Allopurinol-induced xanthinuria: a prospective and retrospective study.

Author: MP Sengalrayan (BSc) Supervisor: Dr CJ Piek

Abstract

Introduction – Allopurinol is used to treat Canine Leishmaniasis (CanL), a chronic systemic disease caused by the protozoa *Leishmania infantum* and characterized by exfoliative dermatitis and renal failure. Allopurinol is a parasitistatic drug which is known to interfere with the purine metabolism of the dog, causing xanthinuria as a side effect. Few studies are conducted investigating xanthinuria in dogs with CanL. Aim of this research was to determine the incidence of xanthinuria and find possible correlations with dosage, length of treatment, specific gravity and pH in dogs with CanL. Another aim was to investigate the effect of storage time on xanthinuria. The final aim was to investigate the incidence of xanthine-uroliths.

Material and methods – Prospective study: Urine samples of client-owned dogs with CanL receiving allopurinol treatment were collected and limited urinalysis including specific gravity, pH and sediment analysis was performed within 3 hours after sample collection. Urinalysis was repeated after 24h and 48h. Retrospective study: Medical records of 145 dogs with CanL were retrospectively reviewed. Dogs were included if medical records reported at least one complete urinalysis performed during allopurinol treatment. In order to investigate the incidence of xanthine-uroliths, all included dogs were screened on reports of abdominal ultrasounds performed during allopurinol treatment.

Results – Prospective study: A total of 10 dogs were included in this study. Six out of 10 dogs (60%) presented with xanthinuria. Minimum dosage of allopurinol and minimum length of treatment resulting in xanthinuria was 16,2 mg/kg/day and 68 days, respectively. There was a correlation found between dosage and incidence of xanthinuria (p=0,015). Strong correlations were found for both specific gravity and pH between day 1, 2 and 3 (p=<0,001 for all). Amount of xanthine was significantly different between day 1 – day 2 (p=0,047) and day 1 – day 3 (p=0,031). Retrospective study: A total of 57 dogs were included in this study. Thirty-three out of 57 dogs (58%) presented with xanthinuria during at least one urinalysis. Minimum dosage of allopurinol and length of treatment resulting in xanthinuria was 15 mg/kg/day and 13 days, respectively. Xanthinuria was present in 59/155 urinalyses (38%) performed during allopurinol treatment. A strong correlation was found between specific gravity and incidence of xanthinuria (p<0,001). Fifteen out of 57 dogs (26%) had reports of abdominal ultrasounds during allopurinol treatment. Xanthine-uroliths were reported in 12/15 dogs (80%) with ultrasound reports and clinical signs developed in 5/12 dogs (43%) with xanthine-uroliths.

Conclusions – An incidence of xanthinuria of 58-60% was found in this research, although an incidence of 45-55% is presumably more accurate. Because of the strong correlation between specific gravity and incidence of xanthinuria, stimulation of water intake is recommended. Because storage time significantly affected the incidence and amount of xanthine, xanthinuria present in stored samples should be re-evaluated in fresh urine. Because 80% of dogs with ultrasound reports presented with xanthine-uroliths and 43% of dogs with xanthine-uroliths on ultrasound developed clinical signs due to these uroliths, this study strongly encourages clinicians to perform an abdominal ultrasound if xanthinuria is detected.

Introduction

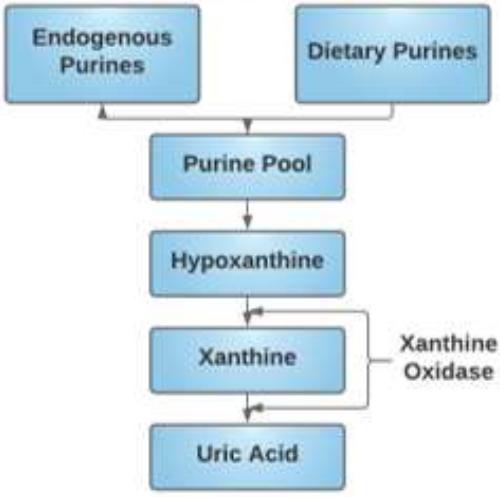
Background of the study

Canine Leishmaniasis (CanL) is a chronic systemic disease endemic in Mediterranean countries caused by the protozoa *Leishmania infantum* and characterized by exfoliative dermatitis and renal failure. A major component in disease outcome and progression is the type of immune response of dogs against *L. infantum*. A strong cellular (Th1) response usually results in a subclinical infection in dogs, while a strong humoral (Th2) response leads to dogs with clinical leishmaniasis (Alexander and Brombacher, 2012; Cortese et al., 2015). High levels of antibodies are characteristic for a strong Th2 response and may lead to auto-antibodies and antibody-antigen immune complexes causing symptoms like anaemia, thrombocytopenia, glomerulonephritis, arthritis and ear rim necrosis (Solano-Gallego et al., 2011).

