

Diagnosis, Prediction and Mineral Analysis of Uroliths in Canines

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Abstract: The study was conducted on 21 clinical cases of dogs suffering from urinary tract calculi to compare radiography and ultrasonography in diagnosis of urinary tract stones. Also, to assess the possibility of predicting the composition of uroliths based on urinalysis, radiopacity, type of bacterial infection and blood biochemistry. Radiopacity, urinalysis, physical characteristics of stones and blood chemistry were used as guide in prediction of the types of uroliths. Under general anesthesia the uroliths were surgically retrieved by cystotomy and urethrotomy (n=18) and from kidney at necropsy (n=1). Out of these, the chemical composition of uroliths was predicted in ten randomly selected cases. Combined urinalysis, blood chemistry, radiography and ultrasonography were able to diagnose all cases of urolithiasis in the urinary bladder, urethra and kidney. Radiography diagnosed 19 out of 21 urolithiasis cases in the urinary bladder and 12 out of 13 in the urethra and 1/1 in the kidney while ultrasonography confirmed 17 out of 21 cases in the urinary bladder and 0/1 in the kidney. Majority of the cases were associated with bacterial infection and most common bacterial isolates found were *Escherichia coli* followed by *Staphylococcus aureus*. The prediction matched in 60% cases when compared with the results of uroliths analyzed by Fourier Transform Infra-red spectroscopy. The mineral analysis showed that most of the stones were composed of magnesium ammonium phosphate followed by calcium oxalate. Ultrasonography and radiography were complementary to each other for the diagnosis of urinary tract problems. Prediction can serve as an alternative to distinguish urolith mineral composition whenever performing surgery is difficult to retrieve the urolith in an unstable patient to choose method of medical treatment.

Key words: Dog • Urolithiasis • Urolith prediction • Radiography • Ultrasonography • Kidney • Urinary bladder

INTRODUCTION

Urolithiasis is a worldwide medical and surgical issue in dogs [1]. Both ultrasonography and radiography can be used to image the urinary tract. Survey and contrast radiography are frequently utilized for diagnostic evaluation of the urinary system. Information regarding the mineral type of the urolith is also required in order to initiate medical dissolution of uroliths to prevent its reoccurrence [2]. Evaluation of urine crystals and serum chemistry values were described to help in the identification of underlying abnormalities responsible for various urinary tract affections such as renal diseases and obstructive lower urinary tract problems including uroliths

[3]. Moreover, with the advent of effective medical protocols to induce dissolution of struvite uroliths in dogs and cats and ammonium acid urate uroliths in dogs, the clinical importance of crystalluria as an indicator to urolith composition in veterinary medicine has increased. However, it was suggested that microscopic evaluation of urine crystals alone should not be relied upon as a definitive index of the mineral composition of macroliths [4]. Consequently, information about the radiographic characteristics of uroliths such as size, shape, radiodensity and surface features for the prediction of type of urolith is emerging. The number of factors reported in the literature, that could help in predicting the mineral composition of canine uroliths are urine pH,

crystal appearance, presence of bacterial infection, radiographic density, radiographic contour, serum abnormalities, breed, gender and age were described [2, 5]. Therefore, the above mentioned data advised to consider degrees of radiopacity of different mineral types of urolithiasis and their characteristic shapes in predicting the type of urolith. However, there is scarcity of literature describing the accuracy of such technique in the diagnosis of the type of uroliths. Therefore, the present study was undertaken to compare radiography and ultrasonography in the diagnosis of urolithiasis and to assess the usefulness of urinalysis, radiopacity, type of bacterium infection and blood chemistry in the prediction of the types of uroliths.

MATERIALS AND METHODS

Study Animals: The study was conducted on 21 adult dogs of eight different breeds, ranging in age from one and half to eleven years, 20 male and one female, with body weight ranging from 9-40 Kg and presented for the treatment at The Small Animal Teaching Hospital.

Urinalysis: Urine samples were collected aseptically by cystocentesis for gross and microscopic examination. In gross examination, urine was evaluated for pH, specific gravity, protein, blood, bilirubin and colour using Multiple Reagent Strips and visual inspection. In microscopic examination, a well mixed sample in graduated conical tube was centrifuged and a small amount of the sediment examined under microscope. The light microscope at low power (10X) and high power (40X) was used first to assess the quantity and type of casts, cells and sediments.

