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Occurrence of canine urolithiasis in Thrissur district, its medical and surgical management, and Fourier Transform Infrared (FT-IR) spectroscopic analysis of uroliths

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Abstract

Fifteen dogs presented over a period of twelve months to University Veterinary Hospitals at Mannuthy and Kokkalai, with symptoms of obstructive urolithiasis were included in the study. Every animal had a history of difficulty in urination, accompanied by clinical manifestations such as pollakuria, stranguria and haematuria. Depending on the location of obstruction, either a urethrotomy, cystotomy or a combination of both were opted as the surgical method, performed under general anaesthesia. Depending on the condition of the patient, postoperative pH modulators, antispasmodics, antibiotics and analgesic therapy were prescribed. All the animals recovered uneventfully. The calculi retrieved through surgery were subjected to Fourier Transform Infrared Spectroscopy (FT-IR) to identify the chemical composition. Nine samples were identified as struvite and five were calcium oxalate monohydrate and one sample was a mixture of struvite and ammonium urate. Identification of the specific composition of calculi/calculus using spectroscopy was helpful in preventing the recurrence of the condition by management practices and postoperative therapeutic diet. The study failed to establish any correlation between the calculi composition and the various risk factors considered in this study.

Keywords: *Canine urolithiasis, urethrotomy, cystotomy, Fourier Transform Infrared Spectroscopy*

According to Koehler *et al*. (2009), uroliths were collections of crystalline and occasionally noncrystalline solid materials that develop in one or more locations in the urinary tract. Uroliths could form when urine becomes oversaturated with lithogenic substances, which can impede frequent and thorough urination. In dogs, urolithiasis is a recurring and a relatively common condition. Diagnosis of urolithiasis is done based on clinical symptoms and imaging techniques like radiography and ultrasonography. Radiopaque calculi are visualised on routine survey radiographs of the abdomen. Acoustic shadowing caused by the uroliths is helpful in diagnosis during ultrasonography. Urinary stones that are radiolucent complicate the diagnosis of cystic calculi, although radiography can still be used to diagnose the condition (Saini and Singh, 2002; Larson, 2009). Though the primary course of treatment for uroliths is surgery, the condition

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requires comprehensive medical, nutritional or managerial strategies to prevent its incidence or recurrence. Calcium oxalate (CaOx) and struvite were the two most commonly found mineral types in canine uroliths (Low *et al*., 2010). Age, sex, etiological factors such as metabolic diseases, ion transport in the kidney and intestinal tract, nutrition, particularly fluid intake, location, climate and in certain cases the presence of a urinary tract infection (UTI), particularly when struvite-containing uroliths and urease-producing bacteria are present, all affect the development of canine urolithiasis and associated symptoms (Osborne *et al*., 2009; Roe *et al*., 2012).

Fifteen dogs exhibiting symptoms of obstructive urolithiasis were studied in the current investigation. In every patient, haematobiochemical and radiological evaluations were performed prior to surgery. Urethrotomy, cystotomy or a combination of the two were used for surgical management, based on the site of calculi. The calculi that were retrieved were then subjected to FT-IR spectroscopy, a precise, quick and adaptable analytical method that offers both quantitative and semi-quantitative characterisation of the composition of urinary stones. A study was undertaken to elucidate the possible risk factors associated with canine urolithiasis.

Materials and methods

Fifteen dogs with signs of obstructive urolithiasis presented to University Veterinary Hospitals, Mannuthy and Kokkalai, Kerala Veterinary and Animal Sciences University, Kerala over a period of twelve months (April, 2023 to April, 2024) formed the subjects for the present study. Age, breed, sex and body weight of the animals were recorded at the time of presentation. Detailed anamnesis including duration of illness, severity of the condition, whether the obstruction was complete or partial, previous ailments and medications if any and clinical symptoms including anorexia, lethargy, depression, vomiting, crouching stance, abdominal distention, stranguria, pollakiuria, dysuria and haematuria were recorded. Thorough clinical examination of the patients was carried out to record the respiratory rate, pulse rate, rectal temperature, colour of mucous membrane, hydration status and appearance of prepuce and penile region. Complete blood count and serum biochemical analysis were conducted preoperatively using an automated haematology analyser* along with right lateral survey abdominal radiographs (Fig.1) to visualise the site of obstruction.