Clinical canine leishmaniasis is initially treated with 20 mg/kg/day allopurinol PO for at least 6 months (or 15 mg/kg/day when glomerular filtration is reduced). When both blood- and urinalysis have normalized and all clinical symptoms are absent, it is considered a full recovery. If there is no full recovery 3 months after initiation of allopurinol treatment, therapy should be intensified with glucantime or miltefosine (Piek, 2018).

Allopurinol is a purine analog of adenosine nucleotides and is considered a parasitistatic drug. The pharmaco-dynamics of allopurinol are based on blocking RNA synthesis in *Leishmania* parasites, leading to a disruption in protein translation and resulting in inhibition of parasite multi-plication (Segarra et al., 2017). However, the pharmaco-kinetics of allopurinol create a disruption in the purine

Figure 1. Purine metabolism. Modified from Osborne et al., 2009



metabolism of the dog by inhibiting xanthine oxidase, the enzyme which converts hypoxanthine into xanthine and xanthine into uric acid (figure 1). This leads to a decrease of uric acid concentrations combined with an increase of xanthine concentrations in both serum and urine. As a result, xanthine, the least soluble purine, is excreted in large quantities in urine, which may lead to xanthinuria and the formation of xanthine-uroliths (Osborne et al., 2009; Mireaux et al., 2014; Torres et al., 2016; Piek, 2018).

Xanthinuria and xanthine-uroliths

The definition of xanthinuria is the presence of xanthine-crystals in urine sediment. The definition of xanthine-uroliths is the presence of uroliths on abdominal ultrasounds in dogs

with CanL receiving allopurinol treatment (Torres et al., 2016). So far, few studies have been conducted or published investigating the incidence of xanthinuria caused by long-term use of allopurinol in dogs. However, xanthinuria is quite commonly observed by clinicians of the UCCA (University Clinic for Companion Animals; Utrecht, the Netherlands) in dogs with CanL, most likely due to allopurinol therapy.

Osborne et al. (2009) investigated all canine uroliths submitted to the Minnesota Urolith Center between 1998 and 2007. In this study 362/373.612 (0.1%) were composed of at least 70% xanthine and at least 316/362 (87%) of these were retrieved from dogs given allopurinol. This percentage could be as high as 96% because 34/362 uroliths were submitted without a drug history. This article has no mention of CanL, but states allopurinol is commonly used to treat uroliths containing sodium and calcium salts of uric acid and ammonium urate.

Allopurinol induced xanthinuria and xanthine-uroliths

Torres et al. (2016) published a retrospective study on the medical records of 320 dogs diagnosed with CanL in an endemic area from 2009 to 2012. All dogs had been treated with 100 mg/kg meglumine antimoniate SC for 28 days and had received a mean dosage of 14,2 mg/kg allopurinol PO BID (range 7,7 – 18,8). Forty-two out of 320 (13%) dogs developed xanthinuria after receiving allopurinol treatment. The median period between initiation of treatment and presence of xanthinuria was 1 year (range 3 weeks – 9 years). Of these 42 dogs, 9 (21,4%) dogs had no further complications, 11 (26,2%) also developed nephrolithiasis, 9 (21,4%) also developed urolithiasis and 13 (31%) developed both urolithiasis and nephrolithiasis. Of these 42 dogs, 19 (45,2%) developed clinical signs regarding the urinary tract.

Segarra et al. (2017) conducted a prospective study including 29 naturally infected dogs with clinical leishmaniasis receiving 10 mg/kg allopurinol PO BID during 180 days and 50 mg/kg N-methylglucamine antimoniate SC BID for the first 28 days. Urinalyses including sediment analyses were performed at day 0, 30 and 180 after treatment onset. Zero out of 29 dogs presented with xanthinuria at day 0, 10/29 (34,5%) dogs presented with xanthinuria at day 30 and 2/29 (6,9%) additional dogs presented with xanthinuria at day 180 of the study. So a total of 12/29 (41,3%) dogs developed xanthinuria during this study.

