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Mineral composition and clinical aspects of urolithiasis in cats in Brazil

[*Composição mineral e aspectos clínicos da urolitíase em gatos no Brasil*]

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ABSTRACT

Between October 2016 and October 2017, 63 feline uroliths were analyzed at Universidade Federal de Goiás (UFG) by using both chemical analysis and energy dispersive spectroscopy (EDS). The most frequent mineral type found was struvite (53.9%), followed by urate (39.7%), calcium oxalate (30.1%) and calcium phosphate (25.3%). Calculus containing xanthine, cystine and silica were not observed. Uroliths classified as simple, comprised a total of 34/63. Amongst the 42 animals present in the study, 26 were male and 16 were female. Pure breed animals comprised 14.4% of the total, and the breeds observed within the study were the Persian, Himalayan, Siamese, and Angora. Cats between 25-72 months old were more frequently diagnosed with uroliths. The clinical signs varied between systemic and urinary signs and the most found were anorexia, vomiting, hematuria and dysuria. All patients were either spayed or neutered and 34 patients had no outdoor access. Familial information was unknown in almost 100% of the cases. The results observed in the present study serve as a basis for future comparisons related to the epidemiology of urinary lithiasis in Brazil, especially for the feline species.

Keywords: chemical analysis, spectroscopy, struvite, urate, uroliths

RESUMO

Entre outubro de 2016 e outubro de 2017, 63 urólitos felinos foram analisados na Universidade Federal de Goiás (UFG), usando-se tanto análise química quanto espectroscopia por energia dispersiva (EDS). O tipo de mineral mais encontrado foi o estruvita (53,9%), seguido pelo urato (39,7%), oxalato de cálcio (30,1%) e fosfato de cálcio (25,3%). Cálculos contendo xantina, cistina e sílica não foram observados. Urólitos classificados como simples comprometeram um total de 34/63. Entre os 42 animais presentes no estudo, 26 eram machos e 16 eram fêmeas. Dos animais comprometidos, 14,4% eram de raças puras, sendo observadas as raças Persa, Himalaio, Siamês e Angorá. Gatos com idade entre 25-72 meses foram mais frequentemente diagnosticados com urólitos. Os sinais clínicos variaram entre sinais sistêmicos e urinários, sendo anorexia, vômito, hematúria e disúria os mais encontrados. Todos os pacientes eram castrados, e 34 deles não tinham acesso à rua. Histórico familiar era desconhecido em quase 100% dos casos. Os resultados observados no presente estudo servem como base para comparações futuras relacionadas à epidemiologia da litíase urinária no Brasil, especialmente em espécies felinas.

Palavras-chave: análise química, espectroscopia, estruvita, urato, urólitos

INTRODUCTION

Urolithiasis is a worldwide distributed disease, characterized by the presence of uroliths along the urinary tract. In cats that present with lower urinary tract signs, approximately 15-23% will have urolithiasis (Lekcharoensuk *et al.*, 2001a; Gerber *et al.*, 2005). The majority of these uroliths are mainly located in the urinary bladder (Cannon *et al.*, 2007; Picavet *et al.*, 2007; Osborne *et al.*, 2009; Hesse *et al.*, 2012; Houston and Moore, 2009; Houston *et al.*, 2016; Kopecny *et al.*, 2021).

There are several factors that can increase the chance of developing urolithiasis. Some of these

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risk factors are: breed, age, gender, diet (Lekcharoensuk *et al*., 2000; Lekcharoensuk *et al*., 2001a; Houston and Moore, 2009; Lulich *et al*., 2016), genetics (Bannasch and Henthorn, 2009; Mizukami *et al*., 2016; Mizukami *et al*., 2015), lifestyle, body score (Lekcharoensuk *et al*., 2000; Albasan *et al*., 2012; Palm and Westropp, 2011), regional factors, demographic factors (Bartges, 2016; Rogers *et al*., 2011; Balázs and Tibor, 2015; Gomes *et al*., 2018). The urinary pH, water intake and frequency of urination also can trigger crystal precipitation and urolith formation (Langston *et al*., 2008; Dijcker *et al*., 2011; Gomes *et al*., 2018).

Uroliths are classified according to the mineral(s) present in their composition, and the mineral types most observed are calcium oxalate and struvite uroliths (Cannon *et al*., 2007; Picavet *et al*., 2007; Osborne *et al*., 2009; Hesse *et al*., 2012; Houston and Moore *et al*., 2009; Gerber *et al*., 2016; Houston *et al*., 2016; Mendoza-López *et al*., 2019; Minnesota Urolith Center, 2019; Burggraaf *et al*., 2021; Kopecny *et al*., 2021). In a lesser extent, urate, cystine, xanthine, calcium phosphate, silica, and solidified dry blood uroliths are observed (Hesse *et al*., 2012; Houston and Moore, 2009; Houston *et al*., 2016; Rogers *et al*., 2011; Balázs and Tibor, 2015; Escolar and Bellanato, 2003; Ángel-Caraza *et al*., 2012; Appel *et al*., 2010; Dear *et al*., 2011; Mizukami *et al*., 2015, 2016; Westropp *et al*., 2006).

The calculus can be divided into the following regions: the nidus, considered the initial portion of development; the body, which is the major portion of the calculus; the shell, which is the outer layer that surrounds the body; and the surface crystals which are an incomplete outer lamination of the urolith. The calculus can be considered simple when in its composition there is more than 70% of a certain mineral type. When no component reaches 70% of the total and there is not the presence of layers, it can be classified as mixed. An urolith can be considered compound when it has two or more layers of different mineral types (Ulrich *et al*., 1996, 2009).

