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Characteristics associated with bacterial growth in urine in 451 proteinuric dogs (2008-2018)

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Abstract

Background: Urine cultures are frequently recommended to rule out infection as a postrenal cause of proteinuria.

Objective: Identify characteristics associated with bacterial growth in urine in proteinuric dogs.

Animals: Four hundred and fifty-one dogs admitted to a teaching hospital between January 2008 and January 2018 with urine protein-to-creatinine ratios (UPCs) >0.5.

Methods: Retrospective study included dogs with a UPC, urinalysis, and quantitative urine culture (QUC) performed within a 72-hour period by searching electronic records. Dogs with recent antimicrobial therapy, urine collected by methods other than cystocentesis, or UPC ≤0.5 were excluded. Signalment, comorbidities, serum BUN and creatinine concentrations, urinalysis findings, and QUC results were recorded. The association between these characteristics and presence of bacterial growth in urine was assessed by univariable and multivariable analysis.

Results: Thirty of four hundred fifty-one dogs (6.7%) had bacterial growth in urine. Of these, 18 (60.0%) had active urine sediment. Bacterial growth in urine was associated with pyuria (odd ratio [OR] 25.1, 95% confidence interval [CI] 7.9-79.6, $P < .001$), bacteriuria (OR 11.1, 95% CI 3.2-39.1, $P < .001$), and lower urinary tract disease (OR 6.7, 95% CI 1.9-23.0; $P = .0028$). If QUC was prompted based on these criteria, 8/451 (1.8%) of proteinuric dogs would have had undetected bacterial growth.

Conclusions and Clinical Importance: The proportion of proteinuric dogs with both inactive urine sediment and bacterial growth in urine was low, suggesting that QUC might not be necessary in the evaluation of all proteinuric dogs. An active urine sediment or lower urinary tract disease should prompt QUC for proteinuric dogs.

KEYWORDS

bacteriuria, proteinuria, pyuria, urinary tract infection

Abbreviations: AKI, acute kidney injury; CFU, colony forming units; CKD, chronic kidney disease; IRIS, International Renal Interest Society; QUC, quantitative urine culture; UA, urinalysis; UPC, urine protein-to-creatinine ratio; UTI, urinary tract infection.

1 | INTRODUCTION

Proteinuria, the presence of an excessive amount of protein in the urine, can be an indicator of underlying renal disease. Renal proteinuria is an important marker of renal disease progression and response to treatment, and is associated with an increased risk of adverse outcomes in dogs and cats with renal disease.[1,2] As such, early identification and intervention is recommended.

Prior to intervention, it must be demonstrated that the proteinuria is persistent and renal in origin. Prerenal and postrenal causes of proteinuria need to be ruled out before initiating an extensive work-up and treatment for renal proteinuria. This step-wise evaluation is well-described in the 2004 ACVIM Consensus Statement on this topic.[3]

Bacterial cystitis, as well as other causes of lower urinary tract inflammation, are well-recognized causes of postrenal proteinuria.[4] As such, quantitative urine cultures (QUCs) are often recommended in the routine diagnostic evaluation of a proteinuric dog.[5] At many practices and institutions, including the practice at which the authors work, QUC is routinely performed when evaluating a proteinuric dog, even in the absence of an active urine sediment or signs of lower urinary tract disease. Some diseases that cause renal proteinuria, such as diabetes mellitus or hyperadrenocorticism, also predispose dogs to bacterial growth in urine, which is frequently subclinical.[6,7] Additionally, a lack of pyuria and cytologic detection of bacteria cannot rule out bacterial growth in urine in dogs with dilute urine samples, which is a reason urine cultures are still recommended despite an inactive sediment.[8] However, the prevalence of bacterial growth in urine in proteinuric dogs with and without active urinary sediments has not been reported. Therefore, the value of routinely performing QUC in proteinuric dogs is not known.

There is poor agreement between the presence/absence of bacteriuria and proteinuria and, when comparing dogs with or without clinical signs associated with bacterial growth in urine, there is no significant difference in UPCs.[9] A wide range of UPCs are represented in both categories, likely due to the fact that a large number of diseases, beyond bacterial growth in urine, can contribute to proteinuria.

