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An investigational study on urinalysis of geriatric dogs with urolithiasis

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Abstract
The present study was carried out to investigate the physical, chemical and microscopical changes of urine in geriatric dogs with urolithiasis. The total number of adult dogs (>6 years) brought to the medicine outpatient ward of Veterinary Clinical Complex, College of Veterinary Science, Rajendranagar with various medical problems during the study period (January 2021 to April 2022) was 7280. Out of these total adult dogs, 620 were geriatric, and out of which, 362 dogs that were showing the clinical signs indicative of lower urinary tract disorders (LUTD), such as haematuria, pollakiuria, stranguria, dysuria, periuria, urethral discharge, dribbling of urine, straining while defecation, tenesmus, lameness and stiffness of limbs etc., were taken up for detailed study. Subsequently, 246/362 geriatric dogs were diagnosed with various lower urinary tract disorders *viz*., bacterial lower urinary tract infection (134/246), urolithiasis (71/246) prostate diseases (29/246) and transitional cell carcinoma (12/246) of urinary bladder. Urine was collected by using catheterisation or USG guided cystocentesis. The colour of the urine was dark yellow to reddish, transparency was cloudy to turbid and odour was slight ammonical to bad foul smell and specific gravity in the range of 1.000-1.015. Dipstick (Chemical) examination revealed alkaline pH in 45 and acidic pH in 26 geriatric dogs. Haematuria was a common finding that was observed with different intensity and also noticed pyuria and proteinuria. Microscopic examination of the urine sediment showed more number of erythrocytes (RBC-++++), leucocytes (WBC-+++), epithelial cells (+++), crystals (struvite, calcium oxalate and bilirubin) and bacteria (++).

Keywords: CUE, urine, urolithiasis, geriatric dogs

1. Introduction
Uroliths are formed in all the species of domestic animals, and is one of the important lower urinary tract diseases in dogs. Formation of uroliths is not a disease but rather a complication of several disorders, which is pretty often a result of a combination of both pathological and physiological factors (Watson *et al.*, 2010) [1]. Uroliths are aggregates of crystalline and occasionally non-crystalline solid substances that form in one or more locations within the urinary tract. For the formation of stones, urine must be supersaturated. Degree of super saturation depends on the ionic strength of urine; an increase in ionic strength reduces the level of super saturation, and subsequently crystal precipitation (Lekcharoensuk *et al.*, 2002; Williams and Wilson, 1990) [2, 3]. Canine urolithiasis is a common cause of emergency urinary tract disease requiring a rapid definitive diagnosis for immediate surgical and/or medical therapy (Houston *et al.*, 2004 and Hunprasita *et al.*, 2017) [4, 5]. Generally, dogs with urolithiasis are presented with serious clinical conditions such as occult haematuria and partial or complete obstruction. Haematuria, pollakiuria, stranguria and dysuria are common clinical signs of lower urinary tract disease that are non-specific to cystic calculi (Tion *et al.*, 2015) [6]. In dogs affected with urocystoliths are difficult to palpate and physical examination findings are often normal unless the urethral obstruction is present, complete blood cell count (CBC) and serum biochemical analysis are usually normal and the clinical signs are not definitive. Reports indicate that haematuria can occurs in more than 50% of dogs with urinary bladder and renal neoplasia or other disorders are known to damage the mucosal surfaces of the urogenital tract such as infection, inflammation, trauma, vascular disease, and coagulopathies. Although haematuria is one of the most common clinical signs exhibited by dogs with

urolithiasis, it can lead to wrong etiological diagnosis due to its multiple causes but physical, chemical and microscopical urine sediment examination along with history, clinical signs, hematology and biochemical gives proper diagnosis (Bartges *et al.*, 2004 and Forrester, 2004) [7, 8]. Urine pH is one of the important factors which favour the formation of urinary calculi. In general, alkaline pH (pH>8.0) favours the formation of phosphate, carbonate and struvite calculi whereas acidic pH (pH<7.0) favours the formation of urate and silicate calculi. The present study was conducted to evaluate the physical, chemical and microscopical changes of urine among geriatric dogs with urolithiasis.