Biochemical Profile: Urea nitrogen, creatinine, calcium and phosphorous were measured and expressed as mg/dL of plasma. Urea nitrogen and creatinine were estimated using Bayer's diagnostic reagent kits with help of RA-50 Autoanalyzer based on enzymatic and picrate methods, respectively. Calcium was analysed using PHOS DT slide on Vitros DT60 autoanalyzer and phosphorous was analysed using CA DT slide on Vitros DTSC autoanalyzer.

Radiography: Right lateral and ventrodorsal survey abdominal radiographs were taken for visualization of uroliths in the urinary bladder, urethra and kidney. Radiographic factors used were 60-80 mAs and 70-95 kVp at focal film distance of 32 inches.

Ultrasonography: Ultrasonography was carried out on an animal in the dorsal or lateral recumbency using a Concept/MVC veterinary ultrasound Scanners, with 3.5 MHz micro convex or 7.5 MHz linear array transducers. The examination was started at the cranial aspect of the abdomen by evaluating the liver and gall bladder and then proceeded in a circular fashion around the left abdomen, next imaging the spleen, left kidney, urinary bladder and the prostate caudally. Similarly, the right kidney was scanned using liver and gall bladder as landmarks. Organs of interest were scanned in transverse, sagittal and/or frontal planes to evaluate the internal architecture, boundaries, organ size, shape and position.

Surgical Procedure and Gross Physical Features of Uroliths: Cystotomy was performed in all dogs with cystoliths. In addition, urethrotomy was also performed in four cases where hydroextraction and retrograde hydropropulsion had failed. In these four animals both cystotomy and urethrotomy were done. Before surgery, all dogs were premedicated with IM injection of butorphanol (0.2 mg/kg), acepromazine (0.05 mg/kg) and atropine sulphate (0.04 mg/kg) body weight. Fifteen minutes later anaesthesia was induced with thiopentone sodium (2.5% solution) to "till effect". The IV line was maintained with 0.9% normal saline solution (10-12 mL/kg) body weight per hour. After endotracheal intubation, maintenance of surgical plane of anaesthesia was done with halothane.

For urethrotomy and cystotomy standard procedures were followed and uroliths were retrieved [6]. The uroliths retrieved from urethra and urinary bladder during surgery and from kidney at necropsy were assessed for size, shape, colour, weight and gross physical features.

Qualitative Bacterial Culture: Qualitative bacterial culture was performed on the samples of urine, urinary bladder mucosal biopsy and uroliths retrieved during surgery.

Urine Culture: Urine was collected aseptically in sterile syringe either by cystocentesis using the ultrasound guidance or by direct cystocentesis during surgery to identify the causative organism. The urine samples were centrifuged in sterile tubes and a loop full of sediment was inoculated on blood agar and MacConkey Lactose agar plate. Individual colonies were identified on the basis of colony morphology, Gram's smear and standard biochemical reactions were used to identify the bacteria [7].

