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Prevalence of positive urine culture in the presence of inactive urine sediment in 1049 urine samples from dogs

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Abstract

Background: Urinalysis (UA) is often used to screen for bacterial cystitis, regardless of sediment results, and followed up by quantitative urine culture (UC) for definitive diagnosis.

Objectives: Determine prevalence of positive UCs in dogs with inactive urine sediments on routine UA.

Animals: A total of 1049 urine samples with inactive urine sediments and UCs collected from dogs presented to a veterinary specialty hospital between January 2018 and February 2020.

Methods: Retrospective study of dogs with an inactive urine sediment on routine UA and follow-up UCs. Signalment, UA findings, proteinuria, and UC results were recorded. Associations among these findings were assessed using multivariate logistic regression carried out using a backward stepwise method.

Results: Overall prevalence of positive UC was 3.4% (95% confidence interval [CI], 2.4-4.8). *Escherichia coli* was the most commonly isolated bacteria. Only naturally voided samples were associated with increased prevalence of positive culture when compared to collection by cystocentesis or a non-specified method. No statistically significant association with culture positivity was found for urine protein-to-creatinine ratio, urine specific gravity, urine pH, breed, age, or sex.

Conclusions and Clinical Importance: Based on the low prevalence (3.4%) of positive culture in urine samples from dogs with inactive sediment on routine UA and the relatively high cost of UC and sensitivity, cost-benefit analysis including clinical suspicion of lower urinary tract disease should inform testing decisions, rather than routinely performing cultures on urine samples without active sediments.

KEYWORDS
bacterial cystitis, proteinuria, pyuria, urinary tract infection

1 | INTRODUCTION

Urinalysis (UA) is an important component of the diagnostic minimum database in healthy and ill patients alike and should be included in

both routine wellness screening and disease investigation.[1] Bacterial urinary tract infection is a common cause of morbidity in dogs and UA is a straightforward, inexpensive tool for detection of bacteriuria, with quantitative urine culture (UC) being the gold standard for diagnosis.[1,2] In some cases, positive culture results are obtained without accompanying evidence of bacteriuria, hematuria, or pyuria noted on urine sediment evaluation.[3] Given the additional expense associated with quantitative UC, the diagnostic utility of routine UC in dogs without evidence on sediment examination of urinary tract infection should be better elucidated.

A 6% prevalence of positive quantitative UCs recently was reported in 100 dogs with inactive urine sediments.[3] Our first objective was to perform a similar prevalence assessment but with a 10-fold increase in cohort size. Our second objective was to determine if concomitant proteinuria, other UA variables, or signalment was associated with positive culture in dogs with inactive urine sediments. It was hypothesized that signalment and UA results may predict UC results and therefore be informative to clinicians deciding whether or not to submit a urine sample for culture.

2 | MATERIALS AND METHODS

2.1 | Sample population

Electronic medical records from the Veterinary Specialty Hospital of San Diego (VSHSD) dated January 2018 to February 2020 were searched. A total of 2869 UAs from dogs were identified during that time period. The presence of either quantitative or qualitative UC from the same urine sample then was determined and samples with no UC (n = 1182) were excluded from the study. Age, breed, and sex were recorded for dogs with included samples. For each urine sample, collection method, urine specific gravity (USG), urine pH, urine protein concentration, presence of bacteria, and urine protein-to-creatinine ratio (UPC, when available) also were recorded. According to hospital policy, a UPC was performed automatically by the hospital's laboratory for all urine samples with trace or higher concentrations of protein (≥15 mg/dL) detected on the initial UA. Patients were excluded from analysis if they had an active urine sediment (n = 633). For the purpose of the study, an active urine sediment was defined as the presence of pyuria (>5 white blood cell [WBC] per high power field [hpf]), hematuria (>5 red blood cell [RBC]/hpf), or bacteriuria. Our institution performs 2 types of UCs: quantitative UC with speciation and minimum inhibitory concentrations (MIC) of antibiotics, and a qualitative (growth/no growth) culture; either was acceptable for inclusion. Patients that met initial inclusion criteria were segregated based on presence or absence of proteinuria. Patients with a UPC of ≤0.5 comprised the low protein group with the remainder comprising the high protein group. This UPC cutoff was based on International Renal Interest Society[4] guidelines. Clinical signs or presenting complaints of the dogs associated with the examined urine samples were not within the scope of the study.

