Ehrlichia*. Data is reported for each of the past 6 years and monthly for the current year. Being seropositive for any tickborne disease (TBD) is a marker for tick and wildlife exposure. There is a long list of coinfections which may cause proteinuria that need to be considered.[4-8] Since migratory birds bring infected ticks to new areas and their reservoir small mammals, even dogs in fenced in yards (without deer contact) are still at risk for exposure to TBDs. And wildlife urine can cause risk of leptospirosis exposure in yards, on balconies, or in basements even in apartments or urban environments. Most leptospirosis cases are in humid/moist states, but even dry/desert areas may have cases, especially near waterways or during flooding conditions.

2. **Have ticks been seen on the dog?** If ticks were removed, were they flat (not much feeding) or engorged? Ticks can be identified by visualizing the anal groove (*Ixodes*), scutum, color/size, or by sending a picture to the University of Rhode Island website (free at www.tickencounter.org/tick_identification). There are many kinds of TBDs carried by various kinds of ticks, and different transmission times for each type. One tick can carry more than one type of organism (see **Slide A** below). If an engorged tick was just removed last week and now the dog is lame, its lameness is probably not due to Lyme disease, at least not from last week's tick bite, since experimentally, dogs did not show any Lyme arthritis signs until 2-5 months after the tick bites. There are other TBDs with shorter incubations though. The incubation for Lyme nephritis is unknown because there is still no model of Lyme nephritis for study.

3. **Has preventive tick control been used, what kind, and has it been properly used with good compliance?** Ticks are active whenever the temperature is greater than 40F (4C), so we recommend tick control year-round. Collars should be on tightly enough to have contact with skin, not just fur. Topical products should be put directly on the skin, so as not to wick up on the fur. Dogs that swim often may have incomplete protection with some topical products. Topical

products with permethrins prevent attachment of ticks, whereas fipronil does not kill each tick until after it has fed for perhaps a day. Permithrins must not be used on cats (exception: the Seresto collar (Bayer) is safe for cats as well as dogs). New isoxazoline chewables for dogs such as afoxolaner (Nexgard-Merial), fluralaner (Bravecto-Merck, also topical for cats), sarolaner (Simparica-Zoetis), and lotilaner (Credelio-Elanco) kill ticks soon after they begin feeding. Simparica has been shown to prevent seroconversion for Lyme and also *Anaplasma*, which is transmitted faster than Lyme spirochetes. The website www.capcvet.org is a useful resource to compare tick products and see which ones also act against fleas and other parasites. Revolution does not kill *Ixodes* ticks.

4. **Has the dog been vaccinated?** Which product was used and when was it last given? Inconsistent efficacy and duration of immunity indicate that infection cannot be completely ruled out, even in dogs "up-to-date" with annual vaccinations. Also, vaccinal antibodies may interfere with the interpretation of some diagnostic serologic tests and make diagnosis of natural exposure more difficult. The type of vaccine and the date when it was last given are important questions in your history-taking.

a. **Vaccine Efficacy and Duration of Immunity:**

(1) Leptospirosis: The canine leptospirosis vaccines are killed bacterins of 4 serovars of pathogenic *Leptospira*, including *L. canicola, L. icterohemorrhagiae, L. pomona,* and *L. grippotyphosa*. I consider leptospirosis vaccines to be CORE vaccines in endemic areas. But the vaccines may not be 100% protective. In the case series at PennVet, 9/51 cases were diagnosed with leptospirosis despite being up-to-date for annual leptospirosis vaccination.[8] Dr. Peter Jezyk used to say that if it were easy to make a good vaccine for a spirochete, they would have made one for syphilis years ago. Indeed, researchers are still trying to make a leptospirosis vaccine for people (7-10 million affected/year, 1 million seriously, with 59,000 deaths[10]).

