Lyme nephritis

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INTRODUCTION

The spirochete *Borrelia burgdorferi* is the causative agent of Lyme disease, one of the most prevalent tick borne diseases found worldwide.[7,12,14,16] While the disease was originally documented in the United States in Lyme, Connecticut, it now has migrated and is endemic in the Northeast region and Midwest.[3,6,7,12,16] Twelve states in the U.S. currently account for 95% of all reported human cases, with canines seen as a sentinel for the presence of the disease. [8,12] Transmission directly from animals to humans is undocumented.[11] It is established that Lyme disease is indirectly zoonotic and is transmitted transstadially via tick exposure.[4,7,12] In endemic areas, exposure is common with rates as high as 70-90% with most seropositive dogs never developing clinical Lyme disease .[7,11,14] High antibody titers can persist for years; however < 5% of those exposed develop signs of acute or chronic Lyme infection.[3,7,11,14]

B. burgdorferi is a helical-shaped gram negative spirochete that cannot survive outside of a host.[3,4,6] It is a unicellular flagellate, allowing it to move throughout connective tissue and is vectored by tick arthropods of the genus *Ixodes.*[6,10] All *Borrelia* spp. that are transmitted by *Ixodes* tick and are associated with Lyme infections are grouped in the *B. burgdorferi* complex, which consists of 15 species.[3,4,6] The *B. burgdorferi sensu stricto* species is most commonly found in humans and dogs in Lyme endemic areas of the United States.[3,4,6]

Lyme nephritis is the most severe manifestation of Lyme disease, seen in < 1-2% of Lyme seropositive canines.[8,11] The average onset is approximately 5-6 years of age with Shetland Sheepdogs, Bernese Mountain Dogs, Labradors and Golden Retrievers predisposed.[8,11,14,16] Lyme nephritis is an acute, progressive protein-losing nephropathy (PLN) with membranoproliferative immune-mediated glomerulonephritis, lymphocytic-plasmacytic interstitial nephritis, and renal tubular necrosis and regeneration.[5,8,11,16]

Lyme nephritis can present as acute, chronic, stable, or progressive with varying degrees of protein losing nephropathy.[8,11] Canines with Lyme nephritis may present with emergent signs including thromboembolic events, edema or effusions (especially after generous crystalloid fluid administration), acute kidney failure, or hypertension.[6,8,11,16] Hypertension may be associated with possible blindness due to retinal detachment or with a heart murmur.[8] Many patients are hypoproteinemic which can lead to hypercoagulability, vasculitis, decreased antithrombin, and damage to the endothelium.[8,11] Thromboembolic events leading to sudden collapse, hind end weakness, or death are not uncommon.[11] Conversely, patients may present only for gastrointestinal or pancreatitis signs.[8,11] Patients have also presented for neurologic signs such as seizures or nystagmus.[6,8] The use of diagnostic testing is to stage the level of disease as well as rule out other diagnostic differentials.[8,11] Treatment includes therapy for protein-losing nephropathy, long-term antibiotics, and potentially the use of immunosuppressive therapy.[8,11]

HISTORY AND PRESENTATION

Crosby, an 8 year old spayed female Yorkie-poo, presented to Pittsburgh Veterinary Specialty and Emergency Center (PVSEC) on December 2, 2016 for intermittent vomiting of two weeks duration, lethargy, and inappetance. During the two week period, Crosby experienced intermittent bouts of vomiting lasting 2-3 days at a time. Her appetite initially decreased, then stopped completely. Her owners noted a foul smell from her mouth that was not previously noted. Crosby had no previous history of illness and was up to date on all core vaccinations. As a primarily indoor dog, she had never been on any flea, tick, or heartworm prevention and was purchased by her current owners from a breeder as a puppy. She had not

seen her primary veterinarian for treatment prior to her presentation to the emergency department.

