

Search for *Borrelia burgdorferi* in Kidneys of Dogs with Suspected “Lyme Nephritis”

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Background: “Lyme nephritis” is a poorly characterized condition associated with proteinuria and often fatal renal failure in dogs with serological evidence of infection with *Borrelia burgdorferi*.

Objective: The aim of this study was to determine if intact *B. burgdorferi* organisms were present in the kidneys of serologically Lyme-positive dogs with histopathologic features of Lyme nephritis.

Animals: Twenty-six affected and 10 control dogs were identified over an 8-year period (1996–2004) in databases at Cornell University’s College of Veterinary Medicine. Case inclusion required serologic evidence of natural exposure to *B. burgdorferi* and availability of renal tissue (frozen or paraffin embedded) exhibiting pathology consistent with Lyme nephritis.

Methods: Renal tissue samples were assessed using modified Steiner (silver) (MS) staining, immunohistochemistry (IHC), polymerase chain reaction (PCR) using 4 primer sets (eubacterial, *B. burgdorferi*, *Bartonella*, and canine genomic DNA), and fluorescence in situ hybridization (FISH) using a 5'-cy3-eubacterial probe for 16S rRNA.

Results: MS stain was positive in 1 case; IHC was negative in all cases. None of the *B. burgdorferi* or *Bartonella* PCR reactions was positive. Two of the *B. burgdorferi* FISH analyses were positive.

Conclusions and Clinical Importance: Minimal evidence of the presence of intact *B. burgdorferi* or any other bacterial organism was found in the renal tissue of dogs with suspected Lyme nephritis. Direct renal invasion by *B. burgdorferi* organisms does not appear to be responsible for this syndrome.

Key words: FISH; Glomerulonephritis; PCR; Proteinuria; Renal failure.

In recent years, much attention has been focused on *Borrelia burgdorferi* and the pathogenesis of Lyme disease in dogs. The introduction of a point-of-care ELISA for the infection-specific C6 antigen^a has made Lyme disease an increasingly common diagnosis in companion animal medicine. However, much about the clinical syndrome in dogs that are seropositive remains to be proven.

The association between *B. burgdorferi* and a syndrome of fever and arthritis has been proven via experimental infection with a tick-attachment model.^{1,2} Other studies have documented that after experimental infection of dogs, quantitative polymerase chain reaction (PCR) for *B. burgdorferi* DNA is positive in skin biopsy samples, and that spirochete numbers correlated with the presence of clinical signs of arthritis.³ However, this is the only syndrome associated with *B. burgdorferi* infection in dogs for which Koch’s postulates have been fulfilled.⁴

In several studies, an association has been made between the presence of serum antibodies to *B. burgdorferi* and the development of a unique renal syndrome, often referred to as “Lyme nephritis.”^{5–7} This syndrome is char-

acterized by persistent pathologic proteinuria and often fatal renal failure in dogs in Lyme endemic areas. The renal lesions in this syndrome are characteristic of membranoproliferative glomerulonephritis and also include tubular necrosis and interstitial inflammation. These lesions could arise as a consequence of direct renal infection with *B. burgdorferi* organisms or possibly secondary to Lyme antigen-associated immune complex deposition. It is also possible that this syndrome is unrelated to infection with *B. burgdorferi* and that the association is merely coincidental. In that case, an additional coinfecting agent occurring in Lyme endemic areas could also be a potential cause of the disease. Monoclonal antibody stains for *B. burgdorferi* antigens were positive in the renal tissue of 2 dogs afflicted with this syndrome, but it is impossible to draw general conclusions regarding etiology from these isolated case reports.^{6,7} (Although a definitive association between protein-losing nephropathy and *B. burgdorferi* infection has not been made, current ACVIM consensus recommendations dictate that proteinuric dogs in *B. burgdorferi* endemic areas be screened for evidence of natural exposure [serum antibodies], and that *B. burgdorferi* seropositive dogs also be assessed for proteinuria.⁸)

