Surgical Management of Obstructive Urolithiasis and Fourier Transform Infrared (FT-IR) Spectroscopic Identification of Uroliths in Dogs: A Report of Six Cases

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Abstract

Six dogs with signs of obstructive urolithiasis presented to University Veterinary Hospitals Mannuthy and Kokkalai, Kerala Veterinary and Animal Sciences University over a period of two months formed subject for the present study. All the animals were having history of difficult urination with clinical signs like stranguria, pollakuria, anuria and/or haematuria. Haemato-biochemical investigations, blood gas analysis, urinalysis, survey radiographs and ultrasonography were performed to arrive at a conclusive diagnosis. Surgical interventions were done under general anaesthesia using either urethrotomy, cystotomy or a combination of both, depending upon the site of obstruction. Post-operative pH modulators, anti-spasmodics, antibiotics and analgesic therapy were employed depending upon the condition of patient and all the animals had an uneventful recovery. When stones retrieved through surgery were subjected to Fourier Transform Infrared Spectroscopy (FT-IR) to identify the chemical composition, three samples were identified as a combination of struvite, calcium oxalate dihydrate and calcium apatite crystals and three samples were a mixture of calcium oxalate monohydrate and calcium phosphate. Identification of specific calculi composition using spectroscopy was helpful in prevention of recurrence through management practices and therapeutic diet postoperative.

Introduction

Urolithiasis is a general term referring to the causes and effects of stones anywhere in the urinary tract, causing urinary tract disease demanding rapid definitive diagnosis for immediate surgical and/or medical interventions (Osborne et al. 1986). The precipitation- crystallization theory of urolithiasis explains that supersaturation of urine with ions resuts in precipitation and formation of particles, that grow into small crystals and aggregates into larger crystals and ultimately results in calculi formation if the microenvironment is favourable (Dibartola et al. 1981). The factors influencing development of canine urolithiasis and its characteristics are age, sex, etiological factors such as metabolic disorders, ionic transport within the intestinal tract and in the kidney, diet including fluid intake, geographical location and climate (Osborne et al. 2009). Definitive theraputic management of urolithiasis should be directed at early confirmation and effective interventions including, surgical therapy, post-operative medications, medical dissolution and urinary pH modulation in conjunction with dietary modifications and theraputic diets (Koehler et al. 2008)

The understanding of the chemical composition of urinary stones is of interest for proper identification of the type of stone, to provide clues to etiopathogeny and prevention of recurrence. Fourier Transform Infrared Spectroscopy (FT-IR) is based on unique wave patterns generated when infrared waves encounter a sample, where some waves get absorbed by the sample (absorbance) and some waves pass through the sample (transmittance). The resulting spectrum is a molecular finger print of the sample which can be compared with a known reference spectra for identification (Koehler et al. 2008). Though numerous studies in the past focused on FT-IR Spectroscopic characterization of kidney stone compositions in clinical urology, there are only limited literature on FT-IR quantification of uroliths in small animals. By virtue of its accuracy and expeditiousness, this method proves to be a valuable technique for the analysis of urinary calculi composition, applicable in veterinary urology.

The present study was conducted in six dogs presented with signs of obstructive urolithiasis varying in the sites of obstruction. Haematobiochemical, blood gas analysis and radiographic examinations were conducted in all the cases and surgical management was done using either urethrotomy, cystotomy or a combination of both. Stones thus collected were subjected to FT-IR Spectroscopy, a specific, rapid and versatile analytical technique which provides quantitative as well as semi-quantitative analysis of urinary stone composition.

Materials And Methods

Six dogs with signs of obstuctive urolithiasis presented to university veterinary hospitals, Mannuty and Kokkalai, Kerala Veterinary and Animal Sciences, University, India over a period of 2 months (November and December, 2019) 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, pollakuria, dysurea and haematuria. Thorough physical examination of the patients were carried out to record the Respiratory rate (RR), Pulse rate (PR), rectal temperature, colour of mucous membrane, hydration status, abdominal palpation and appearance of prepuce and penile region. Complete blood count, serum biochemical analysis and blood gas analysis were conducted along with right lateral survey abdominal radiographs (Fig.1) to visualize the site of obstruction and ultrasonography to evaluate the extent of bladder distension/rupture/cystitis. An ECG was recorded to monitor the patient for the presence of hyperkalemia induced cardiac dysarrhythmia. Aseptically collected urine samples were subjected to urinalysis to determine the pH value and centrifuged to obtain the sediments for microscopical analysis.

Surgical Technique

Cystotomy, urethrotomy or combination of both were the surgical options depending on the site of calculi once the retrograde urohydropropulsion failed. All the animals were pre-medicated with atropine sulfate at the dose rate of 0.045 mg/kg bodyweight followed by xylazine hydrochloride at 1.0 mg/kg bodyweight intramuscularly. Anaesthesia was induced with intramuscular injection of ketamine hydrochloride and midazolam (1mg/ml) at the dose rate of 5.0 mg/kg body weight and 0.05 mg/kg bodyweight respectively and anaesthesia was maintained with two percent isoflurane. Cystotomy was performed though caudal midline laparotomy in the female animal (Case I) to remove the single large cystolith which was constantly irritating the bladder mucosa. Penile urethrotomy was performed in cases III, IV and VI to remove uroliths present behind the os penis, followed by urethrostomy (Fig. 2a). A combination of cystotomy and urethrotomy were performed in case II to remove multiple number of stones present throghout the course of urethra and in the bladder. There was cystorrhexis in case V along with focal necrosis of bladder wall and mucosal hypertrophy (Fig. 2b) which was managed by partial cystectomy. The uroliths retrieved through surgery are depicted in the Fig.3.

