ORIGINAL ARTICLE

The relationships between sediment findings and culture results and the presence of proteinuria in canine urine samples

E. A. Fulton *,[1], W. Weir*, M. Czopowicz † and A. R. McBrearty‡

*School of Biodiversity, One Health and Veterinary Medicine, The University of Glasgow Small Animal Hospital, 464 Bearsden Road, Glasgow, G61 1QH, Scotland
†Division of Veterinary Epidemiology and Economics, Institute of Veterinary Medicine, Warsaw University of Life Sciences–SGGW, Nowoursynowska 159c, 02-776, Warsaw, Poland
‡The University of Glasgow Small Animal Hospital and Vets Now, Glasgow, Scotland
A. R. McBrearty's current address is Vets Now Hospital Glasgow, 123-145 North Street, Glasgow, G3 7DA

[1]Corresponding author email: e.fulton.2@research.gla.ac.uk

Objectives: To assess relationships between urine sediment and microbial culture findings and the presence of proteinuria in canine urine samples, and to assess the change in the percentage of proteinuric samples and urine protein-to-creatinine ratio when urine abnormalities resolve.

Materials and Methods: Canine urine samples collected *via* cystocentesis and submitted for culture and contemporaneous urinalysis (including urine protein-to-creatinine ratio) were retrospectively identified. Dogs receiving corticosteroids were excluded. Associations between haematuria (red blood cells>5/high-power field), pyuria (white blood cells>5/high-power field), presence of microorganisms on microscopy, active sediment, and positive culture and proteinuria (urine protein-to-creatinine ratio>0.5) were investigated. Patient characteristics were considered possible confounders. In dogs with repeat urinalysis, the associations between active sediment and positive culture resolution on proteinuria and urine protein-to-creatinine ratio were assessed.

Results: One hundred and ninety-two of 491 samples were proteinuric (39.1%). Age was positively associated with proteinuria. In the multivariable analysis corrected for age, active sediment was the only variable significantly associated with proteinuria (adjusted odds ratio: 2.12; 95% confidence interval: 1.44 to 3.11); however, only 49.8% of samples with active sediment were proteinuric. Neither resolution of active sediment nor positive culture were associated with reduced proportions of proteinuric samples (from 57.9% to 42.1% and from 40.0% to 25.0%, respectively) or significant reductions in urine protein-to-creatinine ratio (median change: −0.16 and −0.14, respectively).

Clinical Significance: Attributing proteinuria to urinalysis abnormalities or a positive urine culture in canine cystocentesis samples is not supported by our findings, and could result in alternative causes of proteinuria (*e.g.* renal proteinuria) being overlooked.

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INTRODUCTION

Proteinuria is generally taken to mean detection of abnormal amounts of protein in the urine (Lees *et al.* 2005, Vaden & Elliott 2016) and falls into three categories. It may develop due to glomerular filtration of high concentrations of normal or abnormal low-molecular weight plasma proteins which overwhelm normal tubular reabsorption (prerenal), abnormal renal handling of normal plasma proteins (renal) or leakage of protein into the urine in or after the renal pelvis (postrenal) (Lees *et al.* 2005, Harley & Langston 2012, Vaden & Elliott 2016). In practice, proteinuria is often first detected *via* semi-quantitative dipstick analysis, but results are affected by urine concentration and pH (Harley & Langston 2012). Therefore, to confirm proteinuria and provide a quantitative assessment of the magnitude of protein loss, the urine protein-to-creatinine ratio (UPC) is used (Lees *et al.* 2005, Harley & Langston 2012). This has been shown to correlate closely with 24-hour urine protein excretion in dogs (White *et al.* 1984, Center *et al.* 1985, Grauer *et al.* 1985). Proteinuria is defined as a UPC greater than 0.5 (IRIS 2019).

When proteinuria is detected, determination of its origin is important (Lees *et al.* 2005, Vaden & Elliott 2016). Persistent renal proteinuria is particularly significant as it has been associated with disease progression and reduced survival and is thought to be a mediator of renal injury (Jacob *et al.* 2005, Lees *et al.* 2005, Wehner *et al.* 2008). In addition, in dogs with naturally occurring glomerular diseases, interventions that reduce the magnitude of proteinuria have been associated with improved outcomes (Valli *et al.* 1991, Grauer *et al.* 2000).

Postrenal, extra-urinary causes of proteinuria may be largely excluded by evaluation of cystocentesis samples and prerenal causes may be excluded based on evaluation of plasma proteins (Lees *et al.* 2005, Harley & Langston 2012). In the remaining cases, in the absence of the evidence of inflammation or haemorrhage, proteinuria is considered renal in origin (Lees *et al.* 2005). However, in cases with evidence of inflammation, haemorrhage or positive urine culture, the origin of proteinuria is less clear and may be either postrenal urinary, renal or both.

Various studies have investigated associations between the presence of haematuria, pyuria, microscopic bacteriuria or positive urine culture with UPC and proteinuria. The addition of blood to urine samples in vitro has shown that gross haematuria (resulting in dark pink/red urine discolouration) often results in proteinuria (Bagley *et al.* 1991, Vaden *et al.* 2004, Vientos-Plotts *et al.* 2018, Jillings *et al.* 2019). However, the addition of blood at lower dilutions, resulting in >5 red blood cells (RBC) per high-power field (HPF) but minimal colour change (yellow or dark-yellow colouration), rarely resulted in proteinuria, although it caused a significant increase in UPC above baseline (Vientos-Plotts *et al.* 2018, Jillings *et al.* 2019). The effect of haematuria on UPC in vivo is less clear. In pyuric urine samples, there was no significant difference in UPC between samples with and without haematuria (Vaden *et al.* 2004). In dogs undergoing experimentally induced acute bladder injury (caused by cystotomy or *Escherichia coli* infection) in which haematuria, pyuria and bacteriuria developed, most subsequent urine samples were proteinuric but no correlation was found between the categorised RBC count and UPC (Bagley *et al.* 1991).