2.2 | Urine assays

Urinalyses were performed using an automated urine chemistry analyzer (CLINITEK Atlas 10 Reagent Pak, Siemens, Tarrytown, New York) within 4 hours of sample collection. The USG was determined using the same analyzer and verified by hand-held refractometer.

The UPCs were calculated after measurement of urine protein and creatinine concentrations on a single sample using a Beckman Coulter AU analyzer (Beckman Coulter, Inc., Brea, California). Urine protein concentration was measured by colorimetric method and urine creatinine concentration using a kinetic modification of the Jaffe procedure.

All UCs were performed using the same method in a single laboratory. Calibrated loops were used to inoculate 1 μL of urine onto tryptone soya agar with 5% defibrinated sheep blood (Fisher Scientific, Pittsburgh, Pennsylvania). Plates were incubated at 37°C ± 2°C in air and then examined 12 to 24 hours later. Speciation was performed on positive UCs using a mass spectrometer (Vitek MS, bioMèrieux, Durham, NC) that uses matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) technology to identify a given bacterial species based on charge and molecular mass of its proteins. Susceptibilities were determined using a microbroth dilution method to obtain MICs (Vitek 2, bioMèrieux, Durham, North Carolina).

2.3 | Statistical analysis

Statistical analyses were performed using commercially available software packages within Rstudio (Vienna, Austria). First, normality was tested for all continuous variables (USG pH, and age) using a Shapiro-Wilks test. Variables were considered normally distributed if the test statistic (W) was >0.95. Categorical variables were assumed to be non-normally distributed. All normally distributed variables are reported as a mean ± SD. Non-normally distributed continuous variable summary statistics are reported as median accompanied by the minimum and maximum. Categorical variables are reported as the number of individuals and their percentage of the respective culture groups (n%).

All variables first were tested using univariate logistical regression models. Any variable with an unadjusted P-value < .5 was included in the full model. Multivariate logistical regression was carried out starting with a full model and removing variables that did not achieve significance. The least parameterized model then was tested against previous models using a likelihood ratio test. Once the least parameterized model was identified, all of the removed variables were tested for confounding using a likelihood ratio test. The outcome of interest for the models was the presence or absence of growth on culture. Age and sex were forced to stay in both models because of differences in biology between sexes and the aging process. Sex was tested in 2 groups, male (M) and female (F) because of the 26 dogs with unknown reproductive status. The reference for sex was female. A voided sample was set as the reference for collection method. Breed

was separated out into the 5 most common breeds and the remaining dogs grouped into the other category. This "other" category then was used as the reference for testing the effects of breed. A P-value of <.05 was considered statistically significant for the full multivariate models.

3 | RESULTS

All UAs performed between January 2018 and February 2020 were examined (n = 2869). Initially, 1182 samples were excluded because of lack of an associated UC. An additional 633 samples were excluded because of the presence of an active urine sediment as previously defined. Five additional dogs were excluded because of missing age data, bringing the total number of patients to 1049. Patients with UPC ≤0.5 were included in the low protein group (n = 687) and proteinuric patients, defined as having a UPC > 0.5, were included in the high protein group (n = 362).

3.1 | Modeling

Based on the results of univariate modeling, only protein, sex, collection method, and age were tested in the full model. Of those, collection method was the only variable not forced in based upon our hypothesis. Modeling results are presented in Table 1. Mean age in years ± SD for dogs with positive and negative cultures were 9.97 ± 2.97 and 8.69 ± 4.07 years, respectively. Sex distribution of dogs included 548 (52.2%) females and 501 (47.8%) males. Twenty-five female dogs had positive cultures, whereas 523 had negative cultures. Eleven of the 501 male dogs had positive cultures. Increases in age (odds ratio [OR], 1.08; 95% CI, 0.99-1.20; P = .12) and being male (OR, 0.50; 95% CI, 0.23-1.01; P = .06) were not significantly different.

