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Study of major and minor components of uroliths in canine urolithiasis: Risk assessment in different breeds of dogs

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Abstract
Urolithiasis is relatively common disease in dogs. The most common form of mineral crystals that precipitate in urine are calcium oxalate monohydrate (whewellite), calcium oxalate dehydrate (wedelite), calcium phosphate as hydroxyapatite (HAP), cystine urate (ammonium urate or uric acid) and magnesium ammonium phosphate hexa hydrate (struvite). In the reported study struvite is the most commonly found mineral components of urolith seen both in male and female patients. An accurate analysis of urinary calculi is essential for understanding the etiology of urolithiasis and establishing the correct medical treatment to prevent recurrence or therapeutic management. The purpose of study reported here was to analyze major and minor elements in the stone and their percentage (%) and quantity (g/g) of major and minor minerals in the urinary calculi in different breeds of 26 numbers of dogs. i.e. GSD, Doberman, Spitz, Dalmatian, Boxer and Dachshund.
The crystallographic results were used to classify the prevalence and type of mixed uroliths. The term crystallographic analysis is used for petro graphic microscopy and physical methods including AAS, UVS and flame photometry.

Keywords: Urinary calculi, canines, crystallographic studies, AAS, UVS, flame photometry

Introduction
Urolithiasis is relatively common and recurrent disease in dogs. Surgical removal of uroliths has been the main treatment modality although in some cases dietary, managemental or medical measures were advised to prevent their recurrence. The uroliths after being formed either can interfere with the partial/complete and frequent voiding of urine. It is a common and recurrent problem in dogs and cats [6]. During uroliths formation sustained alteration in urine composition which promote the over saturation of one or more substances eliminated in urine and result in their precipitation and subsequent growth. The uroliths formation is eratic and unpredictable which emphasizes that several inter related pathological and physiological facts are often involved and uroliths are formed in all the species of domestic animals.
Definitive identification of uroliths composition requires their analysis by polarizing light microscopy, infra red spectroscopy, X-ray diffraction (XRD) and other methods of quantitative analysis such as SEM, EDX/EDS/XEDS/EDXA/EDXMA, AAS, UVS and other different biochemical estimations. The study was done with uroliths and cystoliths retrieved from 26 clinical canine patients were examined and analyzed by the above said methods and studied their mineral composition and structure as per the age, breed & gender and locality of animal.

Materials and Methods
Canine uroliths were collected (surgically) from 26 numbers of clinical canine patients of different (six) breeds like GSD [6], Doberman [6], Dalmatian [2], Boxer [3], Spits [7] and Dachshund [2]. The selected samples were subjected to different quantitative studies like X-ray diffraction, atomic absorption spectro-photometer (AAS), flame photometry, UV photometry, SEM studies using scanning electron microscope and EDX (energy dispersive X-ray) analysis.
For estimation of different mineral constituents the samples were subjected for wet chemical analysis by AAS (Atomic absorption spectrometry) and UV spectrometry. About 0.2 to 0.3gm of well pulverized urinary stone samples was taken in a 250 ml glass beaker and 20 ml of 1.1%

of HCl was added to the sample. The beaker was warmed over a hot plate for 20 minutes to get a clear solution. The solution was filtered through a Whatman no. 40 filter paper to separate any siliceous turbid material. The volume of the solution was made 100ml in volumetric flask & kept aside for analysis of trace elements like Copper(Cu), Cobalt(Co), Lead(Pb), Cadmium(Cd), Chromium(Cr), Manganese(Mn), Iron(Fe), Nickel(Ni), Zinc(Zn), Strontium(Sn), Molybdenum(Mn), lithium(Li), Aluminum(Al) and Titanium(Ti).

The acid digested solutions were fed to SIMADZU make atomic absorption spectrophotometer (AAS) model AA6300. All trace metals like Cu, Co, Pb, Cd, Cr, Mn, Fe, Ni, Zn, Sn, Mo, Li, Al and Ti were analyzed by feeding original solution to the AAS. For some major components like Mg and Ca, the acid solutions were diluted for feeding to AAS. In the AAS analysis the solution of a sample was suctioned into a high temperature flame with the help of compressed air. In the flame, the metal ion in the solution was atomized and absorbed an externally supplied energy of its characteristic wavelength from the intensity of energy absorbed; the concentration of metal ion in the solution is measured. The energy of the characteristic wave length was supplied from a hollow cathode lamp. The fuel for the flame was acetylene gas and the supporting gas either compressed air or nitrous oxide.

