

STANDARD ARTICLE OPEN ACCESS

Small Animal Internal Medicine Nephrology/Urology

Estimates of Urinary Calcium Excretion in Dogs With and Without Calcium Oxalate Urolithiasis

Danielle E. LaVine  | Emily L. Coffey  | Jody P. Lulich  | Jennifer L. Granick  | Eva Furrow 

From the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota, USA

Correspondence: Danielle E. LaVine (dlavine2018@gmail.com)**Received:** 22 January 2025 | **Revised:** 8 August 2025 | **Accepted:** 14 August 2025**Funding:** This work was supported by Veterinary Clinical Sciences Department, College of Veterinary Medicine, University of Minnesota. National Center for Advancing Translational Sciences, K12TR004373, UL1TR002494. Morris Animal Foundation, D12CA-031, D17CA-017.**Keywords:** calciuresis | cystoliths | hypercalciuria | nephroliths | stones | urine calcium-to-creatinine ratio

ABSTRACT

Background: Fractional excretion of calcium (FeCa) and urine calcium-to-creatinine ratios (UCaCr) estimate hypercalciuria, but more data are needed on how well they discriminate between dogs with and without CaOx urolithiasis.**Objective:** To determine the performance of FeCa and UCaCr in predicting CaOx urolith status.**Animals:** One hundred twenty-one client-owned, normocalcemic dogs: 42 CaOx stone formers (cases) and 79 controls.**Methods:** Analytical, retrospective, cross-sectional study. FeCa (%) and UCaCr (mg/mg) were calculated using measurements from urine and blood and were compared by urolith status with Wilcoxon rank-sum tests. Performance was determined with receiver operating characteristic curves; “optimal” thresholds were selected to maximize sensitivity and specificity. Potential predictors of FeCa and UCaCr (e.g., urolith status, sex, breed, age) were modeled with multivariable regression. Spearman's rank correlation was run for FeCa and UCaCr.**Results:** FeCa and UCaCr were greater in cases than controls ($p < 0.001$ for both); medians were 0.81 (0.12–2.47) and 0.060 (0.008–0.176) in cases and 0.50 (0.08–2.61) and 0.032 (0.005–0.131) in controls. Optimal thresholds for FeCa (0.56) and UCaCr (0.056) had moderate sensitivity (74% and 60%, respectively) and specificity (58% and 75%, respectively). FeCa and UCaCr were strongly correlated ($\rho = 0.94$, $p < 0.001$) and lower in males than in females (estimate = -0.70 and -0.64 , $p = 0.002$ and 0.005 , respectively).**Conclusions and Clinical Importance:** FeCa or UCaCr perform moderately well for identifying CaOx cases; dogs with high values might benefit from therapy to reduce hypercalciuria. Their high correlation makes the determination of both unnecessary. Lower values in males support the development of sex-specific thresholds.

1 | Introduction

Calcium oxalate (CaOx) uroliths are common in dogs, comprising 48% of urolith submissions from the United States to the Minnesota Urolith Center [1]. Not only is CaOx urolithiasis

common, but an estimated 50% of dogs will have a reoccurrence within 2–3 years [2]. Idiopathic hypercalciuria, defined as increased urine calcium excretion with a normal blood or serum calcium concentration, is a risk factor for CaOx urolithiasis in dogs [3–6]. Knowledge of how well urinary calcium

Abbreviations: BUN, blood urea nitrogen; CaOx, calcium oxalate; CI, confidence interval; FeCa, fractional excretion of calcium; iCa, ionized calcium; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic; UCaCr, urinary calcium-to-creatinine ratio.

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excretion predicts CaOx urolithiasis can inform selection of preventative therapies such as hydrochlorothiazide, which reduces urinary calcium excretion by ~40% in CaOx stone forming dogs [7].

In humans, the gold standard approach to estimating urinary calcium excretion is a 24-h urine metabolite evaluation [8]. In dogs, this requires repeat urinary catheterization to obtain urine specimens and housing in metabolic cages for 24 h [4, 9]. This process is expensive and can be technically challenging in female dogs. An alternative approach is measuring a urine calcium-to-creatinine ratio (UCaCr) in random fasting urine samples. In adult humans with calcium urolithiasis, UCaCr in fasting random urine samples is correlated with 24-h urine calcium excretion ($R=0.73$) [10]. While its correlation to 24-h measurements in dogs is unknown, UCaCr is significantly higher in dogs with CaOx urolithiasis compared to stone-free controls [3, 5, 6]. However, there is substantial overlap in UCaCr between dogs with and without CaOx urolithiasis [3, 6]. This might reflect biological variability but could alternatively be due to limitations of the UCaCr to detect hypercalciuria. Another option for estimating calcium excretion is to measure fractional excretion of calcium (FeCa), which is the amount of filtered calcium that escapes renal tubular reabsorption and is excreted in the urine [11]. In humans, FeCa is increased in adult patients with calcium urolithiasis compared to healthy individuals [12]. Data on FeCa in dogs with CaOx urolithiasis are lacking. If FeCa is increased in dogs with CaOx urolithiasis relative to non-stone formers, it might offer an alternative, or potentially superior, option for predicting the presence of hypercalciuria.