In a recent study,⁹ quantitative PCR was performed on formalin-fixed tissues from dogs that were positive or equivocal for Lyme borreliosis (based on appropriate clinical signs, serologic testing, and histopathology). Limited evidence of the presence of *B. burgdorferi* DNA was found in the renal tissue (1 dog had a positive PCR result). However, only 16% (9/58) of the dogs in this study were classified as definitively positive for Lyme borreliosis, and the only dog with a positive PCR result from renal tissue was seronegative for natural exposure to *B. burgdorferi*. The aims of this study therefore were to (1) identify a population of dogs with renal pathologic lesions consistent with the previously described syndrome of Lyme nephritis, (2) determine the Lyme status of these dogs by appropriate serologic testing, (3) employ

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contemporary molecular techniques, including PCR (on both frozen and paraffin-embedded tissues) and fluorescence in situ hybridization (FISH), to determine if intact *B. burgdorferi* organisms are present in the kidneys of dogs with Lyme nephritis, and (4) to assess for the presence of other bacterial organisms in these kidneys as possible alternative or concurrent infectious agents with eubacterial PCR and FISH as well as a specific primer set for *Bartonella* organisms.

Materials and Methods

Case Selection

The databases of the College of Veterinary Medicine at Cornell University were searched over an 8-year period (1996–2004) for case identification. In addition, cases also were submitted by referring veterinarians. Inclusion criteria were as follows: (1) diagnostic renal sample(s) in paraffin blocks or frozen with histopathology consistent with previous published reports of Lyme nephritis^{5–7} and (2) serologic evidence of natural exposure to *B. burgdorferi* (Western blot or C6 antibody test). The same databases were searched for control dogs over the same time period. Inclusion criteria for the control dogs were as follows: (1) diagnostic renal sample(s) in paraffin blocks with glomerular lesions and (2) serologic documentation of lack of natural exposure to *B. burgdorferi* (ELISA, Western blot, or C6 antibody test). All cases that met the histologic criteria for the renal tissue (initially based on existing histopathology reports) and had the results of an appropriate serologic test for *B. burgdorferi* on record were considered for inclusion for this study.

Histopathology

Cases were included if their primary histopathologic lesion was glomerular in origin and the glomerular lesion was described as inflammatory in nature. The following histopathologic diagnoses were accepted: membranoproliferative glomerulonephritis, membranous glomerulonephritis, membranous glomerulopathy, exudative glomerulonephritis, and mesangioproliferative glomerulonephritis. In 33 cases (23 affected and 10 control), the renal sections then were evaluated independently by a pathologist (BLN) in a blinded fashion (ie, without knowledge of clinical data or Lyme status). New sections were recut from the original paraffin blocks at a thickness of 4 μ m and mounted on glass slides. The sections were evaluated by routine hematoxylin-eosin stain for the presence of morphologic changes in the glomerulus, tubules, and interstitium. The pathologist reviewed the histopathology and confirmed that the biopsy results were consistent with the syndrome of Lyme nephritis according to previous published descriptions.⁵ In the 3 remaining cases, the routine histopathology could not be reviewed by our pathologist (BLN), and in these cases, we relied upon the histopathology report provided to us by the referring institution's pathologists (University of Pennsylvania) and confirmed that the report was consistent with previous published descriptions.⁵

Formalin-fixed, paraffin-embedded 4- μ m sections were placed on Probe-on slides^b and stained with modified Steiner (silver) (MS) staining and immunohistochemistry (IHC) with a rabbit polyclonal purified IgG antibody raised against *B. burgdorferi* diluted at 1 : 500 (antigen retrieval not necessary) to evaluate for the presence of spirochete organisms.^c

PCR

The presence of *B. burgdorferi* bacteria in kidney tissue (both frozen and paraffin-embedded) was analyzed. Two 10- μ m sections were