2. Materials and Methods

A total of 71 geriatric dogs that were presented with the clinical signs indicative of urolirhiasis to the Veterinary Clinical Complex, College of Veterinary Science., Rajendranagar, PVNR TVU, Hyderabad were taken up for the present study. After detailed history and clinical examination, the urine was collected by ultrasonography guided cystocentesis or by catheterization in a clean sterile vial and used for complete urine examination (CUE). However, urine sample was also collected from apparently healthy adult dogs for comparison. Ultrasound-guided cystocentesis was used to safely obtain a urine sample. Sterile saline or alcohol was applied over the site of collection. Needle was inserted close to the transducer; the needle was kept within the plane of the ultrasound beam. After verification that the tip of the needle has advanced to the desired position within the bladder lumen, urine was aspirated into the syringe (Lulich *et al.*, 2000) [9]. As a part of urine collection by catheter, at the time of clinical examination the genitalia of the dog was cleaned properly and a spot sample of mid stream urine was collected using sterile catheter in a sterile test tube to decrease potential bacterial, cellular contamination., and these sample were analysed within 1-2 hours of collection to minimize in-vitro or iatrogenic crystal formation. The urine sample was examined manually immediately after collection for its colour, transparency and odour, as the physical examination parameters. Similarly, pH, bilirubin, urobilinogen, glucose, proteins, blood, leukocytes, nitrate, ketone are determined under chemical examination parameters using dipstick. Further, the collected urine sample was processed to obtain urine sediment and microscopic examination was carried out by the procedure mentioned by Sink and Weinstein (2012) [10]. The sample of well-mixed urine (usually 10-15 mL) was centrifuged in a test tube at relatively low speed (about 2-3,000 rpm) for 5-10 minutes until a moderately cohesive button was produced at the bottom of tube. The supernatant is decanted and a volume of 0.2-0.5 mL was left inside the tube. The sediment was re-suspended in the remaining supernatant by flicking the bottom of the tube several times. A drop of re-suspended sediment is poured onto a glass slide and cover slip placed over it. The slide was examined and the routine sediment results were recorded. The sediment content was determined with minimum of ten fields under both low (10x objective) and high (40x objective) power. Since the urine was collected by cystocentesis, less than three cells / hpf of RBC, WBC and epithelial cells were considered as normal in dogs. Air-dried slides were stained with the Giemsa after fixation with methanol for cytology evaluation (Neil *et al.*, 2013) [11].

3. Results

The urine samples from the dogs with urolithiasis were deep yellow in colour among 28 (39.44%) dogs, followed by reddish in 22 (30.99%), deep amber in 11 (15.49%) and brown coloured urine among 10 (14.08%) dogs. The urine sample was clear in 56 (78.87%) dogs, whereas cloudiness and turbidity were seen in 11 (15.49%) and 4 (5.63%) dogs, respectively. The odour of the urine from 47 (66.2%) dogs with urolithiasis was similar to healthy dogs (slight ammonical), where as, strong ammonical in 16 (22.54%) and bad foul smell in 8 (11.27%) dogs, respectively. The urine samples from dogs with urolithiasis had a specific gravity in the range of 1.000-1.015 (Table 1 and Fig. 1). Chemical examination of the urine as shown in table 2 and fig. 2 revealed, alkaline pH in 45 (63.38%) and acidic pH in 26 (36.62%) geriatric dogs with urolithiasis. Haematuria was the common finding that was observed with different intensity, *viz*., mild 26 (36.62%), moderate 18 (25.35%) and severe 12 (16.90%). Proteinuria was observed with mild 22 (30.99%), moderate 16 (22.54%) and severe in 6 (8.45%) dogs. Pyuria with the intensity of mild 25 (35.21%) to moderate 6 (8.45%). Among dogs with urolithiasis, 16 had concurrent bacterial urinary tract infection that showed moderate (++) proteinuria and mild (+) pyuria. Crystalluria 66 (92.96%) and haematuria (RBC) 45 (63.38%) were the major findings on microscopic examination of urine in dogs with urolithiasis. Out of 71 dogs with urolithiasis, 41 (57.75%) dogs showed struvite crystals**,** 23 (32.39%) were calcium oxalate crystals and where as, bilirubin crystal was seen in 2 (2.82%) dogs. Struvite crystals known as magnesium ammonium phosphate or triple phosphate crystals, appeared as colourless, 3-dimensional, prism like crystals “coffin lids”. Calcium oxalate monohydrate crystals varied in size and had a spindle, oval, or dumbbell shape or “hemp seed”. Calcium oxalate dihydrate crystals were typically colourless squares whose corners were connected by intersecting lines (resembling an envelope). Bilirubin crystals form from conjugated bilirubin (water-soluble) and were needle-like to granular crystals in yellow colour. Remaining five dogs did not show crystalluria. Along with crystals, RBC 45 (63.38%), WBC 24 (33.80%) and epithelial cells 20 (28.17%) were also observed on sediment examination of urine. The details are given in table 3 and fig. 3-7.

