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Physicochemical techniques for determining the composition of canine and feline uroliths: a literature review

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ABSTRACT: *Urinary lithiasis has been reported as a problem that affects humankind since ancient times and has been described in several animal species. The condition is a consequence of other diseases that may be present in the urinary system or related to other body disorders. The stone composition needs to be analyzed to identify the possible causes that led to the formation and development of uroliths. For this, several techniques are currently available, some of which are promptly accessible, while others are not. Thus, this literature review aimed to perform a brief introduction on urolithiasis, present the most used techniques in the analysis of the composition of canine and feline uroliths and point out the advantages and disadvantages of each technique.*

Key words: *companion animals, crystallography, microscopy, spectroscopy, urolithiasis.*

Técnicas físico-químicas para determinação da composição de urólitos em caninos e felinos: uma revisão de literatura

RESUMO: *A litíase urinária foi reportada como um problema que atinge a humanidade desde a antiguidade e já foi descrita em diversas espécies de animais. A doença é considerada consequência de outras enfermidades que podem estar presentes no sistema urinário, ou relacionados com outros distúrbios do organismo. Para identificar as possíveis causas que levaram à formação e desenvolvimento de urólitos é importante analisar a composição dos cálculos. Para isso, existem várias técnicas disponíveis atualmente, algumas de mais fácil acesso e outras, nem tanto. Dessa forma, objetiva-se com a presente revisão realizar uma breve introdução sobre a urolitíase e apresentar as técnicas mais utilizadas na análise da composição de urólitos, em caninos e felinos, bem como apontar as vantagens e desvantagens de cada uma das técnicas.*

Palavras-chave: *animais de companhia, espectroscopia, cristalografia, microscopia, urolitíase.*

INTRODUCTION

Urolithiasis is a disease known to humankind since 8000 BC (DOMINGOS & SERRA, 2004). In the past, surgical removal of uroliths was the treatment performed in patients affected by lytic formations. The surgical procedure is only one of several alternatives for managing the disease with advances in research in this field. However, obtaining stones is not the final process concerning the disease, which goes far beyond its removal.

Urinary lithiasis is a multifactorial cause considered a consequence of other conditions. For this reason, identifying the causes is important to use the appropriate approach. Recognizing the possible causes that led the patient to develop stones is not always an easy process and hence determining the urolith composition facilitates the investigation process and assists in the adoption of preventive measures (KOEHLER et al., 2009; LULICH et al., 2016).

Several qualitative and quantitative analysis techniques can be used to determine stone composition. Quantitative techniques are preferable to those qualitative, as they allow the analysis of uroliths and indicate the concentration of each substance present in the sample (KOEHLER et al., 2009; KALINSKI et al., 2012; FIALOVÁ et al., 2017). Polarized light microscopy, X-ray diffraction, infrared spectroscopy, and energy dispersive spectroscopy are among the quantitative techniques (MARICKAR et al., 2009; OLDENBOURG, 2013; SHAMEMA et al., 2015). The techniques differ in terms of the equipment,

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sensitivity, and specificity, and their association is recommended (RACEK et al., 2019).

Stones are classified according to their composition, such as struvite, calcium oxalate, urate, calcium phosphate, xanthine, cystine, and silica. These components can be found in the various urolith layers, i.e., nucleus, stone, and wall. Determining the exact composition of each urolith layer is only possible when using quantitative techniques (KOEHLER et al., 2009).

Thus, this literature review aimed to present the most used techniques in the analysis of the composition of canine and feline uroliths, the ways of carrying out the analyses, and the advantages and disadvantages of each technique.

Urolithiasis

The term urolithiasis refers to the presence of uroliths in one of the portions of the urinary system (kidneys, ureters, bladder, and urethra). Uroliths, also known as calculi or stones, have already been reported in several animal species (ROBINSON et al., 2008), but more commonly observed in humans (SINGH & RAI, 2014), canines (ROE et al., 2012), and felines (HOUSTON et al., 2016). In humans, kidney stones are the most frequent (SINGH & RAI, 2014), while the bladder and urethra are the most observed sites in canines and felines (OSBORNE et al., 2009; BARTGES & CALLENS, 2015; HOUSTON et al., 2016).