The purposes of this study were to identify factors that predict a positive urine culture in proteinuric dogs (urine protein-to-creatinine ratio > 0.5), characterize the types of bacterial growth present, and, ultimately, draw conclusions regarding the utility of routinely performing QUC as part of a diagnostic evaluation for proteinuric dogs.

2 | MATERIALS AND METHODS

Electronic records from the Texas A&M Veterinary Medical Teaching Hospital from January 2008 to January 2018 were searched for dogs that had a quantified urine culture, UPC, and urinalysis (UA) performed.

The records of dogs were retrospectively reviewed. Dogs for which a QUC had been performed on a urine sample collected by cystocentesis were included. If the method of collection was not specified, the cases were included in the analysis as long as their corresponding urinalysis was listed as being collected by cystocentesis, as cystocentesis is the standard collection method for urine cultures at the authors' hospital and it would have been unusual to have collected urine by 2 separate means. If multiple visits met inclusion criteria for a single dog, only the earliest visit within the 10-year time frame was included. Dogs were excluded if the diagnostics (QUC, UPC, and UA) were not performed within 72 hours of each other. This 72-hour time period was chosen for 2 reasons. First, it is standard practice of the authors' institution's microbiology laboratory to store refrigerated urine samples for up 72 hours in case culture is later requested. Additionally, it is standard for most clinicians at our institution to save a sample for culture when obtaining urine, typically in a urine transport tube with preservative if culture within 24 hours is not anticipated. For this reason, while there is no definitive way to retrospectively determine if diagnostics were performed on 1 or more urine samples, it highly likely that for a majority of samples, diagnostics were performed on a single, divided urine sample within this period. Second, there is not expected to be significant changes in UPC in urine stored for up to 72 hours.[10] Dogs were also excluded if they had received antimicrobial therapy within 1 month prior to the tests. Dogs receiving oral tylosin were not excluded, as this was a commonly encountered medication and was thought to be unlikely to affect urine culture results due to predominant excretion in bile with documented low recovery of the drug in urine.[11] Lastly, dogs with a UPC ≤0.5 were excluded. This UPC cutoff was based on current substaging guidelines provided by the International Renal Interest Society.[12] This case selection process is illustrated in Figure 1.

From each record, the dogs' signalment, UA, UPC, and QUC results were recorded. If a recent (within 1 month) biochemistry panel (Vitros 4600 Chemistry System, Ortho-Clinical Diagnostics, Raritan, NJ) had been performed, blood urea nitrogen (BUN; reference interval: 5-29 mg/dL) and creatinine (reference interval: 0.5-1.5 mg/dL) concentrations were also recorded. Dogs were then classified as having renal azotemia (creatinine ≥1.4 and USG <1.030) or as non-azotemic, based on IRIS staging guidelines.[12]

Urinalyses were performed using a standard dipstick (Multixstix 10 SG Reagent Strips, Siemens, Norwood, MA), USG was determined by manual refractometry, and microscopic examination of sediment was performed in-house by trained laboratory technicians, typically performed within 4 hours of submission. Samples are routinely refrigerated between collection and analysis. Dogs were designated as having pyuria (>5 white blood cells per high-power field), hematuria (>100 or too numerous to count red blood cells per high-power field), or bacteriuria (any bacteria seen). This definition for hematuria was set because microscopic hematuria is unlikely to cause significant proteinuria.[13] For the purposes of this study, an active sediment was defined as the presence of pyuria, hematuria, bacteriuria, or any combination of those findings. Inactive sediment was defined as a lack of pyuria, hematuria, and bacteriuria.

UPCs were calculated after measuring the urine protein and urine creatinine of a single urine sample using the Vitros 4600 chemistry analyzer via the Vitros slide method. Urine protein was measured by reaction with pyrocatechol violet-molybdate complex, which was then measured spectrophotometrically. Urine creatinine was measured using an enzymatic assay in which creatinine is hydrolyzed to creatine in the rate-determining step.