Upon presentation, Crosby was quiet, alert, and responsive. Her heart rate (150 bpm), respiratory rate (30 brpm) and temperature (100.1F) were within normal limits. Crosby weighed 2.9kg, with a BCS of 5/9. Her systolic blood pressure was 220 mmHg (hypertensive). A grade II/VI parasternal heart murmur with quiet lung sounds was noted during auscultation. Her mucous membranes were tacky with a pink color and a CRT of < 2 seconds. She had a moderate skin tent. She was estimated to be 5% dehydrated. Her abdomen was soft and easily palpated with no abnormalities detected. A CBC, serum chemistry, and urinalysis were performed. Her CBC revealed mild anemia (36.5%) and a mild thrombocytopenia (158 K/µl). Her serum chemistry revealed mild hypernatremia (153.5 mmol/L), a severe decrease in alkaline phosphatase (4 U/L), a severe hyperphosphatemia (18.6 mg/dl), a moderate hypoalbuminemia (1.9 g/dl), a severe hyperglobulinemia (5.3 g/dl), a severely elevated BUN (168 mg/dl), severely elevated creatinine (6.69 mg/dl), mildly elevated glucose (137 mg/dl), mild hypocalcemia (8.7 mg/dl), and mildly elevated CK (760 U/L). A urinalysis revealed a urine specific gravity of 1.016, severe hematuria (3+ blood), trace glucose, and severe proteinuria (3+). Additional urine tests were ordered based upon initial minimum database results. A urine microabumin test revealed microalbuminuria at > 30 mg/dl, and her urine protein: creatinine ratio was 9.5 (severely elevated). A urine culture was submitted. With the diagnostic results obtained, it was determined that a 4DX Plus SNAP test and thoracic radiographs were needed. Thoracic radiographs revealed no evidence of neoplasia or pleural effusion, and a normal cardiac silhouette. The 4DX SNAP test was positive for *Borrelia burgdorferi*. The results of all testing performed supported a presumptive diagnosis of Lyme nephritis.

PATHOPHYSIOLOGY

The most common modes of transmission of *B. burgdorferi* to a vertebrate host is by the bite of an infected tick (*Ixodes scapularis*).[8,10,16] The *Borrelia* organism remains in the tick midgut for the first 12-24 hours.[16] *B. burgdorferi* uses host specific proteins for immune system evasion and to complete its transmission cycle. The OspA protein allows for binding of *B. burgdorferi* to the tick midgut.[16] During this time, the spirochetes remodel outer surface proteins which allow for later survival in the mammalian host, transforming OspA to OspC.[10,16] This transformation allows for the exit of the spirochete from the midgut to the salivary gland, allowing it to potentially evade the tick's host immune system as it waits for transmission to a mammalian host.[10,16] Once the tick attaches to a host, the transmission of *B. burgdorferi* takes approximately 48-72 hours.[3,8,11]

In the mammalian host, the OspC protein switch is activated by the temperature of the blood.[3,8,10,11] It then binds with plasminogen and helps *B. burgdorferi* to disseminate within the mammalian host.[10] *Borrelia* organisms may migrate in the bloodstream; however they most commonly migrate via tissue, finally settling in collagen and connective tissues such as skin, perineurium, and joint capsules.[10,16] It is suspected that *B. burgdorferi* may undergo metamorphosis into a spherical shape when encountering unfavorable host conditions, further allowing for evasion from the immune system.[10,16]

The mammalian host's immune response is species dependent.[3,10] In canine species, the initial infection time is unable to be determined due to the lack of erythema migrans.[8,10,16] Not all dogs that are infected develop disease; as many as 95% of exposed dogs remain asymptomatic despite infection.[8,11] Clinical signs typically develop within 2-5 months of infection.[8,11] The most common presenting sign is non-specific lameness (polyarthritis);

however, other more rare forms of illness may present including renal, cardiac and neurologic forms.[8,11,16] Acute phase illness may consist of lethargy, fever, and lameness due to neutrophilic polyarthritis; malaise, thrombocytopenia, and lymphadenopathy which may resolve over several days.[8,11]