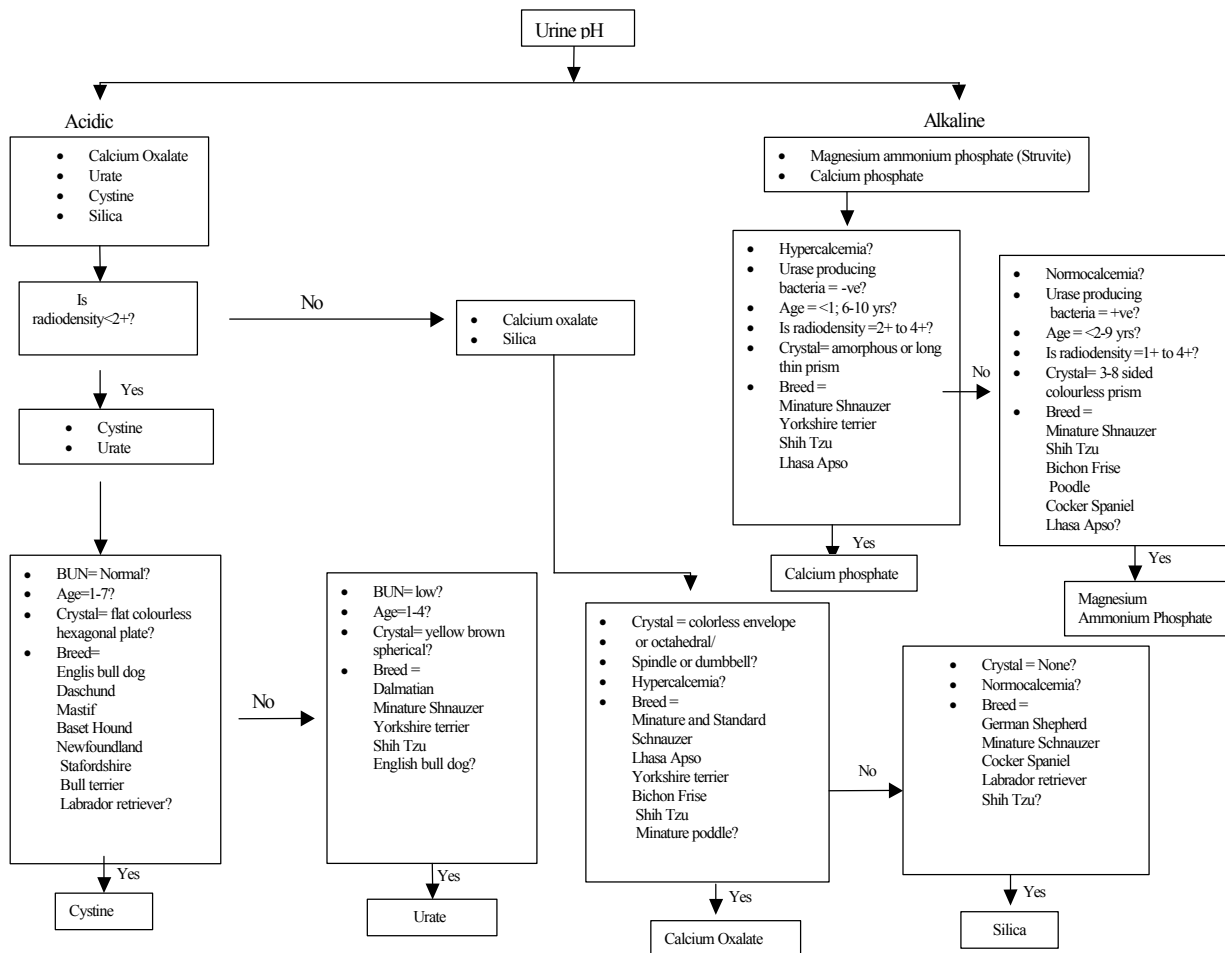


Fig. 1: Algorithm for Urolith Prediction (Based on Osborne *et al.*, [5] and Feeney *et al.*, [2])

Urinary Bladder Mucosal Biopsy Culture: In each animal the urinary bladder mucosal biopsy sample collected during surgery (Gatoria *et al.* 2006) was macerated in 2 ml of brain-heart infusion broth and incubated at 37°C for six to eight hours after which subcultures were inoculated to the blood agar and MacConkey Lactose agar plates. Thereafter, standard procedures as described by Quinn *et al.* [7] were followed.

Uroliths Culture: The representative sample from uroliths retrieved during surgery was placed in absolute alcohol for two hours after which these were washed with sterile saline four to five times and crushed with a sterile pestle and mortar. Sterilized saline of 1 ml was added to the crushed urolith and the 1 ml saline suspension was added to 4 ml sterilized trypticase soya broth. The broth tubes were incubated at 37°C for six to eight hours, after which subcultures were inoculated to blood agar and MacConkey's agar plates.

Thereafter, standard procedures as described by Quinn *et al.* [7] were followed for identification of type of bacteria.