Urolithiasis is considered a consequence of other diseases. Therefore, it is necessary to investigate the possible causes that led the individual to develop the stone, such as a low protein diet, anatomical changes, and metabolic disorders (BARTGES & CALLENS, 2015; LULICH et al., 2016). Also, there are the so-called risk factors, which enhance the chance of a patient developing the disease. Race, age, sex (OSBORNE et al., 2009; ROE et al., 2012; HOUSTON et al., 2016), body score, water intake, urination frequency (BARTGES & CALLENS, 2015; BARTGES, 2016), and even climate and geographic location (ÁNGEL-CARAZA et al., 2010; SHAMEMA et al., 2015) are among the risk factors.

Affected patients may be asymptomatic or manifest different clinical signs, such as dysuria, periuria, and hematuria. The severity and intensity of clinical signs vary according to the size, quantity, shape, and location of the stones (BARTGES & CALLENS, 2015; OKAFOR et al., 2013; BARTGES, 2016). Complementary exams such as abdominal radiography and ultrasound allow the visualization of uroliths in the urinary tract but do not determine their cause (OSBORNE et al., 2009; LANGSTON et al., 2008). Thus, clinical or surgical treatment should not be considered the final outcome of the disease (LULICH et al., 2016; KALINSKI et al., 2012; JOSHI et al., 2014; BARTGES & CALLENS, 2015).

Urolith is defined as a solid concretion composed of crystalloid material and organic matrix (LULICH et al., 2011). Its is classified according to the mineral in its composition, which can be struvite (magnesium ammonium phosphate or triple phosphate), calcium oxalate (monohydrate and dihydrate), urate (ammonium urate, uric acid, and sodium urate), cystine, xanthine, calcium phosphate (brushite, hydroxyapatite, and apatite carbonate), and silica (ÁNGEL-CARAZA et al., 2010; ROGERS et al., 2011; HOUSTON et al., 2016). Some calculi may have drugs (fluoroquinolones, tetracyclines, and sulfonamides) in their composition (OSBORNE et al., 2008). Foreign bodies, such as suture threads, may also be present in the stone composition, which may be the formation nucleus (ULRICH et al., 2009; LULICH et al., 2011).

The components can be found in the various calculus layers. The first urolith layer to be formed, from the center to the edge, is called nucleus or nidus, but it is not always the geometric center of the calculus. The body is the second layer and has the largest portion. Finally, there is the wall region, which is the outer urolith layer and may show surface crystals, being an incomplete layer (KOEHLER et al., 2009; ULRICH et al., 2009; LULICH et al., 2011).

Each part of the urolith can contain different compounds. A urolith is classified as simple when it has more than 70% of a mineral in its composition, receiving the name of the element present in a higher amount. On the other hand, the urolith is classified as mixed when no layer is identified and none of its components reaches 70% of the total. Moreover, it is classified as composite when it has layers interspersed with different compositions (KOEHLER et al., 2009).

Confirmation of the composition is only possible through analytical techniques (ULRICH et al., 2009; KALINSKI et al., 2012), allowing the investigation of the underlying cause and the lithogenesis process (KALINSKI et al., 2012; LEE et al., 2012), in addition to the correct disease treatment (BASIRI et al., 2012; CLOUTIER et al., 2014; KOCADEMIR et al., 2016).

Analysis techniques

The chemical composition of uroliths was first reported in the late 18th century, and the most diverse substances present in lytic concretions can be currently identified with studies and development of techniques (BASIRI et al., 2012; LEE et al., 2012; CLOUTIER et al., 2014; JOSHI et al., 2014; KOCADEMIR et al., 2016).