4. Discussion

In the present study, colour of the urine sample from the dogs with urolithiasis frequently showed deep yellow colour (39.44%) followed by reddish (30.99%), deep amber (15.49%) and brown coloured urine (14.08%). Reddish and brown colour is due to haematuria caused by the irritation and agitation of the uroepithelium by uroliths and these dogs had a symptom of haematuria when they presented to hospital. Appearance of urine was clear in 78.87 percent of dogs, as there was no concurrent infection in the lower urinary tract in these dogs, whereas cloudiness and turbidity was observed in 15.49 percent and 5.63 percent, respectively, which could be due to presence of large concentrations of mucus and desquamated cells along with debris seen in few dogs and also bacteria in dogs which had urinary tract infection. The odour of the urine from 66.2 percent dogs with urolithiasis was similar to healthy dogs (slight ammonical), where as, strong ammonical odour in 22.54 percent dogs which may be attributed to urine retention due to partial or complete obstruction of lower urinary tract by urolith and bad foul smell in 11.27 percent dogs due to bacterial infection of lower urinary tract (cystitis and or urethritis). The urine samples from dogs with urolithiasis had a specific gravity in the range of 1.000-1.015. The findings of the present study regarding physical examination of urine sample from dogs with urolithiasis are in close conformity reports of Tion *et al.* (2015) [6] and Abebe and Narinder (2019) [12]. Chemical examination of the urine revealed, alkaline pH in 63.38 percent and acidic pH in 36.62 percent dogs with urolithiasis. The pH of the urine plays an important role in the formation of the urinary stones. Struvite calculi are formed in alkaline pH and calcium oxalate stones were formed in acidic urine. Infection of the lower urinary tract by urease- positive bacteria by *Staphylococci* spp. and *Proteus* spp. plays the most important role in the pathogenesis of struvite uroliths in dogs. Hydrolysis of urea by urease - positive bacteria liberates ammonia and carbon dioxide, which alkalinizes the urine and increases availability of ammonium and phosphate ions for struvite crystal formation. Under acidic pH, oxalic acid has more ability to link with calcium and forms insoluble salt crystals, together to form calcium oxalate stones. In the present study, incidence of struvite crystals were found in 41 (57.75%), whereas the infection of lower urinary tract with struvite urolith was 22.43 percent with urease positive bacteria *Staphylococci spp.* (14.08%) and *Proteus spp.* (8.45%). Though research reports opined that 95 percent of dogs with struvite urolithiasis will have infection as an underlying cause, in our study the infection in struvite urolithiasis was less, which might be due to decreased struvite solubility in animals with persistently alkaline urine in the absence of UTI as stated by Chew *et al*. (2011) [13]. Other predisposing factors for struvite calculi in the absence of UTI in dogs are associated with family history of struvite stones, alkaline urine due to diet based on vegetable proteins etc. The results of the present study are in accordance with the findings of Alguacil *et al*. (2007) [14] who reported that struvite uroliths were found in the urine of alkaline pH. Radiograph appearance and urine pH are considered the main tools to predict urolith composition (Lulich and Osborne, 2017) [15]. Haematuria was the common finding which was observed in 56 (78.87%) dogs with different intensity, *viz*., mild 26 (36.62%), moderate 18 (25.35%) and severe 12 (16.90%). Proteinuria was observed in 44 (61.97%) dogs, with severity in 30.99 per cent dogs. Pyuria in 31(43.36%) dogs with the intensity of mild (35.21%) to moderate (8.45%). Among dogs with urolithiasis, sixteen had concurrent bacterial urinary tract infection showed moderate (++) proteinuria and mild (+) pyuria. Mechanical irritation of bladder mucosa by change in positions of cystolith due to gravity causes distortion and erosions of bladder lumen and lead to haematuria, severe inflammation and pyuria. Increased concentrations of uroliths affect the mucins glycosaminoglycans produced by bladder mucosal lining cells which reduces their protective ability. This damage to the mucosal lining causes seepage of plasma protein and result in proteinuria. Pyuria indicates concurrent bacterial infection of lower urinary tract in dogs with urolithiais. In support of the present study, the studies conducted by Raila *et al.* (2011) [16], Palma *et al.* (2013) [17] on urinary calculi showed the similar findings. Crystalluria (92.96%) and haematuria (63.38%) were the major findings on microscopic examination of urine in dogs with urolithiasis. Out of 71 dogs with urolithiasis 41 (57.75%) dogs showed struvite crystals, 23 (32.39%) dogs showed calcium oxalate crystals and bilirubin crystals seen in 2 (2.82%) dogs. Remaining five dogs did not show crystalluria, and were diagnosed based on clinical symptoms and imaging techniques, which indicates crystalluria may be found in dogs without urolithiasis and may be absent in dogs with urolithiasis. Other than urolithiasis, crystalluria is also associated with pathological conditions such as urolithiasis, acute uric acid nephropathy. Also crystalluria is a frequent finding of the routine examination of urine sediments. In most instances the precipitation of crystals of calcium oxalate, uric acid, triple phosphate, calcium phosphate and amorphous phosphates or urates is caused by transient super saturation of urine, ingestion of foods, or by changes of urine temperature and / or pH which occur upon standing after micturition as reported by Fazili *et al.* (2010) [18]. Absence of cystalluria does not rule out urolithiasis and mere presence of crystals in the urine sediment cannot confirm the disease condition (Chew *et al.*, 2011) [13]. These are in agreement with Houston *et al.* (2004) [4] who reported that 80-91 percent of all uroliths are struvite and calcium oxalate. Lulich and Osborne (2017) [15] who documented that the radiographic appearance and urine pH are considered as the main tools to predict urolith composition. Upon sediment examination, haematuria was seen in 63.38 percent dogs with urolithiasis, these dogs had same clinical symptom on the day of presentation and had normal Hb levels. WBC were seen in 33.80 percent of dogs with urolithiasis, which is due to variable extent of stress induced by bacteria in the urinary tract, also as a sign of manifestation of body defence mechanism against bacterial infection. Though there was pyuria, the TLC were within the normal range, which might be attributed to local response by immune system to variable extent of damage to the epithelium by uroliths in the lower local urinary tract (Seguin *et al.*, 2003) [19]. Epithelial cells were seen in 28.17 percent of dogs, which could be attributed to the agitation of bladder mucosa by urolith. The findings of our study are in agreement with Dolinsek (2004) [20] and Alguacil *et al.* (2007) [14] who reported that the cytology of urine from dogs with urolithiasis revealed large number of WBC and epithelial cells.