Urolith Prediction and Analysis: The composition of uroliths was predicted before analysis using the predictor parameters given by Osborne *et al.* [5] and Feeney *et al.* [2] such as urine pH, radiographic density, urine crystals, gross physical features, urinary tract infection and frequency of occurrence in the breed. The predictor parameters were factored in the algorithm developed based on the above reports (Fig. 1). Due to limitation of finance, mineralysis of uroliths collected during surgery was done in only ten randomly selected dogs by Fourier Transform Infra-red Spectroscopy at a commercial lab (Dr Lal Path Lab Pvt Ltd, 'Eskay House', 54 Hanuman Road, New Delhi-110 001, India) and the prediction was compared with these results. The uroliths were identified based on the percentage composition of

minerals in them. When the urolith contained 70% or more of one mineral type, it was identified by that mineral type but when a urolith had less than 70% of one mineral, it was identified as mixed urolith.

RESULTS

In this study the median age of affected dogs was 6 years with a range of 1.5 to 10 years. There was a predominance of male with a ratio of 20:1. The breeds affected were Spitz (4), German shepherd (4), Mongrel (4) Doberman (3) Labrador (3), Dalmatian (2) and Boxer (1).

Gross and Microscopic Urinalysis: Dogs affected with urolithiasis had variable urine pH ranging from 5 to 8.5. Among the 21 dogs eight (38.1%) had an alkaline urine (pH = 7.5) and eight (38.1%) had an acidic urine (pH = 6.5) while five (23.8%) had neutral urine pH. Urine crystals were detected in seven (33.3%) of the cases where four (17.4%), two (8.7%) and one (4.3%) of the dogs were having triple phosphate, calcium oxalate and cystine crystals, respectively (Table 1).

Biochemical Analysis: In biochemical evaluation of dogs affected with urolithiasis, both BUN and Creatinin levels were increased above normal in 5 dogs with partial or complete urethral blockage while it was within the normal range in the remaining dogs. Serum calcium and phosphorus levels were within the normal range in the all animals (Table 1).

Radiographic and Ultrasonographic Evaluation: Radiography diagnosed majority of urolithiasis cases, 19/21 in the urinary bladder, 12/13 in the urethra while ultrasonography confirmed 17 out of 21 cases of urolithiasis in the urinary bladder. Both ultrasonography and radiography failed to detect upto 3 mm sized radiolucent uric acid stones in the urinary bladder of one dog but the largest stone that blocked the urethra in the same case was slightly radiopaque. In one case single radiolucent stone was detected by ultrasonography alone, but ultrasonography failed to detect uroliths less than 3 mm in three dogs. In a dog with kidney stone, the size of kidney was small and had hyperechoic medulla with loss of corticomedullary junction (Table 2).

Among the 21 dogs diagnosed with urolithiasis, 13 (61.9%) were in the urinary bladder and urethra both, seven (33.3%) were in the urinary bladder alone and

one (4.8%) was in the urinary bladder and kidney. The majority, (n=18, 85.7%) of the stones were radiopaque (++ to +++) relative to the soft tissue density. In one (4.8%) case it was totally radiolucent undetectable on radiography while in two (9.5%) cases it was slightly radiopaque (+).

Gross Physical Features of the Stones: The physical features of the stones were highly variable (Fig.2). The different colors of stones were grayish white (n=9, 47.4%), light green (n=7, 36.8%), yellowish brown (n=2, 10.5%) and reddish brown (n=1, 5.3%). The most common shapes of uroliths retrieved in this study were oval and round (n=5), followed by pyramidal (n=2), faceted pyramidal (n=2), botryoidal (n=1), ovoid and pyramidal (n=1), round and pyramidal (n=1), elongated-irregular (n=1). The most common surface type found was smooth (n=12, 63.2%) followed by rough and spiculated (n=3, 15.8%) and smooth in one side and rough in the other side (n=1 5.3%). The uroliths retrieved were multiple (n=16) and solitary (n=3). The mean and SE of the aggregated weight of the stones was 2.9266 ± 0.6947 g with 95% CI of (1.3550-4.4982 g). The minimum and maximum aggregated weights recorded were 0.39 and 6.999 grams, respectively. The size varied from 1 mm to 32 mm.