Intraoperatively electrolyte imbalance was corrected based on blood gas analysis with 0.9 percent normal saline, 25 percent dextrose and 7.5 percent sodium bicarbonate injections. Post operatively the animals were given amoxycillin clavulanate at the dose rate of 20 mg/kg for ten days, meloxicam at the dose rate of 0.2 mg/kg body weight as analgesic and cyclopam 10 mg total dose as antispasmodic orally for four days. Post operative dietary management and urinary pH modulators were adviced on a case to case basis depending on the specific urolith composition and urine pH. In cases having abnormally acidic urine, disodium hydrogen citrate was adviced for urine pH correction and DL-methionine and ascorbic acid were adviced for acidification of urine in animals with an increased urine alkalinity usually accompanied by urinary tract infections.

Fourier Transform Infrared Spectroscopic analysis of uroliths

The specific chemical composition of stones retrieved surgically were analyzed quantitatively using FT-IR Spectroscopy. The samples were ground to fine homogenous powder, mixed with Potassium Bromide (KBr), an inert carrier which has no absorption in the spectral region to be investigated. It was then pressed in to a pellet using high pressure in a hydraulic pellet press, loaded in the IR sample holder and spectroscopy was performed using FT-IR spectrometer to obtain the characteristic scan profile.

Results

The breed, sex, age, body weight and physiological parameters of the animals under study are enlisted in Table 1. The average age of animals presented was 8.5 years and five out of six animals were male dogs. All the animals had history of not passing urine for past one to two days except the female which had history of haematuria for past 3 months. The severity of clinical signs depended on the duration of obstruction and whether the obstruction was complete or partial. The animals exhibited anorexia, lethargy, vomiting crouching stance and one animal had cyanotic and inflammed penis. Five out of six dogs had multiple uroliths and radiography confirmed the site of obstruction as behind the os penis in four cases, thoughout the course of urethra and in the urinary bladder in one case and a single large cystolith in the female dog (Fig. 1). Ultrasonography revealed intact bladder in all cases except one with free fluid in the abdomen suggestive of bladder rupture. The mean haematological parameters are represented in Table 2, which were within the normal range except for mild neutrophilic leukocytosis and anaemia in three out of six cases. Serum biochemical analysis revealed elevated blood urea nitrogen and creatinine values indicating post renal azotemia in cases with complete urinary obstruction. Hyperkalemia and primary metabolic acidosis were typical of obstructive urolithiasis in blood gas analysis in four out of six cases (Table 3). An electrocardiogram also revealed increased amplitude of T wave suggestive of hyperkalemia in cases with obstructed outflow. Urinalysis showed alkaline pH of 7.5 to 8.0 and microscopic analysis of urine sediments revealed struvite crystals in first three cases where as pH was towards acidic side (6.0 to 6.5) in other cases which showed presence of calcium oxalate monohydrate crystals on microscopic analysis (Table 1).

Table 1
List of animals in the study with general observations on breed, gender, age, body weight, rectal temperature, respiratory rate, pulse rate, pH of urine and surgical procedure undertaken

Case No.	Breed	Gender	Age (Years)	Weight (kg)	Rectal temp. (°F)	Respiratory rate (per min.)	Pulse rate (per min.)	Urine pH	Surgical procedure
I	Spitz	Female	4.5	7	101.8	38	118	8	Cystotomy
II	Pug	Male	5	10	102.4	41	120	8	Cystotomy and urethrotomy
III	Pomeranian	Male	15	16	101.1	35	107	8.5	Urethrotomy
IV	Non descript	Male	9	15.8	102.8	32	101	6.5	Urethrotomy
V	Doberman	Male	8	26	100.9	20	112	6	Partial cystectomy and urethrotomy
VI	Non descript	Male	11	14.8	103.1	27	109	6.5	Urethrotomy
Abbreviations: Temp, temperature; min, minute									

Table 2
The mean values of haematological parameters

Haematological Parameters		Values
Hb (g/dl)		10.92 ± 1.72
Volume of Packed Red Cells (%)		29.49 ± 3.46
Total Erythrocyte Count (16^6/µL)		4.39 ± 0.50
Total Leucocyte Count (10^3/µL)		13.32 ± 1.57
Differential Leucocyte Count	Lymphocytes (%)	19.83 ± 4.06
	Monocytes (%)	6.25 ± 1.51
	Granulocytes (%)	73.92 ± 5.49
Abbreviations: Hb, haemoglobin; g, grams; dl, decilitre; µL, microlitres		

Table 3
Values of analytes on Blood Gas Analysis and kidney function test of all the animals included in the study

