PROTEIN-LOSING NEPHROPATHY ASSOCIATED WITH *LEPTOSPIROSIS* IN A GREAT DANE: RESPONSE TO THERAPY

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A 1.5-year-old, female Great dane was presented to the University Veterinary Hospital Kokkalai in August 2017 for investigation of undefined enlargement of abdomen, decreased appetite, and weakness reported to be of 2 wk duration. Initial diagnostic work-up included a complete blood (cell) count (CBC), serum biochemistry profile, urinalysis and abdominal ultrasound revealed protein-losing nephropathy, hypoalbuminemia, hyperglobulinemia, and positive for leptospira by polymerase chain reaction. Structural and functional alterations of glomeruli result in increased protein leakage across the filtration membrane and overt renal proteinuria. The presence of proteinuria and hypoalbuminemia without hypoglobulinemia, in association with a negative urine culture and without concurrent liver dysfunction or gastrointestinal disease is consistent with PLN. Urine protein:urinecreatinine ratios > 2 are most compatible with glomerular disease. Following doxycycline therapy along with supportive treatment the urine protein loss decreased significantly and serum albumin concentration regained the reference value.

Keywords: Leptospirosis, Nephropathy, Protein-losing, Hypoalbuminemia Urine protein: urine creatinine ratios.

Protein-losing nephropathy (PLN) is a condition of glomerular protein loss resulting from many different causes. Structural and functional alterations of glomeruli result in increased protein leakage across the filtration membrane and overt renal proteinuria. Protein-losing nephropathy associated with leptospirosis is reported in dogs and typically progresses rapidly to renal failure. (Harley & Langston, 2012). Hypoxia secondary to renal ischemia may be the fundamental alteration causing nephropathy in patients with leptospirosis. (Wohl, 1996).Significant improvement of the PLN and serum albumin concentration was achieved following doxycycline therapy (Mcclainet al, 1984). The presence of proteinuria and hypoalbuminemia without hypoglobulinemia, in association with a negative urine culture and without concurrent liver dysfunction or gastrointestinal disease is consistent with PLN (Littman et al., 2000). Urine protein:urinecreatinine ratios > 2 are most compatible with glomerular disease (Harley & Langston, 2012. The lack of significant blood or WBCs in the urine is also supportive of glomerular protein loss

Major infectious agents causing protein losing kidney diseases includes Brucellosis, Ehrlichiosis, Leptospirosis, Borelliosis, chronic bacterial infections, and heartworm disease. The kidney is one of the principal target organs of Leptospira and are considered as one of the cause of protein losing nephropathy in animals (Lulich et al., 1996) by causing renal interstitial or parenchymal disease to result in proteinuria. Proteinuria associated with leptospirosis is often caused by tubular damage or vasculitis (Sykes et al., 2010).

This case report describes a 1.5-year-old female Great danethat was presented with PLN, marked hypoalbuminemia, and positive for leptospira.

Case History and Observation

A 1.5-year-old, female Great Dane was presented to the University Veterinary Hospital Kokkalai in August 2017 for investigation of undefined enlargement of abdomen, decreased appetite, and weakness reported to be of 2 week duration. Routine vaccination and deworming were up-to-date (Rabies, Parvo and Canine distemper). Physical examination showed that the dog had a dull, thin hair coat, distended abdomen. Temperature and mucous membrane, blood

smear and fecal sample couldn't reveal any abnormality.