Sodium (Na) and Potassium (K) metal ions were analyzed with the help of flame photometer make Systronic, model MEDIFLAME 127. The sample solution was aspirated to a flame with the help of compressed air. The flame color intensity given by the analytic were measured by the detector for concentration of metal ion in the solution, LPG used as fuel for flame and compressed air used as support gas. Colored gas filters were used to measure energy intensity of the selected band. Phosphorus estimation was done by UV spectrometry. Data were evaluated statistically by ‘F’ test (significance level 0.05).

Results

Mg analysis showed Doberman, Boxer, Daschund and Spitz breed were having more or less equal% in their urinary calculi but Dalmatian showed least value. Ca was highest in Dalmatian (Fig. 1). Boxer breeds having more Sn than other breeds (Fig. 2). Boxer and GSD were having more Mo (Fig. 3). Boxer and Dachshund were almost same with respect to K (Fig. 4). Na content was highest in Dalmatian (Fig. 5). Ti was highest in Boxer (Fig. 6). Al was highest in GSD (Fig. 7) followed by Boxer & Dalmatian. The Dalmatian contains high Fe value than other breeds. Boxer containing high Cr (Fig.8), Cd (Fig. 10) and Li (Fig.11), Calcium was high in Dalmatian (Fg.12) and phosphorus high in boxer breed (Fig.13) while more or less same in all other breeds (Fig. 9 & 10).

Ca plays a significant role in the formation of urinary calculi similarly Mn, Cd, Li, Ca & P were also significant in the urinary calculi found between six breeds. Mg was found to be non significant in urinary calculi of different breeds. Similar trend was also observed with respect of K, Na, Sn, Ti, Mo, Al, Cr, Fe, Ni, Cu, Co, Pb, Zn and Ca in the calculi. It was found that, the GSD, Doberman, Boxer, Spitz and Dachshund were not differing significantly with respect to their means. However, Dalmatian was differing significantly ($P<0.05$) from other breeds. The GSD and Doberman also differed with respect to Ca.

Table 1(a): Showing concentration of Na, K, Sn, Ti, Mo, Cr, & Fe in uroliths of different species (in%)

Breed	Na	K	Sn	Ti	Mo	Al	Cr	Fe
GSD	0.07 ± 0.01^b	0.20 ± 0.02^b	1.09 ± 0.1^b	0.12 ± 0.03^d	0.10 ± 0.07^a	0.15 ± 0.03^a	5.88 ± 1.97^c	266.83 ± 10.68^c
Doberman	0.02 ± 0.00^b	0.19 ± 0.02^b	0.89 ± 0.08^b	0.23 ± 0.06^c	0.04 ± 0.00^b	0.02 ± 0.00^b	10.76 ± 5.22^b	119.34 ± 13.89^d
Dalmatian	0.12 ± 0.04^a	0.14 ± 0.05^c	1.63 ± 0.14^a	0.34 ± 0.01^b	0.05 ± 0.02^b	0.12 ± 0.05^a	5.30 ± 0.09^c	456.53 ± 56.30^a
Boxer	0.06 ± 0.01^b	0.31 ± 0.12^a	1.35 ± 0.18^a	0.56 ± 0.04^a	0.14 ± 0.02^a	0.13 ± 0.04^a	40.15 ± 13.20^a	268.72 ± 9.54^c
Spitz	0.05 ± 0.01^b	0.15 ± 0.03^c	1.58 ± 0.01^a	0.16 ± 0.03^d	0.02 ± 0.00^b	0.01 ± 0.00^b	11.64 ± 3.56^b	311.10 ± 20.20^b
Dachshund	0.04 ± 0.00^b	0.34 ± 0.01^a	1.46 ± 0.12^a	0.12 ± 0.01^d	0.03 ± 0.00^b	0.03 ± 0.00^b	6.49 ± 1.14^c	119.73 ± 13.90^d

