UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

XANTHINURIA SECONDARY TO ALLOPURINOL THERAPY IN DOGS WITH CANINE LEISHMANIOSIS: CURRENT PERSPECTIVES OF THE IBERIAN VETERINARY COMMUNITY

LAURA CAPARICA FERREIRA DE JESUS

ORIENTADOR:
Doutor Rodolfo Assis Oliveira Leal

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XANTINÚRIA SECUNDÁRIA AO TRATAMENTO COM ALOPURINOL EM CÃES COM LEISHMANIOSE: PERSPETIVA DA COMUNIDADE MÉDICO VETERINÁRIA IBÉRICA

Resumo

A xantinúria é o maior efeito adverso urinário em cães com leishmaniose tratados com alopurinol. Apesar das medidas preventivas e de maneio serem essenciais aquando desta terapêutica, a informação atualizada acerca do maneio da xantinúria é escassa.

Este estudo visou investigar a abordagem médica da comunidade médico veterinária ibérica (IVC) na prevenção e maneio da xantinúria secundária ao tratamento com alopurinol na leishmaniose canina (CanLeish).

Foi realizado um estudo transversal que teve como base o desenvolvimento de um questionário online e anónimo o qual foi difundido nas redes sociais da IVC. Este questionário detalhou as características gerais dos inquiridos; os regimes de prescrição do alopurinol; a interrupção do alopurinol e os seus efeitos adversos; a deteção, complicações e diagnóstico da xantinúria, assim como as medidas preventivas e reativas da xantinúria.

Foram obtidas 230 respostas (131 de Portugal e 99 de Espanha). A maioria dos inquiridos segue as recomendações internacionais quando usa o alopurinol no tratamento da CanLeish. Um total de 54.6% destes afirmou já ter interrompido a terapêutica antes da sua duração ideal devido ao aparecimento de efeitos adversos além da xantinúria. Cerca de 71.6% dos inquiridos já detetou xantinúria, sendo o aparecimento de sinais clínicos urinários, a complicação mais comum. O método de diagnóstico mais usado para xantinúria é a urianálise. Considerando a prevenção da xantinúria, 75.1% dos clínicos informam os donos sobre a possibilidade de surgirem efeitos adversos associados ao alopurinol, mas apenas 28.4% consideram fazer a transição para uma dieta com baixo teor em purinas. A realização de urianálise e controlos imagiológicos é considerada por 71.2% e 31% dos inquiridos, respetivamente, para monitorizar o tratamento com alopurinol.

Após ser detetada xantinúria, a abordagem terapêutica dos inquiridos consiste na interrupção do alopurinol, na diminuição da sua dose, no aumento da frequência de administração ou na sua substituição. Cerca de 72.1% tomam outras medidas, destacando-se a transição para uma dieta com baixo teor de purinas. A frequência estimada de xantinúria na prática clínica diária foi considerada inferior a 25% por 91.7% dos veterinários.

Estes resultados revelam que a IVC está consciente da xantinúria como uma complicação comum do tratamento com alopurinol na CanLeish. Apesar das medidas preventivas serem por vezes negligenciadas, os Médicos Veterinários Ibéricos aparentam conhecer as diversas opções que podem ser usadas no maneio da xantinúria.

Palavras-chave: xantinúria; maneio; alopurinol; leishmaniose canina; questionário

XANTHINURIA SECONDARY TO ALLOPURINOL THERAPY IN DOGS WITH CANINE LEISHMANIOSIS: CURRENT PERSPECTIVES OF THE IBERIAN VETERINARY COMMUNITY

Abstract

Xanthinuria is the major urinary adverse effect in dogs with leishmaniosis under allopurinol therapy. Although preventive and management measures are essential for its treatment, updated information about xanthinuria management in clinical practice is lacking.

This study aimed to investigate the current medical approach of the Iberian Veterinary Community (IVC) on prevention and management of xanthinuria secondary to allopurinol therapy in canine leishmaniosis (CanLeish).

A cross-sectional study was conducted based on an online anonymous survey which was diffused via Iberian social network veterinary groups. This questionnaire detailed: general characteristics of the respondents; allopurinol prescription regimens; allopurinol withdrawal and adverse effects; xanthinuria detection, complications, and diagnosis; xanthinuria preventive and reactive measures.

A total of 230 answers were obtained from the IVC (131 from Portugal and 99 from Spain). Most clinicians follow international recommendations when using allopurinol in CanLeish therapies. A total of 54.6% of clinicians stated that they had stopped the therapy before its ideal duration due to the appearance of adverse effects other than xanthinuria. About 71.6% of clinicians have detected xanthinuria, being the appearance of urinary clinical signs, the most common complication detected. Urinalysis was the preferred diagnostic method to detect xanthinuria. Considering its prevention, 75.1% of clinicians inform owners about possible adverse effects of allopurinol therapies, although only 28.4% consider an appropriate dietary change to a low purine diet. Urinalysis and imaging controls are used by 71.2% and 31% of clinicians, respectively, to monitor allopurinol therapies.

When facing xanthinuria, measures concerning allopurinol therapy are considered, such as discontinuing it, reducing its dosage, increasing its administration frequency, or replacing it. Also, 72.1% of clinicians take other measures, with emphasis on the transition to a low-purine diet. Finally, the estimated frequency of xanthinuria in their daily practice was considered less than 25%, by 91.7% of veterinary surgeons.

These findings show that the IVC is aware that xanthinuria is a common complication in CanLeish allopurinol therapies. Although preventive measures are often neglected, clinicians seem to be conscious about the different options that can be used to manage xanthinuria.

Keywords: xanthinuria; management; allopurinol; canine leishmaniosis; survey

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List of abbreviations

AHCC - active hexose correlated compound
ALT - alanine aminotransferase
AST - aspartate aminotransferase
BICU - Biological Isolation and Containment Unit
BID - every 12 hours (twice daily)
CT - computed tomography
CanLeish - Canine Leishmaniosis
ELISA - enzyme-linked immunosorbent assay
FMV-UL - Faculty of Veterinary Medicine, University of Lisbon
HEV - FMV - Veterinary Teaching Hospital of the Faculty of Veterinary Medicine, University of Lisbon
IFAT - immunofluorescent antibody test
IVC - Iberian Veterinary Community
mg/kg - milligram per kilogram
MUC - Minnesota Urolith Center
PCR - polymerase chain reaction
SID - every 24 hours (once daily)
TID - every 8 hours (three times daily)
USG - urinary specific gravity

List of symbols

% - percentage

n - size of the sample

® - registered trademark

± - plus-minus

= - equal to

> - superior to

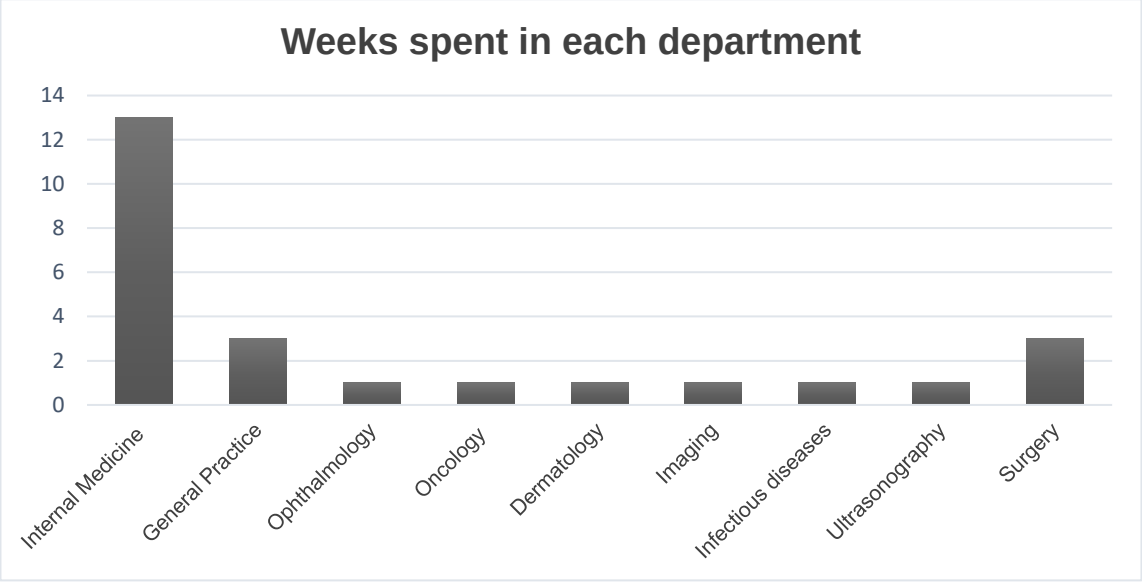
< - inferior to

1. TRAINEESHIP REPORT

This report concerns the hours spent and activities developed in the curricular internship that took place at the Veterinary Teaching Hospital of the Faculty of Veterinary Medicine – The University of Lisbon (HEV – FMV), from 7th September 2020 to 28th February 2021. The internship was based in rotations across the multiple departments of the hospital such as internal medicine, ophthalmology, imaging, surgery, dermatology, oncology, general medicine, inpatient care, surgery, ultrasonography, and infectious diseases, under the supervision of the hospital staff (veterinary surgeons and veterinary nurses). This internship also included weekly presentations on the internal medicine service book club and journal club.

The time spent in each department was variable and it went from one to thirteen weeks and the daily hours varied between 8 to 12 hours, including 12 hours night shifts twice a month. The total amount of weeks spent in each department during the internship can be consulted in Graphic 1. The total internship duration was 6 months (25 weeks), totalizing approximately 1144 hours.

Graphic 1. Total amount of weeks spent in each department during the internship.



Internal Medicine

This was the longest rotation, and it was under the supervision of Prof. Rodolfo Oliveira Leal, board-certified internist, and the supervisor of this thesis. This rotation started every day with our presence in the medical rounds focusing on the inpatient animals and included case discussions (diagnostic plan, complementary exams, surgery, treatments, etc.) from the staff of several departments. During the morning we attended first-opinion, re-evaluation, referral, and second opinion consults from various Internal Medicine subjects (mostly respiratory, gastrointestinal, nephrology, and endocrinology). The consults were performed by Professor

Rodolfo or Dr. Joana Dias (Internal Medicine Resident). We were able to collect clinical history and anamnesis, perform physical examinations, and collaborate in other medical procedures such as blood sampling, cystocentesis, and blood pressure evaluation. In the afternoon, we mainly discussed the morning cases detailing differential diagnosis, results, and treatment options. Once a week the student could also observe complementary exams such as rhinoscopies, bronchoscopies with bronchoalveolar lavages, upper and lower gastrointestinal endoscopies, and bone marrow biopsies.

General Practice

In this rotation, we did 8 hours morning or afternoon shifts over 3 weeks. During morning shifts, we attended the medical rounds of the inpatient animals, and then we proceed to attend first-opinion consults and emergencies. The student was able to perform the anamnesis, physical, neurological, and orthopedic exams and help in various medical procedures. During consultations, all doctors were open to discuss the cases. Some emergency procedures were also performed and discussed. Once a week, the rotation included 12 hours shifts at the inpatient care unit which was a great opportunity to learn even more, follow cases and perform different medical procedures than those performed during consults such as, fluid administrations and management, intravenous drug administrations, oxygen supplementation, urinary catheters placement, blood transfusions, and nutritional support.

Ophthalmology

This rotation had a one-week duration and included attending consultations and ophthalmology surgeries. During this period, the student had the opportunity to learn and perform the ophthalmologic exam under the supervision of the veterinary surgeon responsible for the service, and attend first-opinion, second-opinion, re-evaluation, and referral consultations. The student had the opportunity to discuss differential diagnoses, treatment options, and prognoses of different cases. Some specific medical procedures were also performed detailing Schirmer's test, fluorescein test, and fundoscopic exam. The follow-up of the surgery's patients was also conducted.

Oncology

This rotation had a one-week duration and included attending first-opinion, second-opinion, referral, and re-evaluation consults. Additionally, the student was able to attend and help with the chemotherapy sessions. During this period, the student could perform physical exams, collect anamnesis, discuss, and learn more about chemotherapy drugs, protocols, and side effects. The follow-up of these protocols and animals were also attended.

Dermatology

Over one week, the student attended the dermatology service and their first-, second-opinion, re-evaluation, and referral consultations. The shifts lasted 8 hours and included consultations, medical procedures such as video otoscopies, and communication of laboratory results to the owners. Some specific medical procedures such as skin biopsies, cytology, and fine needle aspirations (FNA) of cutaneous nodules/masses were also performed. The different cases and approaches were discussed, and the student was able to collect the clinical history and anamnesis, perform their physical exam, and participate in their treatment plans.

Imaging and Ultrasonography

For one week the student was able to attend the imaging department learning about radiography and computed tomography (CT) exams, and for another week, learning about the ultrasonography exams. During this period, the student was able to improve positioning skills and her interpretation of the different exams and respective differential diagnosis. The shifts lasted 8 hours (morning or afternoon). The student also assisted in specific medical procedures such as cerebrospinal fluid collection, ultrasound-guided percutaneous needle biopsies, cardiac and abdominal ultrasounds. Anesthesia of these animals was also induced and monitored by the student, under the supervision of a senior clinician.

Infectious Diseases

For one week, the student attended the Biological Isolation and Containment Unit (BICU) of the Veterinary Teaching Hospital. All these activities took place under supervision and included animal monitoring, medication preparation and administration, and correct hygienic procedures and equipment. Every day several infectious disease cases were discussed, and the most suitable treatment plans were decided.

Surgery

This rotation lasted 3 weeks and included 8 hours shifts of elective and emergency surgeries. The shift started with the animal admissions by the students and a pre-surgery checklist was filled with the owner's help. Later, all pre-surgical needed procedures were performed and supervised (peripheral venous catheterization, pre-anesthetic drugs preparation and administration, anesthetic induction, trichotomy, and surgical asepsis, animal intubation, monitoring, assistance in the surgical procedures, and post-surgical monitoring). During this rotation, the student learned and observed surgical techniques, increased their anesthetic monitoring skills, and discussed surgical cases, their post-surgical care, and prognosis.

Complementary Activities

Complementary to the internal medicine rotation, the student had a voluntary participation as a chaperone in the local organization of the board exams of the European College of Veterinary Internal Medicine and created a pamphlet for the service about Inhalation Therapy. This client sheet is now given to owners to help them knowing and using inhalation therapy in their animals (Annexe 1). The student also participated in the “Exposição de Caso Clínico – Jornadas de Medicina Veterinária” organized by AEICBAS, with a “Feline gastrointestinal eosinophilic sclerosing fibroplasia” case (Annexe 2) awarded with the first prize of the event and a submission of an abstract for ECVIM 2021 was also performed with this thesis theme (Annexe 3).

2. LITERATURE REVIEW

2.1. Canine Leishmaniosis

Canine Leishmaniosis (CanLeish) is a severe chronic systemic zoonotic disease caused by a protozoal parasite, *Leishmania Infantum*, affecting dogs, humans, and other mammals. Dogs are the main infection reservoir and are responsible for spreading the disease (Miró and López-Vélez 2018). This disease is endemic in many Southern European Countries, including Portugal and Spain, especially due to their environment which is prosperous for the vector (Paltrinieri et al. 2010).

CanLeish is a major public health concern because it is zoonotic, and its epidemiological risk is increasing due to the world climatic changes and global warming. In fact, most vector-borne diseases, like leishmaniosis, are closely associated with the environment where they grow (Alexandre-Pires et al. 2020; Gálvez et al. 2020). Due to these reasons, for instances, some alterations in the minimum and maximum temperature of the world, daily temperature mean, and environmental factors, are sufficient to increase this disease transmission and the host variability (Salomón et al. 2012; Miró and López-Vélez 2018). Even for humans, climatic changes are responsible for the proliferation of already endemic vector-borne diseases and the re-emerging of other ones, as vector control is getting harder (Kholoud et al. 2018; Miró and López-Vélez 2018).

This disease has a high prevalence of infection, especially in dogs from two to four years old and above 7 years old. However, it has a low rate of apparent clinical disease, highlighting the relevance of infected clinically healthy dogs. The different manifestations of CanLeish and their severity varies from dog to dog because dogs mount variable immune responses, leading to resistance or susceptibility (Baneth et al. 2008; Gallego et al. 2013).

2.1.1. Agent, Transmission, and Infection

The etiological agent of canine leishmaniosis in Europe is *L. Infantum*, and dogs are considered the major hosts and reservoirs of this parasite to humans. In dogs, an infection with *L. Infantum* can lead to clinical disease or subclinical infection, since not all infected dogs develop the disease (Baneth et al. 2008). Infected, clinically healthy, or sick dogs are a risk for humans and other mammals because, after all, they can transmit leishmania parasites to sand flies and contribute to the persistence of the parasite life cycle (Gallego et al. 2013; Maia and Cardoso 2015). The subclinical infection has a higher prevalence in endemic areas and, more than half of the infected dogs are apparently clinically healthy, contributing to a continuous infection of sand flies (Gallego et al. 2013; Miró and López-Vélez 2018).

The principal transmission route of *L. infantum* occurs by the hematophagous activity of infected female phlebotomine sand fly insects (the vectors of this protozoan), although

alternative routes of transmission have been reported, with a much lower prevalence. These include transmission in utero, via blood or sexual contact, and they should be considered even though they are less common. Sand flies usually have a seasonal pattern, increasing their activity occurs during spring to fall, associating the heat with high relative humidity and the absence of extreme weather conditions like rain or wind. Adult sand flies are mainly active in the early morning, in the evening and at night, making these the most propitious hours for the infection to occur (Killick-Kendrick 1999; Gallego et al. 2013; Maia and Cardoso 2015; Miró and López-Vélez 2018).