Case No	pH	pCO_2 (mmHg)	pO_2 (mmHg)	HCO_3^- (mm/L)	BE (ect) mm/L	cSO_2 (%)	Na + (mmol/L)	K+ (mmol/L)	Ca++ (mmol/L)	Cl- (mmol/L)	Glucose (mg/dl)	Lactate (mmol/L)	BUN (mg/dl)
I	7.388	35.8	63.5	10.478	-14.8	89	140	3.2	1.10	121	122	2.4	30.47
II	7.296	26.5	83.3	12.9	-13.6	95.2	146	3.5	1.25	120	120	2.91	10.56
III	7.193	37.9	80.6	14.6	-13.6	92.8	14.2	8.6	1.11	122	66	2.14	105
IV	7.218	30.6	75.9	12.9	-14	90.4	135	8	0.95	120	110	2.8	23.66
V	7.4	11.1	135	10.6	-14.4	99.4	135	7.7	0.64	123	241	1.84	55
VI	7.2	34.6	58.1	14.39	-13.9	84.1	142	8.4	0.72	113	305	1.69	8.43

Abbreviations: pCO_2, partial pressure of carbon dioxide; pO2, partial pressure of oxygen; HCO_3^-, bicarbonate concentration; BE, base excess; cSO_2, oxygen sat
BUN, Blood Urea Nitrogen; mmHg, millimeters of mercury; mm, millimeters; L, litres; mmol, millimol; mg, milligrams; dl, decilitre.

Characterization of urinary calculi by Fourier Transform Infrared (FT-IR) Spectroscopy

On FT-IR, samples I, II and III were identified as combination of magnesium ammonium phosphate hexahydrate (Struvite), calcium oxalate dihydrate (COD) and apatite crystals. The absorbance of calculi with their wave number and percentage absorption are enlisted in Table 4 and the FT-IR spectrum of the three samples are depicted in Fig. 4. The presence of struvite in the samples was confirmed by presence of a strong band at 1010 cm^{-1} and other bands at 2377, 1476, 1437, 872, 770 and 574 cm^{-1}. Absorption bands at 600 cm^{-1} suggested presence of apatite crystals and the broad absorption band at 3000 cm^{-1} indcated presence of dihydrate form of calcium oxalate.

Sample IV,V and VI were identified as mixture of calcium oxalate monohydrate (COM) and calcium phosphate. The absorbance values with their wave numbers and percentage of absorptions are presented in Table 5 and the FT-IR spectrum of the calculi is depicted in Fig. 5. The grouping of 5 bands in a staircase pattern between 3477 and 3047 cm combined with other bands at 2291, 1620, 1320, 885, 662, 781 and 517 cm^{-1} confirmed the presence of calcium oxalate monohydrate crystals (COM). The presence of calcium phosphate crystals was confirmed by detecting absorbance at 1040 cm^{-1} specicific to phosphorous.

Table 4 Absorbance values of the specific modes of vibrations of uroliths containing of magnesium ammonium phosphate hexahydrate (Struvite), calcium oxalate dihydrate and apatite crystals

SAMPLE I		SAMPLE II		SAMPLE III	
Wave number (cm^{-1})	Percentage absorbance	Wave number (cm^{-1})	Percentage absorbance	Wave number (cm^{-1})	Percentage absorbance
2973	4.7	2925	0.53	2930	3
2367	27	2376	11.9	2362	26.8
1469	21.8	1467	13	1468	29
1433	14.43	1435	8.5	1435	23.4
1316	55.4	1316	53	1324	55
1007	0.39	1007	0.17	1000	0.67
874	23.1	891	14.4	873	22.1
768	19.9	761	10	762	17
601	31	600	30	601	32
571	9	572	5.2	573.4	6.9
462	42	461	33.8	465.6	29

Table 5 Absorbance values of the specific modes of vibrations of uroliths containing calcium oxalate monohydrate (COM) and calcium phosphate of crystals					
SAMPLE IV		SAMPLE V		SAMPLE VI	
Wave number (cm^{-1})	Percentage absorbance	Wave number (cm^{-1})	Percentage absorbance	Wave number (cm^{-1})	Percentage absorbance
3487	15.7	3487	8.8	3487	10
3433	15	3433	8.5	3431	12
3337	19	3338	12.11	3341	14
3258	24	3258	17	3259	20
3061	24	3061	15	3064	22
2291	78	2292	79	2290	73
1621	0.06	1633	0.08	1620	0.05
1316	0.4	1317	0.04	1316	0.05
1041	30	1043	36	1039	31
950	57	950	55	950	54
884	55.5	884	51	884	52.5
780	3	781	0.9	781	2
663	17	664	10.4	661	15
517	11	518	6.5	516	8

Discussion

Urolithiasis is considered a surgical emergency as they may complicate with cystorrhexis and uroabdomen when urine is retained for more than 32 to 48 hours. Clinical cases of urolithiasis are mostly recorded in male animals and the incidence is more in older animals compared to younger age groups (Fromsa et al. 2019). The average age of animals presented was 8.5 years and five out of six animals were male dogs. The severity of clinical signs depends on the duration of obstruction and whether the obstruction is complete or partial. In the present study, animals exhibited signs of uremia viz. vomiting, lethargy, dehydration and symptoms pertaining to the lower urinary tract pathology including stranguria, pollakiuria, hematuria, pain and overflow incontinence which may be attributed to long-standing obstruction (Gisselman et al. 2009). Haematological evaluation revealed mild anaemia and neutrophilic leucocytosis, which might be due to concurrent urinary tract infections and cystitis in three out of six cases. A similar observation was recorded by Fromsa et al. (2019) that the mean total leucocyte count value was elevated significantly in partial and completely obstructed animals. Serum biochemical analysis revealed elevated blood urea nitrogen and creatinine in cases with complete obstruction indicating post renal azotemia (Sithanukul S et al. 2010). The values were within normal range in unobstructed and partially obstructed dogs.