cut from existing paraffin blocks with standard methods to limit contamination (eg, wearing gloves during handling, changing the blade before cutting each block). DNA was isolated with the QIAamp DNA Purification Mini kit^d according to the kit's recommended protocol. Three separate PCR amplifications were performed on DNA collected from each sample, using 3 unique primer sets. The 1st primer set (sequences: forward 1 – GCTCAGATTGAACGCTGG, forward 2 – GCTCAGGAYGAACGCTGG, reverse – TACTGCTGCCCTCCGTA) was designed to test for the presence of eubacterial 16S ribosomal DNA.¹⁰ The samples were cycled 25 times for 30 seconds at 94°C, for 1 minute at 63°C, then for 1 minute at 72°C. The 2nd primer set was designed to test for the presence of the *OspA* gene specific to *B. burgdorferi*. Primers targeting a larger (308 bp) amplicon (sequences: forward – AATAGTCTAATATTA GCCTTAATAGC, reverse – CTAGTGTTTTGCCATCTTCTTTG AAAA)¹¹ were used on DNA collected from frozen samples, and primers targeting a smaller (107 bp) amplicon (sequences: forward – ATGTTAGCAGCCTTGACGAGA, reverse – AATTAGATCGT ACTTGCCGTC) were used on DNA collected from paraffin-embedded samples. Amplifications using the primers targeting the large amplicon cycled 30 times for 1 minute at 94°C, for 1 minute at 47°C, then for 1 minute at 72°C. The amplifications using the small-amplicon primers cycled 30 times for 1 minute at 94°C, for 1 minute at 53°C, then for 1 minute at 72°C. The 3rd primer set (forward – GGAGATACGGAATTTTCGTCGAGT, reverse – AAACAAGA CACTCCCACCCTA) targeted a 200 bp region of canine genomic DNA on chromosome 38. These amplifications cycled 30 times for 1 minute at 94°C, for 1 minute at 58°C, then for 1 minute at 72°C. DNA blanks created during the purifications as well as a blank containing nothing but master mix, primers, and H₂O were used as negative controls. The positive control consisted of DNA purified from *B. burgdorferi* bacteria grown in culture.

Products of the PCR amplifications were analyzed on a 1.5% agarose gel. For each sample, 10 μ L was mixed with 2.5 μ L loading dye and loaded onto a 1.5% agarose gel containing 0.006% ethidium bromide. An 8-cm gel was run in 1 \times Tris-Borate EDTA buffer at 90 V for 1.5 hours. Two microliters of O'GeneRuler 100 bp DNA Ladder Plus^c was loaded onto 1 lane for each gel to serve as the ladder. Gels were photographed under UV illumination with a Kodak DC290 digital camera.^f Strong positive PCR product bands were extracted from the gel and cloned using commercially available kits^d and then were referred for sequencing at the Cornell University Biotechnology Resource Center for sequencing using the Applied Biosystems Automated 3730 DNA Analyzer.^g

Bartonella PCR was performed by a commercially available veterinary referral laboratory.^h

FISH

FISH were performed on paraffin-embedded kidney sections. Four-micron-thick sections were cut from the original paraffin blocks and placed on ProbeOn slides.^b Slides were deparaffinized and dried, and then covered with 30 μ L of hybridization solution consisting of 0.9 M NaOH, 20 mM Tris, pH 7.4 with 0.1% sodium dodecyl sulfate and 20% formamide (w/v), 150 ng of Cy3 labeled EUB338 probe, and 225 ng of Cy5 labeled Borr-4 probe.¹² Slides were incubated overnight at 46°C in 50 mL conical tubes containing paper soaked in the same hybridization solution. They were then rinsed in wash solution consisting of 0.9 M NaOH, 20 mM Tris, pH 7.4 and incubated 10 minutes at 48°C in the same wash solution. Slides were then rinsed with deionized H₂O and air dried 1 hour at 46°C. Slides were mounted using 4',6-diamidino-2-phenylindole (DAPI)-containing mounting medium and sealed around the edges with clear nail polish.