The clinical signs of animals with urolithiasis may vary according to the affected segment, quantity, and format of uroliths. Patients usually present signs of urinary tract disease such as stranguria, dysuria and hematuria (Langston *et al*., 2008; Grauer, 2015). Nonspecific signs (vomiting and anorexia) or complete absence of signs may also be observed, especially when the calculus is located within the upper urinary tract (Langston, 2008; Kyles *et al.*, 2005; Lulich *et al*., 2016).

In order to understand the possible contributing factors that led to the development of the uroliths, all cases should be accompanied by a record of signalment (age, sex, breed), current weight and body condition score, results of a thorough history including diet, (Palm and Westropp, 2011; Grauer, 2015; Gomes *et al*., 2018; Lekcharoensuk *et al*., 2001a, 2000) hunting behavior, and treats offered, physical examination, and documentation of all underlying conditions (Lekcharoensuk *et al*., 2001a, 2001; Ángel-Caraza *et al*., 2012). Results of laboratory tests and imaging performed should also be provided (Bartges, 2016; Langston *et al*., 2008; Lulich and Osborne, 2009; Albasan *et al*., 2003). In some cases, for example cats with cystinuria, genetic testing may be recommended (Mizukami *et al*., 2015, 2016; Bannasch and Henthorn, 2009). All retrieved uroliths should undergo analysis. By determining the mineral composition of the uroliths in conjunction with a complete understanding of the individual cat's medical history, the best recommendations can be made for each case to aid in the management and possible prevention of urolithiasis (Houston *et al*., 2016; Gomes *et al*., 2018; Kopecny *et al*., 2021).

Uroliths can be analyzed by either qualitative or quantitative methods (Ulrich *et al*., 1996; Koehler *et al*., 2009; Gomes *et al*., 2022). Qualitative analysis requires no special equipment and thus is easy, quick, and cost effective to perform. The technique involves crushing or pulverizing the urolith and then applying drops of specific reagents to the sample. This method is based on color changes and identifies chemical radicals and ions. This method, by itself, is not recommended (Ruby and Ling, 1986; Ulrich *et al*., 1996; Koehler *et al*., 2009; Gomes *et al*., 2022).

Quantitative methods, which include optical crystallography, x-ray diffraction, infrared spectroscopy, and energy dispersive spectroscopy (EDS), are much more sensitive and specific and allow the identification and composition of each layer of the urolith, making diagnosis of mixed and compound uroliths possible (Osborne *et al*., 1986; Ulrich *et al*., 1996; Kalinski *et al*., 2012; Gomes *et al*., 2022). Not all techniques are readily available in all countries, but the consensus is that quantitative methods are more reliable in confirming mineral composition. Each technique has its advantages and disadvantages, so it is advisable to combine techniques to increase the sensitivity of the result (Bovee and McGuire, 1984; Picavet *et al*., 2007; Escolar and Bellanato, 2003; Ulrich *et al*., 1996; Kalinski *et al*., 2012; Gomes *et al*., 2022).

The epidemiology of feline urolithiasis present regional characteristics. Therefore, it is important to carry out studies to increase knowledge about the disease in Brazil, as well as to improve therapeutic and preventive measures, aiming at minimizing the rate of recurrence. As feline urolithiasis has been poorly studied in this country, it is necessary to perform studies on its pathophysiology, epidemiology, and clinical and laboratory aspects. Thus, the objective of this study is to determine the composition of uroliths in feline patients in Brazil and to analyze epidemiological, clinical and laboratory data of the patients affected.

MATERIALS AND METHODS

The present study consisted of a prospective and descriptive study. The project study was carried out at the Veterinary Medical School and Department of Animal Science of the Federal University of Goias (UFG), Goiania, Brazil. All procedures performed in this study were approved by the Comissão de Ética no Uso de Animais - CEUA (Ethics Committee on the Use of Animals), protocol no. 060/16. All collections and analyzes were performed accordingly to the relevant guidelines and regulations, with the consent of the owners of the animals participating in the study.

A total of 63 uroliths from 42 cats were submitted to the Veterinary Hospital of the Veterinary and Zootechnical School of the Federal University of Goiás (HV/EVZ/UFG) and Veterinary Clinics and Hospitals of different regions of the country were analyzed in the period between October 2016 and October 2017 for analysis. Of the 42 cats, 24 were from veterinary clinics in the state of Goiás, eight from clinics in Rio de Janeiro, six from clinics in the Federal District and four from clinics in Rio Grande do Sul. The uroliths were obtained by surgical removal and/or natural voiding, as described by the veterinarian responsible for the patient. For each animal diagnosed with urinary lithiasis, data were requested regarding the history, clinical signs, and urine test result (when performed).

Both qualitative and quantitative analyses were performed on each submitted urolith. For qualitative analysis, the urolith is crushed or pulverized to sand. The powder from the sanding process was used to perform a qualitative chemical analysis with the use of a commercial renal calculus analysis reagent - K008® (Bioclin, Belo Horizonte, Minas Gerais), following the manufacturer's recommendations. The commercial kit identifies the presence of the substances by chemical reactions, which color changes in the sample. The substances identified by this analysis are ammonium, calcium, carbonate, cystine, phosphate, magnesium, oxalate, and urate. This stage was developed in the Multiuser Laboratory of the Postgraduate Program in Animal Science of the EVZ/UFG.