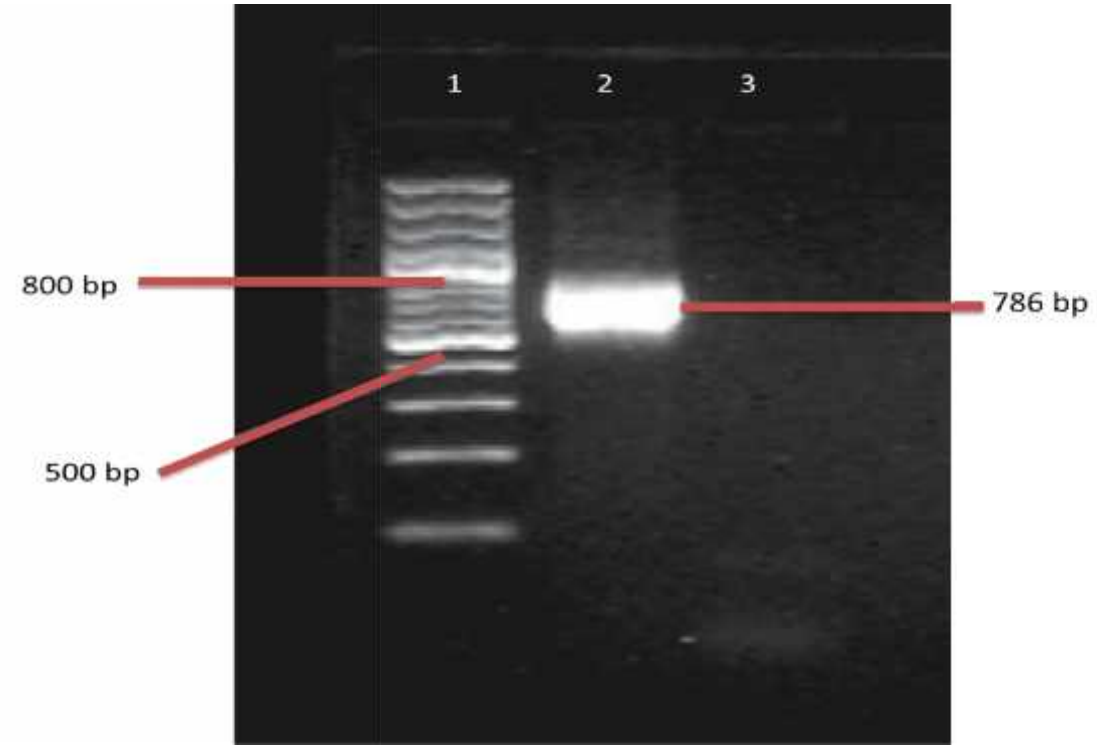
Initial diagnostic work-up included a complete blood (cell) count (CBC), serum biochemistry profile, and abdominal ultrasound. The CBC, serum biochemistry, and ultrasound were performed in the University Laboratory Kokkalai. CBC was almost normal except slight increase in the granulocytes (78%). The serum biochemistry showed a decrease in total protein concentration [3.376 g/dL; reference interval (RI): 5.9 - 7.8 g/dL], hypoalbuminemia at 1.037 g/L (RI: 2.3–3.1g/dL), and a slight hyperglobulinemia at 2.4 g/L (RI: 2.7 to 4.4 g/dL). Abdominal ultrasound was unremarkable except a mild enlargement of liver and presence of ascitic fluid in the peritoneal cavity. Kidney was normal in size with clearcortico-medullary differentiation. Fine-needle aspirates (FNA) of the ascitic fluid were collected, which were slight yellowish clear fluid and used for culture and sensitivity with yielded negative growth.A symptomatic treatment with Lasix 10 ml (frusemide 2.5 mg/kg), inj. Astymine20 ml, InjAmoxirum forte 300mg and injpantocid 40 mg (pantoprazole 1mg/kg) was strated. Dog returned next day without any clinical improvement. Urine was collected by catheterization and send for protein, creatinine and creatinine protein ratio. Urine protein-to-urine creatinine ratio (UPC) was increased at 6.32 [reference limit (RL):< 0.5] with protein and creatinine were 215 mg/dl and 34 mg/dL, respectively. A possible breed-related PLN was suspected although other infectious or immune causes of PLN were considered. Tab envas 10 mg (enlapril 0.5 mg/kg) was added, the other medications were continued and serological evaluation for other infectious disease affecting kidney was recommended in light of the combined history of ascites and current proteinuria. A serum sample was collected for microscopic agglutination test (MAT at Microbiology department) and polymerase chain reaction for the detection of possible incidence of leptospirosis. The MAT revealed presence of agglutinating antibody of *Leptospirainterrogans*serovarIcterohaemorrhagia(titer 1:800) which were confirmed by PCR by using specific primers for pathogenic leptospira. Doxycycline (Dr.Doxy 300mg) 10 mg/kg BW, PO, q24h for 2 wk was added to the therapy along with lacilactone 2 tab per day for 5 days.

The dog returned periodically over the next 4 month for re-evaluation. At 2 wk, the asciteshad resolved and the UPC was improved at 3.41 (RL: < 0.5) although serum albumin (1.641 g/L) and globulin (1.15 g/L) concentrations had not improved. Commercial renal diet and omega-3 fatty acid supplementation were recommended. In October of 2017 (2 mo following initiation of doxycycline treatment), the dog was presented for re-evaluation. Serum biochemistry findings showed improvement in serum albumin and globulin concentrations (2.4 g/dL and 4.882 g/dL, respectively) but these values were still outside the RIs. The CBC was within the RI; the UPC was 2.1 (RL: < 0.5) and aerobic urine culture was again negative. The dog was improved clinically, with improved hair coat, activity level, and appetite.

Discussion

The tests used in this case are microscopic agglutination test (MAT) and Polymerase chain reaction which was widely used method for diagnosing leptospirosis.The MAT is considered the reference immunological test, and detects both immunoglobulin M (IgM) and immunoglobulin G (IgG) class agglutinating antibodies as also mentioned by Niloofa *et al.*, (2015).

Current recommendations are to treat with doxycycline (5 mg/kg/day, IV or PO) for 2 wk. For dogs that cannot tolerate doxycycline, initial therapy with penicillin is appropriate, but this should be followed by a 2-wk course of doxycycline to eliminate the renal carrier phase of infection. Dogs recently exposed to leptospirosis may be treated prophylactically with oral doxycycline for 14 days as also reported by Aiello *et al.*, (2016).



Submerged gel electrophoresis of PCR amplified DNA using primers (Haake et al., 2000) for LipL 32. Lane 1: 1 kb ladder, .Lane 2: sample, .Lane 3: negative control

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