Table 1(b): Showing concentration of micro minerals (ppm) in uroliths of different breeds of dog

Breed	Mn	Ni	Cu	Co	Pb	Zn	Cd	Li
GSD	11.22 ± 2.11^c	23.37 ± 2.31^b	7.36 ± 1.29^d	22.28 ± 7.81^d	249.45 ± 21.94^c	61.48 ± 8.44^c	27.16 ± 3.94^c	24.82 ± 7.43^c
Doberman	8.72 ± 1.48^c	12.41 ± 2.41^c	12.77 ± 5.11^c	25.58 ± 9.43^c	396.77 ± 21.62^b	77.44 ± 21.09^c	35.50 ± 8.57^{bc}	49.75 ± 15.20^b
Dalmatian	66.15 ± 12.14^a	73.96 ± 9.80^a	51.60 ± 6.20^a	55.55 ± 10.15^a	230.45 ± 17.45^c	126.25 ± 23.60^b	56.81 ± 10.99^b	20.36 ± 4.66^c
Boxer	43.70 ± 8.50^b	65.08 ± 8.20^a	19.93 ± 2.77^b	39.15 ± 10.26^b	587.00 ± 41.90^a	268.39 ± 17.62^a	96.51 ± 36.43^a	84.62 ± 9.01^a
Spitz	6.29 ± 0.85^c	18.03 ± 6.93^b	18.79 ± 2.13^b	30.53 ± 9.11^{bc}	342.49 ± 18.20^b	67.80 ± 10.72^c	10.84 ± 2.40^e	33.62 ± 9.51^c
Dachshund	1.38 ± 0.03^d	5.74 ± 1.01^d	17.51 ± 3.61^b	20.84 ± 2.30^d	224.46 ± 13.60^c	46.27 ± 6.58^d	20.00 ± 2.21^d	22.30 ± 6.77^c

Table 1(c): Showing concentration of micro elements (%) in uroliths of different breeds of dog

Breed	Mg	Ca	P
GSD	6.06 ± 0.56^c	10.94 ± 0.56^c	14.66 ± 1.29^c
Doberman	10.63 ± 0.29^a	13.79 ± 0.74^c	13.14 ± 1.21^c
Dalmatian	0.33 ± 0.05^d	24.08 ± 2.23^a	42.82 ± 4.27^a
Boxer	10.01 ± 0.48^a	18.83 ± 1.60^b	25.91 ± 4.67^b
Spitz	9.36 ± 0.17^c	7.08 ± 0.40^d	7.76 ± 0.94^d
Dachshund	11.50 ± 1.00^a	6.95 ± 0.58^d	12.99 ± 1.42^c