The second stage of analysis, which consists of energy dispersive spectroscopy (EDS), was performed in two laboratories. The High-Resolution Microscopy Multiuser Laboratory (Labmic) at the Institute of Physics (IF/UFG) and the Regional Center for Technology and Innovation (CRTI). The samples were firstly prepared for analysis and subjected to the carbon film coating process using the Denton Vacuum, Desk V equipment. After the preparation, the analysis was performed, with the use of microscopy and spectroscopy. Microscopic evaluation in the Labmic was performed by means of a scanning electron microscope (SEM - Jeol, JSM - 6610), equipped with energy dispersive spectroscopy (EDS - *thermo scientific NSS spectral imaging*). In the analysis performed in the CRTI, it was used the SEM (*Jeol JSM-IT300*) and EDS (*Oxford Instruments X-MaxN 80*). Although the laboratories have different models of qeuipment, this doesn't interfere in the result of the analysis.

With the SEM it was possible to identify the different regions of the urolith - nidus, body, and shell - and later the evaluation by EDS was performed in each layer (Fig. 1). In the nidus at least three points were analyzed for their composition, whereas in the body and shell region at least three points were analyzed in more than a part of the layer, according to the sample size. According to the spectrum formed, the urolith composition was defined.

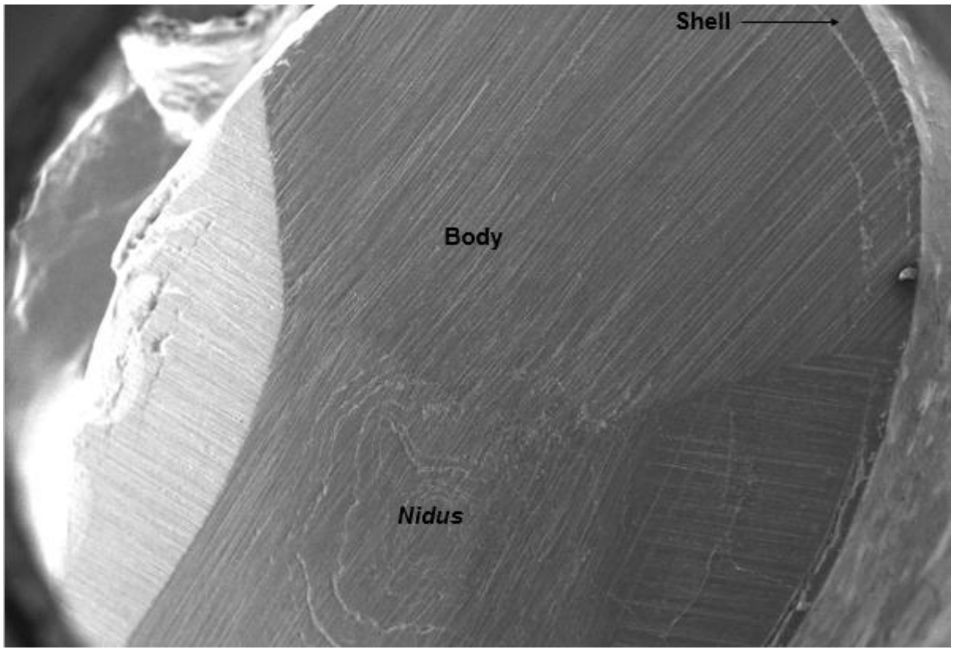

Figure 1. Different regions of urolith - nidus, body and shell - defined by scanning electron microscope.

Struvite uroliths were identified by EDS in the presence of Mg (magnesium) and P (phosphorus) peaks; calcium oxalate mainly by Ca (calcium) peaks; calcium phosphate by Ca (calcium) and P (phosphorus) peaks. In cases where only peaks of C (carbon), N (nitrogen) and O (oxygen) were observed, the urolith was classified as organic composition (Fig. 2). In these cases, the chemical analysis was decisive for the differentiation of urolith into urate, cystine or xanthine. Other peaks such as K (potassium), Na (sodium), Cl (chlorine) were observed, however it is not important for the classification of the stones. For the purpose of this study, an urolith was considered simple if only one mineral type was identified both in the EDS and the chemical analysis. Uroliths with more than one mineral type were considered as being mixed. For the classification of the predominant mineral in mixed uroliths, all the spectrum obtained in all the points analyzed in each of the uroliths (between five and 18 points according to their size) were considered, and the mineral with the highest frequency was considered predominant.

Urinalysis of patients attended at HV/EVZ/UFG were performed at the Multiuser Laboratory of the Postgraduate Program in Animal Science. Urine samples were obtained by cystocentesis.

The variables evaluated by statistical analysis were gender, age, breed, urolith type, urinary tract location, body condition score (BCS), alimentation and urine analysis alterations. For variables with more than two categories, the chi-square test with significance level 5% and Fisher's exact test for pairs analysis (two by two), with relative risk (RR) and *odds ratio* (OR) were used.