Surgical management

Catheterisation and retrograde urohydro-propulsion were tried to establish patency of the urinary tract before proceeding with surgery. When that procedure failed, cystotomy, urethrotomy or combination of both were the surgical methods adopted, depending on the site of calculi. All the animals were pre-medicated with inj. atropine sulphate at the dose rate of 0.045 mg/kg followed by inj. xylazine hydrochloride at 1.0 mg/kg bodyweight intramuscularly. Anaesthesia was induced with intramuscular injection of inj. ketamine hydrochloride and inj. midazolam at the dose rate of 5.0 mg/kg body weight and 0.05 mg/kg bodyweight respectively and maintained with two percent isoflurane.

Cystotomy was performed through a caudal midline laparotomy to remove cystoliths. A combination of cystotomy and urethrotomy were performed to remove multiple calculi present in the bladder and throughout the course of urethra. The surgical wounds were closed in routine manner.

Post operatively, all the dogs were given amoxycillin clavulanate at the dose rate of 20 mg/kg for

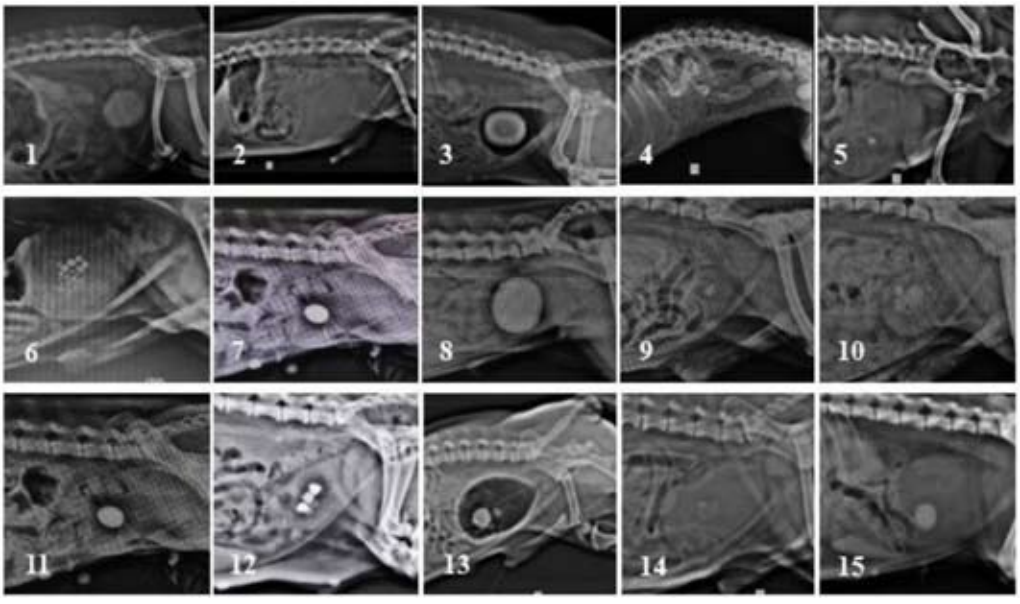

Fig. 1. Right lateral abdominal radiographs of cases 1-15.

ten days, meloxicam at the dose rate of 0.2 mg/kg body weight as analgesic for three days and cyclopam 10 mg total dose as antispasmodic orally for four days. Post operative dietary management and urinary pH modulators were advised on a case-to-case basis depending on the specific urolith composition and urine pH. In cases having abnormally acidic urine, Uripet® syrup was advised for urine pH correction and Uripet intense® was advised for acidification of urine in animals with an increased urine alkalinity, accompanied by urinary tract infections. All the dogs had an uneventful recovery.

Characterisation of urinary calculi

The retrieved calculi were subjected to FT-IR using Spectrum Two™ FT-IR spectrometer (PerkinElmer). It is a modern physico-chemical method suitable for investigation of urinary stone composition (Sofia *et al*. 2010). According to Koehler *et al*. (2009), FT-IR is based on the distinct wave patterns that are produced when infrared waves interact with a sample, some of which are absorbed by the sample (absorbance) and others pass through the sample (transmittance). The resultant spectrum represents the molecular fingerprint of the sample and can be used for identification by comparing it to a known reference spectrum.