Bacterial Culture: UTI was found in 15 (71.4%) cases based on all the three combination of samples used. The most common bacteria isolated were *Escherichia coli* (n=8, 38.1%), followed by *Staphylococcus aureus* (n=3, 14.3%) and one each (4.8% each) of *Staphylococcus intermedius*, *Proteus mirabilis*, *streptococcus spp.* and *Arcanobacterium pyogenes* (Table 1).

Urolith Prediction and Analysis: In this study, magnesium ammonium phosphate uroliths were found in alkaline (n=3), in neutral (n=1) and in acidic (n=1) pH whereas all calcium oxalate uroliths were found at neutral urine pH. One uric acid was found at acidic urine pH. The finding of one magnesium ammonium phosphate in acidic urine was unusual. This may show that pH measurement during presentation might not represent the pH that promoted the stone formation during the course of the disease.

Before the quantitative stone analysis was made, the probable predicted composition of uroliths using predictor parameters by Osborne et al., (1999b) and Feeney et al., (1999) was correct in 6 (60%) cases when

Table 1: Diagnostic laboratory tests employed on dogs with urolithiasis and numbers found positive

Diagnostic laboratory test conducted		No. (%) positive	
Gross urinalysis	Urine colour	Red	16 (76.2)
		Pale yellow	3 (14.3)
		Dark yellow	2 (9.5)
	Urine pH	Acidic	8 (38.1)
		Neutral	5 (23.8)
		Alkaline	8 (38.1)
Microscopic urinalysis	Proteinuria		13 (61.9)
		Bilirubinuria	6 (28.6)
	Crystaluria	Triple phosphate	4 (19.0)
		Calcium oxalate	2 (9.5)
		Cystine crystals	1 (4.8)
Bacterial culture	Haematuria (>10 RBC/HPF)		18 (85.7)
	Pyuria (>5 WBC/HPF)		11 (52.4)
	Epithelial cells/casts		6 (28.6)
	Bacteria		4 (19.0)
	<i>Escherichia coli</i>		8 (38.1)
	<i>Staphylococcus aureus</i>		3 (14.3)
Biochemical analysis	<i>Staphylococcus intermedius</i>		1 (4.8)
	<i>Proteus mirabilis</i>		1 (4.8)
	<i>Streptococcus spp.</i>		1 (4.8)
	<i>Arcanobacterium pyogenes</i>		1 (4.8)
Biochemical analysis	Increased BUN levels		5 (23.8)
	Increased Creatinin levels		5 (23.8)

Table 2: Diagnostic imaging employed on dogs with urolithiasis and numbers found positive

Diagnostic imaging employed	Organ imaged	No. positive/No. imaged	Overall diagnosed
Radiology	Kidney	1/1	19/21
	Urinary bladder	19/21	
	Urethra	12/13	
Ultraonography	Kidney	0/1	17/21
	Urinary bladder	17/21	
	Urethra	Not applied	

Table 3: Prediction of urolithiasis based on predictor parameters and the result of Fourier Transform Infra-red Spectroscopy analysis

Animal case number	PH	Radiodensity	Biochemistry	Microbial growth	Age (yrs.)	Crystal	Breed	Physical characteristic of the stone	Size range (number) of stones	Aggregate weight of the stone (g)	Prediction	FR-IR analysis
1	7.00	++++	Normal	<i>E. coli</i>	6	None	Spitz	Spiculated, oval and white	24mm(1)	2.743	Caox	90% Caox dihydrate and 10% Caox monohydrate
2	7.00	+++	Normal	<i>Staph. intermedius</i>	6	None	Doberman	Smooth, faceted and greenish	4 -14mm (2)	1.043	MAP	90% Caox monohydrate 10% Caox dihydrate
3	7.00	++	Normal	<i>E. coli</i>	3	Triple phosphate	Spitz	Smooth, pyramidal plus round and greenish	12- 15mm (8)	3.52	MAP	80% MAP 20% Amonium Urate
4	8.00	+++	Normal	None	1.5	Triple phosphate	German shepherd	Smooth, pyramidal and white	3-12mm(7)	1.411	MAP	90% MAP 10% carbonate
5	6.50	+	?BUN	<i>Staph. aureus</i>	7.5	None	Dalmatian	Smooth, round and yellowish	1 - 12mm (many)	2.906	Urate	80% uric acid 20 Caox
6	7.50	+++	Normal	<i>E. Coli</i>	5	None	Mongrel	Smooth, oval and white gray	32 mm (1)	5.669	MAP	90% MAP 10% carbonate
7	7.00	++	?BUN	None	10	None	Dalmatian	Irregular, yellowish brown	3-10 mm (16)	0.39	Urate	80% Caox monohydrate 20% Caox dihydrate
8	7.50	+++	Normal	<i>Staph. aureus</i> and <i>Streptococcus spp.</i>	8	None	Mongrel	Smooth, round and light yellow	1 -10 mm (67)	4.005	MAP	90% MAP 10% carbonate
9	7.00	+++	Normal	None	6	None	Doberman	smooth and faceted, pyramidal, greenish	4-6mm (7)	0.58	MAP	70% Caox monohydrate 30% Caox
10	5.00	+++	Normal	<i>Archano bacterium pyogens</i>	6	None	Doberman	One side smooth and other side rough, Oval, white	31 mm (1)	6.999	Caox	90% MAP 10% carbonate apatite