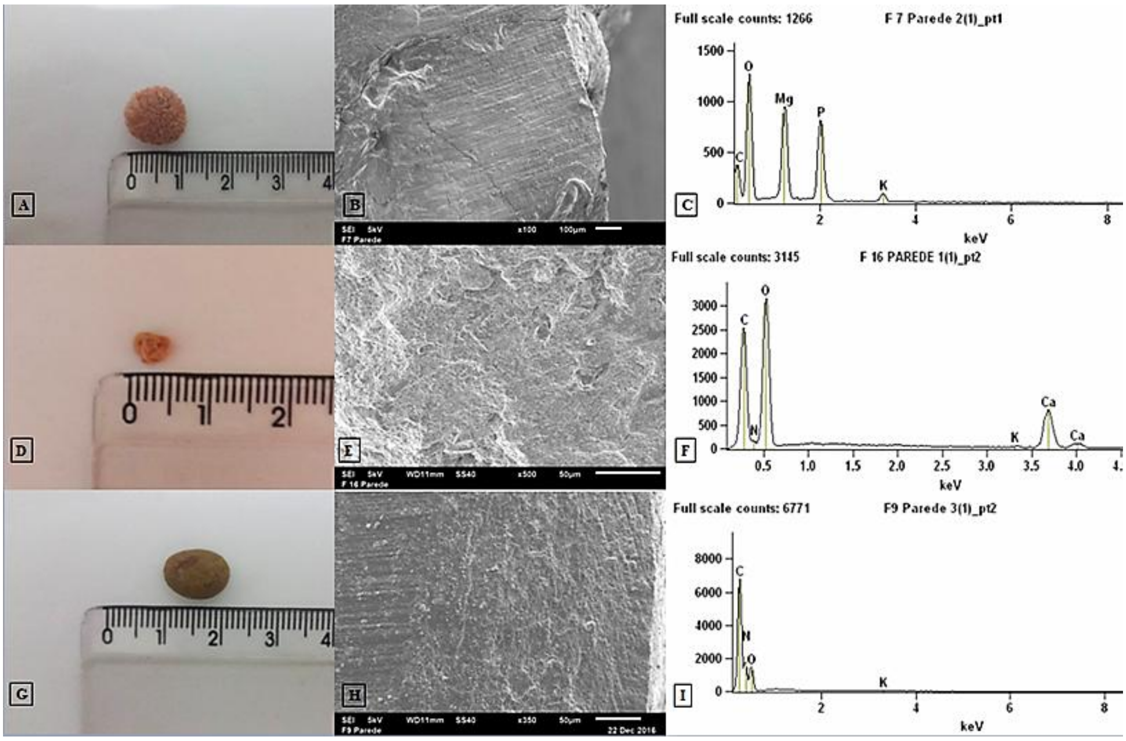

Figure 2. Feline uroliths. Struvite urocystolith (A). Image of the shell layer obtained by SEM (B). Spectrum of EDS from the same region, determining the composition of struvite (C). Calcium oxalate urocystolith (D). Image of the shell layer obtained by SEM (E). Spectrum of EDS from the same region, determining the composition of calcium oxalate (F). Simple organic composition urocystolith, confirmed by chemical analysis as ammonium urate (G). Image of the shell layer obtained by SEM (H). Spectrum of EDS from the same region, determining the simple organic composition (I).

RESULTS AND DISCUSSION

From October 2016 to October 2017, 63 uroliths from 42 felines were analyzed by EDS and chemical analysis. EDS is sensitive and specific and available at the Department of Animal Science of the Federal University of Goias (UFG), Goiania, Brazil. The combination of technologies ensured accurate mineral composition of uroliths and ability to classify as simple, mixed, or compound. A previous study of feline uroliths, using EDS and infrared spectroscopy (IF), showed EDS to be more sensitive in detecting calcium phosphate in uroliths than IF (Escolar and Bellanato 2003). A review on EDS and other urolith analysis technologies has recently been published (Gomes *et al.*, 2022). The choice to perform EDS and chemical analysis was based on a study by Ariza (2014), that showed very good results using this combination of techniques.

Out of the 42 cats, 31 cats (73.9%) had one urolith; four cats (9.5%) had two uroliths; four cats (9.5%) had three uroliths and three cats (7.1%) had four uroliths. Out of the 11 patients who had more than one urolith, in six the mineral composition of all stones was the same. Four of the patients had simple uroliths and two had mixed uroliths. In five cats the mineral composition varied, but in all calculi of the same patient at least one mineral type was present in all uroliths. This means that totally different mineral composition in calculi from the same patient was not observed. In patients with more than one urolith the determination of the type of urolithiasis was based on the predominant mineral.

In all 42 patients, the predominant mineral, including the simple and mixed calculi, was struvite as it was observed in 16 patients (38.1%); followed by ammonium urate, observed in 15 cats (35.7%) and calcium oxalate, observed in 11 cats (26.2%). There was no significant difference in the occurrence of different mineral types (P= 0.4724). In 20 animals (47.6%) there were only simple uroliths, in four (9.5%) there were simple and mixed uroliths and in 18 (42.9%), only mixed uroliths. Simple composition calculi are the most observed (Ulrich *et al*., 2009; Kalinski *et al*., 2012). Probably the greatest number of mixed uroliths observed in this study is related to the technique used that allowed the analysis of many points in the same region of the calculus, which was already observed in another study (Ariza, 2014).

Of the 63 calculi analyzed, struvite was detected in 35 calculi (55.5%), ammonium urate in 25 (39.7%), calcium oxalate in 20 (31.7%) and calcium phosphate in 19 (30.1%). In this study, with a period from October 2016 to October 2017, struvite submissions were the most common submission. Similar findings for 2018 and 2019 were published from two centers offering quantitative urolith analysis in the United States, the Gerald V. Ling Urinary Stone Analysis Laboratory (University of California, Davis) and the Minnesota Urolith Center (MUV) respectively. In 2018, 54.5% of feline submissions to the Gerald V. Ling Urinary Stone Analysis Laboratory were struvite and 37.8% oxalate (Kopecny *et al.,* 2021). In 2019, the MUC reported 52% of submissions were struvite and 35% oxalate (Minnesota Urolith Center, 2019).

Historically, percentages fluctuated at both centers, with struvite predominating in the 1980s through to 1992 and then oxalate surpassing and remaining the number one submission until approximately 2001. Thereafter, struvite submissions increased once again with the MUC reporting struvite at 46% of all submissions by 2013 (oxalate 41%) to 52% in 2019, and the Gerald V. Ling Urinary Stone Analysis Laboratory reporting struvite-containing uroliths increasing significantly from 41.8% in 2005 to 54.5% in 2018 (subsequent decline in oxalate from 50.1% in 2005 to 37.7% in 2018) (Osborne *et al*., 2009; Hunprasit *et al*., 2014; Cannon *et al*., 2007; Kopecny *et al*., 2021).