An Olympus BX51 fluorescent microscopeⁱ equipped with an EXFO X-Cite 120 light source and an Olympus DP70 digital cameraⁱ was used to view the slides. Olympus filter set UMNU2ⁱ was

used to detect DAPI signals, and Chroma Technology^j filter sets 31001 and 41007A with narrow band excitation filter were used to detect 5-FAM and Cy3 signals, respectively. To detect the presence of bacteria, each slide was scanned for Cy3 fluorescence for 15 minutes at 400× magnification. If a slide was too small to be scanned for 15 minutes, the time spent scanning the slide was recorded. Signals were scored as bacteria if they resembled known bacterial forms and showed Cy-3 and DAPI fluorescence without background fluorescence at the 5-FAM spectrum. Signals were further scored as representing *B. burgdorferi* if they resembled the *B. burgdorferi* positive control sample, showed fluorescence for Cy-5, Cy-3, and DAPI, and did not fluoresce at the 5-FAM spectrum. Bacterial controls (slides spotted with suspensions of cultured bacteria including *Escherichia coli* DH5, *Shigella sonnei* [ATCC 25931], and *Salmonella enterica* serovar Typhimurium [ATCC 14028], as well as a section from a natural *Leptospira pomona* kidney invasion, and a section of muscle injected with a live *B. burgdorferi* culture) were first used to confirm Bor-4 probe specificity in preliminary experiments. Bacterial controls run alongside test sections consisted of an *E. coli* DH5 culture slide, a section from a natural *L. pomona* kidney invasion, and a section of muscle injected with a live *B. burgdorferi* culture.

Statistical Analysis

Ages of dogs in the affected and control groups were compared using the two-tailed nonparametric Mann-Whitney *U* test.^k

Results

Signalment Data

Twenty-six affected dogs matched the selection criteria described, with 10 control dogs identified. In the affected group, 12 dogs were deemed to be positive for serologic evidence of natural exposure to *B. burgdorferi* using a point-of-care ELISA for the infection-specific C6 antigen^a or a quantitative C6 antibody ELISA (10 dogs and 2 dogs, respectively), and the remaining 14 dogs were deemed to be positive using a Lyme Western blot. Because the Western blot also detects vaccinal antibody, 2 affected dogs also were identified to be positive for antibodies to vaccination against *B. burgdorferi*. In the control group, 9 dogs were deemed to be negative for serologic evidence of natural exposure to *B. burgdorferi* with a Lyme disease ELISA (which detects both natural exposure and antibodies to vaccination) and 1 dog was deemed to be negative for evidence of natural exposure to, but positive for antibodies to vaccination against, *B. burgdorferi* with a Lyme Western blot.

The median age of the affected dogs was 5.5 years (range 2–12 years), and the median age of the control dogs was 7.5 years (range 1–13 years). The difference in ages between these two groups was not statistically significant ($P = .26$). Within the affected population, there were 5 female intact (FI) dogs (19%), 14 female spayed (FS) dogs (54%), 5 male intact (MI) dogs (19%), and 2 male castrated (MC) dogs (8%). Within the control population, there was 1 FI dog (10%), 4 FS dogs (40%), 2 MI dogs (20%), and 3 MC dogs (30%). Overall, 7 pure breeds were represented in the affected population and 8 pure breeds were represented in the control population; both groups also contained mixed breed dogs. Labrador Retrievers represented 46% (12/26) of the affected pop-

ulation compared with 10% (1/10) of the control population. No affected dogs were known to be serologically positive to other infectious agents, and all affected dogs' histories and course of disease matched that of the previously described syndrome of Lyme nephritis.^{5–7} The vaccination history (including Lyme vaccination) was unknown for most dogs.

MS and IHC Staining

MS and IHC staining was performed in 26 cases, including 20 affected cases and 6 control cases. The remaining 10 cases (6 affected and 4 control) did not have paraffin-embedded tissue available to perform these staining techniques. MS staining was considered to be positive in 1 case from the control population. In this case, organisms were seen in 1 tubule, and the morphology was judged to be consistent with *Borrelia* sp. In a single case from the affected population, organisms were seen within the lumina of a few tubules, but the morphology was compatible with *Leptospira* sp., not *Borrelia* sp. The remaining cases were negative for the presence of organisms with MS. IHC for *B. burgdorferi* organisms was negative in all cases assessed.