2.1.2. Clinical Signs

CanLeish shows as a chronic multisystemic disease with a very wide range of clinical signs from nonspecific ones to its absence, leading to the need for a thorough assessment including a complete medical history, a physical exam, a complete blood count and biochemistry profiles, and various diagnostic techniques to detect the parasite.

It is important to highlight that CanLeish clinical signs and their expression are dependent on multiple factors such as the parasite itself, the host immune status and response, and the host breed, which can lead to resistance or susceptibility in different dogs (Gallego et al. 2013; Miró and López-Vélez 2018; Ribeiro et al. 2018). The resistance or susceptibility status is not static, in fact, stressful situations, immunosuppressive treatments, or concomitant diseases can incite changes in the immune system and consequently have an impact on CanLeish progression (Gallego et al. 2013; Miró and López-Vélez 2018).

The wide spectrum of clinical signs that can be associated with this disease can be displayed with different intensities and they can affect a large variety of organs, tissues, or body fluids (Solano-Gallego et al. 2011). It is possible that many infected dogs do not show any signs in the first infection years while others can have an acute presentation of the clinical signs, leading to a fast progression of the disease and increasing its severity. Therefore, CanLeish can lead to clinical signs within 3 months to several years after infection and can even progress to cure (Alexandre-Pires et al. 2020).

Clinical manifestations can be vague, such as generalized lymphadenomegaly, weight loss, lethargy, splenomegaly, vomiting or diarrhea; cutaneous, such as onychogryphosis or different types of dermatitis; ocular, such as blepharitis or keratoconjunctivitis; other, such as epistaxis, mucocutaneous disease or lameness (Gallego et al. 2013; LeishVet 2018). Among these clinical manifestations, skin lesions are the most frequent ones and may be seen alone or in association with other clinical signs. Apart from skin, other manifestations can show up alone, like for instance, kidney disease which embraces glomerulonephritis, proteinuria,

azotemia, or even renal failure (Solano-Gallego et al. 2011; Ribeiro et al. 2018; Roura et al. 2020).

2.1.3. Diagnosis

As mentioned before, the diagnosis of canine leishmaniosis is complex and can even be more complicated in cases of subclinical infection (which are more frequent than those with clinical infection itself) or when dogs are vaccinated against CanLeish as some of the vaccines available can promote seroconversion which is detected by conventional serological diagnostic tests (Solano-Gallego et al. 2017). Furthermore, the clinical approach should be adapted to each dog and its clinical manifestations so that the diagnosis can be made faster, especially because CanLeish can be associated with other concomitant diseases or other vector-borne diseases (Solano-Gallego et al. 2011).

The diagnosis is necessary in two different situations: in dogs with the presence of clinical signs or clinicopathologic abnormalities consistent with the disease or in clinically healthy dogs screened before vaccination or the onset immunosuppressive treatments (Gallego et al. 2013).

The diagnostic methods for the detection of *Leishmania* spp. are divided into parasitological, molecular, and serological. The parasitological method consists on the observation of amastigotes and can be made by cytology, histology, immunohistochemistry, or culture; the molecular method consists on the identification of the parasitic DNA and can be made by conventional, nested, or real-time polymerase chain reaction (PCR); the serological method relies on the detection of specific antibodies against *Leishmania* spp. and can be quantitative (immunofluorescent antibody test - IFAT and enzyme-linked immunosorbent assay - ELISA) or qualitative (rapid tests – immunochromatographic assays). The most sensitive technique is the real-time PCR and nowadays, it is part of the veterinary diagnostic routine. The first-choice samples for this technique include bone marrow, lymph nodes, spleen, skin, or conjunctival swabs (Solano-Gallego et al. 2017; Ribeiro et al. 2018; LeishVet 2018).

Based on the clinical signs, the LeishVet group (2018) created a guideline system that classifies the CanLeish into four stages (based on the physical exam, clinicopathologic abnormalities, and serology), helping set the prognosis, follow-ups, and therapies for clinically ill dogs. Table 1 summarizes the clinical-stage division suggested by LeishVet (2018) into stage I (mild disease), stage II (moderate disease), stage III (severe disease), and stage IV (very severe disease), according to the severity of the disease and their prognosis. Also, these four stages were decided to cover the wide clinical manifestations and the several degrees of severity found in this disease, suggesting the most useful therapy for each situation (Solano-Gallego et al. 2011).

Table 1. Summary of clinical-stage division considering the severity of the disease and prognosis. Adapted from LeishVet (2018).

Clinical Stage	Serology	Clinical Signs	Prognosis
Stage I	Negative to low positive antibody levels	Mild clinical signs as solitary lymphadenomegaly or papular dermatitis	Good
Stage II	Low to high positive antibody levels	Clinical signs of stage I. Other clinical signs such as diffuse cutaneous lesions, ulcerations, generalized lymphadenomegaly, loss of appetite, or weight loss	Good to guarded
Stage III	Medium to high positive antibody levels	Clinical signs of stage II. Other clinical signs such as signs originating from immune-complex lesions	Guarded to poor
Stage IV	Medium to high positive antibody levels	Clinical signs of stage III. End-stage renal disease, nephrotic syndrome, or pulmonary thromboembolism may be present	Poor

2.1.4. Treatment and Therapeutics

When treating CanLeish, performing a thorough clinical evaluation is essential to establish a classification of the disease, and therefore, achieve the adequate treatment and establish a prognosis (Gallego et al. 2013).

The treatment goal is to improve the dog's quality of life, increase their life expectancy, and achieve the clinical cure. Trying to achieve the parasitological cure can also be a treatment goal, even though it is rare, and relapses are frequent. Also, it is expected that the treatment helps to reduce the parasite load, reducing the infectiousness to sand fly vectors, and prevent clinical recurrences. Therefore, the treatment is essential in dogs showing clinical signs of the disease. On the other hand, clinically healthy dogs do not need immediate treatment and should be monitored for early detection of clinical signs of the disease, initiating treatment in case of worsening (Miró and López-Vélez 2018). The response to treatment usually occurs in the first weeks and it is dependent on several factors namely the owner's compliance, the initial clinicopathological status of the dog (more clinicopathological abnormalities imply a more difficult recovery), and their individual response (Ribeiro et al. 2018; Roura et al. 2020).

CanLeish treatment consists of individual or combined protocols with leishmanicidal or leishmanistatic drugs, the most common drugs used are meglumine antimoniate, miltefosine, allopurinol, and domperidone. Both meglumine antimoniate and miltefosine are recommended in combination with allopurinol. Domperidone can also be a therapeutic option, even though a less frequent one, as it is only an option for dogs in stage I or for prevention (LeishVet 2018). Depending on the clinical staging of the disease, different protocols are recommended by LeishVet group guidelines (2018). The most frequent protocol used is meglumine antimoniate associated with allopurinol and this combination is considered the most effective therapy

(Greene 2012; Torres et al. 2016; LeishVet 2018). The length of therapy depends on the severity of the disease, the dog's individual clinical response, and individual drug tolerance (absence of adverse effects that are associated and reported to each drug) (Ribeiro et al. 2018).

Recently, an alternative to allopurinol has been recommended for dogs who are affected by allopurinol's adverse effects or for those with allopurinol resistance leishmania isolates, mostly due to long-term therapies (Segarra et al. 2017). This alternative consists in the oral administration of nucleotides associated with an active hexose correlated compound (AHCC) - Impromune® - that is targeted to modulate the host immune response and has shown similar efficacy to allopurinol in 6-month oral treatments (Segarra et al. 2017).

Recent fields for *L. infantum* control have made significant advances and are divided into three major areas: chemotherapy, immunotherapy, and immunoprophylaxis. The chemotherapy includes the previously mentioned drugs meglumine antimoniate, miltefosine, and allopurinol, these can reduce the parasite load and can lead to remission, even though the success is not guaranteed. Immunotherapy is an extending area of research because *Leishmania* negatively modulates the canine immune system and immunotherapy has the potential to restore the host immune response; immunomodulators assessed to date include domperidone but other potential therapies are also being assessed. Immunoprophylaxis is used to stimulate an adequate immune response that can avoid the progression of disease after infections, this approach is considered the most essential control measure (Miró et al. 2017).

The vast majority of dogs presents clinical improvements over the first month of therapy. However, some dogs may need long-term therapies before clinical improvement or to get the disease under control (Solano-Gallego et al. 2011).

2.1.5. Monitoring and Prognosis

Dogs with canine leishmaniosis require close monitoring and regular clinical and laboratorial evaluation including: a complete physical exam and clinical history, complete blood count, biochemical profiles ± serum electrophoresis, and complete urinalysis ± urinary protein: creatinine ratio. Less regularly, quantitative serologies and real-time PCRs should also be performed. PCR first choice samples include bone marrow, lymph node, spleen, skin, and conjunctival swabs (LeishVet 2018). Antibody levels are useful for evaluation of the treatment response, and when these have a markedly increased, this should be interpreted as a disease relapse (LeishVet 2018). If the disease is severe, monitoring should be more frequent and should include tests for concomitant conditions. Special attention should be given to dogs with

kidney disease because proteinuria, azotemia, hypoalbuminemia, blood pressure, and hyperphosphatemia must be controlled (Roura et al. 2020).

Meticulous monitoring and treatment have the capacity to improve prognosis. This is getting better as new and more research has been conducted (Roura et al. 2013).

The individual dog's response to the treatment, the absence of more effective drugs, and the absence of better preventive and treatment options turns CanLeish control into a difficult task for veterinary practitioners. For this reason, the prognosis for this disease is variable; it can turn into a parasitological and clinical cure or it cannot be controlled at all, especially due to the progression of the disease and the worsening of organ lesions that can be incompatible with the dog's life or life quality. Therefore, the continuous research in prevention and treatment options is, more than ever, very important to stop or minimize the spreading of canine leishmaniosis and its effects in dogs (Maia and Cardoso 2015; Ribeiro et al. 2018).

2.2. Allopurinol Therapeutics

Allopurinol has become an essential therapeutic tool for the management of Canine Leishmaniosis. It is used around the world as a leishmanistatic drug being administered either alone or in association with other leishmanicidal drugs namely meglumine antimoniate (Solano-Gallego et al. 2009). Apart from CanLeish, it can also be used for the treatment and prevention of recurrent uric acid uroliths and hyperuricosuric calcium oxalate urolithiases (BSAVA 2017). In humans, it is widely used in the control and treatment of primary and secondary hyperuricemia, since the 1960s (Murrell and Rapeport 1986).

2.2.1. Mechanism of Action

Uric acid serum concentration is derived from two different sources, exogenously from food and endogenously from "de novo" purine synthesis which occurs in the liver and reutilizes guanine and hypoxanthine (Osborne et al. 2010).

Allopurinol (4-hydroxypyrazolo(3,4-d)pyrimidine) reduces uric acid synthesis without interfering in important anabolic pathways or normal regulatory functions. Consequently, it is a clinically safe and effective method to decrease uric acid formation (Elion et al. 1966; Elion 1978). It is converted by xanthine oxidase into oxypurinol, its major metabolite, in the liver. Both allopurinol and oxypurinol are structural analogues of hypoxanthine and xanthine, purine bases, and immediate precursors of uric acid. They bind in a competitive way to xanthine oxidase, inhibiting and decreasing the conversion of hypoxanthine into xanthine and xanthine into uric acid (Murrell and Rapeport 1986). This results in a reduction of the uric acid

concentrations and an increase in xanthine concentrations (Bartges et al. 1999). This action mechanism is synthesized in Figure 2.

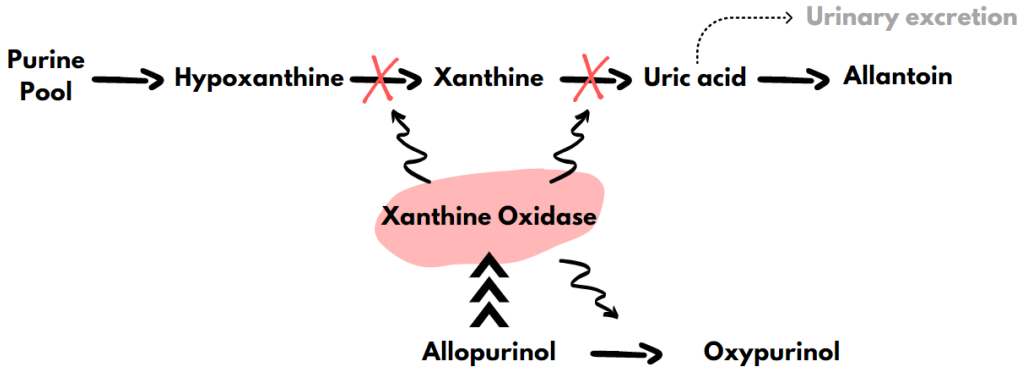


Figure 1. Purine Metabolism and Allopurinol action mechanism. Adapted from (Bartges et al. 1999).
Legend: single arrow – chemical conversion; triple arrowhead – site of action; curvy arrow – site of action; red cross – conversion inhibiting; dotted arrow – urinary excretion.

At the end of this cycle, excess uric acid is converted to allantoin via the hepatic uricase enzyme, the major metabolic end product of this cycle and the most soluble product of the purine metabolic products excreted in the urine. On the other hand, uric acid and xanthine are not highly soluble in urine (Osborne et al. 2010).

Dalmatian dogs have a unique metabolism of purines with different renal and hepatic pathways of uric acid. These dogs have a predisposition to urate uroliths formation because they convert uric acid into allantoin at a reduced rate when compared to other dogs. Moreover, their proximal renal tubules reabsorb less uric acid than other dogs. This results in Dalmatian dogs having a serum uric acid concentration two to four times higher than other dogs. The increased excretion of uric acid is considered to be a risk factor but not a primary cause for urate uroliths formation, meaning that this mechanism remains unclear (Bartges et al. 1999).

The success of allopurinol is mainly due to the oxypurinol properties as a xanthine-oxidase inhibitor, and its persistence in body fluids. The allopurinol capacity to inhibit endogenous purine oxidation leads to a reduction of uric acid levels in urine and serum, increasing the urinary excretion of xanthine and hypoxanthine that can both be accumulated when xanthine oxidase is inhibited. On the other hand, these can also be reutilized for nucleic acid synthesis when their oxidation is inhibited, reducing their excretion (Elion 1978).

Specifically, for its action as a leishmanistatic drug, allopurinol is metabolized by *Leishmania spp.* to produce an inactive analogue of inosine. This analogue leads to defect protein synthesis and inhibits the parasite multiplication, by being incorporated into the leishmanial RNA. Then, allopurinol interferes with purine and subsequent RNA synthesis (Marr and Berens 1977; Greene 2012).

2.2.2. Dosage and Length Recommended for CanLeish treatment

The gastrointestinal absorption of allopurinol occurs rapidly after ingestion, being distributed in tissues and extracellular fluid spaces. Allopurinol is cleared mainly by glomerular filtration and oxypurinol is reabsorbed in kidney tubules in a similar way to the uric acid reabsorption, which means that its elimination is dependent on the kidney function (Elion 1978; Bartges et al. 1999; Greene 2012).

The half-life of allopurinol is dose-dependent, being approximately 3 hours after the administration of a 10 mg/kg dose. Also, it can be administered with food because it does not affect its bioavailability (Bartges et al. 1999).

CanLeish treatment is always a clinical decision and veterinary surgeons should decide the best treatment for each case based on clinical signs, published scientific evidence, and owner factors (Roura et al. 2020). Despite this, the recommended dose of allopurinol (in association with another leishmanicidal drug) for sick dogs is 10 mg/kg, *per os*, every 12 hours, for at least 6 to 12 months (Solano-Gallego et al. 2009; Greene 2012; LeishVet 2018; Roura et al. 2020). However, its length depends on the severity of the disease, the response to the treatment, and the dog's individual response. The current suggestion is that the length of the treatment goes from 6 months to 12 months (Torres et al. 2016; LeishVet 2018). Some highly susceptible dogs are never stable enough to allow veterinary practitioners to stop the allopurinol therapy (Solano-Gallego et al. 2011). Allopurinol treatments can reduce disease relapse, decrease animal infectivity and maintain a reduced parasite load, slow the progression of the disease, avoid relapses, and increase the animal's survival time (Torres et al. 2011; Roura et al. 2013; Roura et al. 2020).

According to Solano-Gallego et al. (2011), some criteria can be used to support allopurinol discontinuation. Therefore, if the presence of a complete physical and clinicopathological recovery and a marked decrease in the dog’s antibody levels are observed, the therapy can be reduced or interrupted. On the other hand, if any allopurinol adverse effects are present, the therapy should also be discontinued or an attempt to control them can be made by reducing the drug dosage or with alternative strategies, this should be made if xanthinuria is present (Roura et al. 2013). An increased interval of administration or dosage adjustment can also be an alternative option if renal or hepatic dysfunction is present (Greene 2012).

As allopurinol was considered without adverse effects and the parasitological cure was not always achieved, in the past the treatment occurred for several years or even lifelong, increasing nowadays the probability to find adverse effects of this drug (Torres et al. 2016; Roura et al. 2020).

2.2.3. Adverse Effects and Therapy Monitoring

Allopurinol is considered a very safe drug. Its major adverse effect is xanthine urolithiasis or xanthinuria, especially in long-term therapies (Torres et al. 2011; Torres et al. 2016; Roura et al. 2020). Other described, but rare, adverse effects in dogs include vomiting and nausea, diarrhea, dermatologic eruptions (pruritus and rash), myelosuppression, cutaneous or non-cutaneous vasculitis, elevated liver enzymes, and hepatopathy (Bartges et al. 1999; Greene 2012; BSAVA 2017). Torres et al. (2016) also described that urinary effects as urolithiasis and renal mineralization can occur under allopurinol treatment and these are sufficient to justify frequent monitoring. Allopurinol resistance is also described as an adverse effect of long-term therapies (Roura et al. 2020).