Several methods are available for analysis of urinary stones, being classified into physical and chemical and qualitative or quantitative (KOEHLER et al., 2009; YAPANOGLU et al., 2010; PRIMIANO et al., 2015; SHAMEMA et al., 2015). Physical methods, quantitative or semi-quantitative, are the most suitable, as they allow evidencing the urolith layers and determining and quantifying the substances of each portion (LANGSTON et al., 2008; KOEHLER et al., 2009). Moreover, these methods allow the classification of the stones as simple, mixed, and composite (KOEHLER et al., 2009) and the differentiation of subdivisions in the mineral group (e.g., calcium oxalate monohydrate and dihydrate) (KASIDAS et al., 2004; SCHUBERT, 2006).

Qualitative chemical analysis

The qualitative chemical analysis technique is performed by several laboratories due to its ease, quickness, and low cost, being a routine analysis (KASIDAS et al., 2004; D'ALESSANDRO et al., 2017). No special instruments are required for this technique (HESSE et al., 2005; SCHUBERT, 2006; PRIMIANO et al., 2015; SIENER et al., 2016; RACEK et al., 2019). The method is based on colorimetric tests that identify individual chemical components, such as inorganic cations and anions and organic components (KASIDAS et al., 2004; KOEHLER et al., 2009; RACEK et al., 2019). The sample is pulverized and a certain amount of drops of specific reagents is added to it (CLOUTIER et al., 2014), leading to color changes (KOEHLER et al., 2009) and the formation of bubbles and/or precipitate (PRIMIANO et al., 2015) in the presence of certain components, such as ammonium, carbonate, calcium, cystine, phosphate, magnesium, oxalate, and urate (MOORE, 2007).

This technique does not allow the identification of layers nor the presence of different constituents per layer because the sample is pulverized (KOEHLER et al., 2009; CLOUTIER et al., 2014). It is also not possible to quantify the percentage of components (CLOUTIER et al., 2014; RACEK et al., 2019), making it difficult to classify the sample as simple, mixed, or composite (CLOUTIER et al., 2014; KOEHLER et al., 2009). The reagents used in this type of analysis do not detect substances in amounts lower than 20% nor components such as silica, xanthine, and drugs (ULRICH et al., 1996; MOORE, 2007; KOEHLER et al., 2009; RACEK et al., 2019). Another limitation for performing this technique is the need for at least 50 mg of material, thus creating problems in the processing of very small stones (KASIDAS et al., 2004).

The method is also little sensitive in detecting calcium and oxalate (KANCHANA et al., 2009; SHARMA et al., 2014), and the technique can present false-positive and false-negative results (KASIDAS et al., 2004; BASIRI et al., 2012) and worse performance in determining the composition of mixed calculi compared to single component calculi (KASIDAS et al., 2004). The evaluation of calculi in children using chemical analysis showed the presence of calcium oxalate associated with cystine, but the infrared spectroscopy technique showed the presence of only cystine, indicating a false-positive result for calcium oxalate (D'ALESSANDRO et al., 2017).

The detection rate of cystine uroliths decreases from 70% to 58% when associated with phosphate (KASIDAS et al., 2004). The chemical analysis of a study carried out with uroliths of human patients showed an error rate of 94% (HESSE et al., 2005) and from 80% to 100%, depending on the substance (SIENER et al., 2016).

The chemical analysis carried out in a study with 55 canine uroliths showed an error in determining the composition of five uroliths, as silica was not detected (ARIZA, 2014). However, the chemical analysis was important to differentiate organic calculi (xanthine and urate).

Quantitative or semi-quantitative analysis

Polarized light microscopy

The polarized light microscopy (PLM) technique is widely used for identifying the composition of urinary stones (RACEK et al., 2019). A polarization microscope, which is based on the interaction of polarized light with the sample crystals, is used in this technique (KASIDAS et al., 2004; SCHUBERT, 2006). The crystalline components of uroliths are identified according to the different optical properties of minerals (KASIDAS et al., 2004; OLDENBOURG, 2013; RACEK et al., 2019).