Pyuria occurs with urinary tract inflammation or infection, and it might be expected that protein leakage would also occur, resulting in increases in UPC or proteinuria. However, in the aforementioned models of acute bladder injury, there was no correlation between number of white blood cells (WBC) per HPF in the urine and UPC (Bagley *et al.* 1991). Similarly, in the study by Vaden *et al.* (2004), 81% of pyuric samples had a UPC <0.4 and UPC did not differ with different degrees of pyuria, suggesting that pyuria is infrequently associated with proteinuria. Also, in the same study, the presence or absence of concurrent microscopic bacteriuria did not affect UPC (Vaden *et al.* 2004). Similarly, in a study by Meindl *et al.* (2019), no association was found between the presence of microscopic bacteriuria and proteinuria, with microscopic bacteriuria identified in 12.7% of samples without proteinuria and 8.0% of those with proteinuria.

The term active sediment has been widely used in the literature and is often, but not always, defined as the presence of at least one of the following changes: RBC>5/HPF, WBC>5/HPF or microscopic bacteriuria (Vaden *et al.* 2004, Sanchez *et al.* 2019, Strachan *et al.* 2022). The only study looking at the effect of active sediment on UPC showed that only 34 of 101 samples (34%) with active sediment had proteinuria; however, this study used an alternative definition of active sediment (RBC≥10/HPF or WBC≥5/HPF, disregarding the presence or absence of microscopic bacteriuria) (Meindl *et al.* 2019).

The effect of positive urine culture on UPC was investigated by Meindl *et al.* (2019). They found poor agreement between proteinuria and the presence of positive urine culture and only a weak correlation between the severity of infection (based on colony-forming units/ml) and UPC. A positive urine culture is not always accompanied by sediment changes and a recent study found no association between a positive urine culture and proteinuria in the absence of active sediment, with only 14 of 36 samples (39%) with positive urine culture and inactive sediment being proteinuric (Strachan *et al.* 2022).

Despite the weak or absent associations reported between urine sediment changes and UPC and the presence of proteinuria, the possibility of these changes causing proteinuria means that UPC may not be routinely performed in samples with urine sediment changes (Vaden & Elliott 2016, Grimes *et al.* 2020). This could lead to significant renal proteinuria being missed and the introduction of bias into retrospective studies. This may be a particular problem in conditions such as diabetes mellitus and hyperadrenocorticism which are associated with both renal proteinuria and lower urinary tract disease (Ortega *et al.* 1996, Hurley & Vaden 1998, Forrester *et al.* 1999, Hess *et al.* 2000, Smets *et al.* 2012a,b, Herring *et al.* 2014, Marynissen *et al.* 2016, Hoffman *et al.* 2018, Dupont *et al.* 2020, Yoon *et al.* 2020). In addition, some conditions such as *Borrelia burgdorferi* infections are associated with both inflammatory changes in urine and glomerulopathy (Grauer *et al.* 1988, Borys *et al.* 2019). A better understanding of the association between urine sediment changes and positive urine culture on proteinuria and UPC is therefore needed.

The principal aim of this study was, therefore, to assess the relationship between urine sediment and culture results and the

presence of proteinuria in canine urine samples (Part A). The secondary aim was to assess the change in the percentage of samples with proteinuria and UPC when active sediment and positive urine culture resolved (Part B).

MATERIALS AND METHODS

Part A: Relationship between urine sediment and culture results and the presence of proteinuria

The database of a University Teaching Hospital was searched for canine urine samples submitted for culture between August 1, 2016 and July 31, 2020 with contemporaneous urinalysis results available. Samples were only included if both urinalysis and culture were performed at the onsite reference laboratory and were collected *via* cystocentesis. Samples were subsequently excluded if they were received by the bacteriology laboratory >24 hours after collection or if urinalysis results were reported >24 hours after submission. Clinical records were retrospectively reviewed and samples from dogs receiving corticosteroids at the time of sampling were excluded as were dogs for which the age was not available. Only the first eligible sample for each dog was included.

Signalment and bodyweight were recorded from the medical records. Urine sediment results for RBC, WBC, and microorganisms on microscopy were recorded. Haematuria was defined as RBC>5/HPF and pyuria as WBC>5/HPF. If any microorganisms were reported on microscopy, microorganisms on microscopy was categorised as positive. Samples were deemed to have active sediment if they were positive for either haematuria, pyuria, or microorganisms on microscopy or any combination thereof. The UPC, which was routinely performed on all samples submitted for urinalysis, was also recorded. Proteinuria was defined as UPC>0.5. The presence of microorganism growth and the microorganism species cultured were also recorded if applicable.

For sediment analysis, 1.5 mL of urine was centrifuged at 1500 rpm for 4 minutes. Four hundred microlitres of supernatant was removed for protein analysis and a further 300 µL removed for urine creatinine measurement. The sediment pellet was then gently resuspended and pipetted onto a slide with a haemocytometer-type grid (Glasstic™ Slide 10, KOVA™ International). The sediment was screened at 10× magnification before at least 10 squares on the slide were assessed at 40× magnification. The average number of RBC, WBC and microorganisms per HPF was recorded semi-quantitatively.