PCR

PCR was performed in all 36 cases, including 23 paraffin-embedded samples (13 affected samples and 10 control samples) and 13 frozen samples (all affected cases). All of the kidney samples (both affected and controls) were negative for *B. burgdorferi* or *Bartonella* DNA using PCR. Among affected cases, 85% (22/26) of the samples were positive for eubacterial DNA; 58% (15/26) of them were weakly positive, and 27% (7/26) were strongly positive (as judged by subjective assessment of the strength of the PCR product band). Among control cases, 90% (9/10) were positive for eubacterial DNA; 80% (8/10) were weakly positive and 10% (1/10) were strongly positive. Three of the strongest eubacterial bands from affected dogs were cloned and sequenced, and no single organism was identified in all of the cases. The identity of the bacterial species included *Weissella viridescens*, *S. sonnei*, *Clostridium perfringens*, and *E. coli* (the last 2 species both being isolated from the same case).

FISH

FISH was performed in 31 cases, including 21 affected cases and 10 control cases. FISH could not be performed in the remaining 5 affected cases because of the lack of available paraffin-embedded tissue for testing. Among affected cases, 10% (2/21) of the kidney samples were positive for spirochete organisms consistent with *B. burgdorferi*. In each of these 2 cases, a single organism was identified in the lumen of a tubule. FISH was negative for *B. burgdorferi* in all control cases. Among affected cases, 48% (10/21) of the kidney samples were positive for eubacterial organisms (including the 2 positive for *B. burgdorferi*); 5% of these cases (1/21) were strongly positive for eubacterial organisms (≥ 100 bacteria identi-

fied). Among control cases, 50% (5/10) were positive for eubacterial organisms, including 20% (2/10) that were strongly positive for organisms. These cases included interstitial as well as glomerular bacterial colonization. An additional 5 samples (4 affected cases and 1 control) had bacteria located on the external surface of the biopsies as identified by eubacterial FISH, thus representing presumptive postbiopsy collection contamination rather than in vivo bacterial colonization.

Four of the samples (3 affected cases and 1 control) that were positive on eubacterial FISH were negative for eubacterial PCR, including one of the samples strongly positive using eubacterial FISH. One of the strongly positive eubacterial PCR results (in an affected case) was negative using eubacterial FISH, and an additional 15 weakly positive eubacterial PCR results were also negative using eubacterial FISH (10 affected cases and 5 control cases). This includes the 5 samples noted previously that had bacteria identified on the external surface of the biopsies using eubacterial FISH (all 5 were deemed positive on PCR). In 66% (20/31) of cases in which both PCR and FISH were performed on the same sample, eubacterial results revealed a discrepancy (positive using one of the techniques but negative using the other technique); this includes 66% (14/21) of affected cases and 60% (6/10) of control cases.

Discussion

Utilizing advanced molecular diagnostic techniques, we found minimal to no evidence of intact *B. burgdorferi* organisms in the kidneys of dogs with serology and histopathology consistent with previously published reports of Lyme nephritis.⁵⁻⁷ IHC and PCR were negative in all cases assessed, and FISH was positive for the presence of a single *B. burgdorferi* organism in only 2 cases. Because visualization of multiple organisms in a single tissue sample is usually required to make a definitive diagnosis of an infectious disease, the significance of these single bacteria in the development of renal lesions is unclear. Although the MS stain was positive for organisms with morphology consistent with *Borrelia* sp. in 1 case, this case actually was a member of the control (Lyme-negative) population. Therefore, the accuracy of interpretation of MS staining in these cases must be questioned.

Interestingly, our eubacterial PCR and FISH assays were frequently positive, indicating that these tests are fairly sensitive for the general detection of bacterial organisms within renal tissue samples. Sequencing of three of these positive bacterial samples revealed no common species among eubacterial positive cases, indicating that no unifying bacterial cause can be identified in association with the histologic lesion. In addition, the distribution of these bacteria within the renal tissue was variable when analyzed by FISH, which also argues against a common bacterial pathogenesis. Ideally, we would also have tested these dogs for other infectious agents with serology, but only renal tissue samples were available from these patients, not blood samples, and many cases were submitted by referring veterinarians, limiting our access to historical information. However,

eubacterial PCR and FISH should detect the presence of most of these agents, including *Leptospira* sp. and rickettsial agents, and these agents were not definitively identified using these techniques.