A total of 150 breeds were represented in the study population, with Labrador Retriever (n = 73), Golden Retriever (n = 44), Yorkshire Terrier (n = 44), Chihuahua (n = 36), and German Shepherd (n = 36) most commonly represented. No significant association was found between breed and UC positivity. Twenty-two (3.2%) of the 687 dogs in the low protein group had growth on culture and 14 (4%) of the 348 dogs in the high protein group had positive cultures. No significant association was found between proteinuria and positive culture growth. Collection methods for samples were reported as voided, catheterized, cystocentesis, or not specified, with 34, 7, 601, and 407 samples, respectively. Dogs with a non-specified collection method were nearly 80% less likely to be culture positive (OR, 0.21; 95% CI, 0.08-0.63; P = .003) and those with cystocenteses performed were almost 90% less likely to be culture positive (OR, 0.11; 95% CI, 0.04-0.34; P < .001) as compared to a sample collected by voiding.

Mean ± SD USG for samples was 1.030 ± 0.02 and did not differ between samples with and without positive cultures.

3.2 | Prevalence

Thirty-six positive UCs were identified, with a 3.4% culture positivity rate (95% CI, 2.4-4.8). Speciation and MICs were performed on 26 of these samples. Of the 36 positive UCs, 10 (27.7%) had positive growth and number of colony forming units (cfu) per mL but no accompanying speciation. Of the positive cultures with speciation and MICs performed, 24 of 26 had a single bacterial species isolated. The most commonly identified pathogens were *Escherichia coli* (n = 11), *Proteus mirabilis* (n = 5), *Enterococcus* (n = 3), and *Staphylococcus pseudintermedius* (n = 2). One culture grew 2 isolates (*Escherichia coli* and *Streptococcus canis*) and another culture grew 3 isolates (*Streptococcus canis*, *Staphylococcus pseudintermedius*, and *Staphylococcus schleiferi*). Of the samples with MICs performed, 2 grew methicillin/oxacillin-resistant strains. The collection methods reported for the samples with growth were cystocentesis (n = 13) and natural voiding (n = 6). The 17 remaining positive samples had no collection method specified.

TABLE 1 Results of logistical regression for retrospective population (n = 1049)

Variable	Summary statistics		Unadjusted		Adjusted	
	With growth	Without growth	OR	P	OR	P
Protein: Low, n (%)	22 (3.2%)	665 (96.8%)	Reference		Reference	
Protein: High, n (%)	14 (3.9%)	348 (96.1%)	1.22 96. (0.6-2.38)	.57	1.04 (0.49-2.15)	.91
Voided collection, n (%)	6 (17.6%)	28 (82.4%)	Reference		Reference	
Catheter collection, n (%)	0 (0.0%)	7 (100%)	0.00 (0.00-4.49 × 10^{12})	.99	0.00 (0.00-5.69 × 10^{12})	.99
Cystocentesis collection, n (%)	13 (2.2%)	588 (97.8%)	0.10 (0.04-0.31)	<.001***	0.11 (0.04–0.34)	<.001***
Collection not specified, n (%)	17 (4.2%)	390 (95.8%)	0.2 (0.08-0.60)	.002**	0.21 (0.08–0.63)	.003**
Age (years) mean (SD)	9.97 (2.97)	8.69 (4.07)	1.09 (1.00-1.20)	.06	1.08 (0.99–1.20)	.11
Male, n (%)	11 (2.2%)	490 (97.8%)	0.47 (0.22-0.94)	.04*	0.50 (0.23-1.01)	.06
Female, n (%)	25 (4.6%)	523 (95.4%)	Reference		Reference	

Note: Summary statistics show the count per group and percentages calculated by row for each variable. Unadjusted results are from the univariate models, and adjusted results are from the multivariate model.

*P < .05, **P < .01, ***P < .001.

Of the 10 urine samples lacking full culture and MIC determination, 2 samples (20%), both collected by cystocentesis, had >100 000 cfu/mL. Of the 26 positive samples with speciation and MICs data available, only 8 (30.8%) had >100 000 cfu/mL. Bacterial quantification for the remaining 26 samples ranged from 4000 to 75 000 cfu/mL.

4 | DISCUSSION

The overall prevalence of positive UC in dogs with inactive urine sediments in our study was 3.4%, which is lower than the previous reported prevalence of 6%.[3] No statistically significant difference in positivity rate was noted between the low protein and high protein groups. These findings corroborate those of prior studies that also failed to show correlation between increased UPC and bacteriuria.[5,6]

Prevalence of bacteriuria previously has been shown to be higher in females and older animals.[1,7] Age and sex did not show significant associations with positive UC in our study. However, based on the small number of positive samples, those variables warrant further investigation in future studies.