Study of risk factors

The location of each case was plotted on a 2D-map of Thrissur district to understand the geographical distribution of cases. The data on quality of ground water in the various locations was taken from the Ground water information booklet of Thrissur district (2013) published by the Central ground water board, Kerala region, Ministry of water resources, Govt. of India. A survey was conducted with the clients, to understand the feeding habits of their pets.

Statistical analysis

Independent t-test was done to study whether the haematological, biochemical and water quality parameters had any influence on the type of calculi composition and p-value less than 0.05 was considered as statistically significant.

Results and discussion

The date acquired in this study are summarised in Table 1.

From this data it could be inferred that smaller/ toy breeds are more affected than the large breeds. In this study the highest incidence was recorded in Chinese Pug (33.3%). The breed wise distribution is given in Fig. 2. Although any breed of dog could be affected with struvite urolithiasis, dogs of toy and small breeds were more commonly affected than dogs of medium and large breeds (Ling *et al*., 1998) which was in accordance with the observations of the present study.

Table 1. Percentage incidence of cases based on breed, sex, age, site of calculi, composition of calculi and feeding habits

Characteristics	Category	Frequency	Per cent
Breed	Chihuahua	1	6.7
	Chinese Pug	5	33.3
	Dachshund	3	20.0
	Fox Terrier	1	6.7
	Golden Retriever	1	6.7
	Japanese spitz	1	6.7
	Labrador retriever	2	13.3
	Lhasa Apso	1	6.7
Sex	Female	7	46.7
	Male	8	53.3
Age	<5 years	11	73.3
	>5 years	4	26.6
Site of calculi	Bladder	10	66.7
	Bladder and Urethra	5	33.3
Calculi composition	Whewellite (Calcium oxalate)	5	33.3
	Struvite	9	60.0
	Ammonium urate and struvite	1	6.7
Type of feed	Home made	7	46.6
	Commercial pet food	4	26.7
	Mixed	4	26.7

The minimum age among the presented animals was two years and the maximum age was 11years with a mean of 5.40 years. Rudejeviene and Karaliute (2022) reported that animals between four and nine years old were considered at greater risk. But in the present study only 26.6% (4/15) of the animals were above the age of five.

A higher incidence was reported in male animals in the present study. Similar findings were reported by Chalpermol *et al*. (2000), who observed that male dogs had greater risk for developing uroliths than female dogs. The occurrence of cystic urolithiasis in females may be

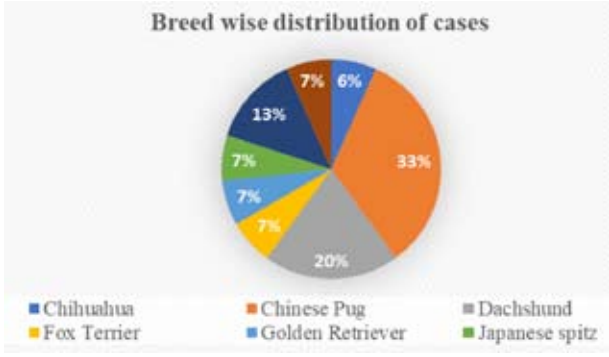

Fig. 2. Breed wise distribution of cases

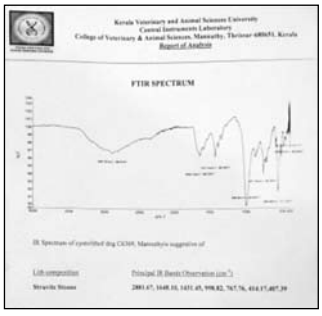

Fig. 3. Struvite calculi

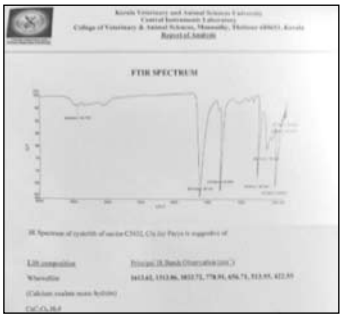

Fig. 4. Whewellite calculi

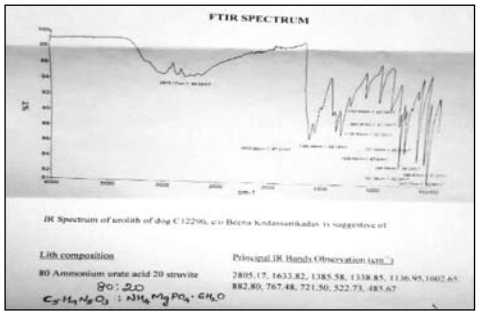

Fig. 5. Ammonium urate and struvite

because females have a relatively short urethra, thus increasing their susceptibility to bacterial urinary tract infections (Seaman and Bartges, 2001).