The polarization microscope differs from a standard transillumination microscope by the addition of a polarizer before the condenser (OLDENBOURG, 2013). Optical properties, such as color, crystal shape, and birefringence, are parameters compared to known standards used to determine the stone composition (SCHUBERT, 2006; BASIRI et al., 2012; OLDENBOURG, 2013).

The analysis through the PLM technique requires the calculus to be sectioned to visualize its internal structure (KASIDAS et al., 2004). After identifying the layers, the material is taken from several points and immersed in a liquid with a known refractive index (AGUILAR-RUIZ et al., 2012) and, subsequently, the identification is carried out using a polarization microscope (KASIDAS et al., 2004; MOORE, 2007; KOEHLER et al., 2009). The difference in the refraction of crystalline components determines the composition and approximate proportion of components per layer, thus providing a quick result (AGUILAR-RUIZ et al., 2012).

Costs, speed of the exam, and the possibility of analyzing small samples are advantages of the technique (RACEK et al., 2019). It also allows the differentiation of the degree of hydration of calcium oxalate uroliths (dihydrate and monohydrate) and the detection of components present at very small quantities (SCHUBERT, 2006).

Among the disadvantages are the difficulty of quantitative analysis in mixed uroliths and the differentiation of calculi derived from purine (such as urate and xanthine) (SCHUBERT, 2006) and formed by drugs (OSBORNE et al., 2008; PRIMIANO et al., 2015). Calcium oxalate monohydrate and calcium carbonate crystals have a similar aspect, which can make it difficult to distinguish between the two, being indicated the association of another technique (HAWKINS et al., 2009).

Its limitations are determined by the optical properties of the material, i.e., fine or amorphous materials cannot be analyzed and smaller mixtures can be neglected. Semi-quantitative evaluation is possible only by subjective evaluation. The standardization of this method is problematic and prone to major subjective error. For this reason, it must be used in association with another method of analysis (RACEK et al., 2019).

X-ray diffraction

This technique consists of analyzing the X-ray diffraction of the sample components (BLASCHKO et al., 2013). The crystalline material upon receiving the X-rays spreads them in a unique diffraction pattern depending on the atomic arrangement of its structure, which is registered, allowing comparison with known reference standards to identify the substances (KUMAR et al., 2006; BUNACIU et al., 2015).

Monochromatic X-rays penetrate the sample and are reflected in characteristic patterns (KASIDAS et al., 2004; SCHUBERT, 2006), such as a “fingerprint” (JOSHI et al., 2014; BUNACIU et al., 2015). The material to be analyzed must be crystalline and mixed amorphous substances are not detectable, which can cause problems when non-mineralogical components (proteins) are present in the sample (KASIDAS et al., 2004; PRIMIANO et al., 2015).

The technique provides information on the analysis of the crystalline phase and chemical characteristics (BHATT & PAUL, 2008; BUNACIU et al., 2015). X-ray diffraction (XRD) is one of the most suitable methods for identifying urolith composition because it has a high degree of reliability (KUMAR et al., 2006; SIENER et al., 2016), being fast and sensitive, and providing accurate information (KOCADEMIR et al., 2016).

The advantages of this method include easy preparation, automatic component measurement, automatic sample exchange, semi-automatic XRD evaluation (automatic search for pattern matching), exact differentiation of all crystalline components (SCHUBERT, 2006), and analysis of very small samples (YAPANOGLU et al., 2010). The disadvantages are high costs, detection of only crystalline components, and 30-minute time for sample evaluation (SCHUBERT, 2006; BLASCHKO et al., 2013). The presence of more than three components in the urolith reduces the effectiveness of the method (BLASCHKO et al., 2013).

A study compared the chemical analysis, infrared spectroscopy, and XRD, and the latter showed higher accuracy in identifying the types of urolithiasis and the subtypes of substances present in uroliths from human patients (SIENER et al., 2016). According to HESSE et al. (2005), the XRD method produced the most accurate results for determining the composition of mixed struvite, apatite, whewellite, and uric acid urinary stones. According to Schubert (2006), different types of phosphates (apatite and hydroxyapatite) were analyzed qualitatively and only XRD detected the two compounds in 100% of the cases.