In Hungary, struvite submissions were also reported as the number one submission (77.3 %) during the period from 2006-2014 but the ratio of calcium oxalate to struvite consistently increased over the 9 years of the survey from 0.13 to 0.8 (Balázs and Tibor, 2015). In other countries including Canada (1998-2014), Mexico (2006-2017), Germany (1981-2008), Switzerland (2002-2009), Belgium (1994-2004), The Netherlands (1994-2004; 2014-2020), Luxembourg (1994-2004), fluctuations in the percentages of struvite and oxalate were noted but oxalate was ultimately reported as the number one submission by the end of the time periods studied with struvite submissions second (Houston *et al*., 2016; Mendoza-López *et al*., 2019; Hesse *et al*., 2012; Gerber *et al*., 2016; Picavet *et al*., 2007; Burggraaf *et al*., 2021). In Spain, struvite was the most common urolith (52.9%) analyzed in a study of 34 uroliths published in 2003. Of interest, urate was second (29.4%) unlike any of the North American or European studies at any period investigated (Escolar *et al.,* 2003).

In this study, the most observed component after struvite was ammonium urate (35.7%). The high occurrence of urolithiasis of ammonium urate observed here does not find an equivalent on other studies. The frequencies of urate calculi found in other studies vary but were less than 10% in the majority of studies with 1% in Hungary (Balázs and Tibor, 2015), 1.7% in Germany (Hesse *et al*., 2012), 2.2% in the Netherlands (Burggraaf *et al*., 2021), 3% in Switzerland (Gerber *et al*., 2016), 4.4% in Canada (Houston *et al*., 2016), 4.9% in Mexico (Mendoza-López *et al*., 2019), 4.8- 10% in the USA (Lekcharoensuk *et al*., 2001a; Osborne *et al*., 2009, Minnesota Urolith Center, 2019; Kopecny *et al* 2021, Cannon *et al*., 2007). Only in the study from Spain, was the percentage of urate uroliths higher at 29.4% of submissions (Escolar and Bellanato, 2003).

Out of the 63 calculi analyzed, 34 (53.9%) presented only one mineral type in their composition being classified as simple and in 29 (46.1%) presented more than one type was identified, classifying them as mixed (Table 1). Two uroliths had a suture thread in the nidus.

Calcium phosphate was only observed in association with the other mineral types, which is common (Ulrich *et al*., 2009). It has been reported a strong association between struvite and calcium phosphate uroliths (Cannon *et al*., 2007), but here the greatest association was with calcium oxalate.

Table 1. Composition and frequency of uroliths classified as simple and mixed

Simple uroliths		Mixed uroliths	
Struvite	N=16	Struvite and calcium phosphate	N=3
Calcium oxalate	N=5	Struvite and calcium oxalate	N=3
Ammonium urate	N=13	Struvite, calcium phosphate and calcium oxalate	N=2
Total	N=34	Struvite, calcium phosphate and ammonium urate	N=4
		Struvite and ammonium urate	N=7
		Calcium oxalate and calcium phosphate	N=9
		Calcium oxalate, ammonium urate and calcium phosphate	N=1
		Total	N=29

Out of the 63 uroliths analyzed, 19 (30.1%) had different compositions in different layers. The differentiation of layers was possible due to the EDS technique, which is corroborated by other studies (Ulrich *et al*., 1996; Koehler *et al*., 2009; Kalinski *et al*., 2012; Ariza, 2014).

For purine uroliths, the observed EDS spectrum consisted of C (carbon), N (nitrogen) and O (oxygen), as described in other studies (Kalinski *et al*., 2012; Ariza, 2014; Lee *et al*., 2012). Thus, the association with chemical analysis allowed the identification of ammonium urate. A similar result was observed in a study with dogs (Ariza, 2014). It should be noted that the carbon peaks appearing in the EDS spectrum may be related to the sample coating. In cases where the chemical analysis determined the presence of more than one compound, the EDS made possible to determine the composition of the different layers and *nidus* and provided subsidies to determine possible causes for the development of the disease (Koehler *et al*., 2009; Kalinski *et al*., 2012; Ariza, 2014, Lee *et al*., 2012).

Out of the 42 cats evaluated, 26 (61.9%) were males and 16 (38.1%) were females. The literature corroborates the higher occurrence in males (Cannon *et al*., 2007; Hesse *et al*., 2012; Houston *et al*., 2016; Rogers *et al*., 2011; Balázs and Tibor, 2015). Table 2 shows the frequency of the urolithiasis type considering the patient's sex.

When comparing sex and the mineral type, the occurrence of urolithiasis in males was significantly higher (P= 0.0052). When comparing pairs, a significant difference was observed only in the ratio of struvite x calcium oxalate uroliths (P= 0.0025, RR= 4.667, OR= 8.944). Therefore, there was no significant difference when comparing struvite with urate (P= 0.2640) and oxalate with urate (P= 0.0975), respectively. In females, no significant difference was observed between the different types of urolithiasis (P= 0.0724).

These findings are different than in several previously published studies in which where males were predisposed to calcium oxalate and females to struvite (Cannon *et al*., 2007; Osborne *et al*., 2009; Houston and Moore, 2009; Houston *et al*., 2016; Rogers *et al*., 2011; Balázs and Tibor, 2015; Mendoza-López *et al*., 2019; Kopecny *et al*., 2021). In the current study, ammonium urate uroliths appeared in greater numbers in males, as described in other studies (Houston *et al*., 2016; Lekcharoensuk *et al*., 2001b; Albasan *et al*., 2012; Appel *et al*., 2010; Dear *et al.*, 2011).