Blood gas analysis was crucial for identification of electrolyte status of the animals. Hyperkalemia and metabolic acidosis were the most significant abnormalities associated with complete urinary obstruction. Impired urinary excretion and defective reabsorption of potassium from the retained urine resulted in hyperkalemia (Balakrishnan et al. 2013). Hyperkalemia might complicate with life threatening cardiac disarrhythmia due to cardiac conduction abnormalities, demonstrated with the help of electrocardiogram (Tag et al. 2008). Increased amplitude of T wave was a consistant finding in ECG of animals with hyperkalemia. Decreased level of HCO3- and negative base excess values indicated primary metabolic acidosis (Freitas et al. 2012) in all the cases except case V. Reduced blood pH value below 7.35 indicated metabolic acidosis due to defective renal function. Radiographic evaluation was helpful to specifically focus the site of obstruction as the stones were radio opaque in nature except cystiene and urate (Lulich et al. 2008). Urine pH was consistently towards alkaline side when the major stone composition was struvite but was acidic in COM and calcium phosphate urolithiasis (Palma et al. 2013).

Treatment was directed to immediately relieve the obstruction, correction of post-renal azotemia, hyperkalemia, acid-base imbalance and dehydration. Alleviation of primary causes of the disease by dietary modification, medical management and fluid therapy were also followed. Cystotomy, urethrotomy or combination of both were the surgical options depending on the site of calculi once the retro grade urohydropropulsion failed. In the present study, three out of six cases were managed with urethrotomy alone, one with cystotomy and two cases by urethrotomy combined with cystotomy.

Characterization of urinary calculi by Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR is a modern physical chemical method suitable for investigation of urinary stone composition (Sofia et al. 2010). The working principle of FT-IR is that every molecule gives a characteristic absorption spectrum in the infrared region depending on the chemical bonding conditions and structure. The vibration motions of atoms in bonds (bond stetching/ contracting/ bond wagging) are measured by passing FT-IR through the powdered stone sample, which has been compressed in to a nearly transparent wafer with adequate quantity of potassium bromide. In the FT-IR region (4000–200cm^{-1}), characteristic attenuation of incident energy occurs depending on the ability of molecules in the compound to oscillate at certain wavelengths (Kanchana et al. 2009).

On FT-IR, 3 samples were identified as combination of struvite, COD and apatite crystals. Struvite has a characteristic infrared spectrum easily recognizable even in mixed stone samples by strong band at 1010 cm^{-1} due to absorption of PO_4 group and presence of other bands at 2377, 1476, 1437, 872, 770 and 574 cm^{-1}. The bands at 1476 and 1435 $cm^{-1}$are because of vibration of NH_4 group (Sofia et al. 2010). Similar absorption values with comparable wave numbers could be obtained for struvite crystals in the present study. When struvite was in association with apatite, the presence of later was deduced by absorption at 600cm^{-1} and by a shift of bands at 1010 cm^{-1} towards higher frequencies. The broad absorption band at 3000 cm^{-1} was due to the presence of O-H group in the dihydrate form of calcium oxalate (Kanjana et al. 2009).

Rest of the three samples were identified as mixture of COM and calcium phosphate. The important spectral characteristics of COM was grouping of five bands between 3477 and 3047cm^{-1}, due to symmetric and asymmetric O-H stretch (Kanchana et al. 2009).The staircase pattern of five absorption bands between 3477 and 3047cm^{-1} in the present study confirmed the presence of COM crystals (Table 5). The absorptions at 2291cm^{-1} and 885$cm^{-1}$was due to C-Cstretching mode, two bands at 662 and 781cm^{-1}were due to the out of plane O-H bending and C-CH bending mode respectively and absorption at 517cm^{-1}arised due to O-C-O in plane bending (Sundaramoorthi et al. 2007). The absorption band at 1620 cm^{-1} and 1320 cm^{-1} was due to the vibration of C = O and C-O respectively (Tsay 1982). Hence, in addition to the grouping of 5 bands, the presence of COM was confirmed with the presence of absorption bands at all the above specified wave numbers for COM. The discrete formation of two bands at 780 and 517$cm^{-1}$was important in differentiating COM from COD (Kanchana et al. (2009). Presence of phosphorous group was detected by absorption at 1040 cm^{-1}(Kanchana et al. 2009), phosphorous groups usually shows an absorption range of 1000-1100^{cm-1}. Thus the presence of Calcium phosphate crystals was confirmed by identifying Calcium and Phosphorous groups together.

All the stone samples analyzed in the present study were composed of mixed compounds, 50 percent being a combination of struvite, COD and apatite and 50 percent COM and calcium phosphate. Quantitative characterization of urolith composition was effective in understanding the etiology, planning proper management and prevent recurrence of the disease. Proper dietary modifications guided by the urolith composition would considerably decrease morbidity and complications associated with recurrent urolithiasis (Bhattacharyya et al. 2014).