Glomerulonephritis is an uncommon but serious form of Lyme disease consisting of a progressive renal disease that may lead to nephrotic syndrome.[2,9] Commonly referred to as "Lyme nephropathy" or "Lyme nephritis," the incidence of occurrence is <1-2%.[8,11] The mean age of presentation and onset is 5-6 years, which is often younger than most animals with a protein losing nephropathy from acquired disease processes.[2,9] Canines with this presentation may or may not have a history of lameness.[6,11] The cause is theorized to be immune-mediated with a possible genetic predisposition, but at this time is poorly understood.[8,11] While the exact pathophysiology is unknown Lyme nephritis may occur because of passive entrapment of cationic antigens or circulating complexes in normal or previously altered glomeruli.[2,11] These complexes then attract inflammatory or glomerular cells to release vasoactive substances, platelet activators, and pro-inflammatory cytokines.[11] This complement cascade attacks cell membranes causing renal damage.[8,11] *Borrelia* specific proteins OspE and OspF bind to the inhibitory protein factor H, removing one renal mechanism that inhibits damage.[11] Secondary tubular changes are often seen including hypertension, efferent arteriole vasoconstriction, toxic proteins in glomerular filtrate, or tubular hypoxia.[8,11] The protein losing nephropathy is characterized by immune-mediated glomerulonephritis that is membranoproliferative, diffuse tubular necrosis with regeneration, and lymphocytic-plasmacytic interstitial nephritis.[5,8,11] Mild to moderate thrombocytopenia may also occur.[8,11]While it is suspected that a particular Lyme antigen may induce glomerular damage, it is at this point unknown and considered unlikely.[2,9] Lyme

nephritis is often rapidly progressive and fatal, with most patients surviving with treatment only weeks to months.[8,11]

DIAGNOSTIC APPROACH AND CONSIDERATION

Four criteria have been established in the establishment of an active Lyme infection in canine species.[3,6,10,11] The typical clinical signs of Lyme should be observed, the patient should have detectable antibodies or organisms, improvement should be noted post therapy, and the patient often lives or has been to an endemic area where there is a real risk of exposure to *Ixodes scapularis* ticks.[6,8,11] Other diseases should also be ruled out as well. A presumptive diagnosis of Lyme nephritis is based on a positive serology and/or history of exposure, findings consistent with a protein losing nephropathy (PLN), and ruling out other causes of disease.[8,11] While finding evidence of Lyme-specific immune complexes within the kidney can strengthen the association, there is a lack of specific IHC stains or elution studies to support this.[11] Response to treatment alone cannot prove Lyme nephritis.[8,11] At this time it is still unknown why some Lyme positive dogs develop PLN.[8] In addition, the true incidence of PLN associated with Lyme disease is still unknown.[8] Diseases to rule out through diagnostic testing include other causes of proteinuria such as lower urinary tract disease, pyelonephritis, infectious causes, genetic nephropathies, or neoplasia.[8,11]

In suspect Lyme nephritis cases, a complete blood cell count (CBC) and serum chemistry should be performed in order to rule out other diseases.[8,11] Urinalysis should be performed to screen for proteinuria.[8,11,15] If protein is detected in the urine, further screening should be done for *B. burgdorferi* including antibody tests, urine protein: creatinine ratio, and urine culture.[8,11,15]

Additional testing may include blood pressure measurements, imaging, tests for infectious causes, and renal cortical biopsy if the canine is an appropriate candidate.[8,11]