The primary objective of this study was to determine the performance of FeCa and UCaCr in dogs for differentiating between idiopathic CaOx stone formers and non-stone formers. We hypothesized that dogs with CaOx urolithiasis will have a higher FeCa and UCaCr than dogs without stones. Our secondary aim was to identify thresholds for both FeCa and UCaCr that optimize discrimination of CaOx stone formers from non-stone formers. We hypothesized that neither FeCa nor UCaCr threshold will achieve both a high (> 90%) sensitivity and specificity for discriminating between CaOx stone formers and non-stone formers.

2 | Materials and Methods

2.1 | Study Population

In this analytical, retrospective, cross-sectional study, data were retrieved from a database of 202 dogs that had participated in previous and ongoing CaOx urolithiasis studies at the University of Minnesota, College of Veterinary Medicine between February 2011 and March 2023 [3, 5, 13]. These studies involved recruitment of CaOx stone formers and stone-free controls using the definitions provided below. Written client consent and approval by the University of Minnesota Institutional Care and Use Committee were obtained for those studies (protocol IDs: 2107-39264A, 2005-38140A, 1807-36213A, 1509-33019A, 1207A17243 and 0908A70802).

Data were reviewed to identify dogs who were 2 years of age or older with contemporaneous urine calcium, urine creatinine, blood ionized calcium (iCa) and blood creatinine concentrations available from a visit where food had been withheld at least 12 h before to sample acquisition. Dogs were classified as CaOx stone formers (cases) or controls. CaOx stone formers were confirmed to have uroliths composed primarily of CaOx (urolith central core composed of > 70% CaOx determined by standard polarizing light microscopy and infrared spectroscopy methods) [14] by the Minnesota Urolith Center. Dogs were classified as controls if they had no history of uroliths and confirmed absence of uroliths on abdominal radiographs, abdominal ultrasonography, or both. Information extracted from medical records included concentrations of urine calcium, urine creatinine, blood iCa, blood creatinine, and blood urea nitrogen (BUN), as well as age, breed, weight, diet, medications and comorbidities. Diet was classified as “therapeutic urinary diet” if it was a prescription diet formulated to prevent CaOx urolithiasis. Prescription diets formulated for dogs with renal disease were included in this classification if the product information contained a statement that the diet was also formulated to prevent CaOx urolithiasis. Dogs were classified as recurrent CaOx stone formers at the time of sample collection if they had a recurrence of CaOx stones after a stone removal procedure.

Dogs were excluded if they had a confirmed diagnosis of another disease that alters urinary excretion of calcium (e.g., diabetes mellitus, hypercortisolism) [15–19]. Dogs were also excluded if they were hypercalcemic, defined as an ionized calcium above 5.9 mg/dL, or azotemic, defined as a creatinine greater than 1.4 mg/dL or a BUN greater than 31 mg/dL. Dogs that received systemic glucocorticoids within the last month [20–22], topical glucocorticoids within the last 7 days [23], or other drugs with known effects on urinary calcium excretion (furosemide [24], hydrochlorothiazide [7], and levothyroxine [25]) were excluded. Dogs were also excluded if they were under general anesthesia at the time of sample acquisition due to the effect of sodium-containing intravenous fluid therapy on increasing urinary calcium excretion in dogs [26].

2.2 | Laboratory Measurements

Urine was collected by midstream voiding or cystocentesis. Urine was analyzed for calcium (spectroscopy and the calcium-sensitive dye Arsenazo II) and creatinine (modified Jaffe procedure) concentrations (Beckman AU480, Beckman Coulter, Brea, CA). The urine creatinine calibrator reagent was changed in August of 2018, which was predicted by internal validation to result in a 13% decrease in urine creatinine concentrations compared to the previous reagent; samples were thus labeled as measured using the original versus new reagent. Venous blood samples were collected, and iCa and creatinine concentrations were determined with a blood gas analyzer (i-STAT 1, Abbott Point of Care Inc., East Windsor, NJ).

UCaCr and FeCa were calculated for each dog. The UCaCr was calculated by dividing urine calcium concentration in mg/dL by urine creatinine concentration in mg/dL, resulting in units of mg/mg. FeCa was calculated using the following formula [11]:

Fractional excretion of Calcium (%) = (urine calcium × blood creatinine concentration)/(blood iCa × urine creatinine concentration) × 100.