Collection by cystocentesis correlated with decreased odds of positive culture compared with samples collected by natural voiding. The same was true of non-specified collection methods compared with voided samples. The standard collection method at VSHSD is cystocentesis and thus it is suspected that the majority of the samples with non-specified collection method were collected by cystocentesis, resulting in the similar ORs between the 2 groups versus the natural voiding group. However, we realize a lack of specified method limits the conclusions that can be drawn from the data and more complete medical records would have benefited our study. The prevalence of urine samples with positive culture growth collected by cystocentesis and catheterization methods exclusively was 2.14%.

Previous reports have shown a possible breed predilection in German Shepherds, miniature or toy Poodles, and Labrador Retrievers for development of urinary tract infection, whereas other studies have shown no breed predilection.[8,9] Our study identified no association between breed and positive UC. In addition, E. coli growth has been found to be higher in dilute and neutral pH samples than in concentrated or strongly alkaline or acidic urine. Our study showed no correlation between pH or USG and positive UC growth.[10]

Escherichia coli was the most commonly isolated bacterial species in samples with quantitative UC, consistent with previous studies that found E. coli to be the most frequently isolated bacterial species from the urinary tract.[1,11,12]

The generally accepted cutoff for clinically relevant bacteriuria in voided urine samples is >100 000 cfu/mL and a cutoff of >1000 cfu/mL is considered clinically relevant for samples collected by cystocentesis.[13] Ten of 36 positive samples had >100 000 cfu/mL. Three of those 10 samples were collected by cystocentesis with the remaining 7 having collection methods that were not specified. Given that 47% of samples with positive growth had no recorded collection method, it is possible that some samples with <100 000 cfu/mL were positive because of contaminant bacteria rather than representing clinically relevant bacteriuria.

Our study had several limitations. Because of the retrospective nature of our analysis, the attending clinician's reasoning for UA submission was not known. In addition, there was no standardization for method of sample collection and in many cases the collection method was not recorded. Samples without a specified collection method and those collected by voiding were intentionally included in the analysis because they represent a large portion of total samples submitted to the laboratory for evaluation. Our goal was to obtain a prevalence that accurately represented the types of urine samples submitted in a private practice setting. Patient medication history at the time of sample collection was not available for a large portion of samples and thus was excluded from the analysis. Concurrent antibiotic administration, existing comorbidities, or corticosteroid use could have affected both the likelihood of developing a urinary tract infection, presence of proteinuria, and characteristics of the urine sediment at the time of UA.[14,15] An additional potential limitation is the duration of time allowed for culture growth. Urine culture samples were evaluated 12 to 48 hours after plating to screen for evidence of growth. The typical growth rate of most uropathogens is 18 to 24 hours. Thus, false negatives could have occurred in the samples evaluated between 12 and 18 hours, contributing to the lower prevalence rate observed in our study. In addition, some organisms such as Corynebacterium spp. and Mycoplasma spp. may require 4 to 7 days of incubation before growth is detected, and thus some samples classified as negative eventually could have had positive growth if allowed to incubate for a full week.[9,12]

Our first aim was to report the prevalence of UC growth in dogs with inactive urine sediments, and presenting complaints, clinical signs, and underlying comorbidities were not evaluated. Further characterization of patient populations with positive UCs is warranted. Future investigation evaluating prevalence of positive UC in samples with hematuria but no pyuria on urine sediment evaluation also is warranted. Furthermore, our study was performed at a large tertiary referral center where patients are more likely to have comorbidities predisposing to urinary tract infection development. Samples collected from a general practice setting with a higher proportion of well patients presented for screening purposes may have resulted in an even lower prevalence of growth from urine samples with inactive sediments. Additional examination of such patient population thus is warranted.

Based on the low prevalence rate (3.4%) of culture positivity in urine samples from dogs with inactive sediments and the relatively high cost of UC and sensitivity, cost-benefit analysis and clinical suspicion of lower urinary tract disease should be considered before recommendation of UC and sensitivity testing for routine UA submissions.

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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