(2) Lyme: There are several types of Lyme vaccines: 1) a recombinant subunit OspA vaccine without adjuvant (Recombitek-Merial), 2) killed adjuvanted bacterins with OspA and one strain of OspC (LymeVax-Zoetis, Duramune-Elanco, Nobivac-Merck), and 3) the new chimeric recombinant adjuvanted vaccine with OspA and 7 types/strains of OspC (Vanguard crLyme-Zoetis). In the 2018 ACVIM Lyme consensus update[1] only 3/6 of the panel recommended Lyme vaccines even in Lyme endemic areas (Lyme vaccines are ELECTIVE vaccines) while all panelists recommended good tick control practice year-round. Lyme vaccines may not protect all dogs completely or for the whole year.[1,2,11,12] Some advocate boostering at 6 months, especially the first year (it is unknown whether every 6 month boostering thereafter is necessary or safe). Vaccine failures were noted only 22 weeks after use of Lyme bacterins or the recombinant subunit OspA vaccine.[12] The new Vanguard crLyme vaccine (Zoetis) has a 15-month license, however, when challenged, although vaccinated dogs were less likely to seroconvert than control (unvaccinated) dogs, the statistics showed 7/16 vaccinated dogs seroconverted (indicating many vaccine failures) compared with 14/16 control dogs.[13]

b. **Interference with Diagnostic Test Interpretations:**

(1) Leptospirosis: Vaccinal antibodies cross-react on all available serologic tests to detect natural exposure antibodies (IgM, IgG, ELISA, and MAT) for leptospirosis, but vaccinal antibodies usually wane fast, between 7-15 weeks post-vaccination. Vaccinal titers are usually negative by 3-4 months post-vaccination. In a study of 32 client-owned dogs, none had a titer of 1:400 or higher by 29 weeks post-vaccination, and only 2/32 dogs had an MAT titer of 1:400 or higher just 15 weeks post-vaccination.[14] Of course PCR tests (blood and urine) are not impacted by vaccination, and if positive, indicate natural exposure and infection with *Leptospira* spp.

(2) Lyme: Vaccinal antibodies against OspC cross-react on tests that measure OspC antibodies (AccuPlex4-Antech, VetScan/FLEX4-Abaxis/Zoetis, and Multiplex-Cornell tests), therefore OspC antibodies can no longer be used as a marker for natural infection in dogs vaccinated with the new bacterins (LymeVax-Zoetis, Duramune-Elanco, Nobivac-Merck) or the new Vanguard crLyme vaccine (Zoetis). It is unknown how quickly vaccinal antibodies wane. The C6 peptide antibody tests (SNAP 4Dx Plus and Lyme Quant C6) are not impacted by any Lyme vaccinations and if positive, indicate natural exposure antibodies, with better sensitivity and repeatability compared with VetScan/FLEX4 (Abaxis/Zoetis) and AccuPlex4 (Antech) and tests.[15,16]

C. Either disease may present with a history that is acute, chronic, or acute on top of chronic. Cases with acute oliguria/anuria may not survive long enough to have convalescent leptospirosis titers done and may be misdiagnosed.

D. Either disease may present with lameness. Only 30% of Lyme nephritis dogs had a history of lameness.[1,2] Leptospirosis cases may show stiffness or lameness due to myositis. High serum AST value with normal ALT (or a high AST/ALT ratio) and high serum CK are indicators of muscle damage. Coincidental degenerative joint disease or coinfection with other TBDs may cause arthritis or polyarthropathy.

E. Either disease may present with polyuria/polydipsia (PU/PD), although primary tubular disease or whole nephron disease is more likely to show PU/PD early, even before azotemia exists. An SDMA test may be helpful to indicate GFR

decline as a cause of PU/PD before azotemia is evident.[17,18] In dogs with leptospirosis, pyelonephritis, or tubular disease, SDMA may be your earliest warning of renal involvement, even before PU/PD and well before azotemia occurs. In primary glomerular disease cases, PU/PD is a relatively late finding.

F. The presence of icterus in these cases is more likely due to leptospirosis or Babesiosis than Lyme disease.

II. PHYSICAL EXAMINATION CLUES: Either disease may show dehydration, lethargy, cachexia (if chronic), hypertension, lameness or stiffness, and rarely, dyspnea. Dyspnea may be due to pulmonary hemorrhage in leptospirosis cases. In PLN cases, dyspnea may be due to a pulmonary thromboembolic event (TE) or pleural effusion in nephrotic syndrome cases.

III. COMMON LABORATORY TEST RESULTS: These cases can mimic one another on CBC, Chemscreen, and urinalysis, with possible anemia, thrombocytopenia, hypoalbuminemia, azotemia, proteinuria, glucosuria, and possible reactive sediment with negative bacterial culture.