MAP- Magnesium ammonium phosphate, Caox- Calcium oxalate



Fig. 2: Comparative size, colour, shape and surface features of ten randomly selected uroliths analyzed. The numbers 1, 2, 3, -----, 9 and 10 in the figure refers to corresponding animal case numbers in Table 3

compared with the total of 10 cases of uroliths analyzed by Fourier Transform Infra-red Spectroscopy. Out of four, three of the miss-predicted uroliths were calcium oxalate monohydrate uroliths in neutral urine (pH=7.0) while one was magnesium ammonium phosphate hexahydrate in acidic urine (pH=5.0) (Table 3).

In ten cases analyzed in this study all uroliths consisted of = 70% of one mineral type. Accordingly, the most common type of urolith found in this study was magnesium ammonium phosphate hexahydrate (n=5, 50%) followed by calcium oxalate monohydrate (n=3, 30%), calcium oxalate dihydrate (n=1, 10%) and uric acid (n=1, 10%). The most common secondary mineral found in urolith composed of = 70% magnesium ammonium

phosphate in this study was carbonate apatite. As a result, four of the five magnesium ammonium phosphate uroliths were composed of 90% magnesium ammonium phosphate and 10% carbonate apatite while the remaining one urolith was composed of 80% magnesium ammonium phosphate and 20% ammonium urate. All of the three calcium oxalate monohydrate stones analyzed in this study were composed of calcium oxalate dihydrates as secondary mineral type ranging from 10 to 30%. Consequently, one stone was composed of 90% calcium oxalate monohydrate and 10% calcium oxalate dihydrate, one composed of 80% calcium oxalate monohydrate and 20% calcium oxalate dihydrate while the remaining one stone was composed of 70% calcium oxalate monohydrate

and 30% calcium oxalate dihydrate. The other single stone identified as uric acid in this study was composed of 80% uric acid and 20% calcium oxalate dehydrate (Table 3).

DISCUSSION

Radiography diagnosed the majority of urolithiasis cases, 19/21 in the urinary bladder, 12/13 in the urethra while ultrasonography confirmed 17 out of 21 cases of urolithiasis in the urinary bladder. Both ultrasonography and radiography failed to detect less than 3 mm size radiolucent stones in the urinary bladder and kidney. Osborne *et al.* [8] concluded that radiodense urocystoliths less than 3 mm in size and radiolucent uroliths were difficult to detect by survey radiography whereas Lang [9] opined that except for single, very small stones, cystic calculi are easy to detect sonographically regardless of their radiopacity.