For urine protein quantification, the spectrophotometer was set at a wavelength of 660 nm with a maximum absorbance of 0.5. Two cuvettes (Sarstedt® cuvettes, Krackeler Scientific Inc.) were used. Six hundred microlitres of distilled water was added to one (to act as a blank) and 600 µL of 3% sulphosalicylic acid (stored at 2 to 8°C) to the test cuvette. Two hundred microlitres of urine supernatant was added to each cuvette. The cuvettes were left to stand for 5 minutes before being gently mixed and placed in the spectrophotometer. The blank cuvette was placed in position 1 and the galvanometer zeroed to negate the effect of the supernatants' yellow colouration. The test sample was placed in position 2 and the optical density determined. The protein content was determined by converting the optical density into a protein concentration in mg/100 mL using a calibration curve. The calibration curve had been generated by assessing serial dilutions of a sample with a known protein concentration (ABX Pentra Multical, Horiba UK Ltd) and verified using urine quality control material (Urine Assayed Control Level 2, Randox Laboratories Ltd). Urine creatinine was measured in µmol/L using a clinical chemistry analyser (Dimension Xpand Plus, Siemens) with the modified kinetic Jaffe method and then converted to mg/100 mL. To calculate UPC, urine protein concentration was divided by the creatinine concentration.

Urine was cultured in an aerobic incubator at 37°C on both 5% sheep blood and MacConkey agar plates. These were initially read after 48-hour incubation and again 48-hours later to detect slow-growing microorganisms. Microorganisms were identified based on colony morphology with additional biochemical testing, as required. Any microorganism growth was reported as positive, unless only one or two bacterial colonies of an undetermined species were grown; these samples were considered contaminated and were therefore classed as negative.

Part B: Effect of active sediment and positive urine culture resolution on the presence of proteinuria and UPC

Dogs included in Part A were eligible for inclusion in Part B if they had one or more follow-up urine samples collected by cystocentesis for urinalysis between 7 and 56 days later. The same exclusion criteria as in Part A were applied, although culture of follow-up samples was not required. If dogs had started medications known to affect UPC before follow-up sample collection or if the dose of these medications had changed, the follow-up sample was excluded. Medications considered to have an impact on UPC were corticosteroids, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and tyrosine kinase inhibitors. When investigating the association between active sediment resolution and proteinuria and UPC, only dogs with active sediment in the initial sample were included. When investigating the association between positive urine culture resolution and proteinuria and UPC, only dogs with a positive urine culture on the initial sample and in which follow-up sample culture was performed and was received by the bacteriology laboratory <24 hours of collection were included. In each case, the first eligible urine sample collected during the follow-up period was included. Urine sediment and culture results were recorded as for Part A.

Statistical analysis

Normality of distribution of continuous variables (age, bodyweight and UPC) was evaluated *via* inspection of histograms and using the Shapiro–Wilk W test. As normality of distribution was violated, the median, interquartile range (IQR), and range were used for descriptive statistics. Categorical variables were expressed as count and percentage and 95% confidence intervals (95% CI) for percentages were calculated using the Wilson score method (Altman *et al.* 2000).

For Part A, the relationships between patient characteristics, urine sediment findings and urine culture results and the presence

of proteinuria were initially evaluated. The Mann–Whitney U test was used for continuous variables and the maximum likelihood G-test or Fisher's exact test (if the expected count in any cell of the contingency table was below 5) was used for categorical variables. Patient characteristics were included as potential confounders. Microorganism species isolated from more than 10 samples were included in the analysis. Crude odds ratios (OR) were used to express the strength of the relationships in the univariable analysis. Variables with P<0.1 on univariable analysis were entered into the multivariable analysis. Multivariable analysis was performed using multivariable logistic regression with a backward stepwise elimination procedure. Proteinuria (UPC>0.5) was the outcome variable. The Hosmer-Lemeshow χ^2 test and Nagelkerke's pseudo-R^2 coefficient were used to assess the goodness-of-fit of the model (Hosmer & Lemeshow 2000). Adjusted (OR_{adj}) were used to express relationship strength in multivariable analysis.

For Part B, to assess the effect of active sediment resolution on proteinuria and UPC, dogs were split into two groups: those in which active sediment resolved and those in which it persisted. The proportion of samples in which proteinuria was present in the initial and follow-up samples was calculated for each group and the change in proportions compared using McNemar's test. Paired analysis of UPC from the initial and follow-up urine samples was then performed for each group using the Wilcoxon Signed Rank test. These tests were repeated to investigate the association between resolution of positive urine culture and proteinuria and UPC. The change of UPC between dogs in which active sediment or positive urine culture resolved and persisted was compared using the Mann–Whitney U test. The significance level (α) was set at 0.05 and Bonferroni correction was applied in the case of multiple comparisons in Part B (denoted by P_{BC}). All statistical tests were two-tailed. Statistical analysis was performed in TIBCO Statistica 13.3 (TIBCO Software Inc., Palo Alto, CA).

This retrospective study was performed at the University of Glasgow and was granted ethical approval from the School of Veterinary Medicine Research Ethics Committee (EA3820).

RESULTS

Part A

During the study period, 1421 canine urine samples from the University Teaching Hospital underwent culture at the onsite reference laboratory and had medical records available for review. Of these, 903 (63.5%) were collected by cystocentesis and thus were preliminarily included; 205 samples were excluded as urinalysis was not performed. Thirty-nine samples were then excluded as they were received by the bacteriology laboratory >24 hours after sample collection and 18 were excluded as urinalysis results were reported >24 hours after urine submission.

Of the remaining 641 samples, 65 were excluded as the dogs were receiving corticosteroids, leaving 576 eligible samples from 492 dogs. One dog was excluded as age at presentation was unknown; hence, the final study population included 491 dogs. Only the earliest eligible sample from each dog was included.