IV. SPECIFIC TESTS FOR LEPTOSPIROSIS: There are antigen (PCR) and antibody (serologic) tests for leptospirosis. Blood and urine samples must be taken **before antibiotics are started for PCR testing** (for leptospirosis and non-Lyme TBDs, e.g., *Anaplasma, Ehrlichia, Babesia, Bartonella*, etc.) and for urine bacterial culture (pyelonephritis is another differential in these cases). *Leptospires* may be in the blood sample the first week of illness, and in the urine sample thereafter. Carriers shed spirochetes in the urine only intermittently, so a negative PCR result does not rule out leptospirosis. For serum antibodies, the Witness Lepto test may pick up IgM antibodies earlier in the course of illness than IgG antibodies would be found,[19,20] but information about specificity and waning time is lacking. The SNAP Lepto ELISA technology detects IgG antibodies against the conserved LipL32 antigen, which is specific to pathogenic *Leptospira* spp., and does not cross-react with Lyme antibodies.[21,22] The MAT test is the gold standard and the only quantitative antibody test. All of the antibody tests cross-react with vaccinal antibodies, but vaccinal antibodies wane within a few months (see above **4b1**). It is **most Important to do convalescent titers for leptospirosis**, at least 10-14 days into the illness, since 45% of cases would not have been diagnosed without convalescent titer results.[8] Convalescent titers may be needed for other differentials as well (acute cases of RMSF, *Anaplasma, Ehrlichia,* etc.).

V. SPECIFIC TESTS FOR LYME DISEASE: There are no PCR tests for Lyme disease, but serologic tests are helpful to differentiate natural exposure antibodies from vaccinal antibodies (see above **4b2**). In sick dogs the Lyme Quant C6 (IDEXX) is useful for all SNAP 4Dx Plus (IDEXX) positive dogs. A low Lyme Quant C6 may rule out Lyme nephritis. High Lyme Quant C6 in dogs with signs that may be due to Lyme disease should be treated and have follow-up Lyme Quant C6 about 3-6 months after treatment is started, to follow waning trends and use as a baseline for future comparisons. The in-house SNAP 4Dx Plus only picks up natural exposure C6 peptide antibodies, has high sensitivity, specificity, and repeatability, and also tests for heartworm antigen and antibodies to other species of *Ehrlichia and Anaplasma.* Without an experimental model for Lyme nephritis, we do not know if convalescent titers are necessary, but based on the Lyme arthritis model, by the time a dog is sick with Lyme disease it will have already seroconverted for Lyme antibodies.

VI. IMAGING: Chest radiographs may suggest pulmonary changes (hemorrhage) in leptospirosis, or effusions or TE events associated with PLN. Abdominal ultrasound in acute leptospirosis cases may show renomegaly, subcapsular fluid, hyperechogenicity of the cortex or medulla, or chronic small kidneys. Lyme nephritis often shows no renal changes.

VII. OTHER TESTS TO DIFFERENTIATE TUBULAR AND GLOMERULAR DISEASE

A. SDS-PAGE, a protein electrophoresis done on a urine sample, sent to Texas A&M (contact Mary B. Nabity at mnabity@cvm.tamu.edu for special instructions). Higher molecular weight proteins indicate albuminuria from glomerular disease while lower molecular weight proteinuria is seen with tubular disease.

B. **Cortical renal biopsy**. The dog must be a candidate (hypertension controlled, adequate platelets, antithrombotics stopped, owner consent) and experienced personnel available to retrieve samples, usually by Tru-cut needle with ultrasound guidance. A special kit is needed for submission to the Ohio State University (contact Rachel Cianciolo at rachel.cianciolo@cvm.osu.edu) for TEM, IF, and thin section LM. There are no validated stains to prove ICGN is due to Lyme specific antigen-antibody complexes, but documentation of ICGN will indicate that an immunosuppressive protocol is warranted. See IRIS guidelines for PLN at https://onlinelibrary.wiley.com/toc/19391676/27/s1.

SUMMARY OF SIMILARITIES AND DIFFERENCES (see **Slide B** below)