Canines with Lyme nephritis often have a mild to moderate nonregenerative anemia, stress leukogram, hypoalbuminemia, thrombocytopenia, proteinuria, a urine specific gravity < 1.022, azotemia, and hypoalbuminuria.[1,8,11,15] They may also have glucosuria, hematuria, hypercholesterolemia, and/ or hyperphosphatemia.[1,8,11,15] An active urine sediment, casts and negative urine culture are common.[1,15] Urine protein: creatinine ratios are generally above 5 and may be as high as 15.[16] Infectious diseases that cause protein losing nephropathy in the geographic area the dog lives and travels in should also be considered as well as possible coinfection with *Anaplasma phagocytophilum*.[8,11,16]

Diagnostic testing for *Borrelia* antigens is considered to be mostly unhelpful in dogs as the organism is found in low numbers if at all in tissues or fluids. Positive results may demonstrate presence of the organism but a negative test does not rule out the disease.[8,11] These tests can include IFA, ELISA, Western blot, C6 peptide antibody or antigen (SNAP 4DX or Lyme Quant C6 by IDEXX), or testing for individual proteins such as OspA.[1,10,11] At this point it is unknown if there are antigens specific to the induction of Lyme nephritis and when they would be likely to be expressed.[1,11] Testing for IgM is unrewarding as canines do not show clinical signs before increased IgG levels occur.[6] However, IgM may be detected throughout the course of disease as new antigens can often be expressed.[11] C6 antigen testing (Lyme Quant C6) is effective at proving exposure but not clinical disease.[1,8] It is 94-100% sensitive and 99% specific and is not positive with vaccination.[1] It is detectable as early as 3 weeks (3-5 week range) and decreases after successful therapy; therefore it is most commonly used to determine efficacy of therapy with decreasing results correlating with successful treatment.[1,6,8,9,16]

However, there is no correlation between the magnitude of C6 results and proving clinical disease.[1,6,8,16]

Antibody testing is directed to testing against whole cell antigens or individual Lyme antigens.[1,8,11] They are less specific for natural exposure antibodies and may cross-react with vaccine antibodies as well as other spirochetes.[11,16] Antibody testing such as ELISA or IFA are the most widely used tests to diagnose Lyme disease.[1,8,16] False negative titers are rare; in endemic areas as many as 50-90% of healthy asymptomatic dogs are seropositive.[1,8,16] High titers may persist from exposure or vaccination and may persist for years.[1,10,11] Paired titers are not found to be helpful.[1,11] A positive titer does not predict development of clinical signs.[1,8,11,16] IFA was one of the first established serologic methods.[6] Specificity is low and IFA can also have false-positive results.[6] ELISA is a highly sensitive test for the detection of *Borrelia* antigen.[8,11] Whole blood is used to characterize whether a dog is infected or vaccinated.[8,11] In clinical practice, an in house SNAP 4DX (IDEXX) may be used as a qualitative test as it tests for the C6 protein antibody, which is a portion of the VIsE lipoprotein of *Borrelia* expressed in the mammalian host.[6,16] It does not cross react with the vaccine and can detect IgG antibodies 3-5 weeks after the initial infection.[6,8,11,16]

Renal cortical biopsies may also be obtained in early PLN cases and is best obtained before end-stage disease changes mask the inciting cause.[8,11] Hypertension must be well controlled and the patient must have an adequate platelet count with antithrombotic therapy discontinued for several days prior to biopsy.[8,11] Elution studies and specific IHC stains for Lyme disease are not readily available or validated and biopsy results do not always indicate the presence of the *Borrelia* organism.[11,16]

Culture is often unrewarding as *Borrelia* has a slow growth rate, is a microaerophilic species, and requires special media (Barbour-Stoenner-Kelly media).[6,8,16] Special stains (silver, acridine orange) and dark field microscopy are required to visualize *Borrelia*.[8] It is considered very insensitive and time consuming (6 weeks to growth) but may have increased sensitivity if the sample for growth is obtained from a skin biopsy at the site where the tick bite occurred.[6,8,11,16] In addition, successful isolation does not imply that *B. burgdorferi* is the cause of the disease.[6,16]