Table 1: Physical examination of urine samples from geriatric dogs with Urolithiasis

Sl. No.	Parameter	Apparently healthy adult animals	Urolithiasis (n=71)	
1	Colour	Pale yellow	Dark yellow	28 (39.44%)
			Amber	11 (15.49%)
			Brown	10 (14.08%)
			Reddish	22 (30.99%)
2	Appearance	Clear	Clear	56 (78.87%)
			Cloudy	11 (15.49%)
			Turbid	4 (5.63%)
3	Odour	Slight ammonical	Slight ammonical	47 (66.2%)
			Strong	16 (22.54%)
			Bad	8 (11.27%)
4	Specific gravity	1.005- 1.015	-	1.000-1.015

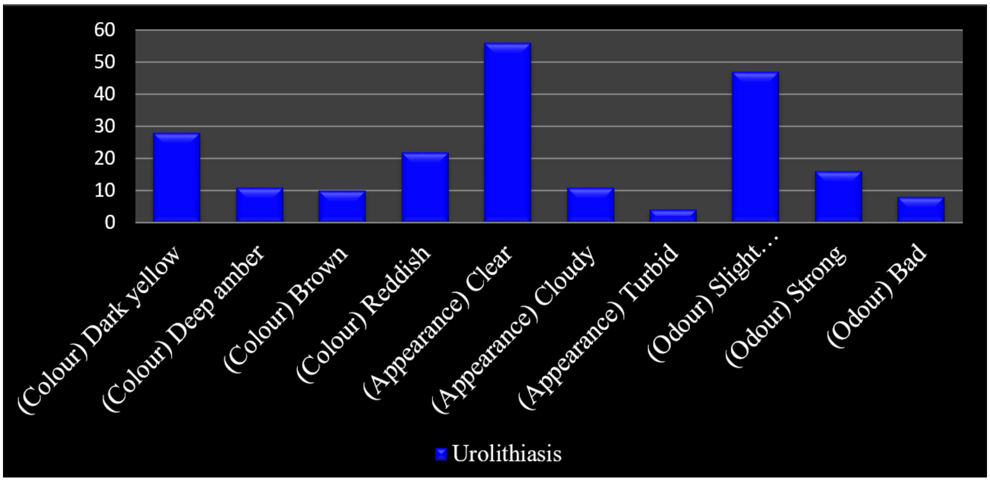

Fig. 1: Physical examination of urine samples from geriatric dogs with Urolithiasis