Infrared spectroscopy

Infrared spectroscopy is one of the most used spectroscopy techniques. The method involves the use of a spectrophotometer that has an infrared light spectrum that interacts and identifies the sample molecules. The light simulates atomic vibrations and, consequently, energy absorption occurs, resulting in absorption or transmittance waves (KASIDAS et al., 2004; SCHUBERT, 2006; BASIRI et al., 2012).

The technique is based on unique wave patterns generated when infrared waves encounter sample molecules. Some waves are absorbed (absorbance) and others cross the sample (transmission), forming a spectrum (SCHUBERT, 2006). The resulting spectrum is such as a molecular “fingerprint” of the sample (KOEHLER et al., 2009; KASIDAS et al., 2004), as no molecular structure produces the same infrared spectrum (BHATT & PAUL, 2008; SELVARAJU et al., 2015). They are compared to known reference spectra for identification (KOEHLER et al., 2009; BASIRI et al., 2012).

A vibrational molecular stretching and flexion of groups of covalently bonded atoms occur in response to excitation at specific wavelengths in samples exposed to infrared radiation (KASIDAS et al., 2004). Quantitative estimates can be obtained because the band intensities are proportional to the compound concentration (PRIMIANO et al., 2015).

Infrared spectroscopy is the most widely used quantitative method in laboratories that perform urolith analysis around the world (BLASCHKO, et al., 2013; SELVARAJU et al., 2015) because the result is relatively fast, specific (KASIDAS et al., 2004; KOCADEMIR et al., 2016), and requires a small amount of sample (KOCADEMIR et al., 2016; D’ALESSANDRO et al., 2017). It is a relatively low-cost technique when considering the value of the equipment and analysis, especially when compared to the X-ray diffraction and scanning electron microscopy with energy-dispersive spectroscopy (BHATT & PAUL, 2008; BASIRI et al., 2012).

This method is useful in the characterization of organic and inorganic matter and drug metabolites (KASIDAS et al., 2004; OSBORNE et al., 2008; D’ALESSANDRO et al., 2017). It also identifies crystalline and amorphous components, such as proteins and fat (HESSE et al., 2005; SIENER et al., 2016), determining the quantities of the different substances in the sample (MOORE, 2007; KOEHLER et al., 2009).

The method also differentiates calcium oxalate monohydrate and dihydrate and identifies the types of calcium phosphate (MOORE, 2007). Despite the high sensitivity of the method, substances such as phosphates and silica are difficult to detect by infrared spectroscopy if calcium carbonate is present. It occurs due to the high absorption of infrared waves by carbonate, which can overlap and prevent the analysis of low constituents. The efficient analysis of calculi with these compositions can associate infrared spectroscopy with energy-dispersive spectroscopy (DIAZ-ESPIÑEIRA et al., 1997). Calcium oxalate monohydrate at low concentrations can be lost in the presence of uric acid due to the superposition of peaks, which does not occur in X-ray diffraction (SIENER et al., 2016).

The Fourier transform infrared spectroscopy (FT-IR) is the most widely used infrared technique (SELVARAJU et al., 2015). The FT-IR technique simultaneously evaluates all frequencies in the infrared spectrum using mobile and fixed mirror that speeds up the sample analysis, which is an advantage compared to the traditional method. The analysis is carried out by identifying the urolith layers and collecting the material (powder) from each layer, being subsequently analyzed by the equipment after pelletizing in tablets containing potassium bromide (SELVARAJU et al., 2015; RACEK et al., 2019).

The disadvantages of infrared spectroscopy techniques include the delay in sample preparation and difficulty in differentiating purine uroliths (SCHUBERT, 2006). Although this method allows for semi-quantitative evaluation of all components present and apparently provides valuable information on the stone composition, it requires special instrumentation (RACEK et al., 2019). Knowing the spectrum of the largest possible number of pure substances is important for the proper composition determination for comparison purposes (CLOUTIER et al., 2014). Also, obtaining a complete database is important for a comparison of the spectra (PRIMIANO et al., 2015).