2.3 | Statistical Analysis

Statistical analysis was performed using R statistical software (v. 4.2.2, www.r-project.org, accessed February 16, 2023). Continuous data were tested for normality with the Shapiro–Wilk test and visualized with Q-Q plots created using the ‘lattice’ package (version 0.20.45) [27]. Data that followed a normal distribution (blood iCa concentration) were reported as mean ± standard deviation and compared between CaOx stone formers and controls with a Student’s *t* test. Data that did not follow a normal distribution (blood creatinine concentration, BUN concentration, FeCa, UCaCr, age, and weight) were reported as median (range) and compared between CaOx stone formers and controls with Wilcoxon rank-sum tests. For FeCa and UCaCr, thresholds were selected in 0.1% and 0.01 mg/mg increments, respectively. Sensitivity and specificity for identifying a CaOx stone former were determined for each threshold. The 95% confidence intervals (95% CIs) for these values were calculated using 2000 stratified bootstrap replicates. Receiver operating characteristic (ROC) curves were used to determine the optimal thresholds for FeCa and UCaCr to discriminate CaOx stone formers and controls (by maximizing both sensitivity and specificity) using the ‘pROC’ package (version 1.18.0) [28]. The area under the curve with 95% CIs was calculated using the default method for the package (DeLong’s method). Data from all dogs that met the study criteria were included; a sample size or power calculation was not performed. The Spearman’s rank correlation was used to evaluate the strength and direction of the relationship between FeCa and UCaCr. Multivariable regression models were built to test predictors of FeCa and UCaCr; these independent variables were natural log-transformed for the regression. Models were built including CaOx stone status (stone former versus control), age (years), sex (male versus female), creatinine reagent (old versus new), blood iCa concentration, and

breed (Miniature Schnauzer versus other). All non-Miniature Schnauzer breeds were grouped together in the “other” category because they comprised less than five dogs in one or both CaOx stone status groups. Models were also created for the subset of CaOx stone formers to test predictors that only varied within this group. The stone former models included predictors found to be significant in the overall model as well as diet (therapeutic urinary versus other) and recurrence status (recurrent versus first-time stone former). Statistical significance was set at a *p* value < 0.05; as stated by the American Statistical Association statement on *p* values, this is not synonymous with importance or clinical significance of a finding [29]. For model predictors reaching statistical significance, least square means and 95% CIs were determined and back-transformed from the natural log scale using the “emmeans” package (version 1.8.7, <https://rvlenth.github.io/emmeans/>, accessed May 11, 2023).

3 | Results

3.1 | Study Group

Data from 157 dogs were reviewed for study eligibility. Thirty-six dogs were excluded (18 under anesthesia at time of sample acquisition, 9 for azotemia, 4 for hypercalcemia, 3 for medications, 1 for diabetes mellitus, and 1 for hypercortisolism). After exclusions, there were 121 dogs in the final study group; data from 68 of the dogs, including UCaCr, were previously published in other studies [3, 5]. The study group comprised 42 CaOx stone formers and 79 controls. Sex, age, and weight data are summarized in Table 1. There was a greater proportion of male dogs in the CaOx stone formers group than in the control group (0.81 versus 0.56, respectively; *p* = 0.0090). Twenty-three of 42 cases (55%) were recurrent CaOx stone formers. All 42 cases had cystoliths, 16 (38%) additionally had nephroliths, and 5 (12%, all males) additionally had urethroliths. Breeds represented in the CaOx stone formers group were the Miniature Schnauzer (20), mixed breed (6), Yorkshire Terrier (5), Bichon Frise (4), Shih-Tzu (2) and 1 each of the following breeds: American Staffordshire Terrier, Australian

TABLE 1 | Comparison of clinical data, including measures of urinary calcium excretion, between 42 dogs with calcium oxalate urolithiasis and 79 control dogs without urolithiasis.

	CaOx stone formers (<i>n</i> = 42)	Controls (<i>n</i> = 79)	<i>p</i>
Age (years)	9 (2–17)	10 (6–16)	0.33
Sex (M, F)	8F (19%), 34M (81%)	35F (44%), 44M (56%)	0.0090
Body weight (kg)	8 (2.4–27.4)	8.18 (1.09–16.8)	0.33
Blood creatinine (mg/dL)	0.8 (0.2–1.2)	0.8 (0.5–1.2)	0.51
BUN (mg/dL)	15 (4–29)	14 (3–26)	0.85
Blood iCa (mg/dL)	5.5 ± 0.30	5.4 ± 0.21	0.036
Urine calcium	7.6 (1.0–19.2)	5.1 (0.7–24.9)	0.027
FeCa (%)	0.81 (0.12–2.47)	0.50 (0.08–2.61)	< 0.001
UCaCr (mg/mg)	0.060 (0.008–0.176)	0.032 (0.005–0.131)	< 0.001

Note: *p* values for Wilcoxon rank-sum tests or Fisher’s exact tests are reported. The laboratory reference interval for blood creatinine is 0.8–1.5 mg/dL, BUN is 8–35 mg/dL, and blood iCa is 5.1–5.9 mg/dL. Results are expressed as median (range) for age, body weight (kg), blood creatinine, BUN, urine calcium, FeCa (%) and UCaCr. Results are expressed as a mean ± standard deviation for blood iCa. Sex is provided as a count (% of group).