The renal samples also were assayed for *Bartonella*, because eubacterial PCR will not always be positive in cases of Bartonellosis (Breitschwerdt, personal communication), and because *Bartonella* also is suspected to be transmitted by the bite of an infected tick.¹³ *Ixodes* ticks coinfecting with *B. burgdorferi* and *Bartonella* sp. have been identified in The Netherlands,¹⁴ and coinfection cannot be ruled out in the current patient population. Bartonellosis has been suggested as being associated with glomerulonephritis in dogs.¹⁵ Inasmuch as *Bartonella* DNA was not identified using a highly sensitive PCR technique, it is unlikely that the renal lesions in these cases were a result of direct renal invasion by *Bartonella* organisms.

One of the limitations of this study is the lack of a 2nd control population consisting of dogs that are Lyme positive and have normal renal histology. We suspect that this population was not successfully identified because animals with normal kidney function are unlikely to undergo renal biopsy, animals with other forms of Lyme disease (eg, polyarthritis) are unlikely to undergo euthanasia and necropsy, and patients presented for necropsy for evaluation of other (nonrenal) conditions are unlikely to have had Lyme serology performed. The addition of this control population would have proven more essential had we identified abundant *B. burgdorferi* organisms within the renal parenchyma of the Lyme positive dogs, in order to prove that the presence of the organisms within the kidney was associated with the development of the renal lesion (and not simply because *B. burgdorferi* organisms are present in the kidneys of all Lyme positive dogs). Because limited evidence of *B. burgdorferi* organisms was found in the sample population, this 2nd control population was not essential to the current study.

Another limitation of this study was that, because of its retrospective nature, we did not have control over the size and quality of the biopsy specimens, or over sample handling. Consequently, some of the biopsies were quite small in size. This precluded us from having enough sample material to perform MS and IHC staining in several cases.

Additionally, because the samples were probably not handled in a sterile fashion when originally acquired, many of our samples were most likely contaminated with bacteria, resulting in some of the positive eubacterial PCR and FISH results. This is corroborated by the fact that 5 cases had a few bacteria identified only on the external surface of the samples via FISH; all of these cases were positive for bacteria via PCR. It is plausible that many (or even all) of the 12 additional cases that were eubacterial positive via PCR but negative with FISH could have been positive because of bacterial contamination during sample handling or processing (because peripheral bacterial contamination may not be identified by FISH), leading to the discrepancies between many PCR and FISH results. For the 4 cases that were eubacterial positive via FISH but negative with PCR, it is

possible that some of this discrepancy was caused by low bacterial numbers, because in 3 of 4 cases, only a small number of bacteria were identified (including a single bacterium in 1 case and 2 bacteria in another case). These bacterial numbers may have been too low to be detected with standard traditional PCR methodology and possibly would have been identified using more sensitive quantitative real-time PCR. Finally, the samples of renal tissue that were obtained for PCR and FISH in each individual case were not always taken from the same location within the biopsy specimen. In some cases, the samples may have even been obtained from different kidneys (in the case of postmortem samples). Because the distribution of eubacteria within an individual kidney sample may have been focal or multifocal in nature, sampling from 2 distinct locations for PCR and FISH analyses may have led to discrepancies between these results in individual cases. Ideally, samples for both of these tests should have been taken from the same location within the renal biopsies.

Several cases were strongly positive for the presence of eubacteria via PCR, FISH, or both, which suggests that a secondary eubacterial infection could have been present in these cases. In the majority of these cases (except 1 case, in which the presence of a neutrophilic pyelitis and ureteritis was noted), routine histopathology did not reveal changes that would have raised suspicion of a concurrent bacterial infection. Ideally, renal biopsy specimens should be handled aseptically, and bacterial culture should be performed on portions of the tissue samples to identify patients with occult secondary pyelonephritis. Regarding the bacteria that were sequenced in the current study, however, three of the species are either unknown to be (*W. viridescens* and *S. sonnei*, though the latter has been documented by isolated case reports in humans¹⁶) or are rarely (*C. perfringens*¹⁷) urinary tract pathogens in dogs, making a diagnosis of bacterial pyelonephritis less likely in these cases. Additionally, future studies may allow us to evaluate the application of these techniques (PCR and FISH) for other naturally occurring renal diseases.