The composition of a human urinary stone was determined by PLM as being a urate urolith, but the infrared spectroscopy identified it as 2,8-dihydroxyadiene, a purine metabolite (KESHAVARZI et al., 2016). The infrared spectroscopy error rate reached 20% while that of XRD reached 30% (HESSE et al., 2005). On the other hand, another study found an infrared spectroscopy error ranging from 4% to 85%, while the XRD technique showed a lower error rate (38%) depending on the substance (SIENER et al., 2016).

Energy-dispersive spectroscopy

Energy-dispersive spectroscopy (EDS) is a technique used for elementary analysis and determination of the chemical composition of samples (LEE et al., 2012), consisting of analyzing the energy of photons generated from the dispersion of electrons in the sample to be analyzed (KALINSKI et al., 2012). The EDS equipment is usually coupled to a scanning electron microscope (SEM), which also allows the analysis of the physical characteristics of a sample (KALINSKI et al., 2012).

The scanning electron microscope uses electrons instead of light to form the image (MARICKAR et al., 2009), which is exposed to an electron beam and emits signals in the form of secondary electrons, backscattered electrons, characteristic X-rays, and Auger electrons, among others, being read by specific detectors. The emitted signals allow obtaining a morphological analysis of the sample through the image (KALINSKI et al., 2012).

Images produced by SEM has a high resolution, which means that very small samples can be examined with high magnification, i.e., in the order of two to five nanometers. It has a great depth of field, allowing a large amount of the sample to be focused at a time (MARICKAR et al., 2009).

The atoms on the sample surface interact with the emission of an electron beam in the analysis by EDS. The electrons in the inner layers of the atoms are excited and ejected. Electrons from the outermost layers tend to fill the space left by the electrons in the inner layer. An electron passing from the outer layer to the innermost layer releases energy in the form of X-rays, which are captured by the equipment. The emitted radiation is characteristic of each of the sample elements (KALINSKI et al., 2012; LEE et al., 2012).

The information generated by the EDS analysis consists of a spectrum that shows peaks corresponding to the elements present in the examined area, thus revealing the stone composition (MARICKAR et al., 2009; KALINSKI et al., 2012). Therefore, EDS can provide a quick qualitative analysis of the elemental composition with a sampling depth of 1 to 2 µm (LEE et al., 2012). In addition, it allows evaluating the different calculus regions and obtaining the percentage of the different elements present in the sample to determine the composition of each layer (SCHUBERT, 2006; MARICKAR et al., 2009; KALINSKI et al., 2012).

Non-conductive energy samples, such as urinary stones, need to be coated with conductive substances, such as carbon, gold, silver, or platinum, in a process called metallization so that the analysis can be carried out (KASIDAS et al., 2004).

The detection of chemical elements using EDS is only possible for those with an atomic number between four (beryllium – Be) and 92 (uranium – U), but some authors have described that elements with an atomic number lower than 12 are difficult to be detected (KALINSKI et al., 2012; LEE et al., 2012). Another influencing factor involves the conditions of the sample surface, i.e., the more irregular, the lower the detection limit. Elemental distribution analysis is used to obtain the percentage of composition of all elements distributed in the sample, being significantly useful for the recognition of crystals not identified by ordinary light microscopy or SEM alone (MARICKAR et al., 2009).

The samples to be analyzed using SEM/EDS analysis undergo little interference in their physical and chemical structures, which makes the analysis repeatable and the use of another technique if necessary (KALINSKI et al., 2012). The analysis can be performed with very small samples (even those smaller than 3 mm in diameter) (RACEK et al, 2019). SEM allows the verification of the stone morphology and its structure on the surface and inside (KOCADEMIR et al., 2016).