Abbreviations: BUN, blood urea nitrogen; FeCa, fractional excretion of calcium; iCa, blood ionized calcium; UCaCr, urine calcium-to-creatinine ratio.

Shepherd, Chihuahua, Fox Terrier, and Kerry Blue Terrier. Breeds represented in the control group were the Miniature Schnauzer (41), Bichon Frise (12), Shih Tzu (9), Standard Schnauzer (8), Dachshund (5), Yorkshire Terrier (2) and mixed breed (2).

The dogs consumed variable diets. Twenty dogs (16 cases and 4 controls) were fed therapeutic urinary diets (including 2 cases fed therapeutic renal diets), and 101 dogs (26 cases and 75 controls) were fed over-the-counter commercial diets or homemade diets. The four controls fed therapeutic urinary diets had housemates with historic urolithiasis.

3.2 | Biochemical Data and Estimates of Urinary Calcium Excretion

Blood measurements and estimates of urinary calcium excretion are summarized in Table 1. Statistically significant differences in blood creatinine and BUN concentrations were not detected between groups. Blood iCa concentration, FeCa, UCaCr, and urine calcium were greater in the CaOx stone formers than controls.

The ROC curves for FeCa and UCaCr are shown in Figures 1 and 2 and had areas under the curve of 0.68 (95% CI: 0.58–0.78) and 0.69 (95% CI: 0.59–0.79), respectively. The optimal thresholds based on the ROC curve analyses to maximize both sensitivity and specificity were 0.56% for FeCa with a sensitivity of 74% (95% CI: 60%–86%) and specificity of 58% (95% CI: 47%–70%) and 0.056 mg/mg for UCaCr with a sensitivity of 60% (95% CI: 43%–74%) and specificity of 75% (95% CI: 65%–84%). The sensitivity and specificity at each of the evaluated thresholds are presented in Table 2 (FeCa) and Table 3 (UCaCr). There was a strong

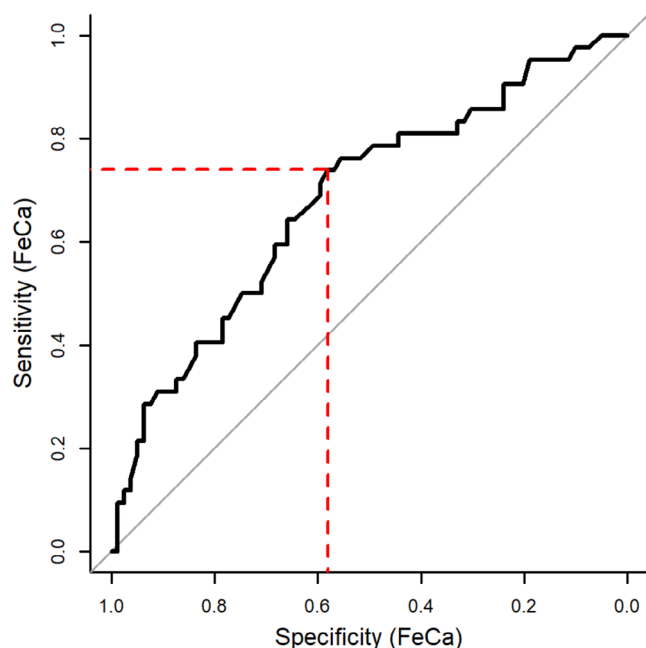


FIGURE 1 | Receiver operating curve of urinary fractional excretion of calcium (FeCa) for the prediction of calcium oxalate urolithiasis in dogs. The area under the curve is 0.68 (95% confidence interval: 0.58–0.78), and the red dashed line indicates the threshold that maximizes sensitivity and specificity (0.56%).