Even though leishmaniosis by itself justifies close monitoring of all infected dogs, the chosen therapy also needs close monitoring because of its potential side effects, mainly in dogs with a high severity disease, including the association of renal and liver disease. This close monitoring is required mainly to reevaluate and optimize drugs and dosages necessary to the treatment (Roura et al. 2020). Additionally, any change in the disease progression can be rapidly detected and treatment adjusted (Roura et al. 2013).

2.2.4. *Leishmania Infantum* allopurinol resistance

Long-term therapies with allopurinol have been described and have resulted in the emergence of resistant *L. infantum* strains, which may magnify an uncontrolled transmission of the disease to humans and other dogs (Suganda et al. 2013). Increased efforts to collect information about this resistance in dogs and their association to disease relapses have been made (Yasur-Landau et al. 2016).

After finding an increased drug resistance in parasites isolated from dogs that have been already on CanLeish treatment, Yasur-Landau et al. (2016) hypothesized that resistance can develop over time under drug treatment selection pressure. Moreover, dissemination and inherent drug-resistant parasites may also be involved in this process. These observations confirmed clinical disease relapses associated with allopurinol resistant *L. infantum*. Later, Yasur-Landau et al. (2017), successfully induced this resistance in-vitro under drug pressure, proving the existence of a genetic basis in this resistance mechanism.

2.3. Xanthinuria

Xanthinuria consists in the urinary excretion of xanthine. Because xanthine is only slightly soluble in urine at any urinary pH, it can lead to and increases the risk of xanthine crystals formation, xanthine shells formation around other uroliths, or even xanthine urolithiasis. These can be responsible for severe urinary clinical consequences like urinary tract obstructions and

can severely impact kidney function (Bartges and Kirk 2008; Jacinto et al. 2013; Roura et al. 2020).

2.3.1. Prevalence

Xanthine crystals and xanthinuria are rare in dogs but their prevalence increases in dogs under allopurinol treatment (Bartges and Kirk 2008).

Torres et al. (2016) described a xanthinuria prevalence of 13% in a group of 320 dogs diagnosed with CanLeish and under allopurinol treatment. In these cases, xanthinuria appeared alone or in combination with other urinary tract conditions/ diseases, namely renal mineralization and urolithiasis.

According to Osborne, Lulich, Kruger, et al. (2009) as well as the sample of uroliths analyzed from 1981 to 2007 in the Minnesota Urolith Center (MUC), in 2007 only 0.1% of the uroliths had a xanthine nature, which means that they were composed by at least 70% xanthine. Almost all were obtained from dogs previously treated with allopurinol. Male dogs, especially castrated, were more often affected than females, and 40 different breeds were identified, including Dalmatians and Cavalier King Charles Spaniel (Osborne et al. 2010).

Since allopurinol is a first-line treatment option in CanLeish, it is expected that xanthinuria and xanthine uroliths prevalence increases due to allopurinol being considered without adverse effects for several years and their prolonged usage to treat this disease. On the other hand, veterinary practitioners are each day more aware of this problem and implement strategies to avoid these adverse effects (Torres et al. 2016; Roura et al. 2020).

2.3.2. Etiopathogenesis

As previously stated, xanthine is a product of the purine metabolism cycle. Therefore, xanthine uroliths are considered as one of the several existent purine uroliths. Xanthinuria and its consequences are one of the most serious adverse effects of allopurinol long-term treatments.

Hereditary or primary occurring xanthinuria is rare. It is mainly described in Cavalier King Charles Spaniel dogs, as a hereditary autosomal recessive disease. Similar to what is already described in humans, where it is a rare hereditary disorder, primary xanthinuria is suspected to be caused by a deficiency in the xanthine oxidase enzyme. This disease can be asymptomatic or can lead to urinary clinical signs and an increase in the xanthine urine concentration as well as the formation of xanthine calculi, due to their poor solubility (van Zuilen et al. 1997; Gow et al. 2011). Even though asymptomatic xanthinuria is described in this breed, Jacinto et al. (2013) could not detect this in a population of 35 Cavalier King Charles Spaniel in the United Kingdom, or an increase in the purine metabolites concentrations when

compared to a control group of 24 dogs. The other form of xanthinuria is also rare and occurs secondary to treatments with allopurinol because allopurinol inhibits the action mechanism of xanthine oxidase and therefore, the conversion of purines, like xanthine and hypoxanthine, to uric acid (Torres et al. 2016).

Regardless of the cause, for xanthinuria to develop it is necessary to have a decreased production of uric acid and an increased serum and urine production, and concentrations of purine metabolites like xanthine and hypoxanthine. For this reason, atypical xanthine quantities are excreted in the urine, urine becomes oversaturated and, because xanthine is poorly soluble, xanthinuria may urge associated with the formation of uroliths (Osborne, Lulich, Swanson, et al. 2009). This can happen due to the allopurinol action mechanism in the pathway of purine degradation, due to a high-purine diet or tissue catabolism (Torres et al. 2016). Even though xanthine urolithiasis is relatively rare, its prevalence arises in the presence of these factors single or combined, especially allopurinol long-term administration and higher dosages (Osborne, Lulich, Swanson, et al. 2009; Torres et al. 2016).

As mentioned above, the increase in the amount of xanthine present in the urine can also be a consequence of the consumption of high-purine diets or tissue catabolism, this occurs because both have the potential to increase the purine catabolism cycle and the amount of xanthine produced (Torres et al. 2016). Purines obtained from the diet can be digested and incorporated into the purine pool like endogenous purines (Osborne et al. 2010).

For xanthine uroliths to form it is necessary that hyperxanthinuria, the excretion of large quantities of xanthine crystals in the urine, and xanthine supersaturation occurs. Also, these uroliths occur because of their low solubility in the urine (Pais et al. 2006; Bartges and Kirk 2008; Jacinto et al. 2013; Torres et al. 2016). Xanthine calculi normally occur in acidic urine and their solubility is lower in acidic pH, which means that the urinary pH influences the xanthine uroliths formation (Pais et al. 2006).

2.3.3. Characteristics and Clinical Signs

Torres et al. (2016) described the urinary adverse effects in dogs under allopurinol treatment. The most frequent ones were xanthinuria, renal mineralization, and xanthine urolithiasis, which can occur isolated or associated among them. Renal mineralization or focal parenchymal calcification can occur due to the renal deposition of xanthine crystals and can lead to renal dysfunction. Other urinary clinical signs detected in association to xanthinuria included ureteral distension, pyelectasia, hydronephrosis, bladder rupture, bacteriuria, urinary obstruction or dysuria, although these are less frequent than those described above (Torres et al. 2016). Clinical signs may be absent until an obstruction of the urinary tract occurs, which can lead to damage into the kidney function or even death. In this study, adverse effects were

frequent in long-term therapies. Urolithiasis was recognized so earlier as after 1 month of treatment until 9 years of treatment. Xanthinuria was early detected up to 3 weeks post-treatment. The authors concluded that even though allopurinol adverse urinary effects are more frequent in long-term treatments, they can be detected also in short-term treatments (Roura et al. 2020).

Canine xanthine uroliths are rare and can be pure (the most frequent ones) or may contain other minerals, such as ammonium urate or sodium and calcium salts of uric acid (Osborne, Lulich, Swanson, et al. 2009). When uroliths are pure they usually have an ovoid and smooth structure and a yellow-brown color. Their diameter can go from 0.5mm to 1cm and their number in each patient can go from 1 to more than 100 with different sizes. Xanthine uroliths are more frequently removed from the lower urinary tract than from the upper urinary tract (Osborne, Lulich, Swanson, et al. 2009; Osborne et al. 2010).

2.3.4. Diagnosis

The diagnosis of xanthinuria can be made using diverse methods like urine analysis, abdominal radiographs, or abdominal ultrasonography. It is also important to consider a thorough physical exam with a complete background history. A complete blood count, biochemistry profile, and an electrolyte panel are essential to aid in the diagnosis and establish a treatment and control plan of an eventual xanthinuria.

Xanthine crystals can be found in a sediment analysis on a routine urine sample, but they are difficult to distinguish from ammonium urate crystals in light microscopy alone. They are usually yellow-brown and may present in the form of spherules of different sizes. Urine analysis can be useful if xanthinuria is suspected and also allows the monitoring of the urinary specific gravity (USG) and the urinary pH, which may increase the suspicion of xanthinuria (Osborne, Lulich, Swanson, et al. 2009).

Xanthine uroliths can be detected by non-contrast abdominal radiography but they need to have a detectable size (their size is variable and the bigger their size, the easier becomes to detect them). They are difficult to identify because they are radiolucent, which means this method has a poor diagnostic value (Osborne, Lulich, Swanson, et al. 2009; Torres et al. 2016). A double-contrast cystography or an abdominal ultrasonography can be performed in order to increase the probability to detect small xanthine uroliths. These are the recommended methods to monitor allopurinol therapies, especially the double-contrast cystography because it is minimally invasive, sedation may not be needed and allows to visualize all uroliths assessing their number, size, and shape. If possible, uroliths can even be removed through the urinary catheter (Osborne et al. 2010).

The definitive diagnosis requires a quantitative analysis of uroliths when they are removed from dogs. If they are small enough to pass the urethra lumen, they could be collected during the voiding phase of micturition, by aspiration using a urinary catheter or by voiding urohydropropulsion. A surgical approach may be needed to collect the uroliths or lithotripsy could be performed to reduce their size before extraction. After this, infrared spectroscopy which is based on unique wave patterns that are generated when infrared waves encounter a sample, can be used to confirm the uroliths nature after being compared to known reference spectra for identification, because the resulting spectrum represents a unique molecular fingerprint (Koehler et al. 2009). High-pressure liquid chromatography of the urine can also be performed to detect xanthine, hypoxanthine, or other purine metabolites because it is a valuable method to analyze purine compounds in urinary calculi (it is more sensitive and more specific for purines) (Safranow et al. 2000; Koehler et al. 2009; Osborne, Lulich, Swanson, et al. 2009; Torres et al. 2016).

The use of urine xanthine-to-creatinine ratios was investigated but not proven to correlate with 24 hours urine xanthine excretions, which means this ratio does not predict xanthine formation and it is not useful in the diagnosis or to control of the presence of xanthinuria (Osborne et al. 2010).

2.3.5. Surgical management

In the presence of xanthine uroliths, a surgical approach becomes essential because their formation is an irreversible process, especially if urinary clinical signs are present. In some dogs, immediate intervention is required (especially when obstruction and big-size uroliths are present) and in other cases, a medical approach is sufficient to control the clinical signs (Osborne et al. 2010). In most cases, combined surgical and medical management is the best option.

According to the data retrieved from Osborne, Lulich, Swanson, et al. (2009) at the Minnesota Urolith Center, the mean age of retrieval of xanthine uroliths in dogs was 5 years.

When surgery is needed, xanthine uroliths are usually present in the entire length of the urinary tract, especially in the lower urinary tract. They can be removed from the renal pelvis, ureter(s), bladder, or urethra by a minimally invasive surgery (via endoscopy - urethrocystoscopy) or not (Osborne, Lulich, Swanson, et al. 2009; Osborne et al. 2010). In some cases, when available, laser lithotripsy can be an option, depending on the location of the stones, because it is highly effective in removing these uroliths from the urethra or in reducing their size in order to make them removable during the voiding phase of micturition, by aspiration by a urinary catheter or by voiding urohydropropulsion (Osborne et al. 2010; Torres et al. 2016).

It is important to state that if additional measures are not taken, surgical management is not a definitive solution and recurrences of xanthine uroliths may occur, especially if xanthinuria remains present, because xanthine uroliths can continue to form and may continue to lead to urinary clinical signs.

2.3.6. Medical Management

There is no effective medical treatment for xanthine uroliths because this process is irreversible and after formation, their medical dissolution is not possible. However, a good chronic medical management can be enough to control the presence of xanthinuria, prevent xanthine uroliths formation or even avoid their adverse effects and consequences.

As previously state, the presence of xanthinuria is influenced by several factors including: allopurinol therapy length and dosage chosen; the consumption of high-purine diets; the rate of endogenous purine precursors production; the rate of purine degradation cycle; and a correct hepatic function because of its influence in the metabolization of allopurinol (Osborne, Lulich, Swanson, et al. 2009). To minimize or prevent the xanthinuria appearance, it is important to act in the possible areas such as the diet or the allopurinol therapy; however, it is also very important to have a close monitoring schedule.

Diet

If an allopurinol therapy is in course, the consumption of high-purine, purine supplemented or high-protein foods can lead to the formation of xanthinuria by increasing the number of available purines for the purine synthesis cycle (Bartges and Kirk 2008; Osborne et al. 2010; Lulich et al. 2016). If this is the case, appropriate caution is needed avoiding this type of foods. Therefore, it is important to stimulate the consumption of purine-restricted commercial veterinary therapeutic foods such as: Purina NF ®, Advance Leishmaniosis ®, Hill's K/D ®, Hill's U/D ®, or Royal Canin U/C Low Purine ®. These diets should be considered in order to minimize the probability of xanthinuria and xanthine uroliths formation. Homemade diets could also be an option if prescribed by a veterinary nutritionist according to each dog's needs. These options can be able to maintain urinary xanthine concentrations below their saturation point (Osborne et al. 2010; Torres et al. 2016; Roura et al. 2020).

If a homemade diet is chosen or preferred, many ingredients should be excluded or considered due to their purine concentration content. Some of these ingredients may be consulted in Table 2 (Osborne et al. 2010).

Table 2. Purine content of several ingredients that may be present in homemade diets. Adapted from Osborne et al. (2010).

PURINE CONCENTRATION	USAGE	FOODS
High purine concentration	Avoid	Anchovies, brain, clams, goose, gravies, heart, kidney, liver, meat extracts, mussels, oysters, salmon, sardines, scallops, shrimp, tuna
Moderate purine concentration	Moderate Use	Asparagus, cauliflower, fish, legumes, lentils, meats, mushrooms, spinach
Low purine concentration	Use	Bread, butter and fats, cheese, eggs, fruits and fruit juices, gelatin, milk, nuts, refined cereals, vegetable soups, water

Water Intake

Associated with the food itself, it is important to increase the dietary water intake, either by increasing the water consumption and availability (guaranteeing the water is fresh and clean to make it more desirable) or by offering moist foods. Even though it can be difficult for some dogs, increasing the water intake throughout the day is essential in order to potentiate the daily urinary volume, producing a more dilute urine, and decreasing the USG to less than 1.020, which leads to a reduced xanthine urinary concentration (Bartges and Kirk 2008; Osborne et al. 2010; Roura et al. 2020).

Urine Alkalinization

As previously stated, there is no effective medical option to dissolve xanthine uroliths. However, urine alkalinization is a common recommendation by several authors to control and prevent their formation because they normally occur in acidic urine and the urinary pH can influence the xanthine uroliths formation (Pais et al. 2006; Bartges and Kirk 2008). To produce more alkaline urine, non-acidifying diets could be selected and, if they are not enough, alkaline agents can be added to the diet (Osborne, Lulich, Swanson, et al. 2009).

Urine alkalinization only modestly increases xanthine solubility. Therefore, it should not be implemented alone in the xanthine uroliths prevention. Urine pH should be regularly monitored and maintained around 7 - 7,5 in order to increase xanthine solubility. The urinary pH manipulation is unlikely to lead to the dissolution of already existing xanthine uroliths and it is only a preventive measure since it has been already shown that xanthine uroliths have minimal dissolution in a physiologic pH range (Pais et al. 2006; Osborne et al. 2010).

If an appropriate diet is already in course without results in the urinary pH changes, an alkaline agent such as potassium citrate can be added to the therapy, particularly if the pH is constantly below 6,5-7. The starting dose is 75 mg/kg every 12 to 24 hours (Osborne, Lulich, Swanson, et al. 2009; Osborne et al. 2010).

Allopurinol Therapy

Nowadays, allopurinol is essential in CanLeish therapy. Since allopurinol is the main reason xanthinuria and xanthine uroliths occur, when they are present, this treatment should be reconsidered. Its withdrawal, reduction, or replacement are beneficial options for dogs with this problem (Osborne, Lulich, Swanson, et al. 2009; Osborne et al. 2010; Torres et al. 2016). When xanthinuria is identified, the therapeutic protocol should be individually adapted to each case and must take into account if the dog is clinically ill or healthy, or if there is an alternative that can be suited to the situation (such as the administration of AHCC associated to nucleotides, Impromune®).

Roura et al. (2020) suggested that in the presence of urinary problems, the allopurinol therapy should be reduced to 10 mg/kg every 24h or less, and other preventive measures like the previously mentioned should be taken in order to keep urinary xanthine concentration below its saturation point (increase water consumption and low-purine food selection). In some cases, as already mentioned, a replacement to AHCC can be considered.

2.3.7. Implications in the allopurinol treatment – prevention and monitoring

Dogs under allopurinol therapy, especially those with a history of xanthinuria or xanthine uroliths require close monitoring to prevent or detect early recurrences and to avoid the devastating consequences that xanthine uroliths can have in dogs.

Close monitoring is an essential tool in dogs before, under, and after allopurinol treatment so that clinical and laboratory changes can be detected early, and their progress can be prevented before severe adverse clinical signs occur. Urinalysis is essential at the beginning of the treatment and in every follow-up assessment because it can detect early changes in the urinary pH, USG, or proteinuria (Torres et al. 2011; Torres et al. 2016; Roura et al. 2020). A sediment analysis can also be useful if xanthinuria is suspected (Osborne, Lulich, Swanson, et al. 2009).

Ultrasonographic evaluation is also essential, mostly after the detection of xanthinuria, to evaluate structural abnormalities, renal mineralization, or the presence of uroliths along the urinary tract (Osborne et al. 2010; Torres et al. 2016). A double-contrast cystography can also be performed regularly instead of an ultrasonography because of the advantages earlier described (Osborne et al. 2010).

