Theses of doctoral (PhD) dissertation

Novel insights into canine proteinuria

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1. Introduction and Objectives

A small amount of protein in the urine of cats and dogs is physiologic. Proteinuria means abnormal loss of proteins in the urine. Persistent proteinuria is defined as an abnormal magnitude of proteins in the urine detected on three or more occasions, two or more weeks apart. Persistent proteinuria found with an inactive urine sediment is generally consistent with renal damage. Proteinuria has high clinical importance. Many studies have proved that proteinuria is associated with shorter survival times, not only in humans but also in cats and dogs. In people, the magnitude of protein loss in the urine is associated with the progression of chronic kidney disease (CKD), and it is a prognostic factor in diabetic nephropathy and some cardiac diseases. Medical reduction of proteinuria prolongs the survival of people, dogs, and cats.

Albuminuria means abnormal loss of albumin in the urine. In people, albuminuria is considered the most important marker of renal damage and an essential predictor of CKD progression into dialysis-dependent kidney failure. Apart from kidney diseases, urinary albumin excretion is a marker of generalized vascular

dysfunction and a risk marker of cardiovascular events (e.g., heart attack, stroke). In human medicine, albuminuria is a negative prognostic factor; its presence and magnitude are associated with increased cardiovascular and all-cause mortality, too.

In veterinary medicine, many studies found that albuminuria can precede proteinuria and thus help diagnose renal damage early. Albuminuria negatively correlates with survival in cats with CKD and critically ill dogs. The measurement of albuminuria is suggested as a screening test for early renal damage in dogs predisposed or suspected to have renal disease (e.g., hereditary nephropathies) and who have hypertension or systemic diseases leading to proteinuria. Although the importance of albuminuria has become apparent recently, the reference interval for albuminuria in dogs has not yet been established. Because of this, previous studies examining albuminuria in different conditions compared urinary albumin values of the diseased animals to healthy control groups. These control groups consisted of 10-40 individuals. To define a reliable reference limit, at least 120 individuals are required.

In dogs, albuminuria has already been detected in many different illnesses: familial glomerulopathies,

leishmaniasis, diabetes mellitus, pituitary-dependent hyperadrenocorticism, CKD, hypertension, critical diseases, systemic inflammatory response syndrome, lymphoma, osteosarcoma. Many studies described membranoproliferative glomerulonephritis in dogs with spontaneous and experimentally induced *Dirofilaria immitis* infection. As a consequence, albuminuria and proteinuria are commonly associated with heartworm disease. In Hungary, the prevalence of *Dirofilaria repens* is higher than *D. immitis*. No studies have examined the possible glomerular damage caused by *D.repens*.

Our first study aimed to establish a reference interval for albuminuria in more than 120 healthy dogs. Our second goal was to define breed-specific reference intervals in case we found significant differences between the albuminuria of different breeds. Sighthounds were a point of interest because they are known to have unique reference values, and greyhounds were previously shown to excrete more albumin than other breeds. Breed-specific reference intervals for Beagle dogs could be helpful because of their frequent use as laboratory animals.

Our second study aimed to investigate whether D. repens is capable of causing similar glomerular lesions as heartworm infection and, thus, whether D. repens

infected dogs have a higher magnitude of proteinuria or albuminuria than noninfected dogs kept under the same circumstances. Our study also aimed to explore whether the magnitude of proteinuria or albuminuria would decrease after topical moxidectin treatment. In addition to our study's primary and secondary goals, we compared some other laboratory variables (hematology, serum urea, creatinine, urine specific gravity) between the infected and noninfected dogs.

2. Materials and Methods

In the first study, one hundred sixty-four clinically healthy dogs were enrolled. The study included two population groups of dogs. The first group consisted of clinically healthy, client-owned dogs from various breeds and sexes, from 1 to 12 years of age. Dogs visiting the Small Animal Clinic of the University of Veterinary Medicine Budapest for their yearly routine health checks were enrolled. All owners signed a consent form — approved by the National Scientific Ethical Committee on Animal Experimentation — before participation.