Our breed results most likely were skewed by the fact that three of the Lyme positive cases were submitted from a guiding-eye dog facility in New York State¹ that utilizes Labrador Retrievers exclusively. If these 3 dogs are removed from the breed analysis, Labrador Retrievers represented 39% (9/23) of the remaining affected population. However, we cannot comment as to whether this represents a significantly increased percentage as compared with the control population, because we do not have data regarding the prevalence of this breed in the overall hospital population during this time period, and because this study included a number of cases contributed by referring veterinarians.

The results of our PCR analysis are consistent with what has been reported recently in a study performing *B. burgdorferi* PCR on renal tissues from dogs.⁹ It is notable that all of the affected cases in the current study were known to be seropositive for natural exposure to *B. burgdorferi*, compared with only 45% of cases that were classified as either positive or equivocal for Lyme borreliosis in the previous study. Additionally, only 55% of

the dogs in the previous study were characterized as having glomerulonephritis, as compared with 100% in the current study. Of the affected cases in this study, PCR was performed on fresh-frozen tissue in 50% of cases compared with 100% on paraffin-embedded tissues in the previous study. *B. burgdorferi* PCR is known to be more sensitive when performed on fresh tissue.¹⁸ Despite the increased sensitivity of PCR in many of the cases in this study because of the use of fresh frozen tissue, *B. burgdorferi* PCR was negative in all cases assessed. The current study also provides the added information of analyzing the tissues via FISH, which has not been previously reported, and by screening for the presence of coinfection via eubacterial PCR and FISH, as well as *Bartonella* PCR.

In conclusion, the use of sensitive molecular techniques (PCR and FISH) failed to consistently identify the presence of intact *B. burgdorferi* or any other bacteria in the renal tissue of dogs with suspected Lyme nephritis. Direct renal invasion by *B. burgdorferi* organisms does not appear to be responsible for this syndrome. Lyme nephritis could be unrelated to infection with *B. burgdorferi*, or the association may be coincidental. In that case, an additional coinfecting agent occurring in Lyme endemic areas is another potential etiology for the disease. However, we failed to consistently identify the presence of a coinfecting bacterial agent in these cases despite sensitive detection techniques. Instead, we propose that this syndrome may occur secondary to Lyme antigen-associated immune complex deposition in the glomeruli of these dogs, leading to a postinfectious glomerulonephritis.¹⁹ Recent work has demonstrated the presence of *B. burgdorferi*-specific circulating immune complexes (CICs) within the serum of Lyme infected dogs, and the concentration of *B. burgdorferi*-specific CICs appears to be increased in dogs with clinical signs of Lyme disease (as compared with asymptomatic seropositive dogs).^m Additional studies should include *B. burgdorferi* antigen-associated immune complex identification in the renal tissue of dogs with Lyme nephritis to make a stronger argument for the immune complex basis of this syndrome.

Footnotes

^a SNAP-3Dx heartworm, *Ehrlichia canis*, and *Borrelia burgdorferi* test, IDEXX Laboratories Inc, Westbrook, ME

^b Fisher Scientific, Pittsburgh, PA

^c BIODESIGN International, Saco, ME; detection performed using a goat anti-rabbit kit from Zymed Laboratories Inc, San Francisco, CA

^d QIAGEN Sciences, Germantown, MD

^e Fermentas International Inc, Burlington, ON, Canada

^f Eastman Kodak Company, Rochester, NY

^g Applied Biosystems, Foster City, CA

^h Tick Borne Diagnostic Laboratory, North Carolina State University, College of Veterinary Medicine, Raleigh, NC

ⁱ Olympus America Inc, Center Valley, PA

^j Chroma Technology Corporation, Rockingham, VT

^k GraphPad Prism Version 4, GraphPad Software Inc, San Diego, CA

^lGuiding Eyes for the Blind, Yorktown Heights, NY

^mGoldstein RE, Atwater DZ. Evaluation of serology and circulating immune complexes in dogs naturally infected with *Borrelia burgdorferi*. *J Vet Intern Med* 2006;20:713 (abstract)

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