Effects of storage time and temperature on crystal formation

Albasan et al. (2003) investigated the effects of storage time and temperature on the number of crystals in urine samples of 31 dogs. In vitro calcium oxalate (CaOx) crystals were formed at room temperature (20°) in 1/9 and 2/9 samples after 6 and 24 hours, respectively. In refrigerated samples (6°C) in vitro CaOx crystals were formed in 4/9 and 9/9 after 6 and 24 hours, respectively. In vitro crystals formed significantly less frequent at room temperature. In vitro formation of magnesium ammonium phosphate (struvite) was also observed in two dogs at both storage times and temperatures. This study also reported storage time and temperature had no significant effect on urine pH or specific gravity.

Risk factors of nephrolithiasis in men

Daudon et al. (2017) described known risk factors of nephrolithiasis in men. Factors which may also apply to allopurinol therapy in dogs are abnormally low or high urine pH, low urine output, high daily dose of drug, long-standing treatment, high urinary excretion of the drug or its metabolites and low aqueous solubility of the drug or its metabolites. Allopurinol is known to induce xanthine-uroliths in men, and prevention is based on administering the lowest effective dosage and performing regular surveillance of xanthinuria.

Aim of the research

The first aim of both the prospective and the retrospective study was to investigate the incidence of xanthinuria in dogs with CanL receiving allopurinol treatment. The second aim of both studies was to investigate possible correlations between xanthinuria and dosage or length of treatment in dogs with CanL receiving allopurinol treatment. The third aim of both studies was to investigate possible correlations between xanthinuria and specific gravity or pH in dogs with CanL receiving allopurinol treatment. The fourth aim of the prospective study was to investigate interobserver bias. The fifth aim of the prospective study was to determine the effects of storage time on xanthine formation. The fourth aim of the retrospective study was to investigate incidence of xanthine-uroliths in dogs with CanL receiving allopurinol treatment.

Material and methods

Prospective study

Urine samples of client-owned dogs with CanL of any age, breed or gender were collected at the UCCA from March to July of 2021. Inclusion criteria were: (1) diagnosis of CanL established by clinical signs and a positive immunoassay or direct observation of amastigotes in biopsies and (2) treatment with allopurinol for at least 30 days prior to sampling. Immunoassays included DAT (Direct Agglutination Test; Sykes, 2013), ELISA (Enzyme-Linked Immuno-Sorbent Assay; Sykes, 2013) and IFA (Immuno-Fluorescent Assay; Sykes, 2013) antibody titres. Dogs carrying co-infections with other vector-borne diseases (*Dirofilaria immitis, Ehrlichia canis, Anaplasma phagocytophilum, Hepatozoon canis*) or receiving intensified treatment with meglumine antimoniate (Glucantime®, Merial) were included. All urine samples were collected by students during or owners prior to outpatient consultation. All owners except one signed an informed consent. The first owner did provide verbal consent, but an informed consent-form was unavailable at the time.

All urine samples were collected during spontaneous micturition and were then transferred into a 10ml syringe. The first urinalysis was performed within 3h after collection. The second and third urinalyses were performed 24h and 48h after the first, respectively. Urinalysis was limited to determination of specific gravity using an RHC 200 ATC refractometer, determination of pH using pH indicator strips with a range from 5 to 8 and sediment analysis. The protocol of the UDVL (University Veterinary Diagnostic Laboratory) was used to obtain and analyse the urine sediment. First, the syringe of urine was gently swirled and 10ml of urine was poured into a centrifuge tube. This tube was continuously centrifuged for 5 minutes at 1500 rpm. Then, the centrifuge tube was tilted with one swift movement into another centrifuge tube and placed upright again. The approximately 0,5ml of sediment was well mixed with a pipette and two individual drops were placed on a glass slide and covered with cover glasses. Next, the glass slides were examined under the microscope at HPF (High Power Field; 10x40) for the presence of xanthine-crystals and if present quantified at LPF (Low Power Field; 10x10) (UVDL, 2016).

After every urinalysis the second centrifuge tube was poured back in the centrifuge tube with the 0,5ml of sediment, closed with a centrifuge tube cap, gently swirled again and stored at room temperature awaiting the second and third analyses. To distinguish xanthine from ammonium urate, all crystals were thoroughly optically analysed at HPF to search for irregular protrusions ("thorn-apples" or "wart-like processes") (UVDL, 2016). If present, crystals were labelled ammonium urate. If not, crystals were assumed to be xanthine, possibly leading to an overestimation of xanthine. However, ammonium urate is typically present in dogs with a portosystemic shunt and these dogs usually have neurological symptoms (Caporali et al., 2015). None of the dogs included in this study displayed neurological symptoms.

In order to investigate interobserver bias, most urinalyses were performed in duplicate by two observers T. Altena (TA) and M. Sengalrayan (MS; author). TA has been a researcher at the UVDL for several years and is responsible for a lot of urinalyses performed at the UVDL; MS is a student at the UCCA and was no expert at urinalysis prior to this study. TA mentored MS during this study, but urinalyses were performed individually at different times and locations.