Study population

The demographic characteristics of the study population are available in Table 1. There were 413 (84.1%) purebred dogs belonging to 74 breeds. The breeds represented by >20 dogs included Labrador retriever (n=44), cocker spaniel (n=32) and border collie (n=22).

Urinalysis results

The median UPC was 0.20 (IQR: 0.06 to 1.45, range: 0.00 to 32.80) and 192 samples (39.1%; 95% CI: 34.9% to 43.5%) were proteinuric. On sediment examination, 97 samples (19.8%; 95% CI: 16.5% to 23.5%) had haematuria, 69 (14.1%, 95% CI: 11.3% to 17.4%) had pyuria, and 115 (23.4%; 95% CI:19.9% to 27.4%) had microorganisms on microscopy. The sediment was active in 202 samples (41.1%; 95% CI: 36.9% to 45.5%).

Culture results

One hundred and fifteen samples (23.4%; 95% CI:19.9% to 27.4%) had positive urine culture. Three additional samples with ≤2 colonies which were not identified were classed as negative. One microorganism species was isolated from 99 (86.1%) samples, two species from 14 (12.2%) and three species from two (1.7%). The isolated microorganisms were *E. coli* (n=62), *Staphylococcus* (n=26), *Enterococcus* (n=13), *Proteus* (n=10), *Streptococcus* (n=8), *Mycoplasma* (n=4), *Corynebacterium* (n=3), *Pseudomonas* (n=2), *Klebsiella* (n=2), *Bacillus* (n=1), *Pasteurella* (n=1) and a yeast (n=1). Of the 115 samples with positive urine culture, 29 (25.2%) had inactive sediment.

Relationship between patient characteristics, urine sediment and culture results and the presence of proteinuria

Table 2 shows the associations between patient characteristics and the presence or absence of proteinuria on univariable analysis; age was the only patient characteristic significantly associated with the presence of proteinuria. Table 3 shows the association between urine sediment and culture results and the presence of proteinuria

Table 1. Comparison of demographic characteristics of dogs included in Part A and Part B of the study

Demographic characteristics	Part A (n=491)	Part B (n=30)	P-value
Males – n (%)	235 (47.9)	10 (33.3)	0.118
Neutered – n (%)	301 (61.3)	18 (60.0)	0.887
Pedigree – n (%)	413 (84.1)	25 (83.3)	0.910
Age (years) – median, IQR (range)	7.1, 4.2 to 10.3 (0.2 to 14.9)	8.2, 5.0 to 11.2 (0.5 to 14.9)	0.642
Bodyweight (kg) – median, IQR (range)	15.0, 9.0 to 26.4 (1.1 to 68.0)	18.5, 9.8 to 27.7 (2.4 to 53.0)	0.451

Demographic information was available for all dogs aside for 37 dogs for which weight was unavailable

Table 2. Association between (a) categorical patient characteristics and (b) continuous patient characteristics and the presence or absence of proteinuria

(a) Variable		Number (%) of non-proteinuric samples (n=299)	Number (%) of proteinuric samples (n=192)	P-value	Crude odds ratio (95% confidence interval)†
Sex	Male (n=235; 47.9)	148 (63.0)	87 (37.0)	0.365	–
	Female (n=256; 52.1%)	151 (59.0)	105 (41.0)		
Neuter status	Entire (n=190; 38.7%)	120 (63.2)	70 (36.8)	0.414	–
	Neutered (n=301; 61.3%)	179 (59.5)	122 (40.5)		
Breed	Crossbreed (n=78; 15.9%)	52 (66.7)	26 (33.3)	0.251	–
	Pedigree (n=413; 84.1%)	247 (59.8)	166 (40.2)		

(b) Variable	Non-proteinuric samples		Proteinuric samples		P-value	Crude odds ratio (95% confidence interval)†
	No. of dogs	Median, interquartile range (range)	No. of dogs	Median, interquartile range (range)		
Age (years)	299	6.10, 2.6 to 9.3 (0.2 to 14.9)	192	9.1, 6.1 to 11.2 (0.2 to 14.9)	<0.001‡	1.17 (1.11 to 1.24)
Bodyweight (kg)	277	15.3, 9.1 to 26.2 (1.5 to 64.9)	177	14.1, 9.0 to 27.0 (1.1 to 68.0)	0.543	–

Part (b): Weight was unavailable for 37 dogs and hence these dogs were excluded from analysis on the effect of weight on proteinuria
†Estimated only if P<0.05
‡Included in multivariable analysis based on the P<0.1 criterion

on univariable analysis. Pyuria, microorganisms on microscopy, active sediment and the isolation of *E. coli* or *Enterococcus* were significantly associated with the presence of proteinuria.

Variables eligible for inclusion in the multivariable analysis were age, haematuria, pyuria, microorganisms on microscopy, active sediment and the isolation of *E. coli*, *Enterococcus* or *Staphylococcus*. Multivariable analysis results are shown in Table 4. When corrected for age, proteinuria was significantly positively associated with active sediment. The odds of proteinuria were 2.1-times higher in samples with active sediment than in samples without active sediment. Given the presence of proteinuria in 31.8% of samples without active sediment, the risk of proteinuria was approximately 1.6 times higher in samples with active sediment.

Part B

Of the 491 dogs in part A, 116 (23.6%) had at least one follow-up urinalysis performed within the study period. Of these, 76 dogs (102 samples) were excluded as urine was not collected *via* cystocentesis (free catch, n=62; catheter, n=9; unknown, n=31). One dog was excluded because the only follow-up urinalysis was reported >24 hours after submission. Two further dogs were excluded as they had started corticosteroid therapy before follow-up urine collection. In total, 30 dogs were eligible for inclusion to assess the association between the resolution of an active sediment (n=26) or a positive urine culture (n=25) and proteinuria and UPC.