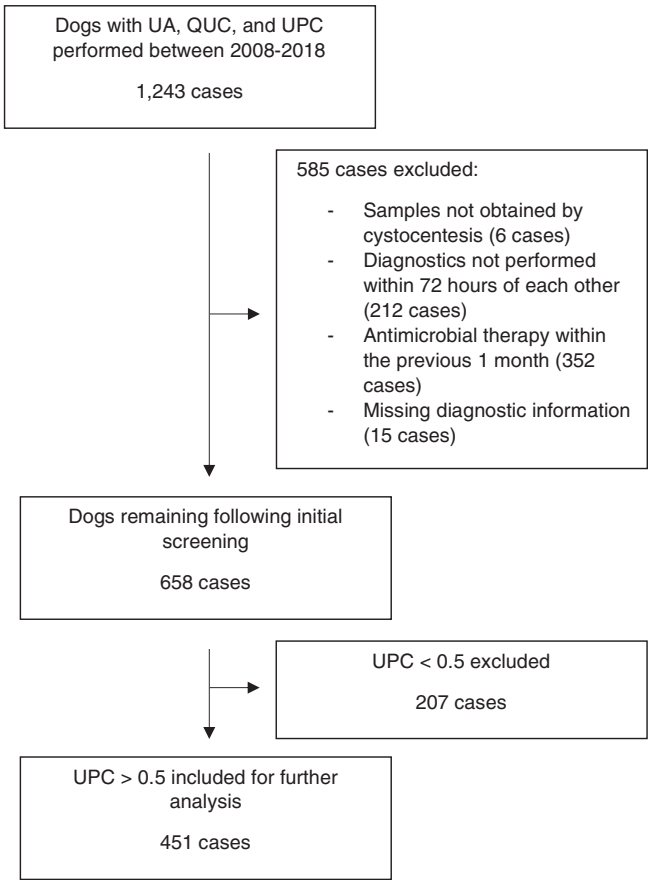

FIGURE 1 Case selection process. QUC, quantified urine culture; UA, urinalysis; UPC, urine protein-to-creatinine ratio

QUCs were performed in-house using the same method over the 10-year time period. Calibrated loops are used to inoculate 1 μL and 10 μL urine onto trypticase soy agar supplemented with 5% sheep's blood (BD, Franklin Lakes, NJ). Plates are incubated overnight at 35°C ± 2°C in an atmosphere with 5% CO_2. A MacConkey agar plate is inoculated with 10 μL urine and incubated in air at 35°C ± 2°C. Urine samples are standardly plated within 4 hours unless collected overnight, in which case they are always plated within 14 hours of the submitted request. Urine samples may be held for up to 72 hours refrigerated pending submission request. These standard practices ensure that a majority of cultures are performed within a 24-hour period and that no culture is performed on a sample older than 72 hours. The results of QUCs were assessed to determine whether the growth was likely to be due contamination or clinically relevant bacterial growth. Bacterial growth was considered consistent with contamination if there were < 1000 colony forming units (CFU)/mL. A distinction between bacterial cystitis and subclinical bacteriuria, defined as a positive urine culture in the absence of clinical signs, was not made for this subset of dogs.[14] Thus, the term bacterial growth in urine is used to refer to clinically relevant bacterial growth.

Known comorbidities were also documented and were divided into the following categories: chronic kidney disease (CKD, if listed as such in the record), acute kidney injury (AKI, if listed or described as such in the record), other renal disease (renal changes other than CKD/AKI such as renal neoplasia or renal cysts), hypertension, hyperadrenocorticism, diabetes mellitus, hypothyroidism, other endocrine disease (hypoadrenocorticism, central diabetes insipidus, hyperparathyroidism), signs of lower urinary tract disease (including any combination of stranguria, pollakiuria, dysuria, or hematuria), lower urinary tract disease (including urinary incontinence, history of recurrent urinary tract infections, ectopic ureters, historic or current cystolithiasis, or urothelial carcinoma), neurologic disease (including seizure disorders, myelopathies), immune-mediated disease (whether or not the dog was being immunosuppressed), cardiovascular disease, hepatobiliary disease, gastrointestinal disease, and neoplasia (any neoplasm outside the urinary tract). It should be noted that, although outdated, the term urinary tract infections (UTIs) will be used exclusively in the context of describing the dogs listed as having recurrent UTIs in their medical record, as in our record system, a distinction was not always made between recurrent bacterial cystitis and subclinical bacteriuria.