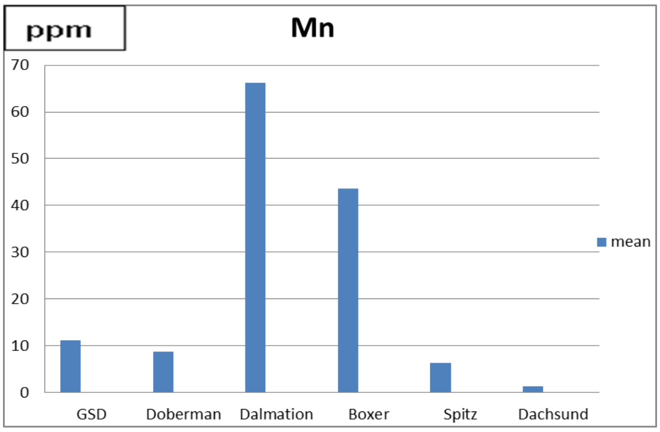

Fig 1: Comparison of 'Mn' in different breeds

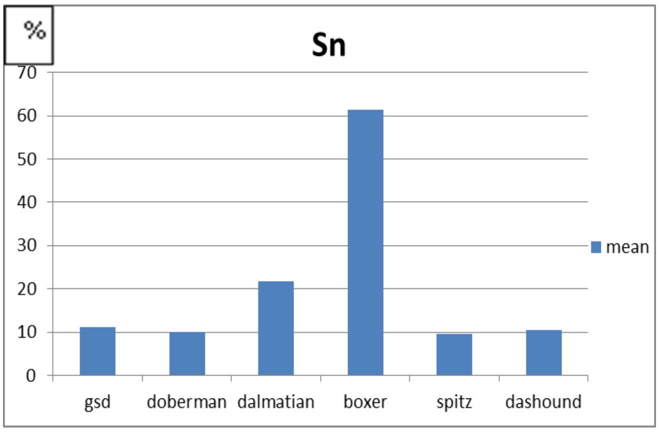

Fig 2: Comparison of 'Sn' in different breeds

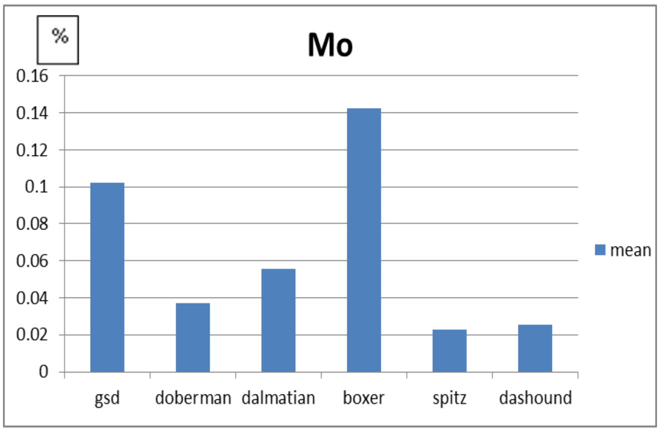

Fig 3: Comparison of 'Mo' in different breeds

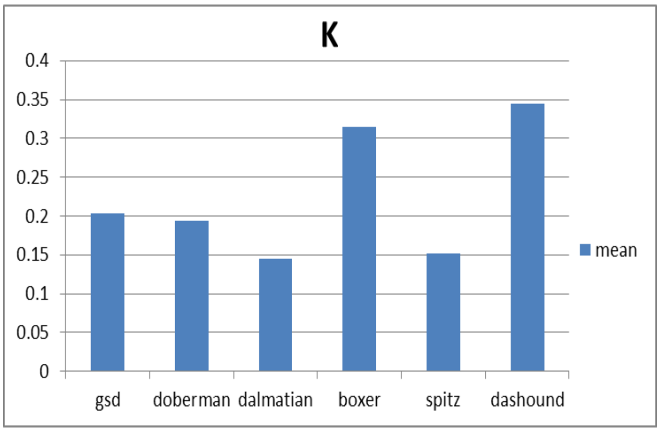

Fig 4: Comparison of 'K' in different breeds

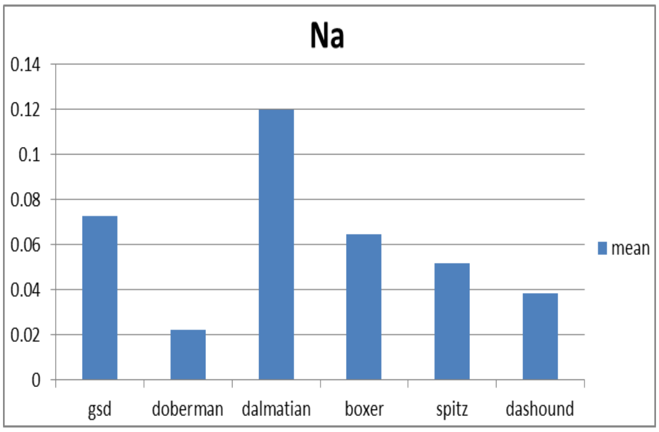

Fig 5: Comparison of 'Na' in different breeds

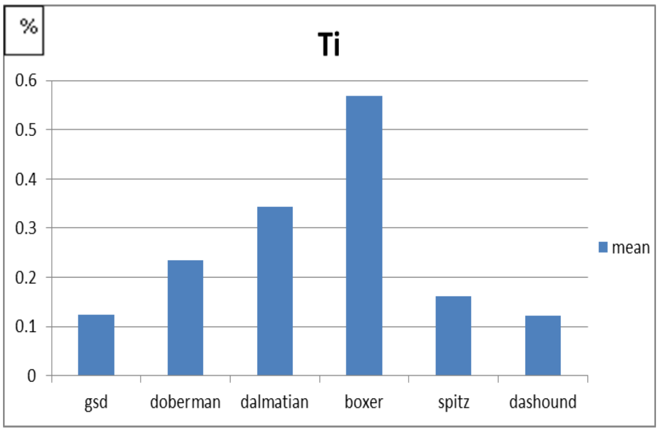

Fig 6: Comparison of 'Ti' in different breeds

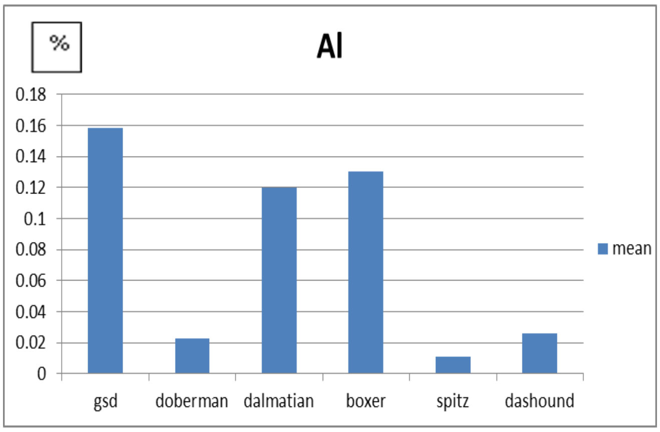

Fig 7: Comparison of 'Al' in different breeds

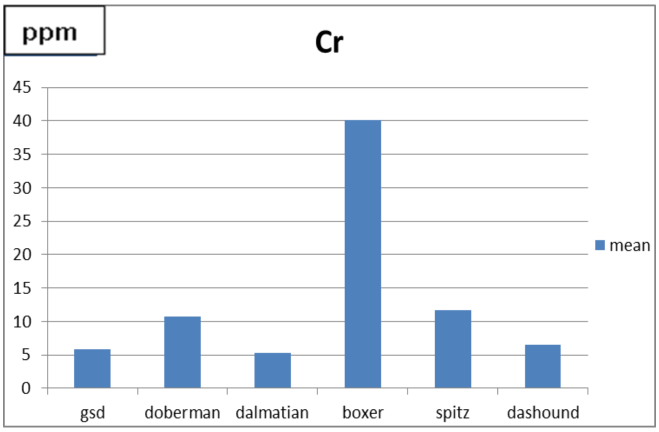

Fig 8: Comparison of 'Cr' in different breeds