Conclusions

Timely diagnosis and aggressive therapeutic interventions are mandatory with urogenital emergencies as it may progress to life threatening complications if not addressed immediately. Urogenital emergencies have fair to good prognosis with early confirmation and effective interventions including, surgical therapy, post-operative medications, medical dissolution and urinary pH modulation in conjunction with dietary modifications and theraputic diets. Reliable analytical informations are crucial for the study of etiology and pathogenesis of stone formation and formulating specific treatment modalities. These data provides clinical guidelines in the prophylaxis, therapy and metaphylaxis of urolithiasis.

Declarations

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Compliance with ethical standards

All procedures performed on animals in the study were in accordance with the ethical standards of the Institutional Animal Ethics Committee at the College of Veterinary and Animal Sciences, Mannuthy. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed and the emergency surgical procedure was carried out with the informed consents from the pet owners.

Conflict of interest

The authors did not declare any conflict of interests, financial or otherwise.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by A. S. Thajunnisa, Laiju M. Philip, S. Anoop, K. M. Dileepkumar and K. A. Abdul Lathief under the supervision of Shyam K. Venugopal, K. D. John Martin and C. B. Devanand. The first draft of the manuscript was written by A. S. Thajunnisa and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability: Not applicable

Consent to participate: Not applicable

Consent to publish: Not applicable

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Figures

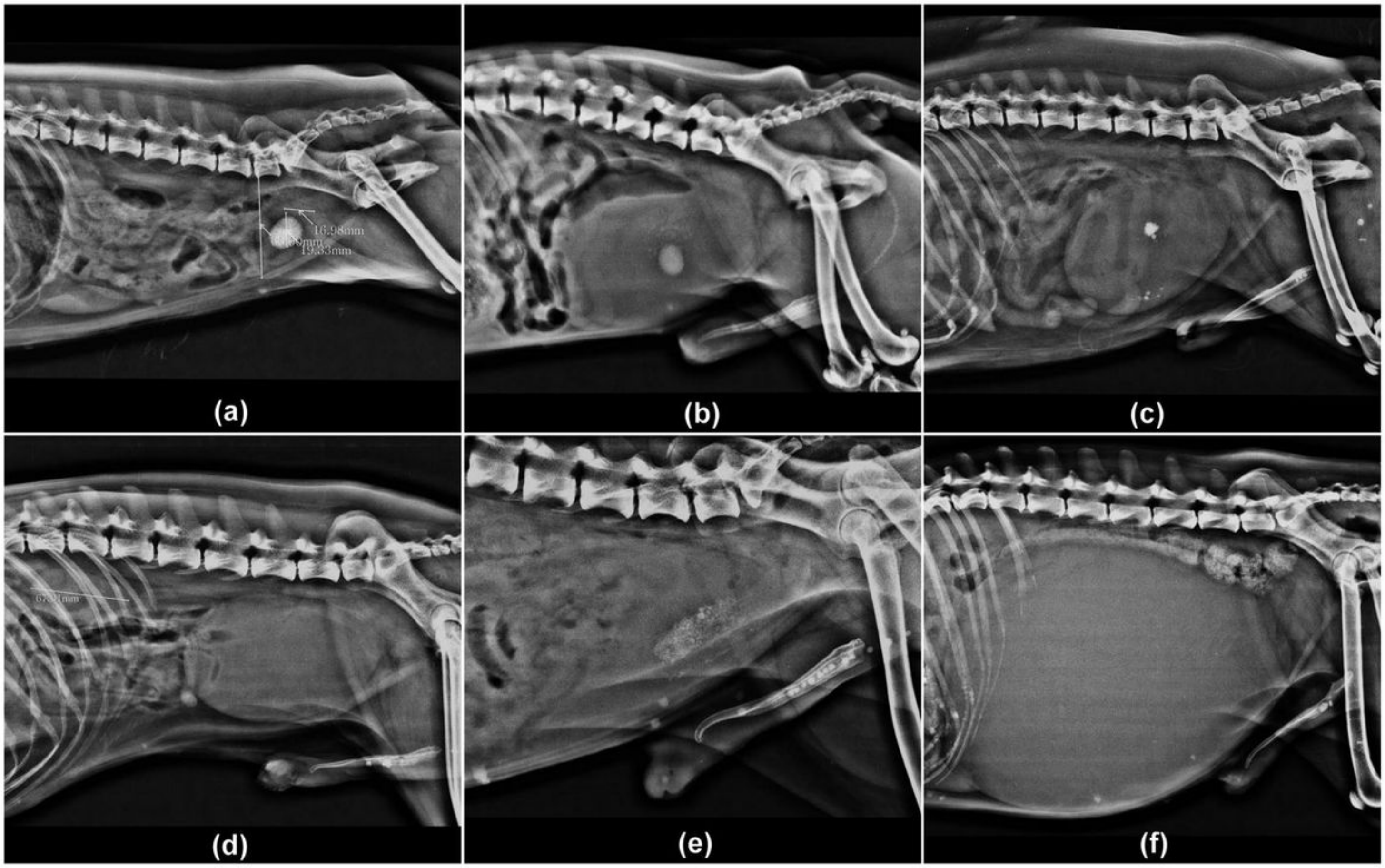

Figure 1

Radiographs representing the site of obstruction in cases I to VI ; a. single large stone (2 cm diameter) in the bladder; b. cystolith along with multiple small stones throughout the course of urethra; c,d and e. uroliths present at the level of os-penis; f. highly distended urinary bladder due to obstruction behind the os-penis