The second group included clinically healthy laboratory beagle dogs of both sexes, from 1 to 12 years of age. The beagles were used as healthy controls in another study without receiving any treatment or interventions. The study was approved by the Government Office of Pest County, Department of Food Safety, Animal-, Plant- and Soil Protection (approval No. PEI/001/1708-4/2015). These dogs' health checks (physical examination, blood, and urine tests) were part of the original study; leftover urine samples were used to measure UAC.

Health status was assessed with histories, physical examinations, hematology, serum biochemistry tests,

and urinalyses. Dogs with abnormalities on the physical examinations or the ones that were microfilaremic or had significant hematologic or biochemical alterations or changes in any of the inflammatory variables (eosinophil/lymphocyte/neutrophil cell count, C-reactive protein, globulins) were excluded. Dogs with proteinuria (UPC ≥0.2), pyuria, or hematuria were also excluded from the study.

Urine samples were taken either by cystocentesis or by the free-catch method. Urinary albumin was determined by the immunoturbidimetric method, and albumin excretion was expressed as the urinary albumin-to-creatinine (UAC) ratio. As healthy dogs may have UAC values close to zero (or even zero itself), the lower reference limit is irrelevant. Hence, we estimated the 95% upper reference limit (that excludes 5% of the reference population) by the nonparametric method, together with its 90% nonparametric confidence interval. As reference individuals were selected randomly from well-defined populations and experienced veterinarians confidently established their health, all reference values were retained, and no outliers were excluded. The results of Sighthounds and Beagle dogs were analyzed as subgroups as well. The results of the subgroups were compared with the Kruskal-Wallis test. The UAC results

of free-catch and cystocentesis samples were compared with the two-sample t-test. The dependence of UAC on age was evaluated by Spearman's rank correlation.

Our second examination was a cross-sectional designed study. Sixty-five 1 to 10 years old clinically healthy laboratory beagle dogs were recruited. Dogs were tested for *D. repens* infection and were grouped as "infected" or " control" dogs. Dogs with abnormalities on the physical examination or those with elevated C-reactive protein (CRP) concentration, white blood cell count (WBC), abnormal renal function, and those with significant hematologic or biochemical alterations were excluded. We also excluded dogs suspected of *D. immitis*, *Ehrlichia canis*, *Anaplasma phagocytophylum*, or *Borrelia sensu lato* infection. Dogs with pyuria or hematuria and dogs with positive urine culture examination results were excluded from the study. Modified Knott's test was performed to detect *D. immitis* and *D. repens* microfilariae. For the detection of *D. immitis* antigens, we used two different tests for all dogs. A PCR test was run for dogs with a positive Knott’s test to differentiate between *D. repens* and *D. immitis* infection. Dogs were considered as "*D. repens* infected" if they were microfilaremic, and their PCR test verified *D. repens* infection, but not *D.immitis* infection, and both of

their *D. immitis* antigen tests were negative. Dogs without microfilaremia and with two negative *D. immitis* antigen tests were used as "control" dogs. Urine samples were taken by cystocentesis. A complete urinalysis was conducted, including microscopic evaluation of the urine sediment, the measurement of urinary protein-to-creatinin ratio (UPC) and UAC, and urine culture examination.

For those dogs where follow-up was feasible, we repeated the measures of UAC and UPC after one or two doses of moxidectin treatment.

Our primary null hypothesis was that *D. repens* infected dogs have the same magnitude of proteinuria and albuminuria as non-infected dogs kept under the same circumstances. The Mann-Whitney U test was used to compare the distributions of the two groups in these respects. The null hypothesis for the secondary outcome of our study was that the magnitude of protein or albuminuria would not change after eliminating *D. repens* infection. The Wilcoxon Signed Rank Test was performed to test this hypothesis. Welch’s two-sample T-test compared other variables between the infected and non-infected dogs with normally distributed data. The Mann-Whitney U test was used when the variables were not normally distributed.

3. New Scientific Results

1. Our study was the first to establish a reference interval of urinary albumin-to-creatinine ratio (UAC) in dogs based on the measurement of UAC in 124 healthy dogs, providing a solid foundation for future research and clinical practice:

   The reference interval for canine UAC is 0 – 19 mg/g. The human reference interval is somewhat higher: 0 – 30 mg/g, but very similar to the upper limit 90% confidence interval of canine UAC 13 – 28 mg/g, found in this study.