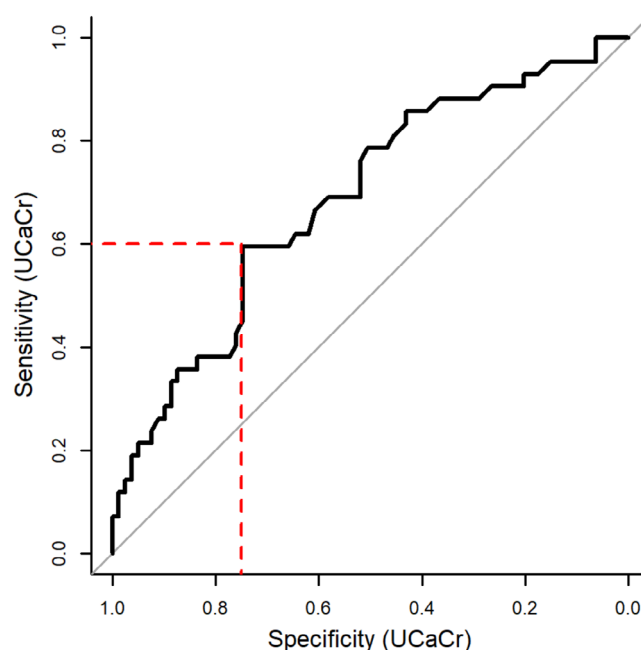


FIGURE 2 | Receiver operating curve of urine calcium-to-creatinine ratio (UCaCr) for the prediction of calcium oxalate urolithiasis in dogs. The area under the curve is 0.69 (95% confidence interval: 0.59–0.79), and the red dashed line indicates the threshold that maximizes sensitivity and specificity (0.056 mg/mg).

TABLE 2 | Sensitivity and specificity for thresholds of fractional excretion of calcium (FeCa) to predict calcium oxalate (CaOx) urolith status in 121 dogs.

FeCa threshold (%)	Sensitivity (95% CI), #True positive/#With disease	Specificity (95% CI), #True negative/#Without disease
0.30	0.86 (0.74–0.95), 36/42	0.24 (0.15–0.34), 19/79
0.40	0.81 (0.69–0.93), 34/42	0.41 (0.30–0.52), 32/79
0.50	0.79 (0.64–0.90), 33/42	0.49 (0.39–0.61), 39/79
0.56	0.74 (0.60–0.86), 31/42	0.58 (0.47–0.70), 46/79
0.60	0.64 (0.50–0.78), 27/42	0.65 (0.54–0.75), 51/79
0.70	0.60 (0.43–0.74), 25/42	0.68 (0.58–0.78), 54/79
0.80	0.50 (0.36–0.64), 21/42	0.72 (0.62–0.82), 57/79
0.90	0.45 (0.31–0.59), 19/42	0.78 (0.70–0.87), 62/79

Note: The FeCa threshold that maximized sensitivity and specificity for prediction of CaOx stone status based on a receiver operating characteristic curve analysis is bolded.

Abbreviation: FeCa, fractional excretion of calcium.

TABLE 3 | Sensitivity and specificity for thresholds of urinary calcium-to-creatinine ratios (UCaCr) to predict calcium oxalate (CaOx) urolith status in 121 dogs.

UCaCr threshold (mg/mg)	Sensitivity (95% CI), #True positive/#With disease	Specificity (95% CI), #True negative/#Without disease
0.030	0.79 (0.64–0.91), 33/42	0.47 (0.35–0.58), 37/79
0.040	0.69 (0.55–0.83), 29/42	0.57 (0.47–0.68), 45/79
0.050	0.60 (0.45–0.74), 25/42	0.70 (0.59–0.80), 55/79
0.056	0.60 (0.43–0.74), 25/42	0.75 (0.65–0.84), 59/79
0.060	0.57 (0.43–0.71), 24/42	0.75 (0.64–0.85), 59/79
0.070	0.38 (0.24–0.52), 16/42	0.84 (0.75–0.91), 66/79
0.080	0.33 (0.19–0.48), 14/42	0.89 (0.81–0.95), 70/79
0.090	0.26 (0.14–0.40), 11/42	0.91 (0.85–0.97), 72/79

Note: The UCaCr threshold that maximized sensitivity and specificity for prediction of CaOx stone status based on a receiver operating characteristic curve analysis is bolded.

Abbreviation: UCaCr, urinary calcium-to-creatinine ratio.

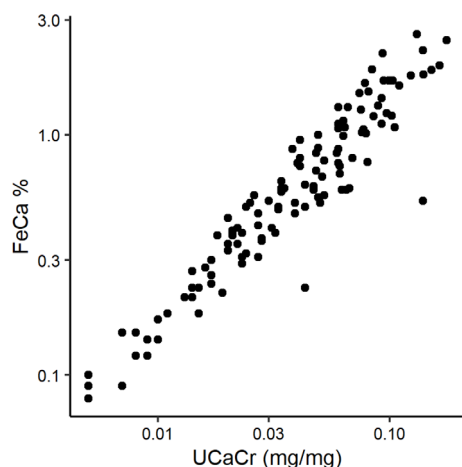


FIGURE 3 | Plot showing the strong correlation (Spearman's rank coefficient of 0.94, $P < 0.001$) between fractional excretion of calcium (FeCa) and urine calcium-to-creatinine ratio (UCaCr) in dogs with calcium oxalate urolithiasis and stone-free controls. Log10 scales are used for both the x and y axes.

correlation (Spearman's rank correlation coefficient = 0.94, $p < 0.001$) between FeCa and UCaCr (Figure 3).