Study population

The demographic characteristics of dogs in Part B are also included in Table 1. Twenty-five (83.3%) were purebred dogs with cocker spaniel (n=4) and Labrador retriever (n=3) most represented. The demographic characteristics of dogs included in part B did not differ significantly from those in part A (Table 1).

Active sediment

Twenty-six dogs had an active sediment on initial urinalysis and an eligible follow-up sample. Of these, active sediment had resolved in the follow-up samples in 19 dogs and persisted in seven. In the group in which active sediment resolved, proteinuria was present in 11 (57.9%) initial samples and in eight (42.1%) follow-up samples; this change was not statistically significant (P_{BC}=0.496). In the group in which active sediment persisted, five samples (71.4%) had proteinuria on initial urinalysis and four samples (57.1%) on follow-up urinalysis; again, this change was not statistically significant (P_{BC}=0.999). Fig 1 shows the change in UPC between the follow-up and initial samples for both groups. The difference between UPC of follow-up and initial samples was not statistically significant in either the group in which active sediment resolved [median UPC change: −0.16 (range: −2.53 to 12.51); P_{BC}=0.226] or the group in which it persisted [median UPC change: −0.26 (range: −5.15 to 1.31); P_{BC}=0.999], nor was the change in UPC significantly different between the groups (P=0.999).

Positive urine culture

Twenty-five dogs had a positive urine culture on initial urinalysis and an eligible follow-up sample. Of these, urine culture was negative (resolved) on the follow-up samples in 20 dogs and persisted in five dogs. Among the 20 dogs in which positive urine culture resolved, eight (40.0%) had proteinuria in the initial samples and five (25.0%) in the follow-up samples. This change was not statistically significant (P_{BC}=0.496). In the five dogs in which positive urine culture persisted, four (80.0%) had proteinuria in the initial samples and three (60.0%) on the follow-up samples. Again, this change was not statistically significant (P_{BC}=0.999). Fig 2 shows the change in UPC between the follow-up and initial samples for both groups. The difference between the follow-up and initial samples was not statistically significant in either the group in which positive urine culture resolved [median UPC change: −0.14 (range: −1.13 to 1.74); P_{BC}=0.224] or the group in which it persisted [median UPC change: 0.00 (range: −5.15 to 12.51); P_{BC}=0.999], nor was the change significantly different between the two groups (P=0.708).

DISCUSSION

The principal aim was to investigate the associations between urine sediment and culture results and the presence of protein-

Table 3. Association between urine sediment and culture results and the presence or absence of proteinuria

Variables	Number (%) of non-proteinuric samples (n=299)	Number (%) of proteinuric samples (n=192)	P-value	Crude odds ratio (95% confidence interval†
Haematuria absent (n=394; 80.2%)	248 (62.9)	146 (37.1)	0.063‡	–
Haematuria present (n=97; 19.8%)	51 (52.6)	46 (47.4)		
Pyuria absent (n=422; 85.9%)	266 (63.0)	156 (37.0)	0.018‡	1.86 (1.11 to 3.10)
Pyuria present (n=69; 14.1%)	33 (47.8)	36 (52.2)		
Organisms absent (n=376; 76.6%)	245 (65.2)	131 (34.8)	0.001‡	2.11 (1.38 to 3.23)
Organisms present (n=115; 23.4%)	54 (47.0)	61 (53.0)		
Inactive sediment (n=289; 58.9%)	197 (68.2)	92 (31.8)	<0.001‡	2.10 (1.45 to 3.04)
Active sediment (n=202; 41.1%)	102 (50.5)	100 (49.5)		
Negative culture (n=376; 76.6%)	233 (62.0)	143 (38.0)	0.380	–
Positive culture (n=115 23.4%)	66 (57.4)	49 (42.6)		
Escherichia coli absent (n=429; 87.4%)	269 (62.7)	160 (37.3)	0.033‡	1.79 (1.05 to 3.06)
E. coli present (n=62; 12.6%)	30 (48.4)	32 (51.6)		
Staphylococcus absent (n=465; 94.7%)	279 (60.0)	186 (40.0)	0.075‡	–
Staphylococcus present (n=26; 5.3%)	20 (76.9)	6 (23.1)		
Enterococcus absent (n=478; 97.4%)	295 (61.7)	183 (38.3)	0.026‡	3.63 (1.10 to 11.95)
Enterococcus present (n=13; 2.7%)	4 (30.8)	9 (69.2)		
Proteus absent (n=481; 98.0%)	294 (61.1)	187 (38.8)	0.523	–
Proteus present (n=10; 2.0%)	5 (50.0)	5 (50.0)		

†Estimated only if P<0.05
‡Included in multivariable analysis based on P<0.1 criterion

Table 4. Multivariable analysis of the association between urine sediment and culture results and the presence or absence of proteinuria after controlling for age

Variables	Regression Coefficient (standard error)	Wald's Statistic	P-value	Adjusted odds ratio (95% confidence interval)
Intercept	−1.95 (0.25)			
Age	0.16 (0.03)	35.6	<0.001	1.17 (1.11 to 1.24)
Active sediment	0.74 (0.20)	14.0	<0.001	2.09 (1.42 to 3.08)
Dropped from the analysis				
Haematuria	0.06 (0.39)	0.02	0.886	1.06 (0.59 to 2.29)
Escherichia coli	0.18 (0.37)	0.23	0.634	1.19 (0.58 to 2.44)
Pyuria	0.26 (0.31)	0.70	0.403	1.30 (0.70 to 2.41)
Organisms	0.33 (0.30)	1.18	0.276	1.39 (0.77 to 2.53)
Enterococcus	1.20 (0.68)	3.14	0.076	3.32 (0.88 to 12.5)
Staphylococcus	−0.77 (0.50)	2.33	0.127	0.46 (0.17 to 1.24)