Retrospective study

Medical records of 145 dogs with CanL treated at the Veterinary Teaching Hospital of the UCCA were retrospectively reviewed from January 2008 to June 2021. Data was acquired by receiving patient numbers of internal patients with a positive DAT titre for CanL at the UVDL. In some patients with CanL receiving monitoring and treatment at the UCCA, immunoassays were performed externally. Therefore the schedule of haematology between January 2008 and June 2021 was manually reviewed for additional CanL patients. Inclusion criteria were: (1) diagnosis of CanL established by clinical signs and serology (DAT, ELISA or IFA) or direct observation of amastigotes in biopsies from skin, lymph nodes, synovial fluids or bone marrow, (2) treatment with allopurinol, either combined with meglumine antimoniate (Glucantime®, Merial) or miltefosine (Milteforan®, Virbac) or as solo treatment and (3) at least one urinalysis including sediment analysis while receiving allopurinol treatment. Patients were excluded when a single urinalysis was performed around the date of diagnosis without follow-up urinalyses or when urinalyses were lacking sediment analyses. Additional urinalyses were excluded if xanthinuria was no longer present after allopurinol treatment was discontinued. In order to investigate the incidence of xanthine-uroliths, all dogs meeting the inclusion criteria were screened on reports of abdominal ultrasounds performed during allopurinol treatment.

Statistical analysis

Statistical analysis was performed using SPSS (IBM® SPSS® Statistics version 27) and a value of $p<0,05$ was used to determine significance. Data were tested for normality by the Shapiro–Wilk test. Scale and ordinal variables with normal distribution were tested for correlations using bivariate Pearson correlation; nominal variables with normal distributions were tested for correlations using Pearson Chi-Square. Means were compared using independent samples t-test with equal variances assumed. Non-parametric tests were used if data did not follow a normal distribution. Ordinal variables were tested for correlations using the Spearman's correlations test. Means were compared using the Mann Whitney U test or the Wilcoxon signed ranks test.

Results

Prospective study

Animal data

A total of 10 client-owned dogs were enrolled in this study. Population consisted of 8 crossbreeds including one crossbreed German shepherd and 2 purebreds; a Bodeguero and a German pointer. Five of 10 dogs were male neutered and 5/10 were female spayed. Median age at time of diagnosis was 3,71 years old (range 1,10 – 10,83) and median weight at time of urinalysis was 22,4 kg (range 11,4 – 31,4). All dogs originated from foreign countries, with the majority of 6 dogs from Spain, 3 dogs from Greece and one dog from Italy.

Eight out of 10 dogs had clinical signs of CanL at the time of diagnosis, the remaining two dogs were tested (mildly) positive using IFA in Spain and were treated primarily based on this titre. Additional DAT titres at the UVDL of these two dogs tested negative (<40). Despite the negative DAT titre and absence of clinical signs during outpatient consultation at the UCCA, these dogs did receive allopurinol treatment for at least 30 days prior to sampling and were therefore included in the study. Clinical signs included exfoliative dermatitis, lymphadenopathy, polyarthritis, anaemia, hypoalbuminemia and hypergammaglobulinaemia. Immuno-assays were performed in all 10 dogs, with 8 titres obtained at the UVDL using DAT and 2 titres obtained at IDEXX Laboratories using ELISA. Positive DAT titres ranged from 1/5120 to 1/40960 and positive ELISA titres ranged from 37,1 to 61,8. Biopsies of lymph nodes were performed in 5/10 dogs, with observations of *Leishmania* amastigotes in 2/5 dogs and absence of amastigotes described in 3/5 dogs.

The median dosage of allopurinol was 19,0 mg/kg (range 6,7 – 21,9). The two dogs receiving treatment based on borderline positive IFA titres received inadequate dosages of 6,7 and 13,3 mg/kg/day allopurinol at time of sampling. One dog with a positive ELISA titre and clinical signs of CanL in the past received a dosage of 9,4 mg/kg/day allopurinol at time of sampling. This dog did receive an adequate dosage of approximately 20 mg/kg for at least one year after diagnosis, but when the physician opted to discontinue treatment due to full recovery the owner was reluctant to discontinue completely, therefore treatment was continued at half the dosage. One dog received a dosage of 16,2 mg/kg/day at time of sampling because of significant weight gain in the first two months of allopurinol treatment. The dosage was altered to 20 mg/kg/day after this visit.