Among the disadvantages, the method is considered expensive and complicated, in addition to not being used for routine analysis of stones (MARICKAR et al., 2009). Another disadvantage of this technique consists of the non-differentiation of organic compounds, such as urate and xanthine, nor the forms of calcium phosphate, such as brushite and apatite (SCHUBERT, 2006; MOORE, 2007).

The method has already been used by several authors in the analysis of urinary stones (ESCOLAR & BELLANATO, 2003; LEE et al., 2012; JOSHI et al., 2014). However, it is usually used as a complementary method (RACEK et al., 2019).

In a study with nephroliths from human patients, the EDS technique showed peaks in carbon, oxygen, and nitrogen in uric acid uroliths, while calcium oxalate uroliths showed peaks in calcium, carbon, and oxygen and struvite uroliths showed peaks in magnesium, phosphorus, oxygen, carbon, and nitrogen (LEE et al., 2012). A study with human urinary stones showed that the thermogravimetry (TG), FT-IR, and XRD techniques were effective in determining stone composition, but some elements were only identified by EDS (JOSHI et al., 2014).

In a study developed by SHAMEMA et al. (2015), the EDS method could detect zirconium and mercury in uroliths, but not detected by XRD and Fourier transform infrared spectroscopy. The presence of the substances was associated with groundwater intake.

A total of 55 canine urinary stones were analyzed by EDS, of which 77% were simple stones (32% of struvite, 29% of calcium oxalate, 7% of cystine, 5% of urate, and 6% of calcium phosphate, i.e., apatite and carbonate) and 23% were composite and mixed calculi (KALINSKI et al., 2012). Ariza (2014) used EDS to evaluate the composition of 55 uroliths obtained from canines. The technique allowed the identification of different mineral types and made it possible to classify the samples in simple and mixed according to the amount of each substance, in addition to differentiating the layers and substances present in each layer of the sample.

ESCOLAR & BELLANATO (2003) analyzed 34 feline uroliths through infrared spectroscopy and EDS. In this case, EDS showed the presence of calcium phosphate in the uroliths, whereas the infrared spectroscopy detected only struvite. The EDS coupled to SEM allows obtaining the maximum simultaneous information on the sample using a single instrument. Therefore, it represents a very effective method for characterizing kidney stones (RACEK et al, 2019).

CONCLUSION

Urolithiasis is considered a multifactorial disease and understanding the composition of uroliths to define the cause of their formation is essential, especially considering treatment and prevention measures. Therefore, the analysis of calculi should always be performed. For this, knowing the available techniques and their advantages and disadvantages is an important factor in choosing the method, in addition to understanding that sometimes it is necessary to associate techniques.

The selection of techniques must be based on availability, cost, and speed, but mainly on the reliability of the result. However, methodologies for quantitative analysis are better and more sensitive compared to qualitative analysis. In cases where there is no possibility of quantitative analysis, the qualitative one can be chosen, but it should not be the first choice.

Among the techniques, the use of infrared spectroscopy and EDS provides us with a good result, as this association allows the classification of the chemical compounds present in the sample and the EDS method allows observing its layers. The analysis methods can be performed in Brazil, at the Universidade Federal de Goiás, and in the United States, at the Minnesota Urolith Center of the University of Minnesota.

Moreover, none of the methods is 100% accurate and, therefore, may not be sufficient to provide information regarding the urolith composition. Thus, the association of two or more techniques is recommended, although it is not always possible in the clinical routine. Urinary lithiasis is an important disease that does not involve only the urinary tract. Therefore, greater attention to the possible causes of the disease is necessary. Also, carrying out studies involving analysis techniques and comparing them aiming at developing faster, more sensitive, and accessible ways to determine the composition of uroliths is important.

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AUTHORS' CONTRIBUTIONS

V.R.G. conducted the experiment, collect datas, analysed the results and wrote the manuscript, P.C.A., L.L.Q. collect and analysed datas, M.C.S.F. designed the research and revised manuscript.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflicts of interest. The founding sponsors had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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