Meticulous and complete monitoring schedules for dogs under allopurinol treatment can improve disease prognosis and have the ability to prevent serious adverse effects before they are irreversible, therefore improving quality of life, delaying the progression of the disease, and increasing survival time (Torres et al. 2016; Roura et al. 2020).

3. XANTHINURIA SECONDARY TO ALLOPURINOL THERAPY IN DOGS WITH CANINE LEISHMANIOSIS: CURRENT PERSPECTIVES OF THE IBERIAN VETERINARY COMMUNITY

3.1. Introduction

CanLeish is a chronic systemic zoonotic disease, and it is endemic in many Mediterranean countries, like Portugal and Spain, especially due to their prosperous environment (Paltrinieri et al. 2010). It has become a major public health concern as their epidemiological risk has been increasing with the world climatic changes. In fact, these changes are reflected in the parasitic life cycle potentiating its impact as a zoonosis (Alexandre-Pires et al. 2020; Gálvez et al. 2020).

Allopurinol has become a crucial therapeutic approach in CanLeish cases (Solano-Gallego et al. 2009). As it has been indiscriminately used over several years and following distinct protocols, its adverse effects have been arising (Torres et al. 2016; Roura et al. 2020). Among them, xanthinuria is the most adverse side-effect and consists in the urinary excretion of xanthine, which can lead to severe urinary clinal consequences (Bartges and Kirk 2008; Jacinto et al. 2013; Roura et al. 2020). Concurrently, renal mineralization and xanthine urolithiasis can also occur (Torres et al. 2016). A previous study from Torres et al. (2016) showed a xanthinuria prevalence of 13% in a group of 320 dogs with CanLeish and under allopurinol treatment. Also, a previous study from the MUC in 2007 showed a prevalence of only 0.1% xanthine uroliths from the total of uroliths sent to the center, being almost all obtained from dogs treated with allopurinol (Osborne, Lulich, Kruger, et al. 2009). The real prevalence of xanthinuria in dogs under allopurinol treatment is not known, but increases in long-term therapeutic protocols (Bartges and Kirk 2008).

Close monitoring is an essential tool in dogs before, under, and after allopurinol treatment, providing early detection of any adverse effects and prevention of their progress. Since xanthine uroliths' formation is an irreversible process, their dissolution through medical management is not effective. However, a good chronic medical management can be sufficient to control or even avoid serious consequences (Torres et al. 2016; Roura et al. 2020). This chronic management includes several measures such as dietary changes, increases in the water intake, urine alkalinization measures, and changes in the allopurinol treatment itself (Bartges and Kirk 2008; Osborne, Lulich, Kruger, et al. 2009; Osborne et al. 2010; Torres et al. 2016).

Although several information is available concerning this topic, most of the literature was published a long time ago, lacking some recent updates. Also, since allopurinol has been used indiscriminately, an increase in adverse effects is expected and more information on how to deal with xanthinuria is necessary and may be useful for many veterinary surgeons.

Also, several recommendations have been made from various authors about the management of allopurinol treatment (Torres et al. 2016; Roura et al. 2020) and the control of xanthinuria. However, up to date, there is no standard protocol to follow, and little is known about how veterinarians react when facing allopurinol treatments and xanthinuria.

Survey-based studies have been frequently used to gather information from wide areas, as a rapid, cost-reduced, and accurate method that reflects the current procedures of the veterinary community facing a defined problem/ clinical condition. Notwithstanding the amount of information available concerning this subject, more information about the management of xanthinuria is important to help clinicians make decisions when managing CanLeish allopurinol treatments, whether the goal is to prevent xanthinuria or to control it.

3.2. Objectives

This study aimed to:

1) Assess the most common allopurinol treatment protocol used by these veterinarians and, in case of withdrawal, what are the reasons for its suspension.
2) Evaluate if these veterinarians have already detected xanthinuria and other associated adverse effects among their patients, and how their diagnosis was performed.
3) Investigate which are the current preventive measures that veterinarians usually take when initiating an allopurinol treatment as well as which monitoring schedules are used.
4) Explore the reactive measures they take when facing xanthinuria and the estimated xanthinuria frequency in their clinical practice.
5) Detail potential differences between Portuguese and Spanish community answers, considering xanthinuria management.

3.3. Materials and methods

3.3.1. Survey development and distribution

In order to collect knowledge about the Portuguese and Spanish veterinary community, a cross-sectional study was conducted in both countries, based on an online anonymous survey, developed using Google Forms® (Annexe 4), with a range of four to twenty-six possible answers, depending on the pathway of answers chosen by the veterinarian. This survey consisted of multiple-choice questions, except for one, which was a short answer. The multiple-choice questions were all closed-ended, but in 11 of them, by selecting the “other option” field, respondents could give a different answer from those enunciated among the listed option.

Due to the anonymous nature of the survey, and because it was web-based, only age and gender were collected in order to ensure privacy.

The content of the questionnaire was segmented into five sections, according to the main subject of the questions. The survey was developed in Portuguese and translated to Spanish being uploaded in the same way, with the same structure (Annexe 5). A translated English version of the questionnaire was also conducted and is available for consult (Annexe 6).

The first section focused on general information about the respondents and on confirmations of voluntarily and anonymously participation, allowing us to characterize the surveyed sample on age and gender.

The second section focused on the common allopurinol prescription regimens, considering dosage and length choices.

The third section consisted of information about the main causes that lead veterinary surgeons to withdrawal allopurinol treatments before its ideal time, including options like owner compliance, financial constraints, and adverse effects. The different adverse effects found by the respondents were also scrutinized.

The fourth section surveyed the respondents about xanthinuria detection in dogs under allopurinol treatment, detection of complications alongside xanthinuria, and diagnosis tools to detect xanthinuria.

Finally, in the fifth section, xanthinuria preventive measures were assessed including owner awareness about xanthinuria and their complications when initiating an allopurinol treatment, dietary changes, and complementary exams used to monitor patients and their frequency. Questions about measures taken after the detection of xanthinuria (reactive measures) were also included, detailing the approach of the allopurinol therapy, and other control measures. At last, the estimated frequency of xanthinuria in dogs under CanLeish treatment was assessed.

After validation by an epidemiologist, the questionnaire was initially distributed throughout the veterinary staff of HEV – FMV, over a period of two weeks, looking for internal validation and rectifications. After these rectifications were discussed and incorporated into the questionnaire, a final version was fulfilled and translated to Spanish. This final version was diffused in Portugal in a second phase, over 18 weeks, around the Portuguese veterinary community, via social network veterinary groups, and diffused in Spain, over 16 weeks, following the same network scheme.

3.3.2. Data processing and statistical analysis

All data were gathered using Google Forms® and downloaded to a database in Microsoft® Excel® 2019. Descriptive statistics were performed in the data using Microsoft® Excel® 2019.

All results were reported as absolute numbers and percentages. For statistical purposes, all choices given in the “Other option” field were considered as one to yield a single frequency.

In order to compare answers between the Spanish and Portuguese veterinary community, about xanthinuria management, Pearson's Chi-squared test was used. Fisher exact tests were used when the expected values were inferior to 5 and it was not possible to perform Pearson's chi-squared tests. Only the results that showed a statistical association were accounted for.

Pearson's Chi-squared tests and Fisher exact tests were implemented using the commercial statistical software IBM® SPSS® Statistics version 25. These tests presented a significance interval of 95%. The significance level was set at p-value <0.05, which means that when the p-value is inferior to 0.05 results are considered statistically significant.

3.4. Results

This questionnaire was completed by a total of 230 veterinary surgeons across the Iberian Veterinary Community (IVC), including 131 answers from Portugal and 99 answers from Spain. All the respondents confirmed that they were veterinary surgeons and their knowledge about the voluntary and anonymous nature of the survey.

3.4.1. General Characteristics of the Respondents

Considering sample characterization, information about gender and age was collected. Answers were obtained from 177 female veterinary surgeons (77%), 52 male veterinary surgeons (22.6%), and one veterinary surgeon who preferred not to answer (0.4%).

Considering the age of respondents, 102 veterinarians were 26 to 35 years old (44.4%), followed by 87 in the 36-45 age group (37.8%), 28 in the 46-55 age group (12.2%), 8 in the 56-65 age group (3.5%), 5 in the <25 age group (2.2%), and none in the >65 age group (0%). These results are summarized in Table 3.

Table 3. Respondent's gender and age group.

Gender	**N**	**%**
Female	177	76.96
Male	52	22.61
Prefer not to answer	1	0.43
Total Respondents	230	
Age Group	**N**	**%**
<25	5	2.17
26-35	102	44.35
36-45	87	37.83
46-55	28	12.17
56-65	8	3.48
>65	0	0
Total Respondents	230	

3.4.2. Allopurinol prescription regimens

From the 230 inquired veterinary surgeons, 229 use allopurinol in CanLeish treatment (99.6%).

Considering the dose chosen to approach this treatment, 162 veterinary surgeons selected 10mg/kg BID (70.7%), 22 selected 10-20mg/kg BID (9.6%), 17 selected 10mg/kg SID (7.4%), 15 selected <10mg/kg BID (6.6%), 9 selected 10-20mg/kg SID (3.9%), 2 selected <10mg/kg SID (0.9%), other 2 selected >20mg/kg SID (0.9%), and finally none selected >20mg/kg BID (0%). These results are summarized in Table 4.

Table 4. Allopurinol usage and dosage chosen for CanLeish treatments.

Usage of Allopurinol in CanLeish	N	%
Yes	229	99.57
Not	1	0.43
Total Respondents	230	
Dosage chosen for CanLeish treatments	**N**	**%**
<10 mg/kg BID	15	6.55
10 mg/kg BID	162	70.74
10-20 mg/kg BID	22	9.61
>20 mg/kg BID	0	0
<10 mg/kg SID	2	0.87
10 mg/kg SID	17	7.42
10-20 mg/kg SID	9	3.93
>20 mg/kg SID	2	0.87
Other	0	0
Total Respondents	229	

Among the 229 veterinarians using allopurinol and considering the mean treatment time for CanLeish, for 92 veterinary surgeons it was 4-6 months (40.2%), for 64 it was 1 year (28%), for 32 it was “other” time period (14%), for 26 it was until the end of the dog’s life (11.4%), for 6 it was over two years (2.6%), for 5 it was two years (2.2%) and for 4 it was 1-3 months (1.8%).

Considering therapeutic monitoring and reasons to reach the end of allopurinol treatments, 149 of the respondents choose the remission/ improvement of clinical signs and a serology’s reduction (65.1%), 34 do it because it is protocol and to avoid long-term adverse effects (14.9%), 22 choose “other option” (9.6%), 14 only take into account the remission/ improvement of clinical signs (6.1%), 7 only take into account the serology’s reduction (3.1%), and 3 do it because they do not see benefits in continuing the treatment after their habitual period (1.3%). These results are summarized in Table 5.

Table 5. Mean CanLeish treatment time and motives to end allopurinol treatments.

Mean CanLeish treatment time	N	%
1-3 months	4	1.75
4-6 months	92	40.17
1 year	64	27.95
2 years	5	2.18
Over 2 years	6	2.52
Until the end of dog's life	26	11.35
Other	32	13.97
Total Respondents	229	
Reasons to reach the end of allopurinol treatments	**N**	**%**
Remission/ improvement of clinical signs and a serology's reduction	149	65.07
Because it is protocol and to avoid long term adverse effects	34	14.85
Remission/ improvement of clinical signs only	14	6.11
Serology's reduction only	7	3.06
Do not see benefits in continuing the treatment after their habitual period	3	1.31
Other	22	9.61
Total Respondents	229	

3.4.3. Allopurinol withdrawal and adverse effects

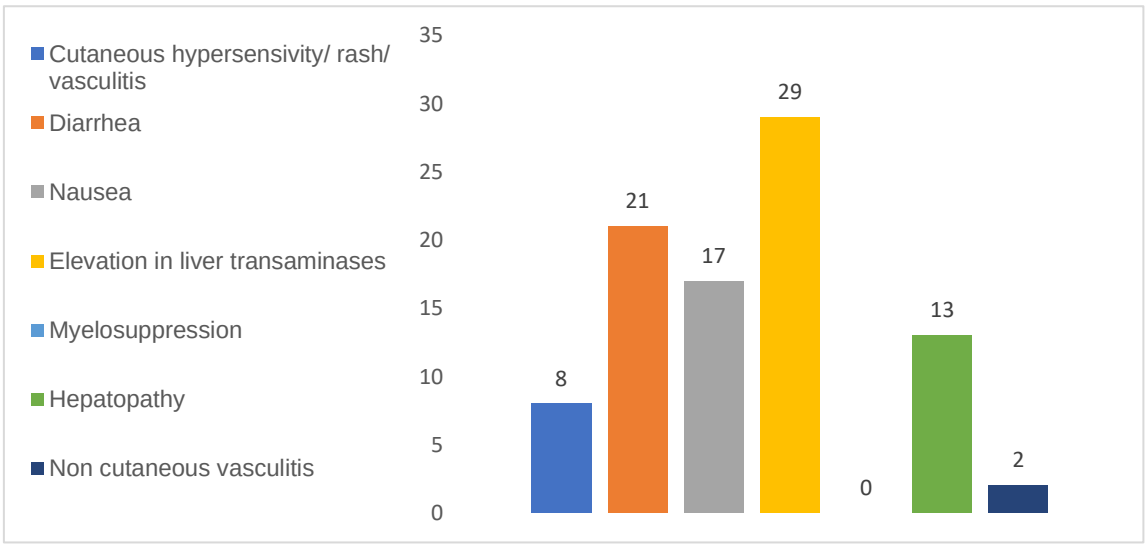
Among the respondents, 125 have already withdrawal allopurinol before the ideal treatment length (54.6%). When asked for the reason that motivated this interruption, 111 claimed adverse effects of the treatment (88.8%), 9 claimed difficulties in compliance (7.2%), 2 referred financial restrictions (1.6%) and 3 veterinary surgeons selected "other option" (2.4%), detailing: the death of a patient, the remission of the disease and the improvement of clinical signs and serology. These results are summarized in Table 6.

Table 6. Allopurinol withdrawal and reasons that motivated this interruption before the ideal treatment length.

Allopurinol's withdrawal before the ideal treatment length	N	%
Yes	125	54.59
Not	104	45.41
Total Respondents	229	
Reason that motivated this interruption	**N**	**%**
Adverse effects of the treatment	111	88.80
Difficulties in the compliance	9	7.20
Financial restrictions	2	1.60
Other	3	2.40
Total Respondents	125	

From those veterinary surgeons who claimed adverse effects, apart from xanthinuria, as a motive to withdrawal allopurinol, 29 of them reported an elevation in liver enzymes (alanine aminotransferase - ALT and aspartate aminotransferase - AST) (50.9%), 21 reported diarrhea (36.8%), 17 reported nausea (29.8%), 13 reported hepatopathy (22.8%), 8 reported cutaneous hypersensitivity/ cutaneous rash/ cutaneous vasculitis (14%), 2 reported non-cutaneous vasculitis (3.5%) and none reported myelosuppression (0%). These results are summarized in Graphic 2.

Graphic 2. Adverse effects that motivated an allopurinol withdrawal.



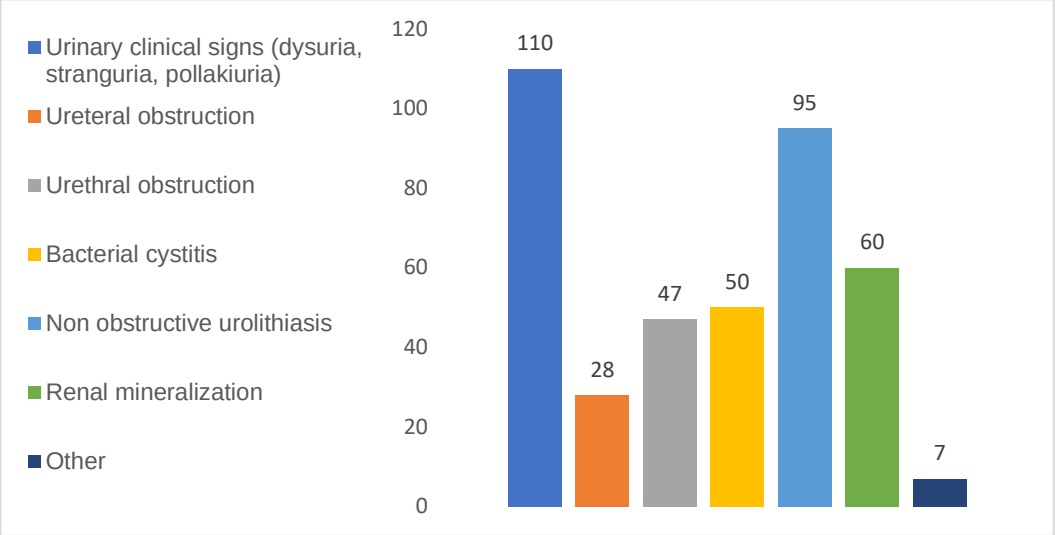
Respondents could select more than one option, therefore the sum of percentages of different options does not equal 100%.

3.4.4. Xanthinuria detection, complications, and diagnosis

Considering the detection of xanthinuria for at least one time in their daily practice, in dogs under allopurinol therapy, 164 veterinary surgeons have already detected it (71.6%).

Among the respondents, and considering the complications detected in association to xanthinuria in allopurinol treatments, 110 have already detected urinary clinical signs like dysuria, stranguria, and pollakiuria (68.8%), 95 have detected non-obstructive urolithiasis (59.4%), 60 have detected renal mineralization (37.5%), 50 have detected bacterial cystitis (31.3%), 47 have detected urethral obstruction (29.4%), 28 have detected ureteral obstruction (17.5%) and 7 (4.4%) have detected other complications doubtfully related to xanthinuria. These results are summarized in Graphic 3.