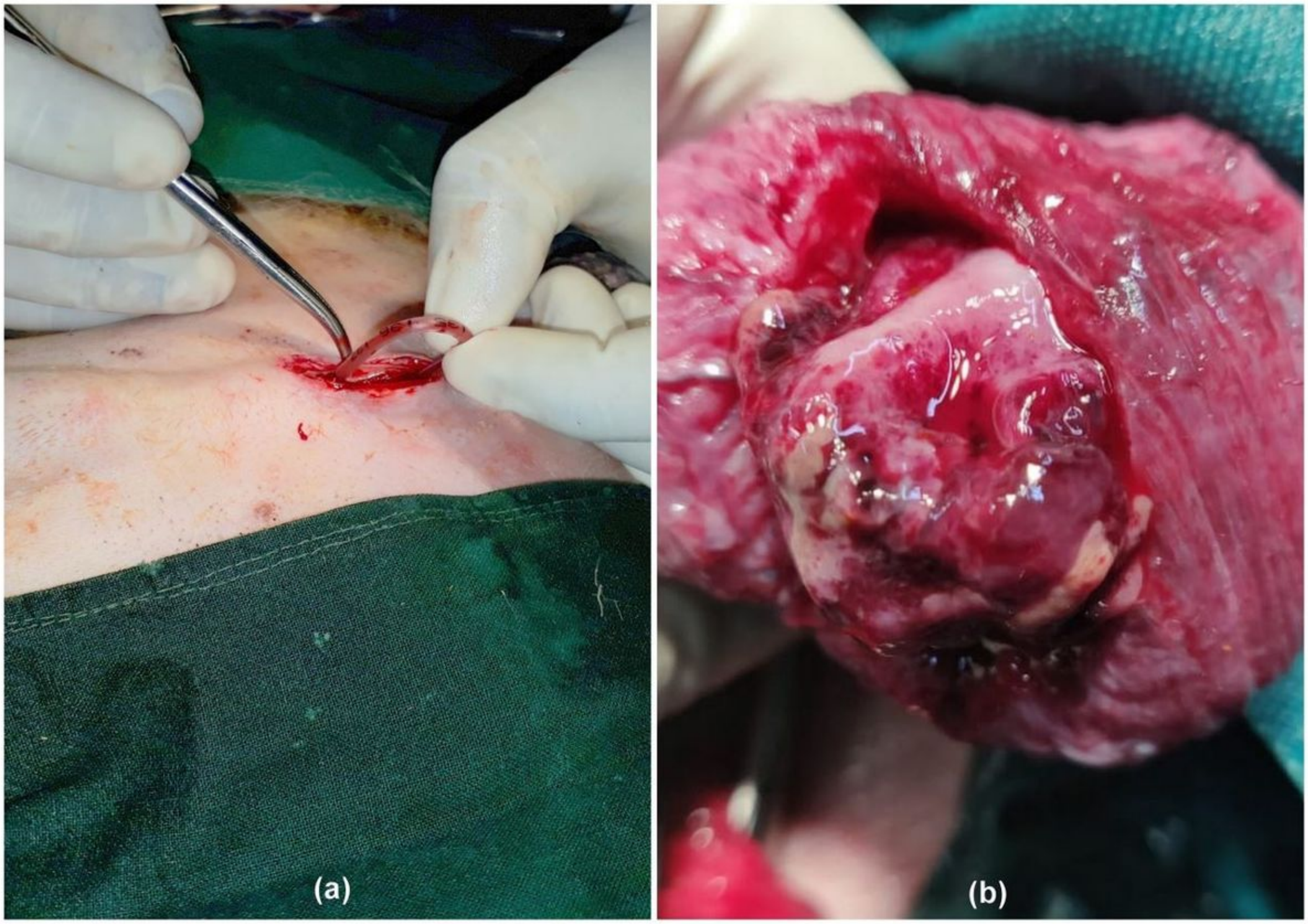

Figure 2

a. Penile urethrostomy in case III; B. Cystorrhexis with mucosal hypertrophy in case V