The mineral composition of a urolith should be known if appropriate medical treatment or dietary management is to be applied to dissolve the urolith [1]. The prediction of mineral composition of uroliths made in this study was correct in 60% cases when compared with uroliths analyzed by Fourier Transform Infra-red Spectroscopy. This is almost in accord with the findings of Weichselbaum *et al.*, [10] that recorded average mineral type accuracy of 69.9% across all mineral types based on the combination of signalment (age, breed, gender) and simulated survey radiographic findings. The lower prediction accuracy rate may be due to the insignificant contribution of the combination of signalment and survey radiographic findings to improve prediction accuracy beyond that achievable with signalment alone. The reported average mineral type prediction accuracy across all mineral types based on signalment alone was 69.8% as compared to 65.7% based on survey radiographs [10]. Feeney *et al.* [1] and Osborne *et al.* [5] had also opined earlier that uroliths mineral composition can be estimated based upon radiographic density, urine pH, urine crystals, urinary tract infection, especially with staphylococcus or proteus and frequency of occurrence in the breed. Weichselbaum *et al.* [11] had suggested that size and shape of uroliths used in conjunction with age, breeds and sex, can facilitate pure urocystolith mineral type prediction.

From the four miss-predicted uroliths in this study, three were calcium oxalate monohydrate uroliths in neutral urine (pH=7.0) while one was magnesium ammonium phosphate hexahydrate in acidic urine (pH=5.0). This

implies that the urine pH at the time of diagnosis may not reflect the urine pH which promoted the formation of the urolith.

The most common type of urolith found in this study was magnesium ammonium phosphate hexahydrate (50%) followed by calcium oxalate monohydrate (30%), calcium oxalate dihydrate (10%) and uric acid (10%). Houston *et al.* [12] reported that uroliths in dogs and cats composed of magnesium ammonium phosphate hexahydrate were those most commonly analyzed (43.8%) where as oxalate close second (41.5%) were followed by urate (4.8%), calcium phosphate (2.2%), silica (0.9%), cystine (0.4%), xanthine (0.05%) and mixed content (6.5%). Osborne *et al.* [5] evaluated uroliths by quantitative methods that included optical crystallography, x-ray diffraction and infrared spectroscopy and reported the different types of urolith composition to be 31.4% calcium oxalate, 49.6% struvite, 8% purine uroliths (uric acid and salts of uric acid, including ammonium urate), 6.6% compound uroliths and 2% cystine, calcium phosphate and mixed uroliths each. Bovee and McGuire [13], reported the different types of stones in dogs to be 69%, 7%, 3%, 10% and 11% of struvite, urate, cystine, oxalate *et al.*, respectively. Osborne *et al.* [14] found that 67% of 839 stones analysed by optical crystallography and x-ray diffraction were composed of magnesium ammonium phosphate hexahydrate. Gatoria *et al.* [15] reported that calcium oxalate and calcium phosphate were predominant, which was observed in 52.38% followed by calcium phosphate 14.29%, uric acid and calcium phosphate 19.07%, calcium oxalate 4.76%, ammonium acid urate 4.76% and magnesium ammonium phosphate and calcium carbonate phosphate 4.76%.

The most common secondary mineral found in urolith composed of = 70% magnesium ammonium phosphate in this study was carbonate apatite. As a result, majority of magnesium ammonium phosphate stones were composed of 90% magnesium ammonium phosphate and 10% carbonate apatite. Osborne *et al.* [14] reported that the most common secondary mineral found in urolith composed of = 70% magnesium ammonium phosphate was calcium phosphate, which was seen in 90.8% uroliths followed by ammonium acid urate seen in 5% and combination of calcium apatite and ammonim acid urate occurred in 3% of such struvite stones. Calcium oxalate monohydrate stones analyzed in this study were composed of calcium oxalate dihydrates as secondary mineral type ranging from 10 to 30%. According to Osborne *et al.* [14] the most common secondary mineral

in urolith composed of 70 – 99 percent calcium oxalate monohydrate were calcium oxalate dihydrate seen in 41.2% of such stones. The stone identified as calcium oxalate dihydrate in this study was composed of 90% calcium oxalate dehydrate and 10% calcium oxalate monohydrate. Osborne *et al.* [14] found that the most common secondary mineral in urolith composed of 70 – 99 percent calcium oxalate dihydrate were calcium oxalate monohydrate seen in 71.4% of calcium oxalate dihydrate stones.

CONCLUSION

Composition of uroliths can be predicted with fair degree of success using predictor parameters such as urinalysis, radiographic density and gross physical features of stones. However, prediction of urolith composition alone cannot be relied upon. Therefore, whenever urolith retrieving is possible, it should be submitted for quantitative mineral analysis.

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