As for the breed, two cats were Siamese (4.8%), two Persians (4.8%), one Himalayan (2.4%) and one Angora (2.4%). Mixed-breed felines represented a total of 36 animals (85.6%), presenting a significant difference (P<0.0001) in relation to the Angora and Himalayan breeds (P= <0.0001, RR= 0.02778, OR= 0.00406), as well as to the Persian and Siamese breeds (P<0.0001; RR= 0.05303; OR= 0.0079).

Among the mixed-breed animals, 31 (86.1%) were short haired and five (13.9%) medium haired (P<0.0001, RR= 6.200, OR= 38.44). In some studies, no significant association between breed and urolith composition was detected (Picavet *et al*., 2007; Mendoza-López *et al*., 2019) while in others, significant breed predispositions were shown for both struvite and oxalate uroliths. For struvite, Domestic Long Haired (DLH) and Domestic Medium Haired (DMH) cats were shown to be at risk in Canada and the USA when compared to Domestic Short Haired (DSH) cats (Houston *et al*., 2016; Kopecny *et al*., 2021). The high frequency in this group of felines in Brazil may be related to the larger population of animals in this category.

In other studies breeds reported to be at risk for struvite included the foreign shorthair, ragdoll, Chartreux, oriental shorthair, Himalayan, Persian, and Siamese cats, (Lekcharoesuk *et al*., 2001b; Osborne *et al*., 2009; Hesse *et al*., 2012). Breeds reported to be at higher risk of developing calcium oxalate uroliths include Tonkinese, Burmese, Devon rex, Himalayan, Persian, Siamese, Ragdoll, British Shorthair, Havana Brown, Chartreux, Scottish fold, Main Coon, (Houston *et al*., 2016; Kopecny *et al*., 2021; Cannon *et al*., 2007; Lekcharoensuk *et al*., 2001b, Osborne *et al*., 2009; Hesse *et al*., 2012; Balázs and Tibor, 2015). Overall, only four pure breeds were observed in the current study. Only the Angora breed that appeared in this study is not reported as susceptible in other studies.

Mixed-breed animals had higher occurrence of stones containing struvite and urate, however, there were no significant difference in the occurrence of different types of stones (P= 0.4169). In several published reports, a variety of breeds, including the Egyptian mau, Birman, Siamese, Bengal, European shorthair, Havana brown, Ocicat, Oriental, Ragdoll, Rex, Snowshoe, and Sphynx breeds have been associated with urate uroliths (Albasan *et al*., 2012; Appel *et al*., 2010; Dear *et al*., 2011; Houston *et al*., 2016). Houston *et al*. (2016), Appel *et al*. (2010), Albasan *et al*. (2012), reported a very significant risk for the Egyptian Mau. Recently, Kopecny *et al*. (2021) reported the Ocicat as the only breed at risk of urate compared to the DSH cat. These breed predispositions were not identified in this study. The data referring to the type of calculus and breed of the affected animals are expressed in table 2.

The patients were grouped by age and were divided into three categories: cats with less than 25 months old; between 25 and 72 months and with more than 72 months. Of the 42 felines in the study, 9 (21.4%) were less than 25 months old, 27 (64.2%) between 25 and 72 months old, and six (14.4%) were more than 72 months old. When comparing the three categories of age, a significant difference was observed (P<0.0001). The age group between 25 and 72 months presented a greater number of cases than less 25 months (P=0.0002; RR=2.857; OR=6.200), as well as patients to the group older than 72 months (P<0.0001; RR=4.500; OR=10.80). The data regarding to the age group and the mineral type identified are shown in table 2.

Table 2. Absolute values (n) and frequency (%) of feline patients according to sex, breed, and age group in the presence of different mineral types

Type of urolith	Struvite	Calcium oxalate	Ammonium urate	Total
Sex				
Males n (%)	14 (53.8%)	3 (11.5%)	9 (34.7%)	26 (100%)
Females n (%)	2 (12.5%)	8 (50.0%)	6 (37.5%)	16 (100%)
Breed				
Angora	0 (0.0%)	0 (0.0%)	1 (100%)	1 (100%)
Himalayan	0 (0.0%)	1 (100%)	0 (0.0%)	1 (100%)
Persian	1 (50.0%)	1 (50.0%)	0 (0.0%)	2 (100%)
Siamese	2 (100%)	0 (0.0%)	0 (0.0%)	2 (100%)
Mixed breed	13 (36.1%)	9 (25.0%)	14 (38.9%)	36 (100%)
Age				
< 25 months	4 (44.4%)	1 (11.2%)	4 (44.4%)	9 (100%)
Between 25 and 72 months	10 (37.0%)	7 (26.0%)	10 (37.0%)	27 (100%)
> 72 months	2 (33.3%)	3 (50.0%)	1 (16.7%)	6 (100%)

None of the cats were younger than 12 months of age and the oldest patient was 12 years old. In general, the literature supports that cats with struvite stones are significantly younger (7 years) (Picavet *et al*., 2007; Cannon *et al*., 2007; Burggraaf *et al*., 2021; Hesse *et al*., 2012; Gerber *et al*., 2016; Houston *et al*., 2016; Kopency *et al*., 2021; Mendoza-López *et al*., 2019; Gerber *et al*., 2016; Lulich and Osborne, 2009; Osborne *et al*., 2009). It would be expected that the older cats had a predominance of calcium oxalate uroliths (Cannon *et al*., 2007; Hesse *et al*., 2012; Houston and Moore, 2009; Bartges, 2016). This age predisposition was not seen in the current study. When compared to the frequency of uroliths within each group, no significant difference was observed.