Continuous data were assessed for normality using a Kolmogorov-Smirnov test and visual inspection of frequency distribution histograms. Differences in continuously distributed data between dogs with and without bacterial growth in urine were assessed using *t* tests for parametric data and Wilcoxon tests for nonparametric data. The association between dog characteristics as well as other categorical data and bacterial growth in urine was initially assessed using univariable analysis with Fisher's exact tests. Characteristics with a univariable *P*-value <.2 were entered into a multivariable logistic regression model. A final multivariable model was then constructed using backwards stepwise elimination. *P*-values <.05 were considered to be statistically significant. Odds ratios (OR) and 95% confidence intervals (CI) were calculated.

3 | RESULTS

The initial search for all dogs that had UA, UPC, and QUC results yielded 1243 cases. Two hundred twelve were excluded because all testing was not performed within a 72-hour time frame, 352 were excluded because of antimicrobial therapy (other than tylosin) within the past month, 15 had missing diagnostic information, and 6 had non-cystocentesis samples, leaving 658 cases. Among these, 207 (31.4%) had a UPC <0.5 and were excluded from further analysis. The remaining 451 cases were included in the study.

Of these 451 cases, 141 (31.2%) were neutered males, 57 (12.6%) were intact males, 235 (52.1%) were spayed females, and 18 (4.0%) were intact females. A wide variety of breeds were represented. The most common breeds were as follows: 48 mixed breed dogs (10.6%), 38 miniature schnauzers (8.4%), 32 Labrador retrievers (7.1%), 22 dachshunds (4.9%), 21 cocker spaniels (4.7%), 21 Yorkshire terriers (4.7%), 18 Shetland sheepdogs (4.0%), 15 shih tzus (3.3%), 14 beagles (3.1%), 12 Chihuahuas (2.7%), 11 rat terriers (2.4%), 10 Pomeranians (2.2%), 10 boxers (2.2%), 9 Pembroke Welsh corgis (2.0%), and 8 Maltese terriers (1.8%).

The most common comorbidities were CKD (119 dogs; 26.3%), cardiovascular disease (98 dogs; 21.7%), neurologic disease (72 dogs; 16.0%), neoplasia (57 dogs; 12.6%), gastrointestinal disease (51 dogs; 11.3%), hepatobiliary disease (43 dog; 9.5%), hypothyroidism (39 dogs; 8.6%), hypertension (38 dogs; 8.4%), hyperadrenocorticism (38 dogs, 8.4%), and

TABLE 1 Univariable analysis of the association between clinical findings and the presence of bacterial growth in urine in 451 proteinuric dogs

Clinical feature	Dogs with bacterial growth in urine (n = 30)	Dogs with no bacterial growth in urine (n = 421)	Odds ratio (95% CI)	*P*-value
Median age (min – max)	11.0 years (5.0-16.0)	10.0 years (0.4-18.0)	NA	0.14
Acute kidney injury	2 dogs (6.7%)	23 dogs (5.5%)	1.2 (0.3-5.5)	0.68
Chronic kidney disease	3 dogs (10.0%)	116 dogs (27.6%)	0.3 (0.1-1.0)	0.033
Other renal disease	0 dogs (0.0%)	7 dogs (1.7%)	NA	1.00
Systemic hypertension	3 dogs (10.0%)	35 dogs (8.3%)	1.2 (0.4-4.2)	0.73
Hyperadrenocorticism	1 dog (3.3%)	37 dogs (8.8%)	0.4 (>0.0-2.7)	0.50
Diabetes mellitus	1 dog (3.3%)	15 dogs (3.6%)	0.9 (0.1-7.3)	1.00
Hypothyroidism	3 dogs (10.0%)	36 dogs (8.6%)	1.2 (0.3-4.1)	0.74
Other endocrine disease	1 dog (3.3%)	11 dogs (2.6%)	1.3 (0.2-10.3)	0.57
Signs of lower urinary tract disease	2 dogs (6.7%)	6 dogs (1.4%)	4.9 (1.0-25.6)	0.093
Lower urinary tract disease	7 dogs (23.3%)	25 dogs (5.9%)	4.8 (1.9-12.3)	0.0029
Neurological disease	6 dogs (20.0%)	66 dogs (15.7%)	1.3 (0.5-3.4)	0.60
Cardiovascular disease	9 dogs (30.0%)	89 dogs (21.1%)	1.6 (0.7-3.6)	0.26
Gastrointestinal disease	3 dogs (10.0%)	48 dogs (11.4%)	0.9 (0.3-3.0)	1.00
Immune-mediated disease	1 dog (3.3%)	18 dogs (4.3%)	0.8 (0.1-6.0)	1.00
Hepatobiliary disease	2 dogs (6.7%)	41 dogs (9.7%)	0.7 (0.2-2.9)	0.76
Neoplasia	4 dogs (13.3%)	53 dogs (12.6%)	1.1 (0.4-3.0)	0.78