In this study, 66.7% animals had cystic calculi, whereas, 33.3% animals had calculi both in the urinary bladder as well as in urethra.

On FT-IR analysis, most of the calculi were struvite (60%) (Fig. 3) followed by 33.3% of whewellite calculi (Fig. 4). In one case (6.7%), the calculi had a mixed composition of ammonium urate and struvite (Fig. 5). Similar findings were reported by various authors (Hoxha and Rapti, 2017; Rudejeviene and Karaliute, 2022). Struvite has a characteristic infrared spectrum easily recognisable even in mixed stone samples by strong band at 2896.30cm^{-1} due to absorption of PO_4 group and presence of other bands at 1431.65, 991.64, 754.54 and 565.79cm^{-1}. Similar absorption values with comparable wave numbers could be obtained for struvite crystals in the study by Thajunnissa *et al*. (2021). The whewellite stone had principal absorption bands at 1610.90, 1313.73, 1031.33, 773.39, 651.09 and 513. 88cm^{-1}. The mixed stone (Ammonium urate and struvite) had principal IR bands at 2805.17, 1633.82, 1385.58, 1002.65, 882.80, 767.48, 721.50, 522.73 and 485.67cm^{-1}.

Uroliths could range in size from sand-like material to large individual stones that might grow to fill the entire cavity in which they form and similar findings were observed under the present study. Uroliths that form in cats, dogs and humans could be grouped into four main mineral types, namely urate (including ammonium urate, sodium urate and uric acid), cystine, magnesium ammonium phosphate and calcium salts (calcium oxalate and calcium phosphate) (Dvorska and Saganuwan, 2015).

Based on a survey of the feeding habits, dogs fed with home made food had the highest incidence (46.6%) of urinary calculi. Dogs fed with commercial pet food and which had a mixed diet had a similar percentage of occurrence (26.7%). The formation of uroliths involved multiple physiological and pathological processes and understanding of the processes involved in the formation and elimination of urine was an essential prerequisite for investigations into the role of diet in urolithiasis (Dvorska and Saganuwan, 2015). The present study could not establish any statistically significant correlation between the incidence of canine urolithiasis and feeding habits. Hence a detailed study needs to be undertaken to study any such correlation.

The most common symptom in urolithiasis was pollakiuria and the second most common symptom was haematuria and similar symptoms were reported in the studies carried out by Rudejeviene and Karaliute (2022).

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 (Parvathamma *et al*., 2020). In the present study surgical management followed by medical management and dietary alterations were resorted to in all the cases.

Descriptive statistics

The descriptive statistics of the haematological and biochemical parameters is presented in Table 2 and their correlation with the type of calculi are given in Table 3.

All the cases under the study were presented with mild, intermittent or bloody urination and no cases of complete obstruction was noticed, so that, there was no discernible rise in the creatinine and BUN levels. Four patients with cystic calculi had marginal increase in creatinine values and Vijaykumar *et al*. (1999) found similar results in their study. According to Sarma and Kalita (2019), these alterations may have been brought on by post-renal uraemia as a result of blockage of the excretory tract by urethral calculi, cystic calculi and cystitis. Leucocytosis was observed in this study which was statistically significant. This finding could be attributed to calculi-induced cystitis. Leucocytosis was also noted by Rajathi *et al*. (2006) in dogs with urolithiasis, which may have been brought on by a urinary tract infection. Of the 15 patients under investigation, anaemia was found in three of them which was not significant statistically. The decrease in red blood cells and haemoglobin in urolithiasis might have been due to the anaemia consequent to cystitis-related haematuria.