2. Breed, age, sex, body weight or collection method does not seem to influence canine UAC values:

   The results of Sighthounds (n=30) and Beagle dogs (n=23) were also analyzed as subgroups. We found no significant differences in the UAC values between Beagles, Sighthounds, and the rest of the dogs. In contrast to previous studies, the UAC did not show a significant association with the age of the animals. For detecting albuminuria, clinicians can decide between free-catch and cystocentesis methods based on which method is technically more accessible, safer, and more comfortable for the animal.

3. *D. repens* infected dogs excrete a higher amount of albumin in the urine than non-infected dogs:

   Albuminuria (UAC >19 mg/g) was detected in 35% of the dogs in the D. repens infected group, while only 12% had albuminuria in the control group. Although there were more overt proteinuric dogs (UPC >0.5) in the infected group (23%) than in control (6%), the UPC values did not significantly differ between the infected and control groups. Albuminuria raises the suspicion of early glomerular damage caused by D. repens infection, similarly to D. immitis.

4. *D. repens* infection causes increased eosinophil granulocyte count and platelet count, while it does not seem to cause azotemia or decreased urine specific gravity:

   Eosinophilia was present in 39%, and thrombocytosis in 42% of the D. repens infected dogs (vs. 12% and 12% in the control group). Our findings of physiologic serum creatinine and urea values and urine specific gravity do not exclude glomerular lesions, as glomerular diseases are often present in non-azotaemic dogs with acceptable urine-concentrating ability.

4. Scientific publications

Publications in peer-reviewed journals related to the thesis

Falus F.A., Szabó K. É., Becker Zs., Müller L., Fok É., Balogh N., Manczur F. 2023. Albuminuria and proteinuria in dogs infected with Dirofilaria repens: A cross-sectional study. Journal of Veterinary Internal Medicine, 37(3), 992-997.

Falus F.A., Vizi Zs. Szabó K.É., Müller L., Reiczigel J., Balogh N., Manczur F. 2022. Establishment of a reference interval for urinary albumin-to-creatinine ratio in dogs. Veterinary Clinical Pathology, 51(4), 585–590.

Falus F. A., Manczur F. 2018. Kutyák és macskák proteinuriája II. rész: Kutyák és macskák proteinuriájának gyógykezelése: Irodalmi összefoglaló. Proteinuria in dogs and cats Part 2. Treatment of proteinuria in dogs and cats. Literature review. Hungarian Veterinary Journal, 140 (3), 135-149.

Falus F.A., Székely D. Manczur F. 2017. Kutyák és macskák proteinuriája: Irodalmi összefoglaló I. rész. Proteinuria in dogs and cats Part 1. Literature review. Hungarian Veterinary Journal, 139(2) 89-100.

Scientific posters, presentations

Falus F. A., Vizi Zs., Török B., Manczur F. 2020. Evaluation of the diagnostic value of urinary albumin to protein ratio in proteinuric dogs. Research Communications of the 29th ECVIM-CA Congress, Journal of Veterinary Internal Medicine 34 (1).

Manczur F., **Falus F. A.**, Kubik N., Müller L., Vizi Zs.; Sterczer Á., Rabnecz Gy. 2018. Microalbuminuria in dogs infected with Dirofilaria repens. Research Communications of the 27th ECVIM-CA Congress, Journal of Veterinary Internal Medicine 32 (1) 531.

Manczur F., **Falus F. A.**, Vizi Zs. 2017. Microalbuminuria in healthy dogs. Research Communications of the 26th ECVIM-CA Congress, Journal of Veterinary Internal Medicine 31 (1) 253.

Falus F. A., Manczur F. 2017. Az albuminuria szerepe kutyák vesebetegségeiben. MTA Áorv. Tud. Bizottsága, Akadémiai Beszámoló, Klinikumok Szekció, 2016, Budapest.

Falus F. A., Manczur F. 2016. A Dirofilaria repens potenciális vesekárosító hatásának vizsgálata. MTA Áorv. Tud. Bizottsága, Akadémiai Beszámoló, Klinikumok Szekció, 2016, Budapest.