Graphic 3. Complications detected in association with xanthinuria in allopurinol treatments.



Respondents could select more than one option, therefore the sum of percentages of different options does not equal 100%.

Concerning the xanthinuria diagnosis, 129 assume diagnosing xanthinuria by identification of crystalluria (78.7%), 20 by post-removal urolith analysis (12.2%), 9 presume it when urolithiasis is observed at abdominal ultrasound (5.5%), 6 by other methods (3.7%) and none presume it when urolithiasis is observed at abdominal radiographs (0%). These results are summarized in Table 7.

Table 7. Methods chosen to diagnose xanthinuria.

Xanthinuria diagnosis	N	%
Identification of crystalluria	129	78.66
Post-removal urolith analysis	20	12.20
Presume it when urolithiasis is observed at abdominal ultrasound	9	5.49
Presume it when urolithiasis is observed at abdominal radiographs	0	0
Other	6	3.66
Total Respondents	164	

3.4.5. Xanthinuria Preventive and Reactive Measures

Concerning xanthinuria prevention, 172 veterinarians advise owners about the adverse effects of allopurinol treatments when they initiate it (75.1%), although only 65 consider an appropriate dietary change for low-purine diets before xanthinuria appears (28.4%). Among these, 33 choose Royal Canin U/C Low Purine® (50.8%), 16 choose Advance Leishmaniosis® (24.6%), 10 choose Hill's U/D® (15.4%), 3 choose Purina NF® (4.6%) and other 3 choose Hill's K/D® (4.6%). These results are summarized in Table 8.

Table 8. Xanthinuria awareness and preventive measures.

Advise owners about allopurinol's adverse effects	**N**	**%**
Yes	172	75.11
No	57	24.89
Total Respondents	229	
Consider an appropriate dietary change for a low-purine diet	**N**	**%**
Yes	65	28.38
No	162	70.74
Other	2	0.87
Total Respondents	229	
Low-purine diets chosen	**N**	**%**
Purina NF	3	4.61
Advance Leishmaniosis	16	24.61
Hill's K/D	3	4.61
Hill's U/D	10	15.38
Royal Canin U/C Low Purine	33	50.77
Total Respondents	65	

About monitoring of urinary adverse effects in dogs under allopurinol treatment, urinalysis controls are prioritized by 163 veterinary surgeons (71.2%). Among these, 20 do it monthly (12.3%), 16 do it every two months (9.8%), 63 do it every 3 months (38.7%), 14 do it every 4 months (8.6%), 41 do it every 6 months (25.2%), 7 do it annually (4.3%) and 2 do it with more than one-year intervals (1.2%). These results are summarized in Table 9.

Table 9. Monitoring of urinary adverse effects in dogs under allopurinol treatment, with urinalysis.

Monitoring of urinary adverse effects with urinalysis	N	%
Yes	163	71.18
No	66	28.82
Total Respondents	229	
Frequency of monitoring	N	%
Monthly	20	12.27
Every two months	16	9.82
Every three months	63	38.65
Every four months	14	8.59
Every six months	41	25.15
Annually	7	4.29
More than one-year intervals	2	1.23
Total Respondents	163	

Also, diagnostic imaging controls are chosen by 71 respondents (31%). Abdominal ultrasonography is preferred by 67 of these (94.4%), followed by abdominal radiographs which are preferred by 4 (5.6%). Among these, 2 do it monthly (2.8%), 7 do it every two months (9.9%), 17 do it every 3 months (23.9%), 5 do it every 4 months (7%), 27 do it every 6 months (38%), 10 do it annually (14.1%) and 3 do it with more than one-year intervals (4.2%). These results are summarized in Table 10.

Table 10. Monitoring of urinary adverse effects in dogs under allopurinol treatment, with imaging controls.

Monitoring of urinary adverse effects with imaging controls	N	%
Yes	71	31.0
No	158	69.0
Total Respondents	229	
Diagnostic Imaging method chosen	N	%
Abdominal radiography	4	5.63
Abdominal ultrasonography	67	94.37
Other	0	0
Total Respondents	71	
Frequency of monitoring	N	%
Monthly	2	2.82
Every two months	7	9.86
Every three months	17	23.94
Every four months	5	7.04
Every six months	27	38.03
Annually	10	14.08
More than one-year intervals	3	4.23
Total Respondents	71	

When facing xanthinuria in dogs under allopurinol treatment, 99 respondents stop allopurinol (43.2%), 55 replaces allopurinol for AHCC (24%), 34 maintains allopurinol but reduces their dosage (14.9%), 12 assume keeping the same therapy (5.2%), 7 maintain allopurinol but increases administration frequency to three times daily (TID) (3.1%) and 22 choose another proceeding (9.6%). Of the 34 veterinary surgeons who reduce the allopurinol dosage, 29 do it in 50% (85.3%), 4 do it in 25-50% (11.8%) and 1 do it in 75% (2.9%). These results are summarized in Table 11.

Table 11. Reactive measures taken by clinicians when facing xanthinuria and allopurinol dosage reduction (in percentage).

Measures taken when facing xanthinuria	N	%
Stop allopurinol	99	43.23
Maintain allopurinol but reduces their dosage	34	14.85
Maintain allopurinol but increases administration frequency to TID	7	3.06
Keep the same therapy	12	5.24
Replace allopurinol for AHCC	55	24.02
Other	22	9.61
Total Respondents	229	
Allopurinol dosage reduction	**N**	**%**
25-50%	4	11.76
50%	29	85.29
75%	1	2.94
Total Respondents	34	

Among the respondents, 165 take additional measures in xanthinuria control (72.1%). From these, 98 choose an appropriate dietary change for low-purine diets, when not previously changed (59.4%), 25 choose the stimulation of water intake (15.2%), 25 choose an increase in the frequency of clinical monitoring to detect earlier possible complications (15.2%), 8 choose an increase in wet food consumption (4.9%), and 9 choose other strategies (5.5%).

Concerning complications associated with forced interruptions of the allopurinol therapy in dogs, 148 veterinary surgeons have not had other complications (64.6%), 41 registered higher or positive serologies for longer periods of time (17.9%), 31 registered clinical leishmaniosis relapses (13.5%) and 9 registered other complications (3.9%).

These results are summarized in Table 12.

Table 12. Additional reactive measures taken, and complications associated with forced allopurinol interruption.

Additional measures taken for xanthinuria control	N	%
Yes	165	72.05
No	64	27.95
Total Respondents	229	
Measures taken	**N**	**%**
Appropriate dietary change for low-purine diets	98	59.39
Stimulation of water intake	25	15.15
Increase in the frequency of clinical monitoring	25	15.15
Increase in wet food consumption	8	4.85
Other	9	5.45
Total Respondents	165	
Complications associated with forced allopurinol interruption	**N**	**%**
Not having other complications	148	64.63
Clinical leishmaniosis relapses	31	13.54
Higher or positive serologies for longer periods of time	41	17.90
Other	9	3.93
Total Respondents	229	

Finally, having into account the estimated frequency of xanthinuria in the IVC daily practice, 114 consider it 0-5% (49.8%), 56 consider it 5-15% (24.5%), 40 consider it 15-25% (17.5%), 14 consider it 25-50% (6.1%) and 5 consider it over 50% (2.2%). These results are summarized in Table 13.

Table 13. Estimated frequency of xanthinuria in clinicians' daily practice.

Estimated frequency of xanthinuria	N	%
0-5%	114	49.78
5-15%	56	24.45
15-25%	40	17.47
25-50%	14	6.11
> 50%	5	2.18
Total Respondents	229	

3.4.6. Assessment of potential differences between Portuguese and Spanish community answers, considering xanthinuria management

A statistical association was found when comparing the respondents' country with the responses about the detection of xanthinuria at least once in clinicians' daily practice ($p<0.001$) (Table 14), xanthinuria prevention, and clinicians' advising owners about possible adverse effects of allopurinol therapies ($p<0.001$) (Table 15), the monitoring of adverse effects with

urinalysis during allopurinol therapies (p<0.001) (Table 16), measures taken when facing xanthinuria considering the allopurinol therapy (p=0.001) (Table 17) and the estimated frequency of xanthinuria in clinicians' daily practice (p<0.001) (Table 18). This association between variables (country and different answers) means that the frequency of responses is dependent on the respondents' country.

Table 14. Answers to the question: Have you ever detected xanthinuria in dogs under allopurinol treatment?

Answers	Portugal		Spain		
	N	**%**	**N**	**%**	**p-value**
Yes	80	61.54	84	84.85	<0.001
No	50	38.46	15	15.15	
Total Respondents	130		99		

Table 15. Answers to the question: When initiating an allopurinol treatment, do you advise owners about the possibility of xanthinuria?

Answers	Portugal		Spain		
	N	**%**	**N**	**%**	**p-value**
Yes	84	64.62	88	88.89	<0.001
No	46	35.38	11	11.11	
Total Respondents	130		99		

Table 16. Answers to the question: Do you routinely perform control urinalysis in dogs under allopurinol treatment?

Answers	Portugal		Spain		
	N	**%**	**N**	**%**	**p-value**
Yes	80	61.54	83	83.84	<0.001
No	50	38.46	16	16.16	
Total Respondents	130		99		

Table 17. Answers to the question: If a dog under allopurinol treatment has xanthinuria, what do you do?

Answers	Portugal		Spain		
	N	**%**	**N**	**%**	**p-value**
I stop the allopurinol treatment	64	49.23	35	35.35	0.001
I maintain allopurinol but reduce their dosage	17	13.08	17	17.17	
I maintain allopurinol but increase their administration frequency to 3 times a day	6	4.62	1	1.01	
I maintain allopurinol, regardless of the presence of xanthinuria	11	8.46	1	1.01	
I replace allopurinol with hexose analogues (Impromune®)	21	16.15	34	34.34	
Other option	11	8.46	11	11.11	
Total Respondents	130		99		

Table 18. Answers to the question: In your experience, what is the estimated frequency of xanthinuria in dogs under allopurinol therapy, in your daily practice?

Answers	Portugal		Spain		
	N	**%**	**N**	**%**	**p-value**
0-5%	87	66.92	27	27.27	<0.001
5-15%	23	17.69	33	33.33	
15-25%	15	11.53	25	25.25	
25-50%	5	3.85	9	9.09	
Over 50%	0	0	5	5.05	
Total Respondents	130		99		

Also, no evidence that suggested a significant statistical association was found when comparing the respondent's country and the method chosen to diagnose xanthinuria (p=0.985), the consideration of an appropriate dietary change for a low-purine diet (p=0.349), the monitoring of adverse effects with imaging controls during allopurinol therapies (p=0.84), the diagnostic method chosen for monitoring of adverse effects (p=1.000), if additional measures are taken to control xanthinuria (p=0.767), additional measures taken to control xanthinuria (p=0.260) and complications associated with forced allopurinol interruptions (p=0.518). This absence of statistical association between variables (country and different answers) means that nothing suggests that differences exist between the frequency of responses between the respondents' countries, meaning these variables are independent.

3.5. Discussion

This study contributed to the assessment of xanthinuria management among the IVC, detailing the most frequent preventive and reactive measures taken.

3.5.1. General Characteristics

To allow a better characterization of the respondents of this study, questions considering this subject were made, exploring the respondent's age and gender.

The number of answers collected (n=230) was relatively small when compared with the entire IVC which is estimated in 40312 members (6560 Portuguese veterinarians and 33752 Spanish veterinarians) (INE 2020; OMV 2021). However, this number of answers is similar to those obtained previously in similar questionnaires conducted in both countries (Oliveira et al. 2010; Bourdeau et al. 2014; Mattin et al. 2014).

The female gender was the most represented one (77%), which is in line with what happens in both Portugal and Spain and reveals a majority of current female veterinarians working in the IVC (INE 2020; Veterinária Atual 2020; OMV 2021).

According to the age of respondents, 84.4% is under 45 years, and from these, 44.4% belongs to the 26-35 age interval which reveals a young IVC population with not much clinical experience. This represents a higher number when in comparison to Portuguese and Spanish numbers (61% under 40 years in Portugal; 47.6% under 45 years in Spain) (INE 2020; Veterinária Atual 2020). This higher percentage of respondents under 45 years may also be associated with the constitution of veterinary social network groups, since younger people usually visit and use social networks more often and in a slightly regular way, which may lead to higher response rates in younger age groups.

3.5.2. Allopurinol prescription regimens

Considering allopurinol’s usage around the IVC, 99.6% of clinicians confess using it as a part of CanLeish therapy, which actually agrees with the actual recommendations for leishmaniosis treatments and with allopurinol being considered the major first-line leishmanistatic drug for CanLeish treatments (Ribeiro et al. 2018; LeishVet 2018).

Even though the allopurinol dosage recommended by LeishVet (2018) is 10 mg/kg BID for at least 6 to 12 months, almost one-third of the respondents do not follow this dosage recommendation. Additionally, 11% of clinicians admit using higher dosages of allopurinol in their CanLeish therapy, which can be associated with a higher prevalence of xanthinuria or other urinary adverse effects (Osborne, Lulich, Swanson, et al. 2009) while a minor percentage prescribe lower dosages, which may be insufficient to control leishmaniosis and may result in

longer treatments since treatment duration depends on the severity of the disease (Ribeiro et al. 2018).

Considering the length of the allopurinol therapy, as previously mentioned, most authors recommend that the treatment lasts 6 to 12 months (Torres et al. 2016; LeishVet 2018; Roura et al. 2020). This is, indeed, the protocol followed by more than two-thirds of the respondents of this study. Despite the recommendations, about 16% of the respondents choose higher treatment periods and most of these follow lifelong allopurinol therapies. On the other hand, a minor percentage (1.8%) choose treatments lasting 1 to 3 months, which may also be insufficient to control leishmaniosis.

The use of higher or lower doses than those recommended and the use of superior or inferior time periods can possibly be explained by the lack of knowledge of the LeishVet recommendations and may be the reason why clinicians do not use them in their daily practice. Also, owners may have the power to change therapeutic decisions due to several reasons such as financial restrictions. Indeed, according to Monteiro (2020), 7% of Portuguese veterinarians were not aware of the existence of guidelines to help manage CanLeish and from those who were aware of their existence, almost one-third admitted not applying these recommendations in their daily practice.

When deciding the end of the treatment, Solano-Gallego et al. (2011) suggested that some criteria can support this decision, especially a remission/ improvement in clinical signs associated with a marked decrease in their serology. Among the respondents, both are chosen to support and decide the end of their CanLeish treatments by the majority of the respondents. Additionally, 6.1% only take into consideration a clinical signs improvement and another 3.1% only take into consideration a decrease in the serology. Also, 14.9% stops allopurinol treatments following the guidelines protocol and in order to avoid long term adverse effects, which shows that even though there is not a special concern with the improvement of clinical signs and serology, clinicians are aware of the potential adverse effects that may be associated to allopurinol therapies (Torres et al. 2016; Roura et al. 2020).

3.5.3. Allopurinol withdrawal and adverse effects

Withdrawing allopurinol before its ideal treatment time may be necessary for various reasons and it is part of the options that veterinary surgeons have when facing allopurinol adverse effects. In fact, and according to our survey, from the 54.6% of veterinary surgeons that have had to interrupt allopurinol before its ideal treatment time, most had to stop it because of adverse effects of the treatment.

Apart from xanthinuria and xanthine urolithiasis, other rare adverse effects like vomiting, diarrhea, cutaneous hypersensitivity/ rash/ vasculitis, nausea, elevation in liver enzymes,

myelosuppression, hepatopathy, or non-cutaneous vasculitis, are described (Bartges et al. 1999; Greene 2012). These were all noticed in minor percentages among the veterinarians that responded to our survey, except myelosuppression which was not reported. Elevation in liver enzymes and gastrointestinal clinical signs were the most common ones, which is in agreement with what is documented and may be due to their hepatic metabolization and gastrointestinal absorption, even though food does not affect its bioavailability (Bartges et al. 1999; Greene 2012).

3.5.4. Xanthinuria detection, complications, and diagnosis

Considering xanthinuria detection, almost three-quarters of the respondents have already detected it at least once in their daily practice. Xanthinuria has been described as the main urinary adverse effect of allopurinol (Koutinas et al. 2001; Solano-Gallego et al. 2009; Torres et al. 2011; Torres et al. 2016). Torres et al. (2016) previously described a xanthinuria prevalence of 13%, in a group of 320 dogs diagnosed with leishmaniosis and under allopurinol treatment, becoming the most frequent urinary adverse effect reported and agreeing with the obtained results in our survey.

When describing the urinary adverse effects of allopurinol in dogs with leishmaniosis, Torres et al. (2016) reported that in association with xanthinuria, the most common adverse effects were renal mineralization (57.1%), urolithiasis (50%), and urinary clinical signs (45.2%). In this survey, 68.8% of clinicians reported urinary clinical signs and this was the most frequent adverse effect found, possibly due to urinary clinical signs being able to be noticed at home by the owners, being a complaint shared in monitoring consults during allopurinol treatment. Also, renal mineralization was already reported by 37.5% of respondents which may have a lower percentage because an ultrasound is needed to detect this adverse effect, and monitoring ultrasounds may not be performed that often, even though they are recommended (Osborne et al. 2010; Torres et al. 2016; Roura et al. 2020). Finally, non-obstructive urolithiasis (59.4%), and obstructive urolithiasis (ureteral (17.5%) and urethral (29.4%) obstructions) were already detected by veterinarians in relative higher percentages and agreeing with the mentioned above study.