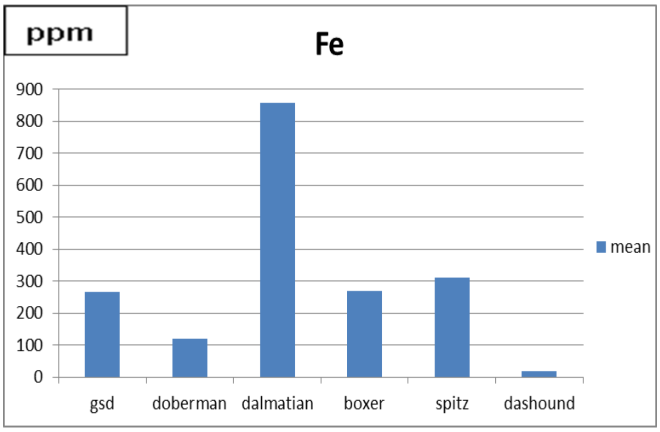

Fig 9: Comparison of 'Fe' in different breeds

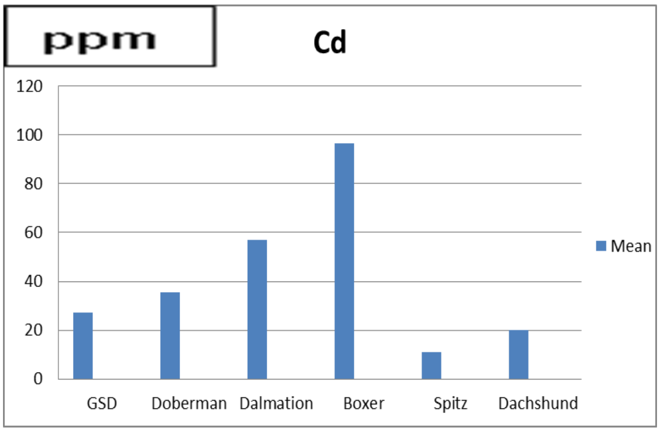

Fig 10: Comparison of 'Cd' in different breeds

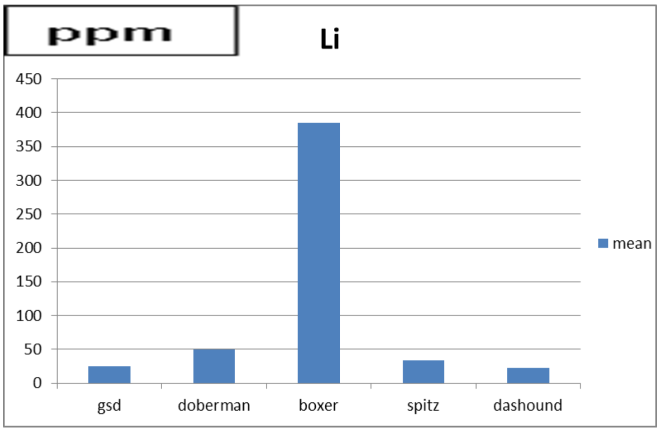

Fig 11: Comparison of 'Li' in different breeds

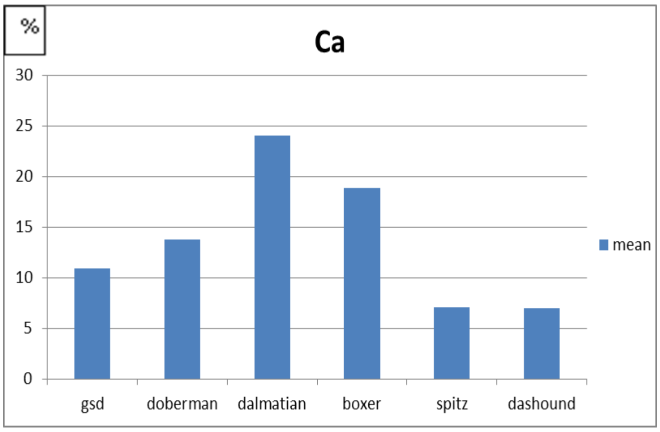

Fig 12: Comparison of 'Ca' in different breeds

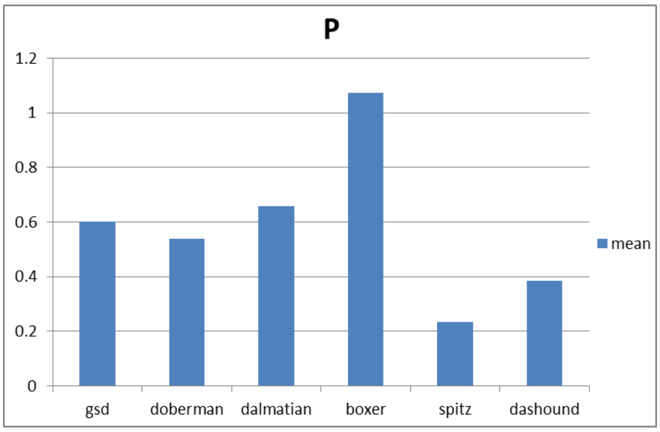

Fig 13: Comparison of 'P' in different breeds

Discussion

The study resulted with all the 14 trace elements in all the stones analysed like others [2] and no statistical significant difference marked between six breeds analysed. But there was difference in some of the elements in some breeds. [3, 5, 7, 8]

The results showed in the study agree with [2] in respect of all the trace elements under investigation were found in urinary stones as long as the latter present in urine. The concentration of major and minor (trace) elements vary to different breeds. Because of mineral concentrations of trace elements compared with the main components of urinary stones the influence on the crystallization process in urine appears to be of secondary importance. Highly significant difference was noted, in the excretion of urinary Ni, Mn and Li as well as in serum concentration of Ni, Mn and Cd between urinary stone patients and the healthy control group.