Only variables with P<0.1 on univariable analysis were included. Hosmer-Lemeshow χ^2 test=7.37, P=0.498; Nagelkerke's pseudo-R^2 coefficient=0.14

uria in dogs. Evidence of a positive association between active sediment and proteinuria was found. When patient characteristics and other urinalysis changes had been accounted for, there was a twofold increase in the odds of proteinuria in samples with active sediment compared to those without (which corresponded to roughly a 1.6-fold increase in the likelihood). However, samples with active sediment were frequently non-proteinuric and, therefore, we suggest caution in overinterpretation of this association. No evidence was found to suggest any association between haematuria or positive urine culture and the presence of proteinuria.

Active sediment was the only urinalysis variable found to be significantly associated with the presence of proteinuria in the multivariable model. However, the age-adjusted OR of 2.1 suggested a weak relationship implying that active sediment explains only a small part of the variability in the occurrence of proteinuria. This is expected as UPC can be increased for many other reasons (Lees *et al.* 2005). In addition, only approximately 50% of samples with active sediment were proteinuric in our population and therefore, although a sample with active sediment was more likely to be proteinuric than a sample without active sediment, there was still a high likelihood that even with an active sediment the sample would be non-proteinuric. Few previous studies have investigated the relationship between active sediment and proteinuria, although an active sediment has been suggested to be a possible cause of proteinuria in earlier studies (Lees *et al.* 2005, Grauer 2011, Vaden & Elliott 2016). Meindl *et al.* (2019) reported that 34% of samples with active sediment were proteinuric but the definition of active sediment used only included the presence of RBC or WBC. The frequency of proteinuria may have been higher in this study if higher cut-offs for haematuria, pyuria and microorganisms on microscopy had been used in the definition of active sediment; however, these cut-offs were chosen as they have frequently been used previously (Vaden *et al.* 2004, Sanchez *et al.* 2019, Strachan *et al.* 2022). Overall, despite the reported association between active sediment and proteinuria, the presence of proteinuria should not be assumed to be due to active sediment in urine samples when both abnormalities are present since half of samples with active sediment in this study were found to be non-proteinuric.

Pyuria was positively associated with proteinuria on univariable analysis; however, it was not found to be significant on multivariable analysis. This is likely due to the interaction between

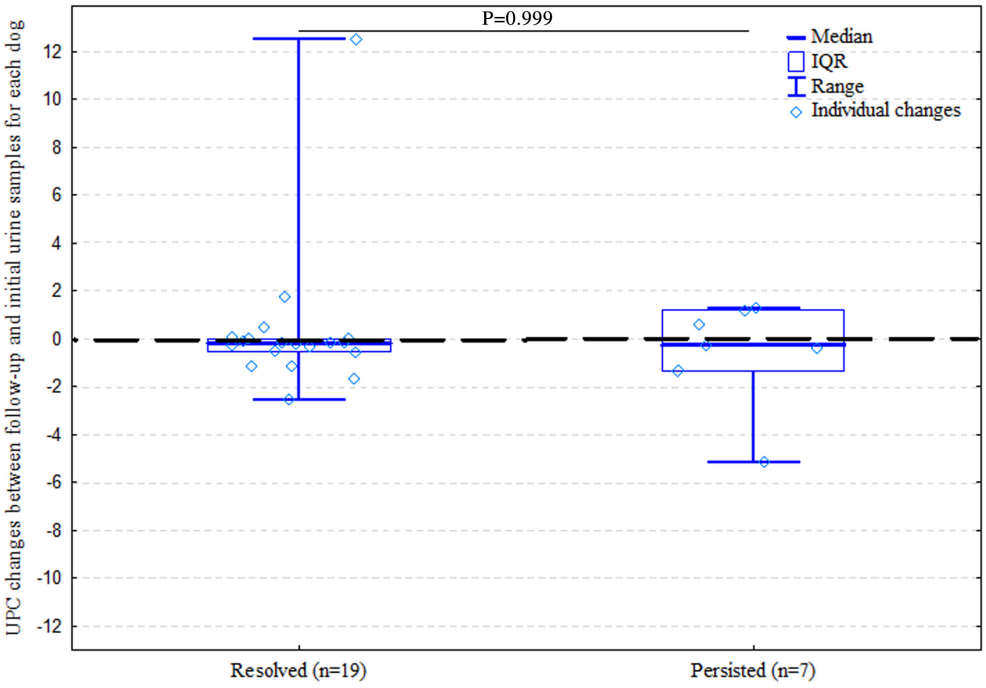

FIG 1. The change in urine protein-to-creatinine ratio (UPC) between the follow-up and initial urinalyses in dogs in which the active sediment resolved or persisted. The change of UPC is presented as the median, interquartile range (IQR) and range of differences between the follow-up and initial urinalysis. The black broken line indicates the line of equality. Negative values represent a decrease in UPC from the initial to follow-up sample

pyuria and active sediment and that active sediment had the stronger association with proteinuria. In addition, only 53% of pyuric samples were proteinuric. This suggests that a single pyuric clinical sample is no more likely to be proteinuric than non-proteinuric and it should not be assumed that proteinuria is due to the presence of pyuria.