Cats with ammonium urate uroliths demonstrated significant differences among the studied age groups (P= 0.0018), with most aged between 25 and 72 months (2.1-6 years). In other studies, age ranges were higher with 4-7 years (Lekcharoensuk *et al*., 2001a), 0.4-17 years with a mean age of 6.3±0.17 y (Appel *et al*., 2010), 4-10 years (Kopency *et al*., 2021) and 1-7 years with a mean age of 4.5±2.2 years (Mendoza-López *et al*., 2019).

Of the 42 cats in the present study, 39 (92.85%) were castrated (24/39 neutered, 15/39 spayed), corroborating with the literature about the gonadectomy as a risk factor for urolithiasis in felines (Lekcharoensuk *et al*., 2001a; Lekcharoensuk *et al*., 2000; Bartges, 2016).

Regarding food, 26 cats received dry food exclusively (61.9%), while 13 (38.1%) received both dry and wet food. Dry diet can be considered a risk factor for the disease (P= 0.0062, RR= 2.000, OR= 4.000), as reported in other papers (Lekcharoensuk *et al*., 2001b; Albasan *et al*., 2012; Dijcker *et al*., 2011).

Regarding housing, of the 42 animals, 30 cats (71.4%) remained indoor, four (9.52%) had access to the outside most of the time, while eight (19.08%) lived free. Animals that live indoors have a greater predisposition to the formation of uroliths, which is related to sedentary lifestyle and consequent obesity (Cannon *et al*., 2007; Lekcharoensuk *et al*., 2001a; Bartges, 2016). The body condition score (BCS) was rated from one to five, with BCS 2 found in five animals (11.9%), BCS 3 in 22 (52.4%), BCS 4 in 14 (33.3%) and BCS 5 in one animal (2.4%). BCS 3 was significantly more frequent (P<0.0001), therefore, for most of the cats in this study, obesity was not the main factor inducing urolithiasis. Unlike our study, Balázs and Tibor (2015) showed 42.4% of the cats were overweight.

Of the 42 cases, in 33 (78.6%) uroliths were removed from the bladder, in five (11.9%) from the ureter, in three (7.1%) from the kidney and one (2.4%) from the urethra. Comparing the anatomical site in which the uroliths were removed, those found in the lower urinary tract, had a higher occurrence than those in the upper tract (P<0.0001; RR= 4.25; OR= 18.06), corroborating other studies (Cannon *et al*., 2007; Hesse *et al*., 2012; Houston and Moore, 2009; Gerber *et al*., 2016; Kopency *et al*., 2021). Table 3 shows the mineral types of the calculi, considering the anatomical site from which they were removed.

Table 3. Frequency of the type of calculus considering the anatomical site of surgical removal

Site	Struvite	Calcium oxalate	Ammonium urate	Total
Bladder	16 (48.4%)	3 (9.0%)	14 (42.1%)	33 (100%)
Kidneys	0 (0.0%)	3 (100%)	0 (0.0%)	3 (100%)
Ureters	0 (0.0%)	5 (100%)	0 (0.0%)	5 (100%)
Urethra	0 (0.0%)	0 (0,0%)	1 (100%)	1 (100%)

When comparing the calculi found in the bladder, there was a significant difference (P=0.0013), however, when compared to the pairs, only struvite *versus* calcium oxalate urolithiasis was significant (P= 0.0008, RR= 5.333, OR= 9.412). Struvite uroliths were found predominantly in the lower urinary tract in 16 cats, as described in the literature (Cannon *et al*., 2007; Houston and Moore, 2009). All urate uroliths were removed from the lower urinary tract, as reported on another study (Lekcharoensuk *et al*., 2001a). In patients whose uroliths were in the kidneys and ureters the composition was of calcium oxalate, corroborating previously published reports (Osborne *et al*., 2009; Kyles *et al*., 2005). However, two additional studies have documented a significant number of struvite (40%) in the ureters (Gerber *et al*., 2016) and a significant portion of dried solidified blood calculi (DSB)-containing uroliths in the upper urinary tract in addition to calcium oxalate uroliths (Kopecny *et al*., 2021). For ureteroliths a significant difference was observed between the occurrence of calcium oxalate X struvite urolith and calcium oxalate X urate (P= 0.0079, RR= 0.2231, OR= 0.0082). It should be emphasized that animals with nephroliths and ureteroliths tend to be asymptomatic or present nonspecific signs, making diagnosis more difficult (Osborne *et al*., 2009; Kyles, *et al*. 2005).

Of the 42 patients in the present study, in nine (21.4%), urolithiasis was recurrent, with less than one year between the first and second episodes. The rate of recurrence was greater than that observed in another study, which was 8% (Picavet *et al*., 2007). In two cases, suture material was observed as the *nidus* of the calculus and may be the cause of recurrence (Ulrich *et al*., 2009; Appel *et al*., 2008). The two uroliths containing suture thread were composed of struvite and ammonium urate.

The clinical signs observed in the patients presented were significantly different (P=<0.0001). Of the 42 animals, 40 presented clinical signs, of which 26 presented dysuria (65.0%), 20 hematuria (50.0%), seven presented vomiting (17.5%), five anorexia (12.5%), three weight loss (7.5%) and two prostration (5.0%). Two patients were asymptomatic even with uroliths in the bladder and in these cases, the calculus was finding in an annual checkup. The clinical manifestations reported here are similar to those described in the literature (Gerber *et al*., 2005; Escolar and Bellanato, 2003; Grauer, 2015).

Physical examination revealed signs in 26 cats (61.9%). The most frequent were dehydration, observed in 24 patients (92.3%) and abdominal pain under palpation in 15 animals (57.7%). The authors believe that dehydration is related to abdominal discomfort and stress, resulting in lower water intake. Other signs consisted of renal pain, arrhythmia, pulmonary rales, lymphadenomegaly, murmur and hypocorous mucosa. Other diseases and clinicals alterations were discarded through specific exams when the responsible clinician deemed it necessary.