Multivariable regression analyses were performed to evaluate CaOx urolith status, age, sex, urine creatinine reagent method, blood iCa concentration, and breed (Miniature Schnauzer versus

other) as predictors of FeCa and UCaCr (Table 4). Being a CaOx stone former was a predictor of greater FeCa and UCaCr ($p < 0.001$), and male sex was a predictor of lower FeCa and UCaCr. The FeCa and UCaCr data are plotted by stone status and sex in Figures 4 and 5, respectively. No statistically significant effects of the other predictors were detected on either estimate of urinary calcium excretion. The back-transformed least square means and 95% CIs for stone status and sex are shown in Table 5.

To test how FeCa and UCaCr are affected by variables with relevance specific to stone formers (being fed a therapeutic urinary diet and stone recurrence status), additional multivariable regressions were performed using only dogs from the case group. Sex was also included in these regressions based on its effect on both FeCa and UCaCr in the overall models. Regression results are shown in Table S1 and did not identify a statistically significant effect of any of these three predictors in the case group.

4 | Discussion

This study demonstrates that FeCa and UCaCr are higher in CaOx stone-forming dogs than in control dogs, though substantial overlap occurs between groups. The FeCa and UCaCr thresholds determined by the ROC curve analyses had only moderate sensitivity (74% and 60%, respectively) and specificity (58% and 75%, respectively). However, different thresholds can be selected to optimize either sensitivity or specificity, depending on the goals of the clinician or researcher. The FeCa and UCaCr were strongly correlated, suggesting that clinical determination of both calculations is unnecessary to predict stone status and the presence of hypercalciuria.

Our finding of elevated FeCa and UCaCr levels in CaOx stone formers compared to controls is consistent with previous research, which shows the majority of dogs with CaOx stones exhibit increased urinary calcium excretion [3, 4, 6]. In a previous study evaluating UCaCr in a small number of male Miniature Schnauzers, the optimal threshold to identify CaOx stone formers was 0.06 mg/mg and had a sensitivity of 56% (95% CI: 21%–86%) and specificity of 93% (95% CI: 80%–100%) [6]. Our study identified a similar optimal threshold of 0.056 mg/mg with comparable sensitivity at 60% (95% CI: 43%–74%) but lower specificity at 75% (95% CI: 65%–84%). However, the confidence intervals for specificity overlapped between our data and the previous study, suggesting that the difference between the two studies might be due to random sampling variability. Alternatively, the lower specificity in this study could be attributed to differences in study design. The present study included both sexes and multiple breeds; this greater variability in the signalment of the study participants might have reduced the specificity. Further, the controls in this study were screened with either abdominal radiography or ultrasonography, whereas all dogs in the previous study underwent abdominal ultrasonography. If some dogs in the present study had small uroliths that were not radiographically apparent and were thus erroneously classified as controls, this might reduce the specificity of the UCaCr threshold.

In this study, FeCa and UCaCr were highly correlated. This suggests that both diagnostics are not necessary to estimate hypercalciuria in idiopathic CaOx stone formers or healthy dogs.

TABLE 4 | Multivariable regression for effects of calcium oxalate (CaOx) stone status, sex, age, urine creatinine calibrator reagent (original vs. new), blood ionized calcium concentration, and breed (Miniature Schnauzer vs. other) on the natural log-transformed fractional excretion of calcium (FeCa) and urine calcium-to-creatinine ratios (UCaCr) in 121 dogs.

Predictor	Log(FeCa)			Log(UCaCr)		
	Estimate	SE	p	Estimate	SE	p
CaOx status, case	0.57	0.15	<0.001	0.60	0.16	<0.001
Sex, male	−0.43	0.15	0.0053	−0.41	0.15	0.0092
Age (per year)	0.03	0.03	0.36	0.04	0.03	0.23
Creatinine reagent, new	0.06	0.24	0.82	0.11	0.25	0.66
Blood ionized calcium	0.45	0.31	0.15	0.53	0.31	0.093
Breed, Miniature Schnauzer	−0.14	0.15	0.36	−0.17	0.15	0.27

Abbreviation: SE, standard error.