PCR is expensive and the assay to run can be difficult and may not be readily available.[2,8] It is best suited for samples obtained from the skin, joint fluid, CSF, or urine; however the organism is rarely found in most of the fluids or blood products sampled.[2,8,16] It is more often found in connective tissue or synovia.[2,8,16] PCR is very sensitive but may possibly detect fragments, remnants, or blebs of *B. burgdorferi*.[2,5,6,8] Positive results do not indicate the organism is alive as certain detectable fragments are nonviable.[2,5,6,8] It also can pick up other forms of Lyme such as L-forms, spheroplasts, or cysts and can cross react with *Leptospira* spp.[2,8]

Other testing that may be considered to rule out other causes of PLN may include infectious disease titers/PCR, genetic testing for appropriate breeds, or tests for immune mediated disease (ANA test); however these should be considered dependent on geographic location and suspicion of disease.[8, 11]

TREATMENT AND MANAGEMENT

In all clinical Lyme cases, the organism cannot be effectively eliminated due to complex immune evasion mechanisms.[6, 8] Despite no validated management protocols due to difficult

diagnostic confirmation, the general consensus is that all dogs with proteinuria and Lyme positive status should be treated for presumed Lyme nephritis.[8,11]

B. burgdorferi is sensitive to tetracyclines, amoxicillin, azithromycin, erythromycin, and 3rd generation cephalosporins.[6,8,11,13,16] As with other tick borne diseases, doxycycline is considered to be the antibiotic of choice due to its lipid solubility and anti-inflammatory properties.[2,8,11,16] It is also potentially effective against susceptible coinfections.[6,11,15] Doxycycline is administered at 10 mg/kg daily.[8,9,11,15,16] Amoxicillin is often a second choice antibiotic and is administered at 11 mg/kg every 12 hours.[15,16] Remaining appropriate antibiotics are considered in refractory cases.[11] Antibiotic therapy should continue for 28-30 days but may be longer if the patient has a protein losing nephropathy.[8,11,16] The current recommendation for recurrent Lyme or chronic Lyme disease is repeated 30 day treatments every 3 months or each time the disease recurs.[9,11] Lyme-specific immune complexes can drop post-doxycycline therapy and will often correlate well with C6 quantitative levels.[1] Clinical response is often rapid, occurring within 1-2 days.[8,16]

Despite proper antibiotic therapy, not all animals may be cleared from infection. The possibility of "chronic" Lyme disease is widely debatable and is yet unproven.[6, 16] In addition, persistent titers can occur and PCR often will still detect the presence of the organism whether it is alive or only cell components.[6,16]

If azotemia or PLN is present (Lyme nephritis), a longer duration of doxycycline therapy is usually warranted as well as crystalloid or colloid therapy.[8,11] In addition, these patients may be treated with adjunctive therapy including ACE inhibitors, low-dose aspirin, omega-3 fatty acids, low protein dietary therapy, fluid therapy, immunomodulating therapy, and additional anti-hypertensives if needed.[1,8,10,11,16] If immune-mediated glomerulonephritis is confirmed via

biopsy or if the patient is deteriorating, immunosuppressants may be warranted which may include mycophenolate, glucocorticoids, cyclophosphamide, or chlorambucil.[8,11]

EXPECTED OUTCOME AND PROGNOSIS

In Lyme nephritis cases, the prognosis is guarded to poor.[1,8,11,16] Many dogs succumb within days to weeks.[1,8,11,16] Death is often due to thrombosis or acute renal failure.[8,11,16] Of the Lyme nephritis cases with longer survival times (months to over a year), many were treated with immunosuppressive drugs in addition to the other therapies.[16]

When monitoring progress with C6 antibody testing, a failure to decrease post-treatment may not be considered prognostic.[1] However, the C6 quantitative ELISA from IDEXX is recommended 4-6 months post therapy; results will typically decrease by 12 weeks post antibiotic therapy.[1]