According to several authors, the xanthinuria diagnosis can be presumed using urinalysis, abdominal radiographs or ultrasonography, and these are particularly valuable when allopurinol treatments are ongoing and xanthinuria is suspected. The definitive diagnosis requires a post-removal urolith analysis (Osborne, Lulich, Swanson, et al. 2009; Torres et al. 2016). In this survey, more than three-quarters of clinicians generally diagnose xanthinuria based on the identification of crystalluria, which follows recommendations of using urinalysis and sediment analysis as an essential tool for monitoring dogs under allopurinol treatment

(Osborne, Lulich, Swanson, et al. 2009; Torres et al. 2011; Torres et al. 2016; Roura et al. 2020). A minor percentage of clinicians usually diagnose xanthinuria by post-removal urolith analysis which is the only method that can provide a definitive diagnosis, but as it requires a urolith retrieval, it is not a very common procedure (Osborne, Lulich, Swanson, et al. 2009; Torres et al. 2016). Finally, a low percentage of clinicians based their diagnosis on the detection of urolithiasis on abdominal ultrasound. This does not reflect the useful role of abdominal ultrasound on the clinical monitoring of dogs under allopurinol treatment, as recommended by some authors (Osborne, Lulich, Swanson, et al. 2009). Most recently, other authors only recommend urinalysis as part of regular follow-ups of dogs under allopurinol therapy, and abdominal ultrasounds as part of follow-ups if xanthinuria is detected (Torres et al. 2016; Roura et al. 2020).

3.5.5. Xanthinuria Preventive and Reactive Measures

Up to date, there are no standard guideline measures to approach xanthinuria, either in a preventive or reactive way. However, several authors have made recommendations of some measures that can be used to medically manage this situation. These measures include a diet change, increasing the water intake, urine alkalinization therapies, and the management of the allopurinol therapy.

As previously stated, xanthinuria has been considered the most common adverse effect of allopurinol treatments (Koutinas et al. 2001; Solano-Gallego et al. 2009; Torres et al. 2011; Torres et al. 2016). Despite this fact, only about three-quarters of clinicians advert owners about the possible adverse effects of an allopurinol treatment, including xanthinuria, and less than one-third consider an appropriate dietary change for low-purine diets, as recommended by diverse authors to prevent xanthinuria (Osborne et al. 2010; Torres et al. 2016). Even though there are several low-purine diets available, Royal Canin U/C Low Purine® and Advance Leishmaniosis® were the most frequent choices among those who change their diet has a preventive measure. A dietary change has been recommended to decrease the number of purines available for the purine synthesis cycle, reducing the probability of xanthinuria (Bartges and Kirk 2008; Osborne et al. 2010; Torres et al. 2016). Even though it may be an effective way to decrease the likelihood of xanthinuria, few veterinarians choose this approach as a preventive measure which may be related to the fact that most of these diets are more expensive when compared to the common ones. Taking into account that the current cost of a leishmaniosis therapeutic protocol may be very elevated, this can in part explain why clinicians do not directly recommend preventive measures for xanthinuria. Although not that common, veterinary-prescribed homemade low-purine diets could also be taken into account

to preventively manage xanthinuria, especially if financial restrictions are a concern for the owners. This option was not mentioned by anyone of the respondents.

Considering the monitoring of allopurinol therapies and prevention of urinary adverse effects, almost three-quarters of the respondents use urinalysis as a routine monitoring method which is in agreement with the recommendations to use urinalysis as a basis of regular monitoring of these dogs (Osborne, Lulich, Swanson, et al. 2009; Torres et al. 2011; Torres et al. 2016; Roura et al. 2020). Despite not existing a recommended time interval for this monitoring, most respondents do it once every three months, some do it every six months (25.15%), and a minor percentage do it monthly. This shows that veterinarians seem to be aware of the serious consequences of xanthinuria and other adverse urinary effects, monitoring them as early as possible. Few veterinarians have chosen large time intervals (1 year or more), which is consistent with the low percentage of veterinarians that do not monitor allopurinol therapies with urinalysis.

Concerning diagnostic imaging controls, only about one-third of clinicians use them as part of the allopurinol therapy. Diagnostic imaging controls are recommended before or after the detection of xanthinuria and this low percentage may be related to the fact that being a more expensive method, it is often avoided for financial restrictions. Among the clinicians who use diagnostic imaging controls, almost all prefer abdominal ultrasonography's, and most clinicians choose to do it in time intervals of every six months, followed by those who do it every three months or every year, in minor percentages. These results show that when facing allopurinol treatments, the vast majority of clinicians prefer monitoring them with urinalysis, choosing inferior time intervals between them, and use diagnostic imaging methods as a second monitoring tool, choosing larger time intervals between them. These preferences actually agree with the most recent recommendations (Torres et al. 2016; Roura et al. 2020).

Taking into account changes in the allopurinol therapy after xanthinuria is detected, stopping allopurinol is the preferred reactive option among our respondents, as it is the main source of its production, and stopping it will prevent future complications. The next preferred option by about a quarter of respondents is replacing allopurinol for AHCC, which does not induce the production of xanthinuria and seems to be a good option with similar efficacy to allopurinol in 6 months treatments (Segarra et al. 2017). Reducing the allopurinol dosage and increasing their administration frequency are also strategies evoked by the respondents, possibly because clinicians know how important allopurinol is in the CanLeish treatment. However, that may represent lower percentages because clinicians may find these therapy changes insufficient to control this problem. On the other hand, a very small percentage of clinicians admit keeping the same allopurinol therapy, showing that they are aware of how important allopurinol is and they are willing to take a chance at possible serious xanthinuria complications or do not think these complications are that serious. Among those who choose

to reduce the allopurinol dosage, the vast majority assume reducing it in 50% of the original dosage chosen, which agrees with the recommendations made by Roura et al. (2020). All these allopurinol regimen changes (replacing allopurinol, maintaining allopurinol but reducing their dosage or increasing their administration frequency) have been suggested by several authors, especially because allopurinol is the main reason xanthinuria appears (Osborne, Lulich, Swanson, et al. 2009; Osborne et al. 2010; Torres et al. 2016).

Assuming that changes in the allopurinol therapy may not be sufficient to stop xanthinuria or to avoid possible serious consequences, clinicians were asked if they take additional measures, besides those concerning allopurinol therapy. Among them, almost three-quarters admit taking additional measures, which shows that they are aware of the benefits additional measures may have in controlling the presence of xanthinuria (Bartges and Kirk 2008; Osborne et al. 2010; Torres et al. 2016; Roura et al. 2020). Among those who take additional measures, an appropriate dietary change to a low-purine diet was by far the most chosen option (59.4%), which shows veterinarians are aware of the importance the number of purines fed have in the production of xanthinuria but prefer to use this measure in a reactive way after xanthinuria shows up when compared to a preventive way. Both stimulation of water intake (15.2%), increasing the frequency of clinical monitoring (15.2%), or increasing wet food consumption (4.9%) were also taken into consideration, but veterinarians do not attribute them as much confidence in their beneficial effects as to an appropriate dietary change. These measures are in agreement with the literature (Bartges and Kirk 2008; Osborne, Lulich, Swanson, et al. 2009; Osborne et al. 2010; Torres et al. 2016; Roura et al. 2020).

Concerning the value of allopurinol in CanLeish treatments, when allopurinol is retrieved or reduced unexpectedly, for instance, adverse effects associated with the treatment, complications may urge in the leishmaniosis control. In these situations, most veterinary surgeons are not facing other complications (64.6%), but clinical leishmaniosis relapses and higher or positive serologies for longer periods of time have been noticed by several of them. These findings show that reducing or interrupting an allopurinol treatment can turn controlling leishmaniosis into a much more difficult task and worsen the prognosis of these dogs.

Overall, it is recognized that xanthinuria is rare in dogs but its prevalence increases in allopurinol presence, which is bringing awareness to this problem to veterinary practitioners (Torres et al. 2016). Among those who responded to our survey and considering the estimated xanthinuria frequency in their daily practice, about three-quarters of them think it is less than 15%. In fact, Torres et al. (2016) described a prevalence of 13% in dogs with leishmaniosis under allopurinol treatment which is actually close to what these veterinary practitioners experience in their daily clinical practice.

3.5.6. Assessment of potential differences between Portuguese and Spanish community answers, considering xanthinuria management

Differences in the frequency of answers of the IVC were found when considering the detection of xanthinuria. These differences are mainly due to a higher proportion of Spanish veterinarians that have already detected xanthinuria when compared to Portuguese ones.

Also, differences were found when considering veterinarians advising owners about the possibility of xanthinuria when initiating an allopurinol treatment, mainly because there is a higher proportion of Spanish veterinarians that advise owners of its possibility while only a few Portuguese veterinarians do it. Furthermore, a higher proportion of Portuguese veterinarians do not use urinalysis (35.4%) when compared to Spanish veterinarians who do use it (83.8%).

All these differences described above are hard to evaluate but may urge due to living/ working in endemic or non-endemic areas within both countries, the number of dogs with leishmaniosis that are seen in the clinicians' daily practice, and the clinical severity of these dogs, which was not possible to assess since this information was not obtained from the respondents. Factors like the clinical experience and years of practice, added to the knowledge about most recent information's on xanthinuria management may influence these response patterns and unfortunately were not evaluated by this questionnaire. Also, Spain has some veterinarians that are highly recognized for their publications and work in the leishmaniosis field, providing many hours of continuous education to the Spanish veterinary community, and this may explain some of the differences found between both countries.

Differences were also found in the measures taken about allopurinol when facing xanthinuria. Spanish veterinarians have higher proportions of answers considering solutions like replacing allopurinol with AHCC and Portuguese veterinarians have higher proportions of considering solutions like stopping allopurinol and maintaining allopurinol regardless of the presence of xanthinuria. The fact that this food supplement was developed by a Spanish laboratory and both studies that showed their efficacy were presented by Spanish veterinarians teams (Segarra et al. 2017; Segarra et al. 2018) can have an impact on the subject. However, the other differences found are harder to explain, but can also be explained by the factors enunciated above (working in endemic or non-endemic areas, number of leishmaniosis cases and their severity, clinical experience and knowledge about most recent information on xanthinuria).

Finally, differences were found when comparing answers related to the estimated frequency of xanthinuria in dogs under allopurinol therapy, mainly because most Portuguese veterinarians believe this frequency is low between 0 and 5% (66.92%), and as an opposite, Spanish veterinarians distribute almost equally their perception of this frequency in intervals up to 25%. These differences may, once again, be explained by the working/ living areas of

the respondents, the number of CanLeish cases veterinarians see, and their severity, but unfortunately could not be assessed by our study.

Overall, although useful and interesting information was collected from this survey, further larger surveys would be important to collect more representative results and to establish useful correlations such as the relationship between the profile of the veterinary surgeon and the trend of answers. More information about leishmaniosis endemic areas, the number of dogs treated per year, and their relationship with the prevalence of xanthinuria may be interesting to further evaluate in the future.

3.6. Limitations

A cross-sectional study was conducted, based on an online anonymous survey and although it allowed us to obtain several data and interesting conclusions, some limitations could be considered for further similar studies.

First, the rate of replies was not calculated due to two reasons: 1) the questionnaire was spread in veterinary Spanish and Portuguese Facebook groups and since it was not sent individually, it is hard to know how many veterinarians actually received the questionnaire; 2) despite restricted to veterinary groups, the link used to spread the questionnaire was public, meaning everyone who wanted to reply had that possibility, making it hard to predict how many veterinarians had access to it.

Another limitation was the relatively small number of replies, although in line with previous studies. A wider promotion via e-mail and some advertising could have increased the number of replies and would allow us to calculate the rate of replies, even though it may be a difficult task gathering e-mails of the entire IVC. In spite of the fact that the questionnaire only had twenty-six questions, a shorter questionnaire could have resulted in more responses. Also, since the link of the questionnaire was public, even though the first question was intended to confirm that the respondents were veterinary surgeons, the possibility of having responses from people with other occupations exists, but it is considered negligible. Also, the sample of the respondents may not be representative of the entire IVC because of the voluntary response bias and the sample may be mainly represented by those respondents who are more concerned, informed, or familiarized with xanthinuria and CanLeish.

Furthermore, while analyzing the questions, some limitations were found considering the structure of the questions. Eleven questions out of twenty-six had an option that allowed the respondents to give a different open and short reply to the question, under the name of “Other option”. This was integrated into these questions considering that veterinarians could proceed in different ways than those contemplated in the options created. However, considering the

type of responses this option provided, it would have been better to have a closed answer because many veterinarians actually followed one of the contemplated options but wanted to add some extra information that in most cases was not relevant to the question itself.

Concerning the questions that did not have a mandatory response because they were dependent on the answer to the above question, unfortunately, multiple mistaken replies were given. This led to the need to individually evaluate each questionnaire to eliminate answers that were wrongly collected, and this resulted in fewer replies than those who were supposed to exist. Therefore, the questionnaire should have been structural changed so that all questions were mandatory, resolving this problem.

When analyzing questions concerning the mean allopurinol treatment time veterinarians usually choose, an option including “six to twelve months” was absent and could have made sense to be included because it is the most recommended length for allopurinol treatments. Even though two different options included this length “4-6 months” and “1 year”, this option could have made the question clearer and reduce some subjectivity.

Moreover, in the questions concerning xanthinuria reactive measures, an option considering urine alkalinization should have been contemplated because it is one option to approach xanthinuria, however, it is not a common approach measure and if veterinarians wanted to reply and use that measure, they could have written it in the “other option” field. Nonetheless, as it was not clearly stated, this can be considered a limitation of this survey.

Also, when complications detected in association with xanthinuria in allopurinol treatments were observed and compared to other studies, a general option presenting “Obstructive urolithiasis” instead of the several obstruction locations that were present in the options available could have been more useful because other studies observed frequencies of obstructive urolithiasis, and this could have allowed easier comparisons to the previous data.

Considering the available information about xanthinuria in general, the data interpretation and comparison was not easy and relied mostly on several authors' opinions and not on standard recommendations, because they do not exist.

Finally, the information collected in this questionnaire was not sufficient to explain some differences that occurred between Spanish and Portuguese veterinary community answers. Questions considering general characteristics of the respondents like their working/ living region, the number of leishmaniosis cases they see, and their severity could have been useful to draw further conclusions about the response patterns and to explain differences that were found. Also, it could have helped us draw conclusions about whether the respondents’ sample is representative of the IVC.

3.7. Conclusion and future perspectives

This study focused on the IVC perspectives on the clinical management of xanthinuria secondary to allopurinol therapy in dogs with leishmaniosis.

In terms of allopurinol prescription regimens, most clinicians follow the actual recommendations of the LeishVet group considering the use of allopurinol on length and dosage, and for the criteria chosen to support and decide the end of an allopurinol treatment. However, the use of longer treatment lengths and higher dosages remains frequent among those who responded to this questionnaire.

Taking into account allopurinol withdrawal and adverse effects, apart from xanthinuria, more than half of the respondents have had to interrupt an allopurinol treatment before its ideal treatment time, and adverse effects were the most common reason that motivated it, especially gastrointestinal and hepatic adverse effects.

Concerning xanthinuria detection, complications, and diagnosis, xanthinuria and other urinary adverse effects have already been detected by most clinicians, being urinary clinical signs, the most common adverse effect noticed. Also, when taking into consideration the xanthinuria diagnosis, most clinicians follow recommendations and use urinalysis as a tool for monitoring these dogs and to presumptuously diagnose xanthinuria. However, some clinicians still prefer to only diagnose xanthinuria by post-removal urolith analysis, the only method that provides a definitive diagnosis.

Considering xanthinuria preventive measures, most clinicians advertise owners about possible adverse effects of an allopurinol treatment, but few approaches it preventively and make an appropriate dietary change to a low-purine diet. Also, many clinicians use urinalysis as a regular monitoring tool and only few uses diagnostic imaging, like abdominal ultrasonography, for that purpose.

Also, considering reactive measures taken after the detection of xanthinuria, stopping allopurinol is the preferred option taken when xanthinuria appears, followed by replacing it with AHCC, reducing allopurinol dosage or increasing their administration frequency. Also, additional measures are considered by many clinicians, including an appropriate dietary change, and both a stimulation of water intake and an increase in the frequency of clinical monitoring. From those who stop allopurinol, clinical leishmaniosis relapses and higher or positive serologies for longer periods of time have been noticed. Finally, most veterinarians thinks that the estimated frequency of xanthinuria in their daily practice is lower than 15%.

Some differences were found when considering xanthinuria management between Portugal and Spain but further studies requiring more information about the respondents and bigger samples are required to value these differences and assess how they are reflected in the IVC clinical daily practice.

Preventive measures (especially an appropriate dietary change to a low-purine diet) are always important and should be accounted for before it comes to a point where allopurinol must be interrupted or reduced, especially because of all the possible consequences of xanthinuria itself or the consequences of stopping the appropriate CanLeish treatment with allopurinol.

This study showed that most veterinarians in the IVC are aware of the high prevalence of xanthinuria as a common complication in allopurinol treatments in dogs with leishmaniosis. Although preventive measures and their importance are often neglected, clinicians seem to prefer reactive measures and they seem to be conscious about the different options to manage xanthinuria after their detection. Guidelines for the management of CanLeish are recognized and followed by many clinicians, showing that the creation of guidelines to prevent and approach xanthinuria would be beneficial to help the veterinary community on making decisions and to bring even more awareness to xanthinuria, their prevalence, and their possible serious urinary adverse effects.

Furthermore, studies conducted on a larger scale, available to more countries and more veterinarians, would be useful to extrapolate these conclusions and bring even more awareness about xanthinuria to clinicians and allowing the creation of standard clinical xanthinuria management measures.

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5. ANNEXES

Annexe 1. Pamphlet created for the Internal Medicine Service.

HOSPITAL ESCOLAR VETERINÁRIO

Serviço de Medicina Interna

O MEU ANIMAL VAI FAZER TERAPIA INALATÓRIA – E AGORA?