Table 2. Descriptive statistics regarding haematological and biochemical parameters

Variable	Category	Frequency	Per cent	Mean	SE
RBC (n = 15)	< 5.50	3	20.0	6.50	0.28
	5.50 - 8.50	11	73.3		
	> 8.50	1	6.7		
HGB (n = 15)	< 12.0	3	20.0	13.57	0.51
	12.0 – 18.0	12	80.0		
TLC (n = 15)	6.0 – 17.0	3	20.0	20.57	1.08
	> 17.0	12	80.0		
HCT (n = 15)	< 37.0	1	6.7	44.36	2.89
	37.0 – 55.0	12	80.0		
	> 55.0	2	13.3		
PLT (n = 15)	200 - 500	15	100	335.0	18.13
BUN (n = 9)	9 - 26	8	88.9	16.89	1.89
	> 26.0	1	11.1		
Creatinine (n = 12)	0.6 – 1.4	9	75.0	1.09	0.10
	> 1.4	3	25.0		

Table 3. Comparison of haematological and biochemical parameters among cases having different type of composition

Variables	Whewellite	Struvite	t-value	p-value
RBC (10^6/µL)	7.21 ± 0.52	6.12 ± 0.32	1.883^{ns}	0.084
HGB (g/dL)	13.64 ± 0.9	13.68 ± 0.71	0.032^{ns}	0.975
TLC (10^9/L)	23.86 ± 1.35	18.99 ± 1.34	2.351*	0.037
HCT (%)	51.68 ± 4.72	40.33 ± 1.82	2.243^{ns}	0.073
PLT (10^3/µL)	321.8 ± 45.49	344.00 ± 18.94	0.532^{ns}	0.605
BUN (mg/dL)	19.18 ± 1.48	17.02 ± 2.93	0.532^{ns}	0.614
Creatinine (mg/dL)	1.18 ± 0.11	1.09 ± 0.14	0.457^{ns}	0.659

** Significant at 0.05 level*

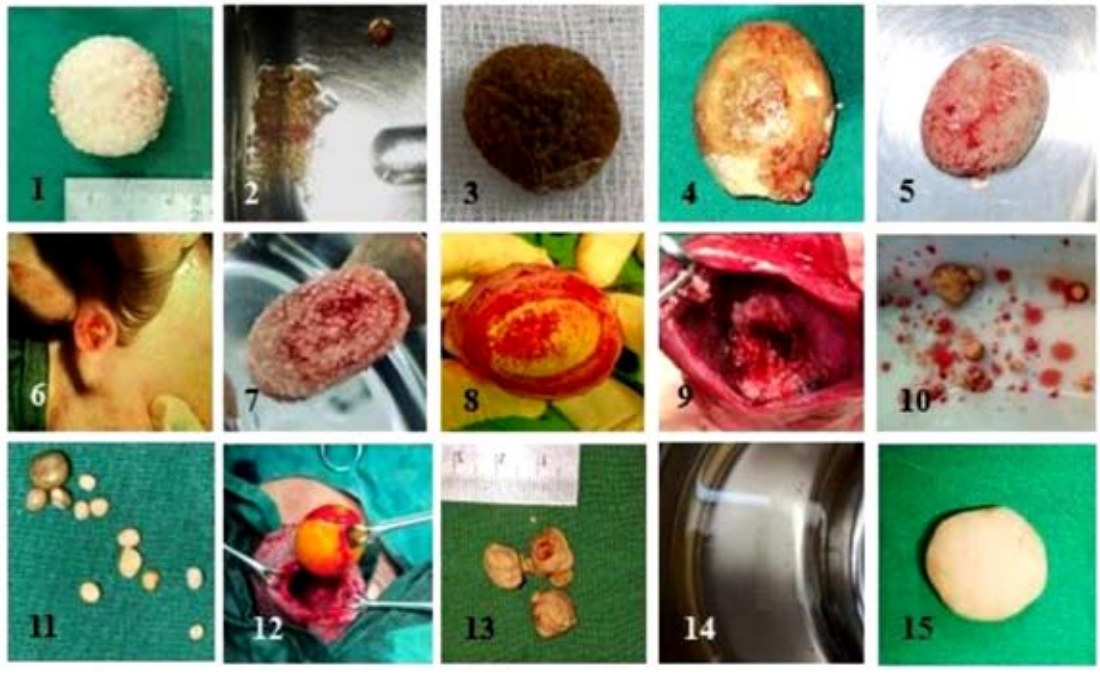

Fig. 6. The recovered calculi of the cases 1-15. Note the differences in size, colour and texture of the different calculi.

Verma *et al*. (2006) also observed similar findings in dogs with calculi induced cystitis.