At a minimum, treatment with doxycycline is recommended if any dog is symptomatic or proteinuric.[1,8,11] It is recommended that any dog who is seropositive for *Borrelia* should be monitored for proteinuria.[1,8,11] No evidence suggests that treatment of subclinical infection is warranted.[1,8,11,16] All dogs in endemic areas should be vaccinated and maintained on an effective tick-control preventative.[1,8,10,11] Vaccination in non-endemic areas is not recommended.[8]

CASE OUTCOME

Crosby was hospitalized overnight in PVSEC's critical care unit. She was placed on intravenous fluids at two and a half times maintenance (15 ml/hr) for diuresis. She was also placed on famotidine (1 mg/kg) every 24 hours intravenously, maropitant citrate (1 mg/kg) intravenously every 24 hours, and doxycycline (10 mg/kg) every 24 hours intravenously. An

additional blood pressure reading was taken at 10pm which showed an increase to 240 mmHg (systolic). At this point it was determined that she should receive amlodipine at 0.2 mg/kg by mouth. Additional blood pressure readings were taken at 2am, 4am, and 6am with the results as follows: 260 mmHg, 208 mmHg, and 208 mmHg. Additional amlodipine doses (0.2 mg/kg) were repeated at 2am and 4am following Crosby's blood pressure readings.

On 12/3/16, Crosby was transferred to PVSEC's Internal Medicine service for treatment of presumptive Lyme nephritis. Crosby's heart rate remained between 180-200 bpm throughout the day, and she was noted to be intermittently anxious. Her blood pressure remained within normal limits at 138 mmHg. Dexamethasone sodium phosphate was added to her treatment for immunosuppression at 0.06 mg/kg intravenously every 24 hours. Her intravenous fluids remained at 15ml/hr for diuresis with 10mg/hr of metoclopramide added for additional gastrointestinal support. Crosby experienced no further vomiting and urinated and defecated appropriately. She remained uninterested in food throughout the day but was syringe fed 6ml Clinicare every 4 hours starting at 9pm. Crosby remained stable overnight and her blood pressure remained between 138-140 mmHg during additional readings.

On 12/4/16, a renal panel was submitted. She remained anemic at 29%. All electrolytes were within normal limits. Her BUN decreased slightly to 163 mg/dl (severely elevated), and her creatinine decreased to 5.7 mg/dl (severely elevated). Her BUN/creatinine ratio was 28.5 mg/mg. She remained on all medication and fluids. Her 24 hour urine culture was negative for bacterial growth. Crosby's blood pressure was maintained at 140 mmHg. Hill's K/D was offered, but she was not interested and was syringe fed Clinicare every 4 hours. She remained comfortable and stable.

On 12/5/16, Crosby showed continued improvement in her bloodwork. Her anemia improved slightly to 33%. Her BUN continued to decrease (156 mg/dl), and her creatinine was 6 mg/dl. Her BUN/creatinine ratio decreased to 26.2 mg/mg. She was moderately hyperkalemic at 6.39 mmol/L. She continued to be fed Clinicare and refused Hill's K/D. Her blood pressure reading was 160 mmHg and amlodipine was discontinued in the morning with her blood pressure being continuously monitored. Additional readings showed that she maintained a reading of 160 mmHg over the next 24 hours. Her 48 hour urine culture was negative with no bacterial growth noted. Overnight, she was noted to have an increase in respiratory rate and effort, with lung sounds being slightly crackled. Her pulse ox on room air was measured at 90% and on 100% O2 was 97%. It was determined that she was potentially hypervolemic. Furosemide was administered intravenously once at 0.2 mg/kg for hypervolemia and hyperkalemia. She experienced no further respiratory issues.