As urinary tract inflammation would be expected to increase urine protein, the proportion of pyuric samples that were non-proteinuric may be considered surprising, however, this finding is supported by those of Vaden *et al.* (2004). In that study, only 19% of pyuric samples had a UPC of ≥0.4. The lower percentage of samples with pyuria having proteinuria in that study may be because known cases of protein-losing nephropathy were excluded and different methodology for UPC measurement was used. Together these studies provide little evidence to assume proteinuria in pyuric samples is due to the presence of WBCs.

The presence of microorganisms on microscopy was positively associated with proteinuria on univariable but not multivariable analysis. As with pyuria, the interaction between microorganisms on microscopy and active sediment and the stronger association between active sediment and proteinuria is likely to explain this finding. Only 53% of samples with microorganisms on microscopy were proteinuric suggesting that, as with pyuric samples, a sample with microorganisms on microscopy is no more likely to be proteinuric than non-proteinuric. Previous studies have not found an association between microscopic bacteriuria and proteinuria (Vaden *et al.* 2004, Meindl *et al.* 2019). Proteinuria was more frequent in this study than in samples with microscopic bacteriuria in the study by Meindl *et al.* (2019) (53% *versus* 8%, respectively). Although we included samples in which either fungi or bacteria were observed (microorganisms on microscopy rather than microscopic bacteriuria), the low prevalence of fungal urinary infections means this is unlikely to explain this difference (Jin & Lin 2005). It is more likely that the study by Meindl *et al.* (2019) was biased against cases with microscopic bacteriuria as UPC was not measured in all cases undergoing urinalysis and samples with microscopic bacteriuria may have been less likely to have had UPC performed and therefore be included. Together, these studies suggest little evidence to assume proteinuria in a urine sample is because of the presence of microorganisms on microscopy. This is not unexpected because it appears unlikely that the microorganisms themselves would substantially increase urine protein, particularly without associated inflammation.

No evidence was found to suggest an association between the presence of haematuria and proteinuria and samples with haematuria were no more likely to be proteinuric than non-proteinuric (48% *versus* 52%). In vitro studies have shown that the addition of blood at dilutions which do not cause colour change rarely result in proteinuria (although UPC may increase from baseline) (Vientos-Plotts *et al.* 2018, Jillings *et al.* 2019). The colours of the samples included in this study were not available; however, 94 of 97 haematuric samples had RBC <50/HPF and would therefore be unlikely to display gross haematuria (Vaden *et al.* 2004). The limited number of in vivo canine studies has also shown no evidence of an association between haematuria and UPC (Bagley *et al.* 1991, Vaden *et al.* 2004). Together, these results suggest haematuria is unlikely to be the reason for proteinuria in a sample, particularly without gross haematuria. It remains possible; however, that more marked haematuria may be associated with proteinuria.

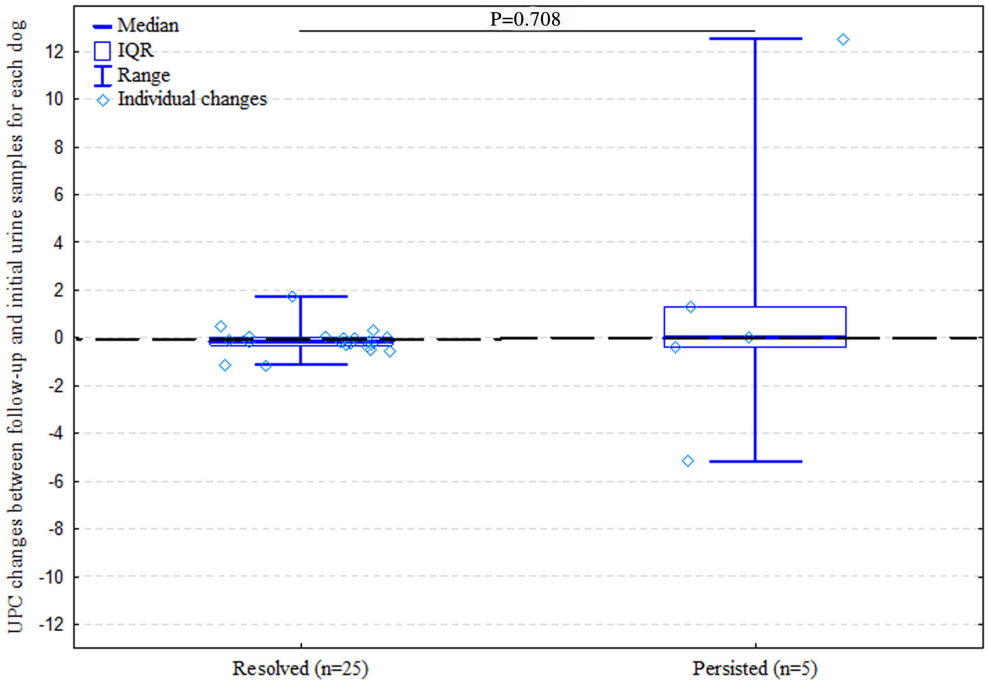

FIG 2. The change of urine protein-to-creatinine ratio (UPC) between the follow-up and initial urinalysis in dogs in which the positive urine culture resolved or persisted. The change of UPC presented as the median, interquartile range (IQR) and range of differences between the second and first urine analysis. The black broken line indicates the line of equality. Negative values represent a decrease in UPC from the initial to follow-up sample

No evidence of an association between positive urine culture and proteinuria was found in this study and only 42% of samples with a positive urine culture were proteinuric. The absence of an association, even on univariable analysis, suggests that this is not just because associated inflammation has a greater effect. The absence of an association is not unexpected as unless the burden of microorganisms was very high, the small amount of protein derived from the microorganisms themselves would not be expected to have an effect. It is possible that categorising all samples with growth of >2 colonies as having a positive urine culture rather than excluding those with sparse cultures resulted in incorrect classification of some contaminated samples; however, as all samples were collected by cystocentesis the likelihood of sample contamination was low (Carter *et al.* 1978). As the laboratory used provides semi-quantitative results for abundance of microorganism growth, previously reported cut-offs to define contamination could not have been used. The lack of an association between positive urine culture and proteinuria also agrees with the findings of Meindl *et al.* (2019) and Strachan *et al.* (2022). Together, these findings suggest that proteinuria is unlikely to be due to a positive urine culture in a sample and this assumption could result in alternative causes of proteinuria being missed.