O QUE É A TERAPIA INALATÓRIA?

A terapia inalatória consiste na administração de um fármaco diretamente no trato respiratório, garantindo assim que se atinjam concentrações locais mais elevadas. Por ser muito vantajosa, o seu uso está a tornar-se cada vez mais frequente nos animais de companhia. Em Medicina Veterinária, e à semelhança da Medicina Humana, é realizada com recurso a câmaras inalatórias adaptadas especificamente a gatos (AeroKat), cães de pequeno porte e cães de grande porte (AeroDawg).

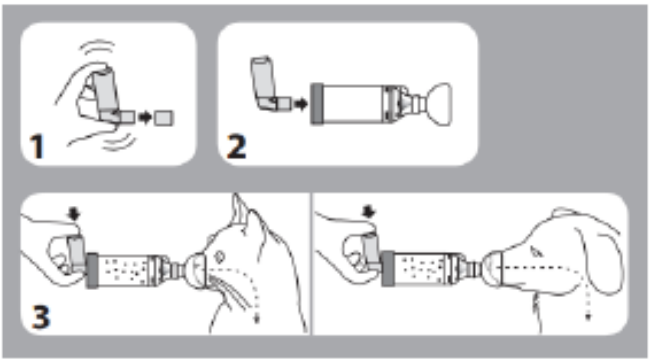
QUAIS AS SUAS VANTAGENS?

Por permitir uma administração local, as grandes vantagens da terapia inalatória consistem na necessidade de administração de menores doses de medicação para atingir as mesmas concentrações locais quando em comparação com a administração oral, tendo assim menos efeitos secundários sistémicos, o que é muito importante particularmente com a administração de corticosteróides.

COMO USAR?

Antes de usar: Para que o uso da terapia inalatória seja maximizado é importante que os nossos cães e gatos estejam completamente familiarizados com as câmaras inalatórias. Para isso, a sua adaptação deve ser feita lentamente, apenas com a máscara e com recurso a estímulos positivos associados à mesma, por exemplo, pode ser usada comida para estimular a colocação do focinho dentro da máscara, durante vários dias. Depois desta adaptação e de garantirmos que o nosso animal aceita a câmara, podemos começar a usá-la para terapia inalatória!

A câmara inalatória pode ser adquirida no HEV e vem com duas máscaras de diferentes tamanhos para que se escolha a que melhor se adapta ao seu animal (deve cobrir o nariz e boca, mas não os olhos). O inalador deve ser agitado imediatamente antes do uso e a sua cavilha/tampa deve ser removida. Depois, o inalador é acoplado na parte de trás da câmara e aplica-se a máscara suavemente sobre o focinho. Finalmente, pressionamos o inalador para libertar a medicação (1 puff) para dentro da câmara e deixamos o nosso animal fazer 5-10 inalações (podemos contar as respirações pelo movimento da válvula na parte superior do dispositivo).



Para mais informações, ou para ver vídeos de como usar, pode consultar a página oficial:

www.aerodawg.com

www.aerokat.com

O serviço de Medicina Interna deseja as melhoras do seu animal e votos de sucesso na terapia inalatória!

Folheto elaborado pela aluna estagiária Laura Jesus 2020/21

Annexe 2. Clinical Case presented at "Jornadas de Medicina Veterinária – AEICBAS".

Fibroplasia Esclerosante Eosinofílica Gastrointestinal Felina: relato de um caso clínico

Laura C. Jesus[1†*] | Miguel F. Carvalho[1*]

[1]Faculdade de Medicina Veterinária da Universidade de Lisboa, Lisboa, Portugal; *Hospital Escolar Veterinário – Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal; †Autor apresentador: Laura Jesus (laurajesus@campus.ul.pt)

Abstract

Introdução: A Fibroplasia Esclerosante Eosinofílica Gastrointestinal Felina (FEEGF) é uma doença inflamatória pouco comum, que afeta gatos e que se caracteriza pelo aparecimento de uma ou mais massas eosinofílicas no TGI e nos seus linfonodos satélite[1,2,3]. Até à data, a sua causa é desconhecida. Os sinais clínicos mais comuns incluem vómito e diarreia crónicos[1]. O diagnóstico definitivo requer uma análise histopatológica[2].

Caso clínico: Foi recebido para consulta no Serviço de Medicina Interna do HEV-FMV, um gato macho castrado, com oito anos, Europeu Comum, por quadro de hematoquézia e perda de peso há cerca de três anos. Como antecedentes médicos, o gato apresentava uma bronquite crónica, estável com terapêutica inalatória, e história de vómito crónico episódico, o qual foi resolvido anteriormente com transição para alimentação hidrolisada. O exame clínico não apresentava alterações, com exceção da distensão das ansas intestinais identificadas à palpação abdominal. Estabelecidos os diagnósticos diferenciais, foram realizadas análises gerais, as quais não revelaram alterações. A realização de uma ecografia abdominal permitiu identificar: um espessamento difuso do intestino delgado e cólon, com camadas mantidas; a suspeita da presença de uma massa parietal heterogénea ao nível da válvula ileocólica (com dimensões de 21x18mm) a envolver a porção inicial do cólon ascendente; linfonodos cólicos normodimensionados mas hipoecogénicos e com reatividade da gordura mesentérica. Foi também realizada uma colonoscopia e colheita de amostras para análise histopatológica e imunohistoquímica, e PAAFs ecoguiadas da massa e linfonodo cólico. Os resultados suportaram o diagnóstico de FEEGF.

Plano de Tratamento: Estando o gato já com dieta hidrolisada (hipoalergénica), após investigação e, enquanto se aguardavam os resultados da análise histopatológica, foi instaurado tratamento com prednisolona (0.5 mg/kg SID PO durante 10 dias, seguido de uma redução progressiva durante 3 meses) e ciclosporina (5 mg/kg durante um período inicial de 30 dias).

Evolução clínica, evolução e prognóstico: Após o início da terapêutica médica, registou-se uma melhoria clínica com resolução da hematoquézia. Nos controlos ecográficos realizados mensalmente, verificou-se uma regressão ecográfica da lesão e do espessamento do cólon identificados anteriormente, o que suportou a redução progressiva da corticoterapia.

Conclusão: Este caso clínico sobre-eleva a FEEGF como causa de hematoquézia em gatos. Apesar da etiologia ser incerta, suspeita-se de uma causa imunomediada. Devido ao facto de existirem poucos casos descritos na literatura, o tratamento é pouco consensual, estando descrito o recurso a imunossupressores, antibioterapia e, em alguns casos, resseção cirúrgica das lesões. Sendo uma doença pouco frequente, este caso clínico visa consciencializar os MV para a FEEGF, uma vez que os respetivos sinais clínicos são inespecíficos e podem mimetizar outras doenças gastrointestinais com expressão crónica.

Palavras chave: FEEGF; gato; vómito; diarreia; hematoquézia.

Abreviaturas: FEEGF - Fibroplasia Esclerosante Eosinofílica Gastrointestinal Felina; TGI – trato gastrointestinal; PO – *per os*; SID – uma vez por dia; BID – duas vezes por dia; QUOD – a cada 48h; MV – médicos veterinários; PAAFs – punções aspirativas por agulha fina.

Annexe 3. Abstract submitted for the European College of Veterinary Internal Medicine – Companion Animals Congress.

Xanthinuria Secondary to Allopurinol Treatment in Dogs with Leishmaniosis: Current Perspectives of the Iberian Veterinary Community

Laura Jesus, Carolina Arenas, Marina Domínguez-Ruiz, Paolo Silvestrini, Xavier Roura, Rodolfo Oliveira Leal

Xanthinuria is an important adverse urinary effect in dogs with leishmaniosis on therapy with allopurinol.

This study aimed to investigate current medical approach of the Iberian veterinary community (IVC) on prevention and management of xanthinuria secondary to allopurinol therapy in canine leishmaniosis (CanLeish).

An online anonymous survey including 4 to 26 questions (depending on the answering pathway chosen) was conducted. The content was divided into five sections focusing on: general information about the respondents, allopurinol prescription regimen, therapeutic monitoring, causes for allopurinol withdrawal, adverse effects, xanthinuria diagnosis, treatment, and preventive measures. After internal validation, the survey was uploaded through an online platform and diffused via Iberic social network veterinary groups. Only answers regarding xanthinuria diagnosis and preventive measures were finally selected.

A total of 230 answers were obtained: 131 from Portugal and 99 from Spain. About 99.6% (229/230) of the clinicians use allopurinol as part of CanLeish treatment. A total of 71.6% (164/229) have identified xanthinuria in dogs with leishmaniosis; 78.7% (129/164) generally diagnose xanthinuria based on identification of crystalluria, 12.2% (20/164) by post-removal urolith analysis, and 5.5% (9/164) based on detection of urolithiasis on abdominal ultrasound. Regarding complications associated with xanthinuria, urinary clinical signs was reported by 68.8% (110/160) of clinicians, non-obstructive urolithiasis by 59.4% (95/160), renal mineralization by 37.5% (60/160), bacterial cystitis by 31.3% (50/160), urethral obstruction by 29.4% (47/160), and ureteral obstruction by 17.5% (28/160).

Regarding xanthinuria prevention, 75.1% (172/229) of clinicians commonly inform the clients of the adverse effects of allopurinol treatment although only 28.4% (65/229) consider a change to a low purine diet. Regarding monitoring of urinary adverse effects, urinalysis and diagnostic imaging are prioritized by 71.2% (163/229) and 31% (71/229), respectively. Abdominal ultrasonography is preferred (94.4%; 67/71), followed by abdominal radiographs (5.6%; 4/71).

When facing xanthinuria, 43.2% (99/229) of clinicians stop allopurinol treatment, 24% (55/229) switch for active hexose correlated compound (nucleotides), 17.9% (41/229) change the frequency or dosage of allopurinol administration, and 5.2% (12/229) keeps the same therapy. Dietary modification (59.4%; 98/165), stimulation of water intake (15.2%; 25/165), increase in wet food consumption (4.9%; 8/165), and increased frequency of clinical monitoring (15.2%; 25/165), were also implemented.

The IVC is aware of the high prevalence of xanthinuria as a common complication in CanLeish. Although preventive measures are often neglected, clinicians seem to be conscious about the different options to manage xanthinuria in dogs with leishmaniosis, under allopurinol treatment.

Annexe 4. Questionnaire provided online (Portuguese version).

Prevalência e maneio da xantinúria em Portugal

Este questionário é destinado a médicos veterinários que exerçam a sua atividade na área de clínica de animais de companhia. A sua participação é voluntária e anónima, sendo que demora cerca de 5 minutos a responder. Os dados serão usados no âmbito de uma tese de mestrado da FMV-UL orientada pelo Professor Rodolfo Oliveira Leal. Todos os dados fornecidos serão tratados de acordo com o Regulamento Geral sobre a Proteção de Dados. Se tiver alguma dúvida, por favor contactar laurajesus@campus.ul.pt.

SECÇÃO 1

1. **Confirmo que sou médico veterinário e que este questionário é voluntário e anónimo.***
   a. Aceito participar

SECÇÃO 2 – CARACTERIZAÇÃO DA AMOSTRA

2. **Qual a sua idade?***
   - <25
   - 26-35
   - 36-45
   - 46-55
   - 56-65
   - >65
3. **Qual o seu sexo?***
   - Feminino
   - Masculino
   - Prefiro não responder

SECÇÃO 3 – USO DE ALOPURINOL

4. **Usa Alopurinol no tratamento da Leishmaniose?***
   - Sim. (Continua o questionário)
   - Não. (Fim do questionário)

SECÇÃO 4 – TRATAMENTO COM ALOPURINOL

5. **Qual a dose de alopurinol utilizada no tratamento da leishmaniose? Se utilizar alopurinol noutra dose que não as indicadas por favor indique na caixa "Outra opção".***
   - Uma dose inferior a 10mg/kg de 12h em 12h.
   - Uma dose de 10mg/kg de 12h em 12h.
   - Uma dose entre 10mg/kg e 20mg/kg de 12h em 12h.
   - Uma dose superior a 20mg/kg de 12 em 12h.
   - Uma dose inferior a 10mg/kg de 24h em 24h.
   - Uma dose de 10mg/kg de 24h em 24h.
   - Uma dose entre 10mg/kg e 20mg/kg de 24h em 24h.
   - Uma dose superior a 20mg/kg de 24h em 24h.
   - Outra opção:
6. **Em média, durante quanto tempo faz o tratamento com alopurinol? Se fizer o tratamento com uma duração que não esteja indicada, por favor indique na caixa "Outra opção".***
   - 1 - 3 meses
   - 4 - 6 meses
   - 1 ano
   - 2 anos
   - Mais de 2 anos
   - Até ao fim de vida do animal
   - Outra opção:
7. **Após esse tempo de tratamento, o que o leva a suspender o alopurinol? Se é outro o motivo pelo qual suspende o tratamento, por favor indique na caixa "Outra opção".***
   - Decréscimo da serologia independentemente dos sinais clínicos
   - É protocolar e faço-o para evitar efeitos adversos a médio-longo prazo
   - Remissão/melhoria dos sinais clínicos, independentemente da serologia
   - Remissão/melhoria dos sinais clínicos e decréscimo da serologia
   - Pela minha experiência, não vejo benefício em continuar após o período habitual
   - Outra opção:
8. **Já interrompeu a terapêutica com alopurinol antes do que considera ideal? ***
   - Sim (continua).
   - Não (secção 6).

SECÇÃO 5 – INTERRUPÇÃO DA TERAPÊUTICA COM ALOPURINOL

9. Por que motivo interrompeu a terapêutica com alopurinol antes do período de tempo que considera ideal? Se o motivo for outro por favor indique na caixa "Outra Opção".*

- Restrições financeiras
- Difícil compliance
- Complicações secundárias ao tratamento
- Outra opção:

10. **Se na questão anterior selecionou a opção "Complicações Secundárias", além da xantinúria, a que complicações secundárias ao tratamento se refere? Selecione todas as opções que se apliquem**.

- Hipersensibilidade cutânea/ rash cutâneo/ vasculite (sintomatologia cutânea)
- Diarreia
- Náusea
- Aumento da ALT e AST (não presentes antes do tratamento)
- Mielossupressão
- Hepatopatia
- Vasculite (não cutânea)

SECÇÃO 6 – DETEÇÃO DE XANTINÚRIA

11. Já detetou xantinúria em animais tratados com Alopurinol?*

- Sim (continua).
- Não (Secção 8).

SECÇÃO 7 – PRESENÇA DE XANTINÚRIA

12. Quais as complicações que já detetou associadas à presença de xantinúria? Selecione todas as opções que se apliquem. Se detetou outra alteração, por favor indique na caixa "Outra opção". Se nunca detetou complicações não responda.

- Obstrução ureteral.
- Obstrução uretral.
- Cistite bacteriana.
- Urolitíase não obstrutiva.
- Mineralização renal.
- Sinais clínicos urinários (disúria, estrangúria, polaquiúria).
- Outra opção:

13. De acordo com a casuística presente na sua prática clínica, como é mais frequente fazer o diagnóstico de xantinúria? Se utilizar outro método, por favor indique na caixa "Outra Opção".*

- Identificação de cristalúria na urianálise
- Não é estabelecido a 100% mas é presumido quando é identificada urolitíase na ecografia.
- Não é estabelecido a 100% mas é presumido quando é identificada urolitíase na radiografia.
- Análise dos cálculos após remoção cirúrgica.
- Outra opção:

SECÇÃO 8 – PREVENÇÃO DA XANTINÚRIA

14. Quando inicia um tratamento com Alopurinol, adverte os donos sobre a possibilidade de existir xantinúria?*

- Sim
- Não

15. Quando inicia um tratamento com alopurinol, altera a dieta com vista a prevenir um possível aparecimento de xantinúria? Se alterar para outra que não as rações mencionadas, por favor indique qual na caixa "Outra Opção".*

- Não altero.
- Altero a dieta para Purina NF ®.
- Altero a dieta para Advance Leishmaniosis®.
- Altero a dieta para Hill's K/D®.
- Altero a dieta para Hill's U/D®.
- Altero a dieta para Royal Canin U/C Low Purine®.
- Outra opção:

16. Realiza controlos de urina II a animais com tratamento com alopurinol em curso?*

- Sim.
- Não.

17. Se respondeu "Sim" na resposta anterior, com que frequência faz esses controlos?

- Mensalmente
- A cada 2 meses
- Trimestralmente
- 3x por ano
- 2x por ano
- Anualmente
- Com intervalos superiores a 1 ano.