Abbreviation: CI, confidence interval.

lower urinary tract disease (32 dogs; 7.1%) (Table 1). The most common reasons for dogs to be classified in the lower urinary tract disease category were urinary incontinence (13/32 dogs; 40.6%), history of recurrent UTIs (10/32 dogs; 31.3%), and cystolithiasis (8/32 dogs; 25%). The cystolithiasis subgroup included dogs with current cystolithiasis or a history of cystolithiasis (4/32 dogs each; 12.5% each).

Of the 430 dogs for which BUN and creatinine were measured, 145 (33.7%) were azotemic. Three dogs with severe azotemia (creatinine ranging from 3.7 to 10.6 mg/dL) were included in this group even though they did not strictly meet the IRIS staging guidelines (USG was not <1.030), as their USGs (which ranged from 1.035 to 1.038) were considered inadequate given the degree of azotemia and thus not attributable to prerenal azotemia alone. Of these 3 dogs, 1 dog had ultrasonographic evidence of nephritis (hyperechoic kidney, bilateral pyelectasia) and casts on urinalysis, 1 dog had glucosuria likely causing interference with refractometry, and the third had both marked proteinuria and ultrasonographic evidence of mild to moderate chronic renal degenerative changes including numerous small cysts.

Out of 451 dogs, 378 (83.8%) had urine cultured within 24 hours, 68 (15.1%) were cultured from 24 to 48 hours, and 5 (1.1%) were cultured from 48 to 72 hours of sample submission. Of these 451 dogs, 44 (9.8%) had positive growth on urine culture. The culture results were further evaluated and 30 (68.1% of all positive cultures; 6.7% of all cultures) were interpreted as clinically relevant, based on growth of ≥1000 CFU/mL. The remainder were considered contaminants. Of the 30 dogs with clinically relevant positive urine cultures, 18 (60.0%) were classified as having an active sediment. Table 2 documents the urinalysis findings of these dogs. Therefore, 12/451 (2.7%) of cases had bacterial growth in urine that would not have been suspected based on sediment analysis alone.

The most commonly cultured organism was *Escherichia coli* (14/30, 46.7%). *Enterococcus* spp., *Staphylococcus* spp., and *Streptococcus canis* were the next most frequently identified organisms, each occurring in 4/30 (13.3%) samples with clinically relevant growth. Mixed infections (>1 bacterial organism) were present in 5/30 (16.7%) of samples with clinically relevant growth.

The results of the univariable analysis were divided into clinical and laboratory findings. Clinical findings included age and comorbidities; the results of univariable analysis are presented in Table 1. Among comorbidities, chronic kidney disease ($P = .033$) and lower urinary tract disease ($P = .0029$) were significantly associated with bacterial growth in urine. Age ($P = .14$) and signs of lower urinary tract disease ($P = .093$) were also entered into the multivariable analysis as $P < .2$.

Univariable analysis of laboratory findings, including renal values and urinalysis results, are presented in Table 2. Serum BUN ($P = .019$) and creatinine ($P = .016$) concentrations were significantly lower in dogs with bacterial growth in urine than those without. Azotemia as a categorization did not reach significance ($P = .096$), but was entered into the multivariable model as $P < .2$. Hematuria ($P = .017$), pyuria ($P < .001$), and bacteriuria ($P < .001$) were significantly associated with urine bacterial growth. There was no significant difference in age, UPC, urine pH, or glucosuria between dogs with and without bacterial growth in urine; however, UPC ($P = .052$) and urine pH ($P = .13$) were included in the multivariable model as $P < .2$.