Table 2: Chemical examination of urine samples from geriatric dogs with Urolithiasis

Sl. No.	Parameter	Apparently healthy adult animals	Severity level	Urolithiasis (n=71)
1	pH	Acidic	Acidic (<7)	26 (36.62%)
		-	Alkaline (≥7)	45 (63.38%)
2	Leukocytes	Absent	Mild	25 (35.21%)
			Moderate	6 (8.45%)
			Severe	0
3	Blood	Absent	Mild	26 (36.62%)
			Moderate	18 (25.35%)
			Severe	12 (16.90%)
4	Bilirubin	Absent	Mild	27 (38.03%)
			Moderate	12 (16.90%)
			Severe	0
5	Ketone bodies	Absent	Mild	2 (2.82%)
			Moderate	6 (8.45%)
			Severe	0
6	Urobillinogen	Present	Mild	26 (36.62%)
			Moderate	41 (57.75%)
			Severe	6 (8.45%)
7	Glucose	Absent	Mild	0
			Moderate	0
			Severe	0
8	Protein	Absent	Mild	22 (30.99%)
			Moderate	16 (22.54%)
			Severe	6 (8.45%)

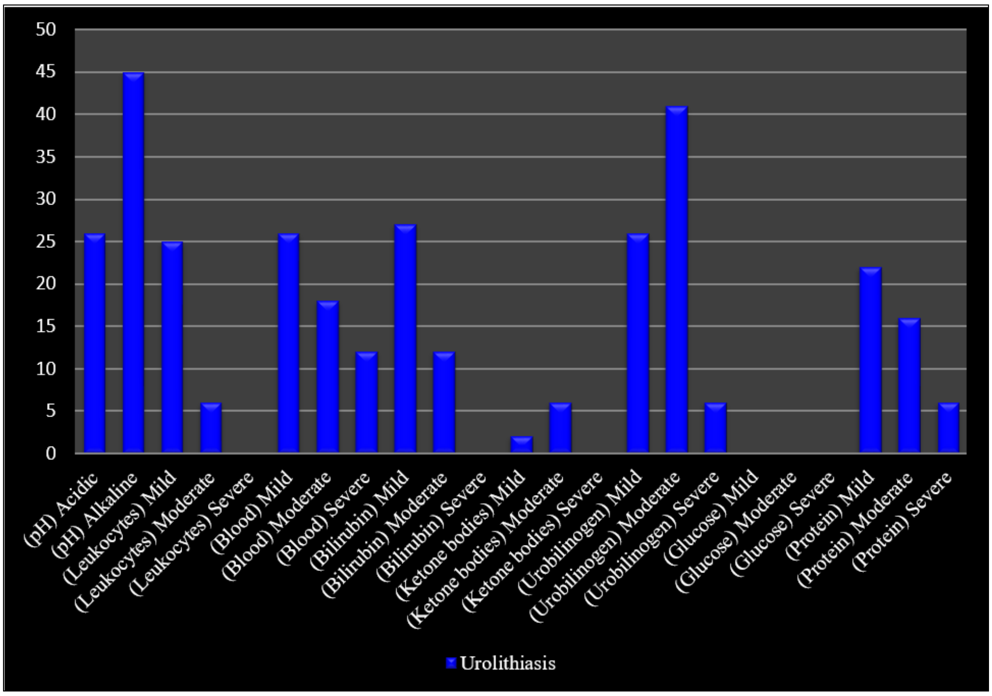

Fig 2: Chemical examination of urine samples from geriatric dogs with Urolithiasis

Table 3: Microscopic examination of urine sediment from geriatric dogs with Urolithiasis

Microscopic findings		Apparently healthy adult animals	Urolithiasis (n=71)
RBC		1 cell/hpf	45 (63.38%)
WBC		1 cell/hpf	24 (33.80%)
Epithelial cells		1- 2 cells/hpf	20 (28.17%)
Casts		Absent	6 (4.48%)
Bacteria		Absent	5 (7.04%)
Crystals	Struvite	Absent	41 (57.75%)
	Calcium oxalate	Absent	23 (32.39%)
	Bilirubin	Absent	2 (2.82%)