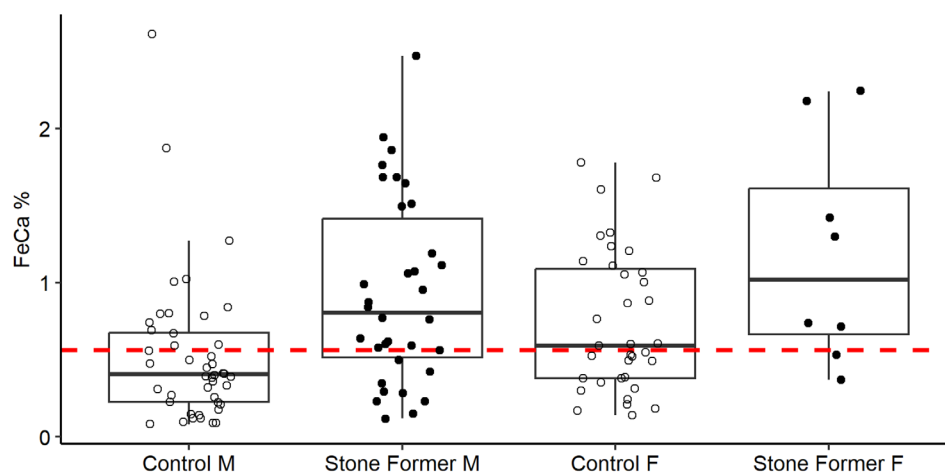


FIGURE 4 | Box and whisker plot of fractional excretion of calcium (FeCa) comparison of control dogs (open circles) and calcium oxalate stone formers (closed circles) separated by sex. The red dashed line indicates the threshold (0.56%) that maximizes sensitivity and specificity as determined by the receiver operating curve. F, female; M, male.

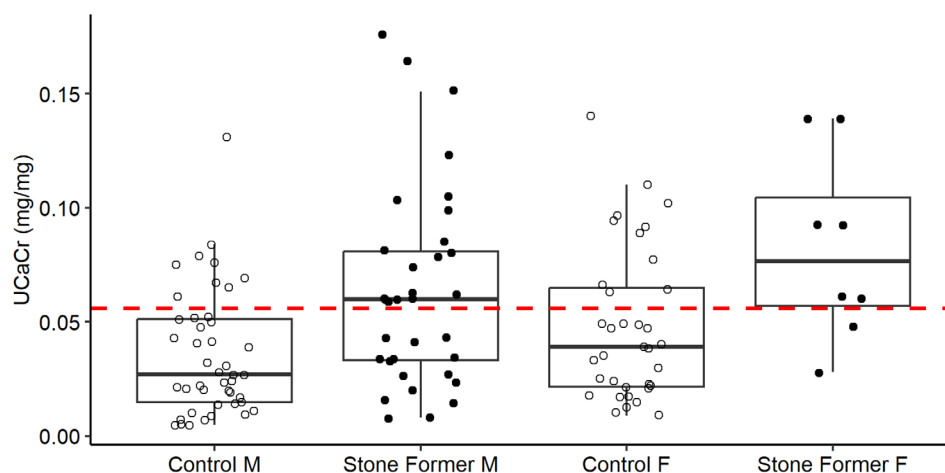


FIGURE 5 | Box and whisker plot of urine calcium-to-creatinine ratio (UCaCr) comparison of control dogs (open circles) and calcium oxalate stone formers (closed circles) separated by sex. The red dashed line indicates the threshold (0.056 mg/mg) that maximizes sensitivity and specificity as determined by the receiver operating curve.

FeCa requires urine and contemporaneous blood for iCa and creatinine concentrations, whereas UCaCr only requires a urine specimen. Therefore, UCaCr is technically an easier and less expensive estimate of urinary calcium excretion than FeCa.

In the multivariable analyses, male sex was found to predict lower UCaCr and FeCa. There are a few potential reasons why females might be protected from forming stones at the same degree of hypercalciuria as a male dog. Since upper urinary tract

TABLE 5 | Least square means (LSM) and 95% confidence intervals (CIs) from the multivariable models for fractional excretion of calcium (FeCa) and urine calcium-to-creatinine ratios (UCaCr) in 121 dogs.

	LSM (95% CI)	
	FeCa	UCaCr
Controls		
Males	0.40% (0.30%–0.53%)	0.027 (0.021–0.036)
Females	0.62% (0.45%–0.84%)	0.041 (0.030–0.056)
CaOx Stone Formers		
Males	0.71% (0.53%–0.96%)	0.049 (0.036–0.067)
Females	1.09% (0.74%–1.62%)	0.074 (0.050–0.110)

Note: Results are shown for the two significant model predictors, calcium oxalate (CaOx) stone status and sex, with results averaged over the other model predictors (age, urine creatinine reagent, blood ionized calcium concentration, and breed).

uroliths do not have a sex predisposition in dogs [30], we postulate that the sex difference in lower urinary tract uroliths is due to anatomic or functional differences in the lower urinary tract. Other considerations for such a protective effect include higher urinary citrate excretion or lower oxalate excretion in females [31, 32]. A limitation of the study is that there were relatively few female dogs in the CaOx stone former group; the lower proportion of females in the CaOx stone former group compared to the control group reflects what is observed in CaOx stone formers overall and our general hospital population [1]. In the multivariate analysis, other variables analyzed including breed, age, iCa, and methodology change (urine creatinine calibrator reagent) were not predictors of FeCa or UCaCr.