The recovered calculi are shown in Fig. 6. They varied in form, from sand like particles to single large crystalline uroliths.

Geographical distribution of cases

Thrissur district can be divided into the high lands on the Eastern side, the plains in the middle including the Kole wetlands and the Western coastal belt. The distribution of cases presented has been depicted in Fig. 7. This figure shows that most of the cases are brought from the plains in the middle, compared to the eastern and western boundaries of Thrissur district. This could be attributed to the accessibility of the hospitals in which the study was conducted rather than differences in the geographical pattern.

Quality of ground water

According to Joji (2013) the pH of water in Thrissur district is alkaline, with soft (<75 mg/L) to moderately high (75-150 mg/L) hardness as per the classification of hardness based on the concentration of $CaCO_3$ mg/L. The water quality parameters of ground water at different locations in Thrissur district from where the cases were brought, did not show any statistically significant correlation with the type of calculi (Table 4).

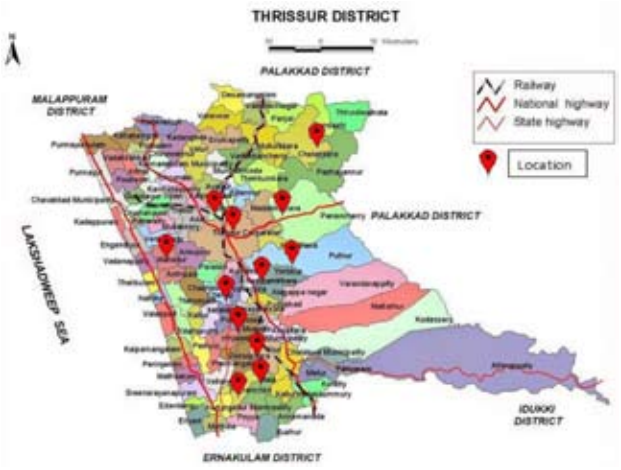

Fig. 7. Geographical distribution of the presented cases

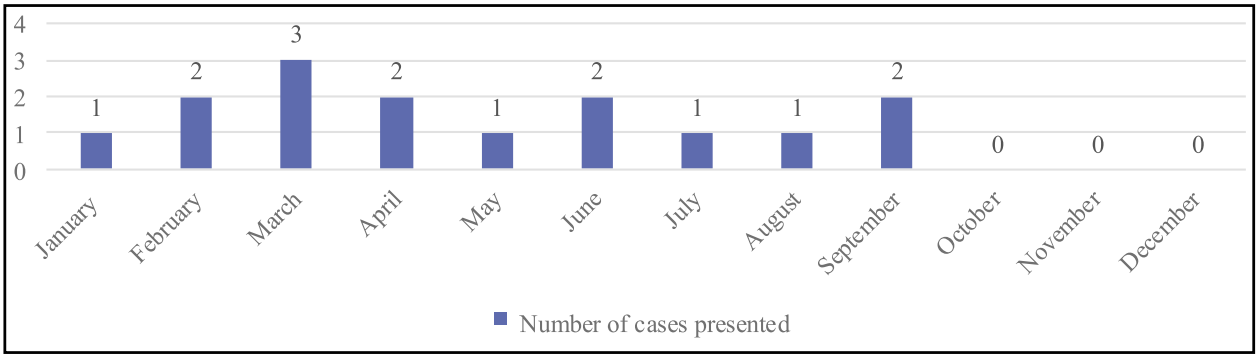

Fig. 8. Month-wise case distribution

Table 4. Comparison of water parameters among cases with different type of calculi composition

Variables	Whewellite	Struvite	t-value	p-value
pH	7.92 ± 0.14	8.00 ± 0.15	0.374^{ns}	0.715
Total Hardness as $CaCO_3$ (mg/L)	55.6 ± 16.7	47.56 ± 14.84	0.341^{ns}	0.739
Ca (mg/L)	15.8 ± 4.34	11.89 ± 3.01	0.757^{ns}	0.464
Mg (mg/L)	3.87 ± 1.56	4.36 ± 1.87	0.174^{ns}	0.865
Cl (mg/L)	34.5 ± 11.95	28.33 ± 8.19	0.437^{ns}	0.670
F (mg/L)	0.30 ± 0.10	0.22 ± 0.05	0.760^{ns}	0.462
NO_3 (mg/L)	20.58 ± 9.72	6.92 ± 5.28	1.359^{ns}	0.199

ns non-significant

Table 5. Calculi composition according to type of food consumed

Calculi Composition	Homemade food	Commercial pet food	Mixed	Total
Whewellite	2 (28.6)	1 (25.0)	2 (50.0)	5 (33.3)
Struvite	5 (71.4)	2 (50.0)	2 (50.0)	9 (60.0)
Ammonium urate and struvite	0	1 (25.0)	0	1 (6.7)
Total	7 (100)	4 (100)	4 (100)	15 (100)