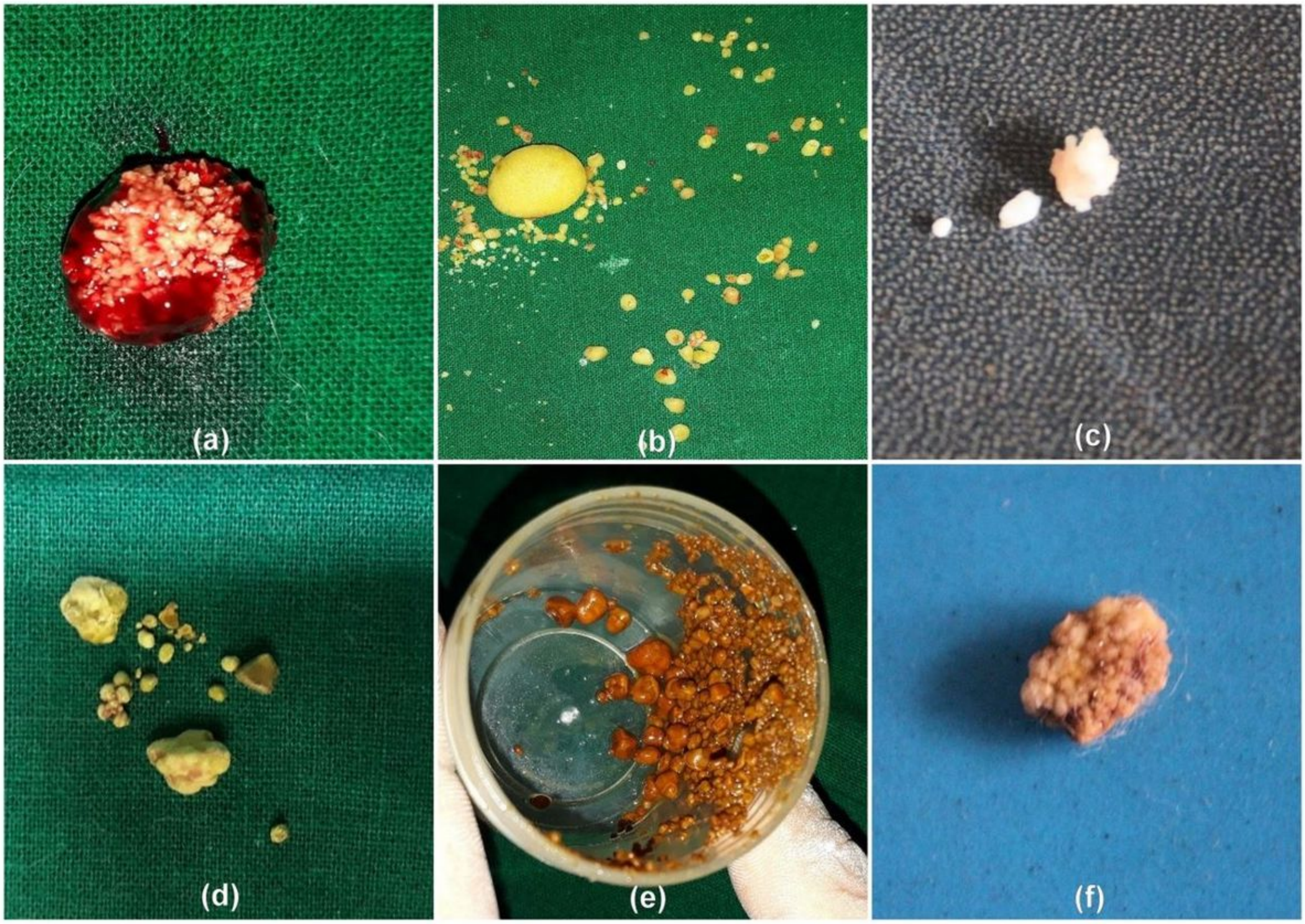

Figure 3

Gross appearance of uroliths retrieved through surgery. a, b and c: Mixture of struvite (magnesium ammonium phosphate), Calcium oxalate dihydrate and apatite crystals retrieved from cases I, II and III ; d, e and f: Mixture of calcium oxalate monohydrate and calcium phosphate from cases IV, V and VI