The substantial overlap in FeCa and UCaCr between cases and controls might represent true biological overlap or might be related to the limitation of using spot urine samples to estimate urine calcium excretion. In humans, the gold standard measurement of urinary calcium excretion is a 24-h urine metabolic evaluation with hypercalciuria [33]. The intraindividual variability in 24-h urinary calcium is sufficiently low in humans that a single measurement is considered adequate for evaluation [34]. In contrast, the intraindividual variability of UCaCr and FeCa in healthy dogs is relatively high [35]. Whether pooling multiple UCaCr samples might reduce this variability and better predict urinary calcium excretion remains to be determined.

For FeCa, differences in the measurement of blood or serum calcium concentration complicate comparisons of values between studies. We used blood ionized calcium concentration, whereas previous papers used serum total calcium concentration in their calculations [18, 35]. We chose to use ionized calcium concentration because the protein-bound calcium fraction is not excreted in urine [36]. While neither measurement for blood calcium concentration is perfect, ionized calcium concentration is highly correlated ($r=0.91$) to the filterable fraction of calcium [37].

The higher FeCa and UCaCr in the CaOx stone-forming dogs relative to the stone-free controls in this study is consistent with idiopathic hypercalciuria. We excluded patients with conditions or medications that have been associated with hypercalciuria, such as patients with ionized hypercalcemia, azotemia, and endocrinopathies. Though all study participants were

normocalcemic, the mean blood ionized calcium concentrations were slightly higher (by 0.1 mg/dL) in CaOx stone formers than controls. As previously theorized, this suggests the origin of idiopathic hypercalciuria is intestinal (absorptive), bone (resorptive), or both. Given that dogs with CaOx urolithiasis have lower serum β -crosslaps concentration (a marker of bone turnover) and a subset have reduced deactivation of calcitriol, absorptive hypercalciuria is more likely [38, 39].

Breeds that are high risk for CaOx uroliths might be more likely to have hypercalciuria [39, 40]. The control group in this study was comprised largely of breeds at risk for CaOx urolithiasis and might not reflect other healthy populations [17, 40]. Although we did not detect a breed effect, this breed imbalance could potentially overestimate urinary calcium excretion; our thresholds for UCaCr and FeCa apply primarily to the breeds studied.

A limitation of this study was that some dogs with confirmed CaOx uroliths had been started on a therapeutic urinary diet and potassium citrate prior to sample collection. Therapeutic urinary diets are formulated to reduce urine calcium concentrations [7]. Potassium citrate also reduces urinary calcium excretion by ~30% in humans; hypothesized mechanisms include decreased bone resorption due to its alkalizing effects, binding of calcium by citrate in the intestines, and increased tubular reabsorption of calcium [41]. Inclusion of stone-forming dogs receiving a diet or supplement with potassium citrate could underestimate their FeCa and UCaCr. Diet standardization was not possible due to the retrospective nature of this study. We did not identify an effect of therapeutic urinary diet on UCaCr or FeCa in the regression analysis for CaOx stone formers in this study, but it is possible that the estimates of UCaCr and FeCa would have been higher on maintenance diets. Therapeutic urinary diets are often the first line of preventative care for CaOx stone formers. Thus, the study group reflects the expected population of CaOx stone formers being managed in clinical practice.

In conclusion, FeCa (calculated using blood ionized calcium) and UCaCr are highly correlated and have comparable performance as estimates of urine calcium excretion to predict CaOx stone risk. Given the relative ease of measurement, UCaCr might be preferable for use by clinicians or researchers. While the thresholds determined by the ROC had only moderate sensitivity

and specificity, performance is reported at a range of different thresholds that can be selected to optimize either sensitivity or specificity, depending on the goals of the clinician or researcher.

Acknowledgments

Data were previously collected through funding by Morris Animal Foundation grants, D12CA-031 and D17CA-017, and a University of Minnesota, College of Veterinary Medicine Signature Program grant. Partial support for Drs. Eva Furrow and Emily Coffey was provided by National Institutes of Health (NIH) National Center for Advancing Translational Sciences grants, UL1TR002494 and K12TR004373, respectively. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Disclosure

Authors declare no off-label use of antimicrobials.

Ethics Statement

Approval by the University of Minnesota Institutional Animal Care and Use Committee; 2107-39264A, 2005-38140A, 1807-36213A, 1509-33019A, 1207A17243, 0908A70802. Authors declare human ethics approval was not needed.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Multivariable regression for effects of sex (male), diet (therapeutic urinary diet vs. other), and urolith recurrence on the natural log-transformed fractional excretion of calcium (FeCa) and urine calcium-to-creatinine ratios (UCaCr) in 42 dogs with calcium oxalate urolithiasis.