(Figures in parenthesis indicate the percentage)

Feeding habits

A survey was conducted among the pet owners to study the type of food they are feeding their pets and to see whether it had any correlation with the incidence of uroliths (Table 5). Even though a higher incidence is shown for dogs fed on homemade food, especially with struvite calculi, it may not be possible to draw any conclusion in the present study since the number of samples was not sufficient for a statistical analysis.

Month-wise occurrence

The month-wise occurrence of cases is depicted in Fig. 8. According to the data the incidence is highest in March and no cases have been reported in the months of October, November and December. The sample size was not sufficient to suggest any statistically significant correlation with the month-wise incidence of cases.

The natural progression of the urine chemistry leading to stone formation is urine saturation, super saturation, crystal nucleation, aggregation and retention of crystals by urothelium and the continued growth of the stone on the retained crystals (Sanderson *et al*., 2001). Most of the calculi in dogs were found either in the bladder or urethra (Hoxha and Rapti, 2017) which was in accordance with the present study. Detection of urocystoliths does not necessarily warrant surgical intervention; however, obstruction of urine outflow, an increase in size or number of calculi, persistent clinical signs and a lack of response to therapy were the indications for calculi removal. Surgery was required in patients with non-dissolvable calculi and clinical signs (Lulich *et al*., 2011).

Determining the composition of uroliths is essential to prevent recurrence. All removed uroliths should be analysed to determine mineral composition, which will aid in developing a successful treatment and prevention plan. Reports of analysis will give the chemical makeup of the urolith. In cases of recurrence, uroliths should be resubmitted, because mineral composition can change from one episode to another (Koehler *et al*., 2009). Between 5% and 15% of uroliths may be mixed or compound stones. Ammonium urate and calcium carbonate (or calcium apatite) may be mixed with struvite as part of infection-induced struvite stone formation (Bartges and Callens, 2015). A similar observation was noticed under the current study in Case No. 6.

Management of diet

Dietary management has an important role in the treatment of urolithiasis. As a result, a combination strategy that includes adjusting the urine pH, volume, and nutritional composition is typically used. Only struvite uroliths can be dissolved by diet alone; urate and cystine uroliths can only be dissolved by diet in conjunction with medication therapy. Calcium oxalate uroliths cannot be dissolved by diet, however they can be less likely to recur with dietary care (Sturgess, 2009). Numerous commercial diets are designed using different approaches to control CaOx, such as adding potassium citrate or consuming a high sodium diet (Bartges *et al*., 1999). In dogs and cats, increasing water intake and diuresis can be achieved by feeding wet foods exclusively (dietary moisture >70%) (Stevenson *et al*., 2003). Precursor-controlled dry and canned veterinary commercial diets with acidifying and/or diuretic qualities effectively dissolve struvite uroliths that arise spontaneously. Dissolution of calculi in dogs can take up to three months and hence procedures need to be used in conjunction with the right kind of antibiotic treatment (Queau, 2019). Appropriate dietary adjustments based on the composition of the urolith would significantly reduce morbidity and problems related to recurrent urolithiasis (Bhattacharyya *et al*., 2014).

Conclusion

Urolithiasis is a multifactorial disease with implied epidemiological aspects influenced by different risk factors. These include breed, sex, age, diet, anatomical and genetic predisposition, behavioural peculiarities, metabolic disorders and urinary tract infection. Surgical management was successful in all the cases. In the present study, the majority of the calculi were struvite, followed by calcium oxalate and one with a mixed composition. The risk factors considered in this study like the geographical distribution, groundwater quality and feeding habits did not show any correlation with the type of calculi recovered, which may be because of the small sample size. Therefore, a detailed prospective study is warranted to establish any such correlation.

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Conflict of interest

The authors declare that they have no conflict of interest

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