SLIDE A: SOME TICKBORNE DISEASES

SLIDE B: SIMILARITIES AND DIFFERENCES BETWEEN LYME NEPHRITIS AND LEPTOSPIROSIS

REFERENCES

1. Littman MP, Gerber B, Goldstein RE, et al. ACVIM consensus update on Lyme borreliosis in dogs and cats. J Vet Intern Med 2018;32:887-903.
2. Littman MP, Goldstein RE, Labato MA, et al. ACVIM small animal consensus statement on Lyme disease in dogs: diagnosis, treatment, and prevention. J Vet Intern Med 2006;20:422-434.
3. Sykes JE, Hartmann K, Lunn KF, et al. 2010 ACVIM small animal consensus statement of leptospirosis: diagnosis, epidemiology, treatment, and prevention. J Vet Intern Med 2011;25:1–13.
4. Slade DJ, Lees GE, Berridge BR, et al. Resolution of a proteinuric nephropathy associated with *Babesia gibsoni* infection in a dog. J Am Anim Hosp Assoc 2011;47:e138-144.
5. Ullal T, Birkenheuer A, Vaden S. Azotemia and proteinuria in dogs infected with *Babesia gibsoni.* J Am Anim Hosp Assoc 2018;54:156-160.
6. Littman MP, Daminet S, Grauer GF, et al. Consensus recommendations for the diagnostic investigation of dogs with suspected glomerular disease. J Vet Intern Med 2013;27:S19–S26.
7. Goldstein RE, Brovida C, Fernandez-del Palacio MJ, et al. Consensus recommendations for treatment for dogs with serology positive glomerular disease. J Vet Intern Med 2013;27:S60–S66.
8.Tangeman LE, Littman MP. Clinicopathologic and atypical features of naturally occurring leptospirosis in dogs: 51 cases (2000-2010). J Am Vet Med Assoc 2013;243:1316-1322.
9. Goldstein RE, Lin RC, Langston CE, et al. Influence of infecting serogroup on clinical features of leptospirosis in dogs. J Vet Intern Med 2006;20:489–494.
10. Costa F, Hagan JE, Calcagno J, et al. Global morbidity and mortality of leptospirosis: a systematic review. PLOS Neglected Tropical Diseases 2015;9:e0003898.
11. Töpfer KH, Straubinger RK. Characterization of the humoral immune response in dogs after vaccination against the Lyme borreliosis agent: a study with five commercial vaccines using two different vaccination schedules. Vaccine 2007;25:314-326.
12. Moroff S, Woodruff C, Woodring T, et al. Multiple antigen target approach using the AccuPlex®4 BioCD system to detect *Borrelia burgdorferi* antibodies in experimentally infected and vaccinated dogs. J Vet Diagn Invest 2015;27:581-8.
13. Zoetis Study B864R-US-12–037
14. Martin LER, Wiggans KT, Wennogle SA, et al. Vaccine-associated leptospira antibodies in client-owned dogs. J Vet Intern Med 2014;28:789–792.
15. IDEXX Laboratories 2018. IDEXX SNAP 4Dx Plus Test provides sensitive and specific detection of heartworm and tick-borne diseases. Available at https://idexxcom-live-b02da1e51e754c9cb292133b-9c56c33.aldryn-media.com/filer_public/83/86/838611fd-fe8c-4e61-b41b-16218f154087/flex4-head-to-head-white-paper.pdf. Last accessed 18Jul2018.
16. Goldstein RE, Eberts MD, Beall MJ, et al. Performance comparison of SNAP®4Dx®Plus and AccuPlex®4 for the detection of antibodies to *Borrelia burgdorferi* and *Anaplasma phagocytophilum*. Intern J Appl Res Vet Med 2014;12:141-47.
17. Finco DR, Duncan JR. Evaluation of blood urea nitrogen and serum creatinine concentrations as indicators of renal dysfunction. A study of 111 cases and a review of related literature. J Am Vet Med Assoc 1976;168:593-601.
18. IRIS Staging of CKD (modified 2016) at www.iris-kidney.com/pdf/3_staging-of-ckd.pdf. Last accessed 18Jul2018.
19. Lizer J, Velineni S, Weber A, et al. Evaluation of 3 serological tests for early detection of *Leptospira*-specific antibodies in experimentally infected dogs. J Vet Intern Med 2018;32:201-207.

20. Lizer J, Grahlmann M, Hapke H, et al. Evaluation of a rapid IgM detection test for diagnosis of acute leptospirosis in dogs. Vet Rec 2017;180:517.
21. Curtis KM, Foster PC, Smith PS, et al. Performance of a recombinant LipL32-based rapid in-clinic ELISA (SNAP®Lepto) for the detection of antibodies against *Leptospira* in dogs. Intern J Appl Res Vet Med. 2015;13(3):182–189.
22. Winzelberg S, Tasse SM, Goldstein RE, et al. Evaluation of SNAP®Lepto in the diagnosis of leptospirosis infections in dogs: twenty-two clinical cases. Intern J Appl Res Vet Med. 2015;13(3):193–198.