Lower urinary tract disease, signs of lower urinary tract disease, CKD, BUN, creatinine, azotemia, hematuria, pyuria, bacteriuria, UPC,

TABLE 2 Univariable analysis of the association between laboratory findings and the presence of bacterial growth in urine in 451 proteinuric dogs

Laboratory finding	Dogs with bacterial growth in urine (n = 30)	Dogs with no bacterial growth in urine (n = 427)	Odds ratio (95% CI)	*P*-value
Azotemia	5/28 (17.9%)	140/402 (34.8%)	0.4 (0.2-1.1)	0.096
Creatinine (mg/dL)[a]	0.9 mg/dL (0.4-4.9)	1.1 mg/dL (0.3-21.7)	NA	0.016
BUN (mg/dL)[a]	20.0 mg/dL (4.0-154.0)	24.0 mg/dL (3.0-289.0)	NA	0.019
Urine specific gravity[a]	1.024 (1.004-1.052)	1.019 (1.001-1.055)	NA	0.57
UPC[a]	2.3 (0.6-13.2)	3.5 (0.5-33.1)	NA	0.052
Urine pH[a]	7 (5.5-8)	6.5 (5-8.5)	NA	0.13
Hematuria	3 (10.0%)	6 (1.4%)	7.7 (1.8-32.4)	0.017
Pyuria	16 (53.3%)	10 (2.4%)	47.0 (18.1-121.8)	<0.001
Bacteriuria	14 (46.7%)	10 (2.4%)	36.0 (13.9-93.2)	<0.001
Glucosuria	1 (3.3%)	20 (4.8%)	0.7 (0.1-5.3)	1.00

Abbreviations: BUN, blood urea nitrogen; CI, confidence interval, UPC, urine protein-to-creatinine ratio.
[a]Non-normally distributed continuous data expressed as median (range), differences tested using a Wilcoxon test.

and urine pH were entered into the initial multivariable model. After backward stepwise elimination, only the presence of pyuria (OR 25.1, 95% CI 7.9 to 79.6, $P < .001$) or bacteriuria (OR 11.1, 95% CI 3.2-39.1, $P < .001$), as well as a diagnosis of lower urinary tract disease (OR 6.7, 95% CI 1.9-23.0, $P = .0028$), remained in the final model and were associated with the presence of bacterial growth in urine. If these criteria (pyuria, bacteriuria, and lower urinary tract disease) were used to prompt QUC in this population of dogs, only 8/451 (1.8%) of dogs would have had an undiagnosed bacterial growth in urine.

4 | DISCUSSION

To help rule out bacterial growth in urine as a postrenal cause of proteinuria, QUC is frequently recommended as a standard diagnostic test for proteinuric dogs.[5] In this retrospective study, we analyzed the results of UA, QUC, and UPC performed on dogs over a 10-year time period (2008-2018). Dog characteristics, including comorbidities and renal values, were also analyzed. We identified pyuria and bacteriuria (but not hematuria) to be predictors of bacterial growth in urine. Additionally, lower urinary tract disease was also associated with bacterial growth in urine.

It is not surprising that our study demonstrated an association between lower urinary tract disease and bacterial growth in urine, as many of the diseases that resulted in dogs being placed in this category affected the anatomy and function of the lower urinary tract (urinary incontinence, scrotal urethrostomy), provided a nidus for infection (cystolithiasis), or suggested an underlying disease process that increased risk of infection (recurrent UTIs). Due to the relatively low numbers of dogs in each of these subgroups, we could not determine which specific disease processes in this category predicted bacterial growth in urine in proteinuric dogs. Thus, based on our results, we would recommend culturing samples from all proteinuric dogs with a history of or current lower urinary tract disease.

Interestingly, no other comorbidities were significantly associated with bacterial growth in urine among the proteinuric dog population in this study. Dogs with hyperadrenocorticism are at increased risk for bacterial growth in urine, with rates of bacterial growth in urine over 40% in dogs with hyperadrenocorticism, diabetes mellitus, or both.[6] In euthanized dogs with hyperadrenocorticism, 6.6% have bacterial growth in urine, which is significantly higher than those without hyperadrenocorticism.[15] Given this predisposition to bacterial growth in urine, in addition to a known association with proteinuria, it is interesting that diabetes mellitus and hyperadrenocorticism were not associated with bacterial growth in urine in this study. Our study design resulted in a population biased toward enrolling dogs with an inactive sediment, thus dogs with hyperadrenocorticism and active sediment might have been excluded. The reason for this bias is that UPCs are generally requested after results of a UA are available and, given that bacterial cystitis is a known cause of proteinuria, clinicians are less likely to request a UPC if active sediment has already been identified. Another possible explanation might be that treatment of the endocrinopathy could influence rates of bacterial growth in urine; the adequacy of control of diabetes mellitus or hyperadrenocorticism was not assessed in dogs in this study.