On 12/6/16, Crosby's bloodwork revealed a continued improvement in her BUN (142 mg/dl) and stable creatinine (6 mg/dl). Her BUN/creatinine ratio was 23.6. She remained hyperphosphatemic at 16.4 mg/dl but had improved since her previous bloodwork. Her blood pressure continued to remain normal on Doppler readings at 110 mmHg. Crosby's owners determined that they were unable to afford any additional days of hospitalization; it was elected that she be discharged with a follow up appointment to perform a renal panel in 5-7 days. Crosby was sent home with mirtazapine 15 mg (1/4 tablet every 24 hours), maropitant citrate 24 mg (1/4 tablet every 24 hours), famotidine 10 mg/ml (0.25 ml every 12 hours), prednisone 3 mg/ml (0.5 ml every 24 hours), doxycycline 50 mg/ml (0.6 ml by mouth every 12 hours) and subcutaneous fluids (100 ml every 24 hours). Crosby's owners elected humane euthanasia shortly after her return home due to her poor prognosis.

REFERENCES

1. Cote, Etienne. “Borreliosis.” *Clinical Veterinary Advisor*. St. Louis, MO. Mosby 2015, 134-136. Print.

2. Chou et al. “Detection of *Borrelia burgdorferi* DNA in tissues from dogs with presumptive Lyme borreliosis.” *Journal of the American Veterinary Association* (2006). Vol 229:8. 1260-65.

3. Fritz, Curtis L. “Emerging Tick-borne Diseases.” *Veterinary Clinics of North America* (2009); Vol 39:2. 265-78

4. Fritz et al. “Lyme borreliosis.” *Journal of the American Veterinary Medical Association* (2003); Vol 223:9. 1261-70.

5. Hutton et al. “Search for *Borrelia burgdorferi* in Kidneys of Dogs with Suspected “Lyme Nephritis”.” *Journal of Veterinary Internal Medicine* (2008); 22: 860-65.

6. Krupka et al. “Lyme Borreliosis in Dogs and Cats: Background, Diagnosis, Treatment and Prevention of Infections with Borrelia burgdorferi sensu stricto.” *Veterinary Clinics of North America* (2010): Vol 40:6 1103-19.

7. Mead et al. “Canine Serology as Adjunct to Human Lyme Disease Surveillance.” *Emerging Infectious Diseases* (2011) Vol. 17:9 1710-12.

8. Littman, et al. “ACVIM Small Animal Consensus Statement on Lyme Disease in Dogs: Diagnosis, Treatment, and Prevention.” *Journal of Veterinary Internal* Medicine (2006): 422-34.

9. Littman, Meryl P. “Protein-losing Nephropathy in Small Animals.” *Veterinary Clinics of North America* (2011) Vol 41:1. 31-62.

10. Littman, Meryl P. “Canine Borreliosis.” *Veterinary Clinics of North America* (2003)Vol 3:4. 827-62.

11. Littman, Meryl P. "Lyme Nephritis." *Journal of Veterinary Emergency and Critical Care* (2013): 163-73. *Www.pubmed.gov*. Wiley-Blackwell, 5 Mar. 2013. Web. 24 Feb. 2017.

12. "Lyme Disease." *www.cdc.gov*. *Centers for Disease Control and Prevention*, 31 Dec. 2015. Web. 04 Mar. 2017.

13. Nelson, Richard W. et al. “Chapter 93 Infectious Diseases.” *Small Animal Internal Medicine*. St. Louis, MO: Mosby/Elsevier; 2009: 1296. Print

14. Stokes, Jennifer et al. “Causes of Acute Renal Failure.” *The Compendium on Continuing Education for the Practicing Veterinarian (*USA) (2006). Vol. 28:5 387-97.

15. Segey, G. and Ettinger et al. “Proteinuria.” Textbook of Veterinary Internal Medicine. St. Louis, MO: Saunders/Elsevier; 2010: 168-171. Print.

16. Sykes, Jane E. "Chapter 51 Lyme Borreliosis." *Canine and Feline Infectious Diseases*. St. Louis, MO: Elsevier/Saunders, 2014. 487-97. Print.