18. **Utiliza rotineiramente exames complementares imagiológicos aquando de um cão sob tratamento de alopurinol para avaliar a possibilidade de urolitíase a xantina?***
    - Sim
    - Não
19. **Se respondeu "Sim" na pergunta anterior, qual o exame imagiológico que privilegia? Se privilegiar outro exame, por favor indique na caixa "Outra opção".**
    - RX abdominal
    - Ecografia abdominal
    - Outra opção:
20. **Com que frequência realiza os controlos imagiológicos nos animais em tratamento com alopurinol?**
    - Mensalmente
    - A cada 2 meses
    - Trimestralmente
    - 3x por ano
    - 2x por ano
    - Anualmente
    - Com intervalos superiores a 1 ano.
21. **Se um cão apresentar xantinúria e estiver sob tratamento com alopurinol, o que faz? Se proceder de outra forma, por favor indique na caixa "Outra opção".***
    - Descontinuo o alopurinol.
    - Mantenho o alopurinol mas diminuo a dose.
    - Mantenho o alopurinol mas aumento a frequência de administração (divisão da dose diária em 3 tomas).
    - Mantenho o alopurinol na mesma dose, independentemente da presença de xantinúria
    - Substituo o alopurinol por análogos da hexose (Impromune®).
    - Outra opção:
22. **Se na questão anterior selecionou a resposta "Mantenho o alopurinol mas diminuo a dose", para quanto reduz a dose?** _____
23. **Além do respondido acima, toma ainda medidas adicionais relativamente à presença de xantinúria?***
    - Sim.
    - Não.
24. **Se na questão anterior selecionou "Sim", que medidas adicionais de controlo da xantinúria toma? Se tomar outras medidas, por favor indique-as na opção "outra".**
    - Transição para dieta com baixo teor de purinas (se não feito anteriormente)
    - Estimulação do consumo de água
    - Aumento do consumo de comida húmida
    - Aumento do número de controlos para detetar possíveis complicações de forma mais precoce
    - Outra opção:
25. **Em cães em que a terapêutica de alopurinol foi interrompida ou reduzida de forma forçada, registou algumas complicações? Se registou outras, por favor indique na caixa "Outra opção".***
    - Não tenho tido complicações.
    - Sim, recidiva de leishmaniose clínica.
    - Sim, serologias positivas ou mais elevadas durante mais tempo.
    - Outra opção:
26. **Na sua prática clínica, qual a frequência estimada de xantinúria em animais tratados com alopurinol?***
    - 0-5%
    - 5-15%
    - 15-25%
    - 25-50%
    - Mais de 50%

Annexe 5. Questionnaire provided online (Spanish version)

Prevalencia y manejo de la xantinuria en España

Este cuestionario está destinado a veterinarios de clínica de pequeños animales. Su participación es voluntaria y anónima, y le costará alrededor de 5 minutos responder. Los datos derivados del mismo se utilizarán para un trabajo de final de carrera de una estudiante de la Facultad de Veterinaria de Lisboa (FMV-UL) dirigido por el profesor Rodolfo Oliveira Leal. Todos los datos facilitados serán tratados de acuerdo con el Reglamento General de Protección de Datos. Si tiene alguna pregunta, se puede poner en contacto con laurajesus@campus.ul.pt.

SECCIÓN 1

1. Confirmo que soy veterinario y que este cuestionario es voluntario y anónimo. *

- Acepto participar

SECCIÓN 2- CARACTERIZACIÓN DEL ENCUESTADO

2. ¿Qué edad tiene?*

- <25
- 26-35
- 36-45
- 46-55
- 56-65
- >65

1. Sexo*

- Femenino
- Masculino
- Prefiero no responder

SECCIÓN 3-USO DEL ALOPURINOL

4. ¿Utiliza alopurinol para el tratamiento de la leishmaniosis? *

- Sí. (El cuestionario continúa)
- No. (Fin del cuestionario)

SECCIÓN 4-TRATAMIENTO CON ALOPURINOL

5. ¿Qué dosis de alopurinol utiliza en el tratamiento de la leishmaniosis? Si usa otra dosis de alopurinol diferente a las especificadas, indíquelo en la casilla "Otra opción". *

- Una dosis menor de 10 mg / kg cada 12 horas.
- Una dosis de 10 mg / kg cada 12 horas.
- Una dosis entre 10 mg / kg y 20 mg / kg cada 12 horas.
- Una dosis superior a 20 mg / kg cada 12 horas.
- Una dosis de menos de 10 mg / kg cada 24 horas.
- Una dosis de 10 mg / kg cada 24 horas.
- Una dosis entre 10 mg / kg y 20 mg / kg cada 24 h.
- Una dosis superior a 20 mg / kg cada 24 horas.
- Otra opción:

6. De manera genérica, ¿durante cuánto tiempo recomienda el tratamiento con alopurinol? Si recomienda el tratamiento por una duración no especificada, indíquelo en la casilla "Otra opción". *

- 1-3 meses
- 4-6 meses
- 1 año
- 2 años
- Más de 2 años
- Hasta el final de la vida del animal
- Otra opción:

7. Después de ese tiempo en tratamiento con alopurinol, ¿por qué motivo suspende el tratamiento con alopurinol? Si es por otro motivo, indíquelo en la casilla "Otra opción".*

- Disminución de la serología independientemente de los signos clínicos
- Por protocolo; lo hago para evitar efectos adversos a medio-largo plazo
- Remisión / mejoría de los signos clínicos, independientemente de la serología
- Remisión / mejoría de los signos clínicos y disminución de la serología
- En mi experiencia, no veo ningún beneficio en continuar después del período habitual
- Otra opción:

8. ¿Ha interrumpido en alguna ocasión el tratamiento con alopurinol antes de lo que considera ideal? *

- Si (continuación).
- No (sección 6).

SECCIÓN 5-INTERRUPCIÓN DEL TRATAMIENTO CON ALOPURINOL

9. ¿Por qué interrumpió el tratamiento con alopurinol antes de lo que consideraba ideal? Si el motivo es diferente, indíquelo en "Otra opción". *

- Motivos económicos
- Dificultad de administración
- Complicaciones secundarias al tratamiento
- Otra opción:

10. Si en la pregunta anterior seleccionó la opción "Complicaciones secundarias", (además de xantinuria), ¿a qué complicaciones secundarias se refiere? Seleccione todas las opciones que correspondan.

- Hipersensibilidad cutánea / eritema-erupción cutáneo / vasculitis (síntomas cutáneos)
- Diarrea
- Náuseas
- Aumento de ALT y AST (no presentes antes del tratamiento)
- Mielosupresión
- Hepatopatía
- Vasculitis (no cutánea)

SECCIÓN 6-DETECCIÓN DE XANTINURIA

11. ¿Ha detectado alguna vez xantinuria en animales en tratamiento con alopurinol? *

- Si (continuación).
- No (Sección 8).

SECCIÓN 7- PRESENCIA DE XANTINURIA

12. ¿Qué complicaciones ha detectado asociadas a la presencia de xantinuria? Seleccione todas las opciones que correspondan. Si detectó otras anomalías, por favor indíquelo en "Otra opción". Si nunca ha notado ninguna complicación, no responda.

- Obstrucción ureteral.
- Obstrucción uretral.
- Cistitis bacteriana.
- Urolitiasis no obstructiva.
- Mineralización renal.
- Signos clínicos urinarios (disuria, estranguria, polaquiuria).
- Otra opción:

13. En su experiencia, ¿cómo es más frecuente diagnosticar la xantinuria? Si utiliza otro método, indíquelo en "Otra opción". *

- Identificación de cristaluria en análisis de orina
- No está 100% probado, pero se asume cuando se identifica urolitiasis en la ecografía.
- No está 100% probado, pero se asume cuando se identifica urolitiasis mediante radiografías.
- Análisis de cálculos tras la extracción quirúrgica.
- Otra opción:

SECCIÓN 8-PREVENCIÓN DE XANTINURIA

14. Al iniciar el tratamiento con alopurinol, ¿advierte a los propietarios sobre la posibilidad de xantinuria? *

- Si
- No

15. Cuando inicia el tratamiento con alopurinol, ¿cambia la dieta para prevenir la posible aparición de xantinuria? Si cambia a cualquier otra dieta que no esté especificada, indique cuál en la casilla "Otra opción". *

- No hago cambios en la dieta.
- Cambio a Purina NF ®.
- Cambio a Advance Leishmaniosis®.
- Cambio a Hill's K / D®.
- Cambio a Hill's U / D®.
- Cambio a Royal Canin U / C Low Purine®.
- Otra opción:

16. ¿Realiza análisis de orina de control en animales que reciben tratamiento continuo con alopurinol? *

- Si.
- No.

17. Si respondió "Sí" en la pregunta anterior, ¿con qué frecuencia realiza estos urianálisis de control?

- Mensual
- Cada 2 meses
- Trimestral
- 3 veces al año
- 2 veces al año
- Anualmente
- Con intervalos superiores a 1 año.

18. ¿Realiza de manera rutinaria pruebas de diagnóstico por imagen cuando un perro está en tratamiento con alopurinol para evaluar la posibilidad de urolitiasis de xantina?*

- Si
- No

19. Si respondió "Sí" en la pregunta anterior, ¿qué prueba de diagnóstico por imagen prefiere? Si prefiere otro tipo de prueba de diagnóstico por imagen, indíquelo en la casilla "Otra opción".

- Radiografías de abdomen
- Ecografía de abdomen
- Otra opción:

20. ¿Con qué frecuencia realiza controles de diagnóstico por imagen en animales en tratamiento con alopurinol?

- Mensual
- Cada 2 meses
- Trimestral
- 3 veces al año
- 2 veces por año
- Anualmente
- Con intervalos superiores a 1 año.

21. Si un perro en tratamiento con alopurinol tiene xantinuria, ¿qué hace?. Si hace otra cosa no especificada, indíquelo en la casilla "Otra opción". *

- Detengo el tratamiento con alopurinol.
- Mantengo el alopurinol pero disminuyo la dosis.
- Mantengo la dosis de alopurinol pero aumento la frecuencia de administración (dividiendo la dosis diaria en 3 tomas).
- Mantengo la misma dosis de alopurinol, independientemente de la presencia de xantinuria
- Reemplazo el alopurinol por análogos de hexosa (Impromune®).
- Otra opción:

22. Si en la pregunta anterior seleccionó la respuesta "Mantengo el alopurinol pero disminuyo la dosis", ¿a cuánto reduce la dosis? _____

23. Además de todo lo anterior, ¿toma medidas adicionales con respecto a la presencia de xantinuria? *

- Si.
- No.

24. Si seleccionó "Sí" en la pregunta anterior, ¿qué medidas adicionales toma para controlar la xantinuria? Si toma otras medidas que no estén especificadas, indíquelas en la opción "otras".

- Cambio a una dieta baja en purinas (si no se hizo previamente)
- Estimulación del consumo de agua
- Mayor consumo de comida húmeda
- Reaizo un mayor número de controles para detectar posibles complicaciones
- Otra opción:

25. En los perros en los que se interrumpió o redujo prematuramente el tratamiento con alopurinol, ¿experimentó alguna complicación con respecto a la leishmaniosis? Si ha registrado alguna otra, por favor indíquelo en la casilla "Otra opción". *

- No he tenido complicaciones.
- Sí, recaída de leishmaniosis clínica.
- Sí, serologías positivas o superiores durante más tiempo.
- Otra opción:

26. En su experiencia, ¿cuál es la frecuencia estimada de xantinuria en animales tratados con alopurinol? *

- 0-5%
- 5-15%
- 15-25%
- 25-50%
- Mas de 50%

Annexe 6. Questionnaire provided online (English version)

Prevalence and management of xanthinuria

This questionnaire is intended for veterinarians who work in small animal clinics. Your participation is voluntary and anonymous, and it takes only about 5 minutes to answer. The data collected will be used in the context of a master's thesis at FMV-UL supervised by Professor Rodolfo Oliveira Leal. All data provided will be treated following the General Data Protection Regulation. If you have any questions, please contact laurajesus@campus.ul.pt.

SECTION 1

1. I confirm that I am a veterinarian and that this questionnaire is voluntary and anonymous.*

- ☐ I agree to participate.

SECTION 2 – RESPONDENTS' CHARACTERIZATION

2. How old are you?*

- ☐ <25
- ☐ 26-35
- ☐ 36-45
- ☐ 46-55
- ☐ 56-65
- ☐ >65

3. What is your gender?*

- ☐ Female
- ☐ Male
- ☐ I prefer not to answer.

SECTION 3 – ALLOPURINOL USAGE

4. Do you use allopurinol to treat leishmaniosis? *

- ☐ Yes. (Continues).
- ☐ No. (End of the questionnaire).

SECTION 4 – ALLOPURINOL TREATMENT

5. What dose of allopurinol do you use to treat leishmaniosis? If you use a different dosage of allopurinol than those specified, please indicate it in the "Other option" box.*

- ☐ A dose lower than 10 mg/kg every 12 hours.
- ☐ A dose of 10 mg/kg every 12 hours.
- ☐ A dose between 10 mg / kg and 20 mg / kg every 12 hours.
- ☐ A dose higher than 20 mg/kg every 12 hours.
- ☐ A dose lower than 10 mg/kg every 24 hours.
- ☐ A dose of 10 mg/kg every 24 hours.
- ☐ A dose between 10 mg / kg and 20 mg / kg every 24 hours.
- ☐ A dose higher than 20 mg/kg every 24 hours.
- ☐ Other option:

6. Generically, for how long do you recommend an allopurinol treatment? If you recommend a treatment length different than those specified, please indicate it in the "Other option" box.*

- ☐ 1-3 months
- ☐ 4-6 months
- ☐ 1 year
- ☐ 2 years
- ☐ More than 2 years
- ☐ Until the end of the animal's life
- ☐ Other option:

7. After that treatment time with allopurinol, why do you stop it? If it is for a different reason than those specified, please indicate it in the "Other option" box.*

- ☐ Decreased serology regardless of clinical signs.
- ☐ For protocol, I do it to avoid adverse effects in the medium-long term.
- ☐ Remission/ improvement of clinical signs, regardless of serology
- ☐ Remission/ improvement of clinical signs and decreased serology
- ☐ In my experience, I don't see any benefits in continuing after the usual period.
- ☐ Other option:

8. Have you ever stopped an allopurinol treatment earlier than you think is ideal?*

- ☐ Yes (Continue).
- ☐ No (Section 6).

SECTION 5 – INTERRUPTION OF ALLOPURINOL TREATMENTS

9. Why did you stop an allopurinol treatment earlier than you thought was ideal? If you have a different reason than those specified, please indicate it in the "Other option" box.*

- Financial restrictions
- Administration difficulties
- Complications secondary to the treatment
- Other option:

10. If in the previous question you selected the option "Complications secondary to the treatment" (in addition to xanthinuria), what secondary complications are you referring to? Select all options that apply.

- Skin hypersensitivity/ erythema-rash / vasculitis (dermatologic symptoms)
- Diarrhea
- Nausea
- Increased ALT and AST (not present before the treatment)
- Myelosuppression
- Hepatopathy
- Vasculitis (non-cutaneous)

SECTION 6 – XANTHINURIA DETECTION

11. Have you ever detected xanthinuria in dogs under allopurinol treatment?*

- Yes (Continues).
- No (Section 8).

SECTION 7 – XANTHINURIA PRESENCE

12. What complications have you detected associated with the presence of xanthinuria? Select all options that apply. If you have detected different complications than those specified, please indicate them in the "Other option" box. If you have never detected any complications, do not reply.

- Ureteral obstruction.
- Urethral obstruction.
- Bacterial cystitis.
- Non-obstructive urolithiasis.
- Renal mineralization.
- Urinary clinical signs (dysuria, stranguria, pollakiuria).
- Other option:

13. In your experience, how do you diagnose xanthinuria most often? If you use another method than those specified, please indicate it in the "Other option" box.*

- Identification of crystalluria in urinalysis
- It is not 100% proven, but it is assumed when urolithiasis is identified on ultrasonography
- It is not 100% proven, but it is assumed when urolithiasis is identified in radiography.
- Analysis of uroliths after surgical extraction.
- Other option:

SECTION 8 – XANTHINURIA PREVENTION

14. When initiating an allopurinol treatment, do you advise owners about the possibility of xanthinuria?*

- Yes
- No

15. When you initiate an allopurinol treatment, do you change your diet to prevent a possible occurrence of xanthinuria? If you choose a different diet than those specified, please indicate which one in the "Other option" box.*

- I do not make a dietary change.
- I change to Purina NF ®.
- I change to Advance Leishmaniosis®.
- I change to Hill's K / D®.
- I change to Hill's U / D®.
- I change to Royal Canin U / C Low Purine®.
- Other option:

16. Do you routinely perform control urinalysis in dogs under allopurinol treatment?*

- Yes.
- No.

17. If you answered "Yes" to the previous question, how often do you perform these urinalysis follow-ups?

- Monthly
- Every 2 months
- 4 times a year
- 3 times a year
- 2 times a year
- Annually
- With intervals greater than 1 year.

18. Do you routinely perform control imaging tests in dogs under allopurinol treatment?*

- Yes
- No

19. If you answered "Yes" to the previous question, which imaging tests do you prefer? If you prefer different imaging tests than those specified, please indicate which one in the "Other option" box.

- Abdominal radiography
- Abdominal ultrasonography
- Other option:

20. How often do you perform these imaging follow-ups in dogs under allopurinol treatment?

- Monthly
- Every 2 months
- 4 times a year
- 3 times a year
- 2 times a year
- Annually
- With intervals greater than 1 year.

21. If a dog under allopurinol treatment has xanthinuria, what do you do? If you do something different than those options specified, please indicate it in the "Other option" box.*

- I stop the allopurinol treatment.
- I maintain allopurinol but reduce their dosage.
- I maintain allopurinol but increase their administration frequency to 3 times a day.
- I maintain allopurinol, regardless of the presence of xanthinuria.
- I replace allopurinol with hexose analogues (Impromune®).
- Other option:

22. If in the previous question you answered, "I maintain allopurinol but reduce their dosage", how much do you reduce the dosage? _____

23. In addition to all the above, do you take additional measures regarding the presence of xanthinuria?*

- Yes.
- No.

24. If you selected "Yes" in the previous question, what additional measures do you take to control xanthinuria? If you take different measures than those specified, please indicate them in the "Other option" box.

- Change to a low-purine diet (if not previously done)
- Stimulation of water consumption
- Increase consumption of wet food
- Increase in the number of controls to detect possible complications
- Other option:

25. In dogs that allopurinol was prematurely discontinued or reduced, did they experience any complications in leishmaniosis? If you have registered different complications from those specified, please indicate them in the "Other option" box.*

- I have not had complications
- Yes, relapse of clinical leishmaniosis
- Yes, positive or higher serologies for longer periods of time
- Other option:

26. In your experience, what is the estimated frequency of xanthinuria in dogs under allopurinol therapy, in your daily practice?*

- 0-5%
- 5-15%
- 15-25%
- 25-50%
- Over 50%