Urine tests were performed only in 30 patients. Of these, only seven (23.3%) cats presented crystalluria. The type of urolith and crystals observed are described in table 4. The low prevalence of crystalluria indicates that not all cats with calculus can develop crystalluria, and when it is present, crystals not always have the same composition of uroliths *In vitro* formation is also possible. These results are similar to those described by other authors (Dear *et al*., 2011; Lulich and Osborne, 2009; Albasan *et al*., 2003).

Table 4. Composition of urolith and type of crystal observed in the urine test in seven cats with uroliths that presented crystalluria

Cats with crystalluria	Type of urolith	Type of crystal
1	Urate	Urate
1	Urate	Oxalate
1	Struvite	Struvite
1	Struvite	Struvite and oxalate
1	Struvite and calcium oxalate	Urate
1	Struvite and calcium phosphate	Urate
1	Struvite, calcium oxalate and phosphate	Urate and oxalate

As for the cells present in the urinary sediment, 100% of the cats (30) presented hematuria; 76.7% (23) leukocyturia/ pyuria; 73.3% (22) epithelial cells and 66.7% (20) bacteriuria. This sediment pattern, with presence of inflammatory cells and bacteria is typical of bladder lithiasis (Lulich and Osborne, 2009; Albasan, *et al*., 2003).

The changes observed in the urinary sediment presented a significant difference (P= 0.0094). Hematuria was the most common finding when compared to leukocyturia (P= 0.0105; RR= 1.304; OR= 19.47), presence of epithelial cells (P=0.0046; RR= 1.364; OR= 23.04) and bacteriuria (P=0.0008; RR=1.500; OR=31.24). For the other comparisons, no significant difference was observed. The presence of bacteria and leukocytes in the urine in amounts greater than 5/field may indicate urinary tract infection. In felines the UTI may be a consequence of urolithiasis and not a primary cause, as observed in dogs (Cannon *et al*., 2007; Lekcharoensuk *et al*., 2001a; Grauer, 2015). Although the prevalence was high, no urinary culture was performed, so it is not possible to state that the infection induced urolithiasis, being more likely to have been a consequence of lithiasis.

The urinary pH was classified into three categories: less than 6.5 (acid), observed in 24 cats (80%), pH between 6.5 and 7.5 in five cats (16.7%) and pH>7.5 (alkaline) in one cat (3.3%), with a significant difference ($P<0.0001$). The occurrence of the type of urolithiasis and urinary pH no presented significance only in those cases with pH below 6.5, where urate uroliths were most observed. As most of the calculi were composed of struvite, more cats with alkaline pH would be expected (Lulich and Osborne, 2009). However, because there was a high amount of urate uroliths, urinary pH could be considered important to the formation of uroliths, since acid urine is a risk factor (Lekcharoensuk *et al*., 2001a; Appel *et al*., 2010; Dear *et al*., 2011).

Although the present study included a relatively limited number of patients, the results found differed from those obtained in international studies. Similar to the USA and Hungary, struvite submissions outnumbered calcium oxalate submissions (Balázs and Tibor, 2015; Minnesota Urolith Center, 2019) but in Canada, Mexico, Germany, Switzerland and the Benelux, oxalate predominated (Houston *et al*., 2016; Mendoza-López *et al*., 2019; Hesse *et al*., 2012; Gerber *et al*., 2016; Picavet *et al*., 2007). The reader is reminded that it is not possible to compare studies based on location alone-the period of investigation is critical. Why struvite predominated in this study and in the USA and Hungary, is surprising as struvite uroliths are generally sterile in cats and easy to medically manage. They often have a spherical shape on radiographs and if the attending veterinarian believes the uroliths to be struvite, dissolution with any number of veterinary therapeutic diets designed for dissolution, is generally successful within a 4-week period. The American College of Veterinary Internal Medicine published a consensus on uroliths in dogs and cats and recommended medical management of feline struvite urolithiasis (Lulich *et al*., 2016). Further studies to understand why cats are developing struvite uroliths and veterinary clinics fail to try dietary dissolution and elect to remove and submit struvite uroliths are warranted.

The high prevalence of urate uroliths observed in this study has no clear explanation. Some authors relate this type of calculus to food, liver, or portosystemic disease (Lekcharoensuk *et al*., 2001b; Appel *et al*., 2010; Dear *et al*., 2011). The portosystemic deviation or liver disease were not considered in these patients by the clinicals, though blood and imaging exams. This high incidence of urate urolithiasis reinforces the need for further studies examining not just dietary and geographical/environmental factors that may be influencing the development of urate uroliths in certain regions of Brazil, but also further evaluating the role of underlying liver disease (Appel *et al*., 2010; Dear *et al*., 2011), and the role genetics may play in their development (Bannasch and Henthorn, 2009). All this information is crucial to establish the most appropriate therapeutic and preventive measures, in order to reduce recurrence.

CONCLUSION

This study demonstrates and characterizes the occurrence of urolithiasis in cats from different regions of Brazil. Even though relatively frequent in feline clinical practice, some aspects of urolith development are not yet completely understood. The higher incidence of urate uroliths observed in this study when compared to similar studies in other regions emphasizes the need to gather regional data on urolith composition. A precise diagnosis of urolith composition is of outmost importance for the selection of the most appropriate therapeutics, aiming to achieve a successful treatment and avoid recurrences. This is one of the first studies of feline urolithiasis in Brazil. Future studies are needed to better understand the development of the disease in the species and the prevalence of different types of stones.

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