CKD was also not associated with bacterial growth in urine, although it did reach statistical significance in the univariable analysis. In 1 cohort of dogs with CKD, 32% had positive urine cultures over the course of their disease.[16] A positive leukocyte esterase dipstick result and presence of microorganisms on sediment examination was associated with positive urine culture results and a majority (80%) of dogs with positive urine cultures had microorganisms present on sediment analysis.[16] Only 8% of these dogs had clinical signs of lower urinary tract disease, supporting that subclinical bacteriuria is the most common diagnosis associated with bacterial growth in urine in dogs with CKD.[17] In our study, CKD was not associated with bacterial growth in urine; however, we only evaluated proteinuric dogs, and our study was biased against dogs with active urine sediment (including bacteria) on sediment examination. Thus, even though dogs with CKD commonly have subclinical bacteriuria, our study might be biased against this population, as clinicians might not have requested a UPC if bacteria were identified on sediment analysis. Additionally, a dog

was only categorized as having CKD if listed as such in their diagnosis list. Thus, it is very possible that dogs with early stage CKD (IRIS Stage I) might not have been identified or listed as such, which would have led to an underestimation of this comorbidity in our study population.

Signs of lower urinary tract disease, as a category, were not associated with bacterial growth in urine. It is possible that this was due to the low number (8 dogs) within this group leading to type II error. However, a number of sterile disease processes can also result in signs of lower urinary tract disease.

There was a relatively low prevalence of bacterial growth in urine in our study population of dogs (30/451; 6.7%). This is likely in part due to the fact that UPCs are generally not performed if an active urine sediment (including bacteria on urine sediment) is identified. For this reason, this population of dogs is biased toward those with inactive sediments and likely underestimates the prevalence of bacterial growth in urine in proteinuric dogs as a whole. That being said, it is still interesting to note that the majority (60.0%) of the cases with positive growth had an active urine sediment. For some cases, the UPC was likely requested prior to having the results of the urine sediment examination. For others, dogs were identified as being proteinuric and the QUC was performed as part of our hospital's routine diagnostic approach. The selected dogs are representative of those that would be tested in a clinical setting in that UPC and QUC are performed for a variety of reasons; however, this study should not be interpreted to indicate the prevalence of bacterial growth in urine in all proteinuric dogs. A prospective study would be better suited to evaluate that prevalence.

All of the dogs with bacterial growth in urine and active urine sediments had bacteriuria or pyuria. None had active urine sediments characterized by hematuria alone. Thus, it is not surprising that hematuria, although considered a characteristic of an active sediment, was not found to be associated with bacterial growth in urine in the multivariable analysis of this study. This is likely due to the fact that a large number of sterile processes, such as cystolithiasis or iatrogenic hemorrhage secondary to cystocentesis, can also result in hematuria. Therefore, a finding of hematuria in the absence of pyuria or bacteria identified on sediment examination does not appear to support the presence of bacterial growth in urine in dogs.