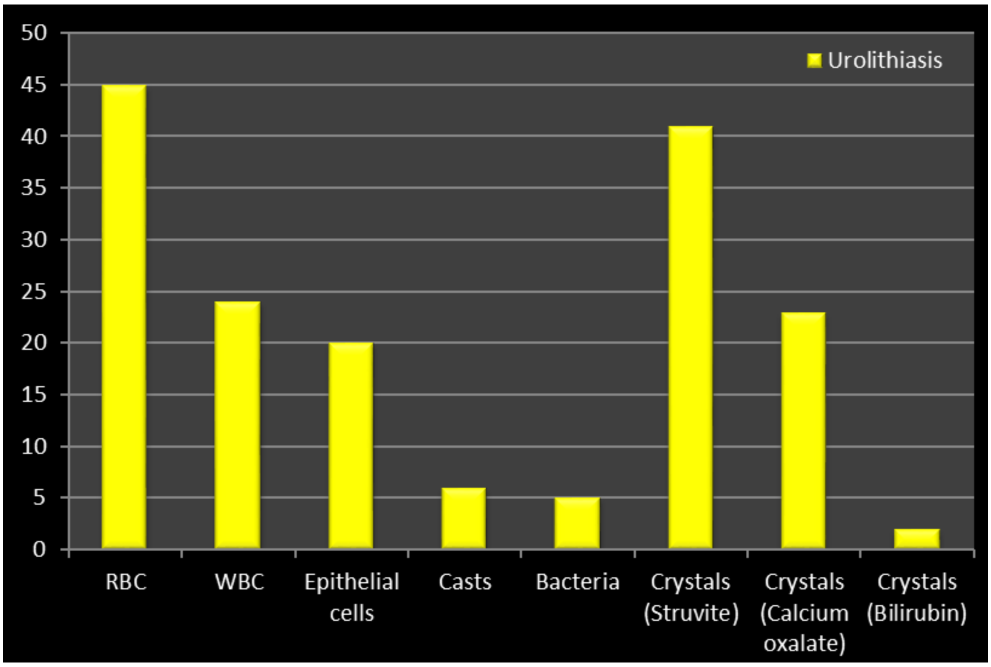

Fig 3: Microscopic examination of urine sediment from geriatric dogs with Urolithiasis

Fig 4: Bilirubin crystals in urine sediment on microscopic examination (40X)

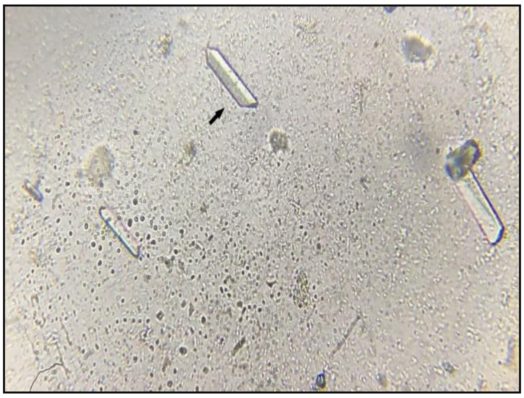

Fig 6: Calcium oxalate monohydrate crystals in urine sediment on microscopic examination (40X)

Fig 5: Struvite crystals in urine sediment on microscopic examination (40X)

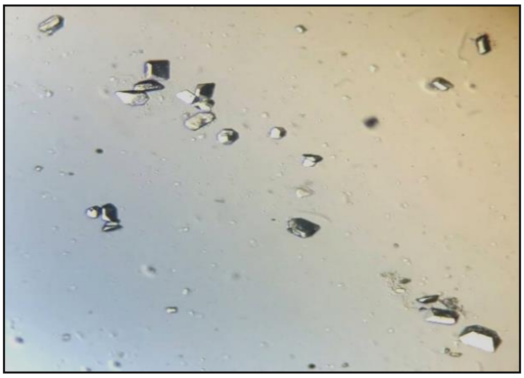

Fig 7: Calcium oxalate dihydrate crystals in urine sediment on microscopic examination (40X)

5. Conclusion
From the present study, it may be concluded that the urolithiasis infections is quite common among the geriatric dogs. Complete urine examination (CUE) that includes physical, chemical and microscopic examination is most effective tools for early diagnosis of urolithiasis infection.

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