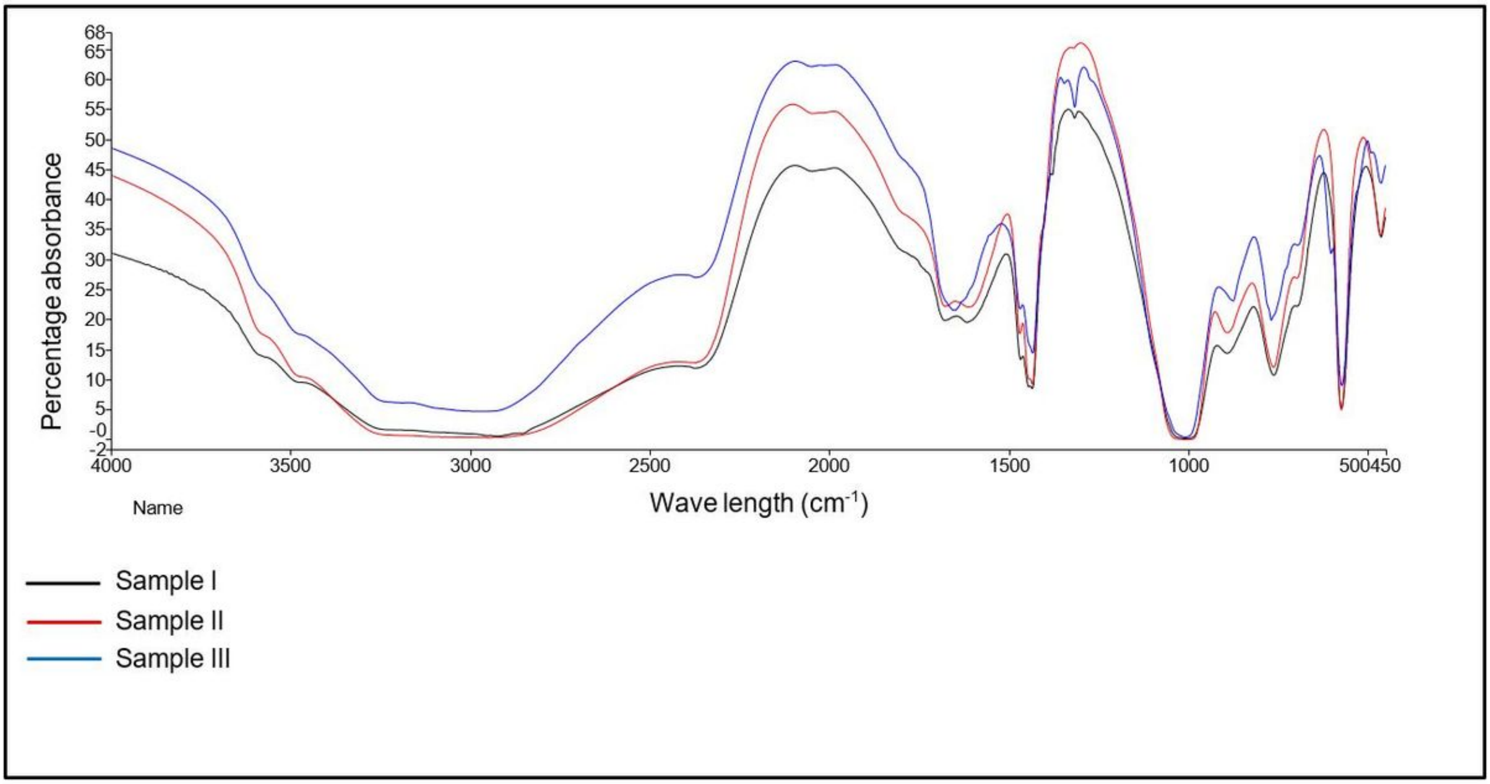

Figure 4

FT-IR spectrum of urinary stone containing magnesium ammonium phosphate hexahydrate (Struvite), calcium oxalate dihydrate and apatite crystals

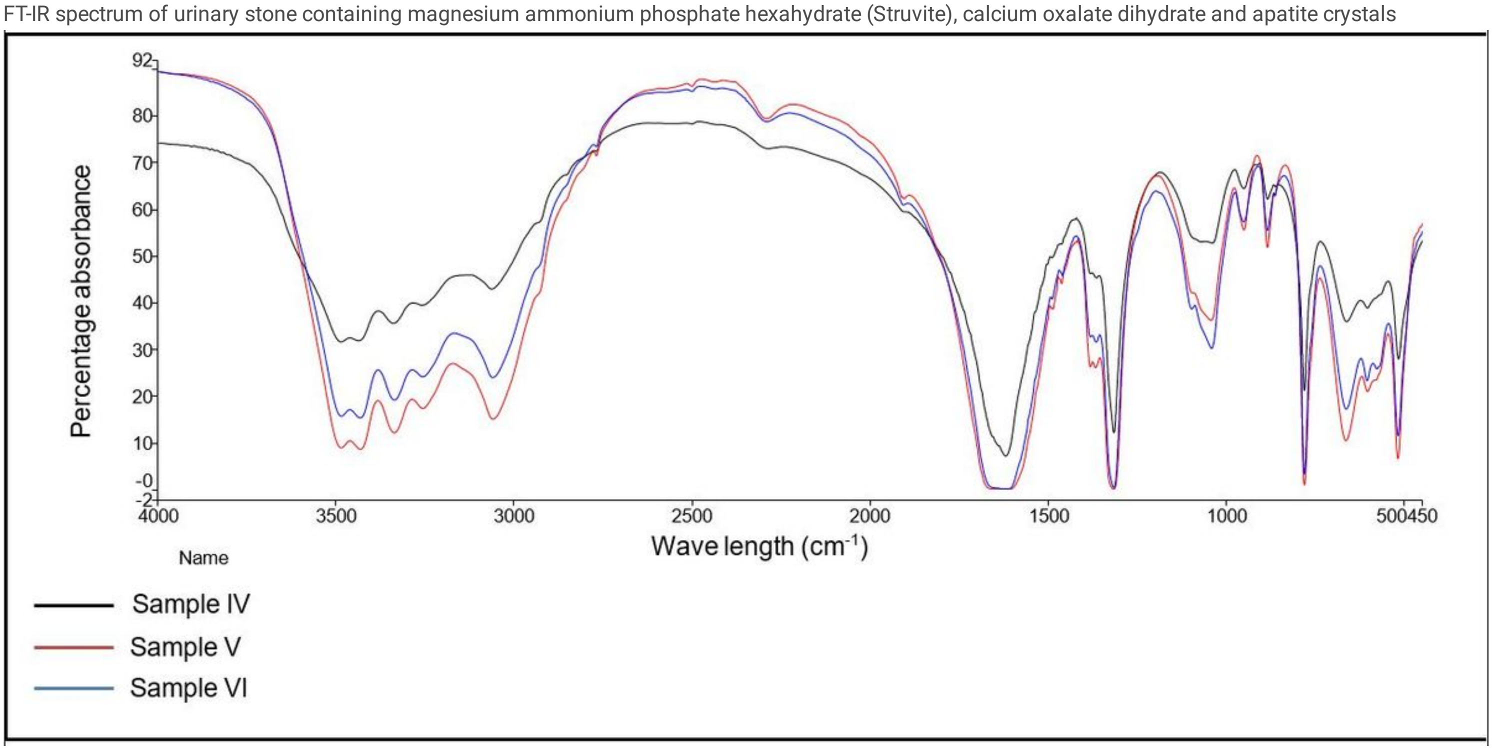

Figure 5

FTIR spectrum of urinary stone containing calcium oxalate monohydrate (COM) and calcium phosphate