In our study population of proteinuric dogs, there was a very low prevalence (12/451; 2.7%) of bacterial growth in urine with an inactive sediment. These represent dogs with bacterial growth in urine that would not have been identified by urinalysis alone. Interestingly, 4 of these 12 dogs were in the lower urinary tract disease category, such that only 8/451 (1.8%) of dogs would have had an undiagnosed bacterial growth in urine if the criteria of lower urinary tract disease, bacteriuria, or pyuria were used to prompt QUC. Of these 8 dogs, 2 had signs of lower urinary tract disease that might have prompted QUC. The 6 remaining dogs included 2 receiving chemotherapy (for mast cell tumor and lymphoma, respectively), a poorly controlled diabetic, a nonambulatory tetraparetic dog with an undefined cervical myelopathy that had an indwelling urinary catheter, and 2 dogs with cardiac disease. The dogs with cardiac disease both had chronic valvular disease: 1 was receiving pimobendan and enalapril; the other was receiving pimobendan, enalapril, and furosemide. Their QUCs grew *Enterococcus* spp. (>100 000 CFU/mL) and *Enterobacter* spp. (13 000 CFU/mL), respectively. The records for the dogs with cardiac disease indicated that the QUC was prompted by proteinuria on UA, and thus these might represent cases of subclinical bacteriuria for which antimicrobial treatment would not have been indicated. The tetraparetic dog with a urinary catheter was classified as having neurological disease and not lower urinary tract disease because of the absence of signs of lower urinary tract disease or primary urinary tract disease; however, had it been categorized with the dogs with lower urinary tract disease, it would have only further strengthened our finding that lower urinary tract disease is an indication for QUC in dogs with proteinuria. In both humans and small animals (cats and dogs) with indwelling urinary catheters, routine screening for and treatment of subclinical bacteriuria is not recommended.[18,19] In our study, the presence of a urinary catheter could have been included as a criteria for the lower urinary tract disease category. However, given that urinary catheter placement is not listed in the diagnosis list and is not always clearly or consistently noted in a specific part of the medical record, this particular risk factor was not evaluated.

As stated above, there was a very low incidence (1.8%) of bacterial growth in urine that would not have been identified if the risk factors determined by this study (lower urinary tract disease, active sediment) were used to prompt QUC. This finding suggests that routine urine culture of proteinuric dogs without these risk factors might be unnecessary. Foregoing a urine culture in a dog deemed low risk for bacterial growth in urine would help save client resources, including time and money.

In this study, we reviewed each QUC result and classified each as indicative of clinically relevant bacterial growth in urine or a contaminant based on a CFU cutoff of 1000 CFUs/mL (<1000 CFU/mL being considered a contaminant). Despite this cutoff, some positive cultures might have been misclassified. Interestingly, when we performed the same statistical analysis on the data and included all positive urine cultures, the same 3 factors (pyuria, bacteriuria, and lower urinary tract disease) remained in the final multivariable model and were significantly associated with bacterial growth in urine. We considered that distinguishing clinically relevant bacterial growth from contaminants was a more rigorous approach, and the respective odds ratio for each variable was higher than when all positive urine cultures were included.

The retrospective design of this study means that it has some inherent limitations. As previously discussed, clinicians are less likely to perform a UPC when bacterial growth in urine is suspected based on clinical signs or urine sediment analysis. This study was not designed to accurately determine the prevalence of bacterial growth in urine in dogs with proteinuria. Additionally, the diagnostic evaluation was clinician-dependent and was not standardized, so we relied on the available medical records to classify dogs by comorbidity. Some dogs might not have been fully evaluated for comorbid diseases. Furthermore, there was variation in the level of detail provided in the dogs' histories, and it is possible that signs of lower urinary tract disease were not recorded in some cases. Therefore, our study might have underestimated the frequency of signs of lower urinary tract disease. Lastly, the method of collection was frequently not stated on the quantified urine cultures. In these cases, a concurrent urinalysis was stated to be collected by cystocentesis and it was assumed the urine cultures were also performed using a cystocentesis-collected

sample, especially as this is standard protocol in our hospital. However, it is possible that voided samples were unintentionally included. For these reasons, prospective evaluation of urine cultures in dogs newly diagnosed with proteinuria is warranted.

While this study reveals characteristics that might help predict positive urine culture results for a proteinuric dog, it does not allow us to determine whether the bacterial growth in urine was the cause of the proteinuria. To determine whether the proteinuria was postrenal and resolved after resolution of the bacterial growth in urine, repeat evaluation for proteinuria would have been necessary.

In conclusion, this study determined that pyuria, bacteriuria, and lower urinary tract disease are associated with the presence of bacterial growth in urine in proteinuric dogs. An association between common comorbidities associated with proteinuria, such as hyperadrenocorticism, and the presence of bacterial growth in urine in this group of proteinuric dogs was not found. Although the prevalence of bacterial growth in urine was relatively low (6.7%) in our study, an active urine sediment or lower urinary tract disease should prompt quantitative urine culture for proteinuric dogs. Furthermore, routine urine culture in proteinuric dogs without these risk factors might not be necessary.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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