These 14 minor elements were significantly higher in certain breeds than others. These results give idea that the trace elements also play significance role in the formation of urinary stones while little is known about the biological significance of these trace elements. However, there are statements in the literature that, Mn is supposed to be the central importance as a coenzyme for the synthesis of mucopolysacharides. Similarly, a reverse relationship to calcium absorption in the gut has been reported [1].

Analysis of calculi revealed that, struvite or magnesium ammonium phosphate (MAP) was the most common urolith mineral type found in dogs accounting to 15/26 (57.69%). Next to MAP was calcium oxalate uroliths 4/26 (15.38%), mixed 3/26 (11.538%), urate 2/26 (7.70%) and silica 2/26 (7.70%). The percentage of male and female animals forming uroliths of each mineral type were calculated and male (21/26) counts 80.7% found more prone to urolithi asis than females (5/26) counting 19.3% under study. Breed distribution indicated that certain breeds of dogs were at increased risk of developing specific urolith types for example Dalmation with urates. It was found that small breeds of dogs were more prone to formation of uroliths. These may be attributed to the smaller size of their urinary tract and also to greater confinement in the house or flat, which may favour urine retention and consequently urinary tract infection (4).

Calcium oxalate uroliths on physical examination appeared hard and brittle with sharp edges protruding from the surface and magnesium ammonium phosphate calculi were yellow to white in color and fairy hard and easily crushed to chalk powder where as ammonium urate calculi found to be yellow colour brittle and smooth. It is possible that difference in the pattern of urolith formation observed among breeds i.e. Spitz tends to be the highest and Dalmatian and Dachshund found to be lowest.

Conclusion
The trends identified in the study may assist in clarifying the groups at increased risk of developing urolithiasis. This will allow researchers to work with “at risk” breeds to identify and reduce primary risk factors and therefore the chances of initial stone formation. The information will also assist the veterinarians in the accurate diagnosis of urolithiasis in dogs, thereby allowing implementation of an appropriate management strategy for the prevention of stone recurrence.

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References
1. Hamdbidge KM. The need for trace element analysis in the medical sciences, in elemental analysis of Biological material current problem and technique with special reference to trace elements, Vienne IAEA Technical reports series.1980; 197:19-28.
2. Hofbauer J, Stessan I, Hobarth K, Vujic G. Trace elements and Urinary stone formation: New aspects of the Pathological Mechanism of Urinary Stone formation Schwet 3 H. The Journal of Urology. 1991; 145:93-96.
3. Joost J, Tessadri R. Trace element investigation in kidney stone patients Eur. Urol. 1987; 13:264-270.
4. Low WW, Uhl JM, Kass PH, Ruby AL, Westropp JL. Evaluation of trends in urolith composition and characteristics of dogs with urolithiasis.25, 499 cases. JAVMA. 2010; 236(2):193-200.
5. Meyer JL, Angino EE. The role of trace metals in Calcium Urolithiasis. Invest Urol. 1977; 14(5):347-350
6. Osborne CA, Sanderson SL, Lulich JP. Canine cystine urolithiasis, cause, detection, treatment and prevention, Vet. Clin North Am Small Anim. Pract. 1999; 29:213-230.
7. Scott R, East BW, Janczyszyn, Boddy K, Yates AJ. Concentration and distribution of some minor and trace elements in Urinary tract stones a preliminary study, Urol. Res. 1980; 8(3):167-169.
8. Wandt MAE, Pougnett MAB. Simultaneous determination of major and trace elements in urinary calculi by micro wave-assisted digestion and inductively coupled plasma atomic emission spectrometric analysis. Analyst. 1986; 111:1249-1253.