In part B, we looked at the associations between resolution of active sediment or positive urine culture and the presence of proteinuria and UPC for the first time. In the small number of cases included in this study, neither the resolution of active sediment nor of a positive urine culture were associated with a significant reduction in the frequency of proteinuria or a significant reduction in UPC. In dogs in which the initial samples had active sediment but this subsequently resolved, the percentage of proteinuric samples was lower in the follow-up samples than it had been in the initial samples, but this was also true for dogs in which active sediment persisted. Similarly, the median UPC decreased when active sediment resolved but the change was very small and a similar decrease was seen in the group in which active sediment persisted. Similar findings were observed in dogs with positive urine culture on the initial samples. No prior studies have investigated the association between resolution of active sediment or positive urine culture and the presence of proteinuria and UPC, therefore, although our study numbers were small our findings further suggest caution in attributing proteinuria to the presence of active sediment or positive urine culture.

Age was the only patient characteristic significantly associated with the presence of proteinuria. While it was not the purpose of this study to investigate associations between patient characteristics and proteinuria, these were included because of their potential confounding effects. An association between age and proteinuria has not previously been reported in dogs. Gizzarelli *et al.* (2019) reported no difference in prevalence of proteinuria in samples from dogs under 6 years old compared to those above in a study of Italian dogs considered unlikely to have been unaffected by diseases capable of causing proteinuria; in another study, the prevalence of proteinuria was no higher in geriatric dogs than senior dogs (Willems *et al.* 2017). In people, the prevalence of microalbuminuria has been reported to increase continually after 40 years of age (Jones *et al.* 2002). In people and cats, the increasing prevalence of proteinuria with age is not considered a physiological change but instead the consequence of underlying pathology (Verma *et al.* 2012, Paepe *et al.* 2013).

Further research is required to investigate the association between age and proteinuria in dogs.

Unlike many previous studies, UPC was measured on all samples sent for urinalysis at the onsite laboratory, additionally, because almost all urine samples collected were sent to this laboratory rather than undergoing point-of-care testing, this population was diverse and minimally biased. It is, however, from one referral centre using a single method of UPC determination and therefore caution is needed extrapolating the findings to other populations. Dogs receiving corticosteroids were excluded because they have been shown to be less likely to have inflammatory cells in the sediment when lower urinary tract diseases (such as urinary tract infections) are present and this could have altered associations between sediment changes and UPC (Ihrke *et al.* 1985).

This study has several limitations. Due its retrospective nature, only dogs in which both urinalysis and culture were requested by the attending clinician were included which may have led to population bias. Samples were not pooled samples and in dogs with renal proteinuria, UPC varies from day-to-day (Nabity *et al.* 2007); however, only limited data exist to suggest daily fluctuations in dogs with postrenal proteinuria (Bagley *et al.* 1991). Despite this, a single sample is often used in a clinical situation initially, particularly when it is collected by cystocentesis, and interpretation of the results is then required. The effect of the clinical signs of lower urinary tract disease on proteinuria were not investigated in this study as this would have been difficult due to its retrospective nature. It is possible that dogs with clinical signs of lower urinary tract disease are more likely to have an associated proteinuria regardless of urine sediment findings; however, this hypothesis requires further study. Finally, despite the initial inclusion of 491 dogs, only a few cases were eligible for inclusion in Part B making this part of the study extremely underpowered to detect significant UPC changes (power of 5% to 10% depending on the number of compared dogs on *post hoc* analysis using the method proposed by Zar (2010)). Additionally, the 30 dogs with proteinuria included in Part B may not be representative of a broader population; a larger, prospective study, investigating changes in the frequency of proteinuria and UPC when urine sediment abnormalities resolve is warranted.

No evidence of a positive association between haematuria or positive urine culture and proteinuria in canine urine samples was detected. While some evidence of a positive association between proteinuria and active sediment was found, this was weak. Only about half of the samples with active sediment were proteinuric and the resolution of active sediment on the follow-up sample was not associated with a significant reduction in the percentage of proteinuric samples nor a significant reduction in UPC. These findings, along with the existing literature, suggest that attributing proteinuria to the presence of sediment abnormalities or a positive urine culture in canine cystocentesis samples is ill-advised and could result in other causes of proteinuria being missed or overlooked. Repeat urine sampling for UPC measurement is recommended in such cases and, if persistent, investigation for other causes of proteinuria such as renal protein loss should be considered.

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Author contributions

Emily Alexandra Fulton: Conceptualization (equal); data curation (lead); formal analysis (supporting); investigation (lead); methodology (equal); project administration (equal); writing – original draft (lead); writing – review and editing (supporting). **William Weir:** Data curation (supporting); investigation (supporting); methodology (supporting); project administration (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Michał Czopowicz:** Formal analysis (lead); methodology (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Alix R McBrearty:** Conceptualization (lead); data curation (supporting); formal analysis (supporting); investigation (supporting); methodology (equal); project administration (equal); supervision (lead); writing – original draft (supporting); writing – review and editing (lead).

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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