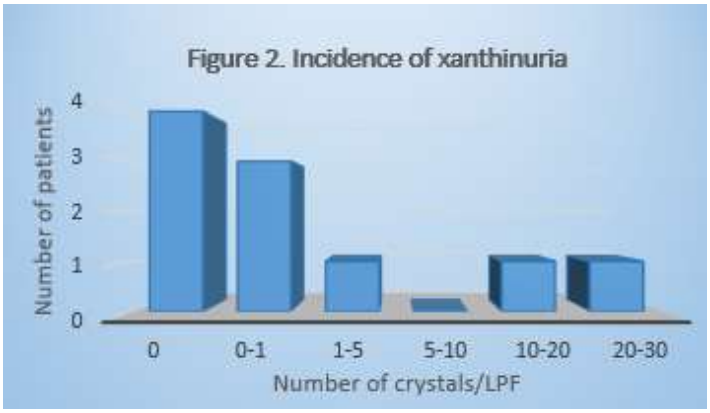
The median duration of allopurinol treatment at time of sampling was 189 days (range 43 – 1389). Four of 10 dogs were on the low end of this range with less than 100 days of treatment at time of sampling; again the two dogs receiving treatment based on the borderline positive IFAT titres and two dogs recently diagnosed with CanL based on very high DAT titres (1/40960) and extensive clinical signs. Only one dog had been continuously treated with allopurinol for almost 4 years. The remaining 5/10 dogs were receiving allopurinol for approximately 6 months to 1 year. All data was normally distributed except for length of treatment. All baseline characteristics and normality tests are summarized in table 1.

Table 1. Baseline characteristics of dogs in the prospective study. Descriptive analysis is described as median (minimum – maximum). Normality is described as yes/no (p-value). MN = male neutered, FS = female spayed, NA = not applicable, ref. = reference interval, pos. = positive.

	Prospective population	Normal distribution
Number of dogs	10	NA
Sex (MN/FS)	5/5	NA
Age at diagnosis (years)	3,71 (1,10 – 10,83)	Yes (0,100)
Weight (kg)	22,4 (11,4 – 31,4)	Yes (0,843)
Country of origin: Spain/Greece/Italy	6/3/1	NA
DAT titre	12800 (<40 – 40960)	Yes (0,074)
ELISA titre (ref. >12 = pos.)	49,45 (37,1 – 61,8)	NA
Amastigotes in lymph nodes: Yes/no/NA	2/3/5	NA
Allopurinol dose (mg/kg/day)	19,0 (6,7 – 21,9)	Yes (0,068)
Length of treatment (days)	189 (43 – 1389)	No (<0,001)
Specific gravity	1.027 (1.013 – >1.050)	Yes (0,138)
pH	6,5 (5,3 – 7,7)	Yes (0,137)

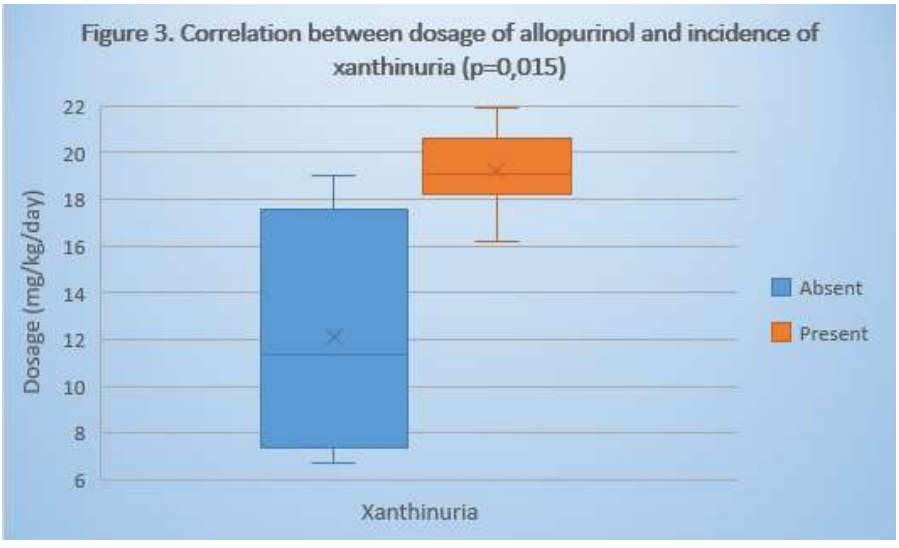
Incidence of xanthinuria

Six out of 10 dogs (60%) presented with xanthinuria; 3 urine samples with 0-1 xanthine-crystals/LPF, one with 1-5 xanthine-crystals/LPF, one with 10-20 xanthine-crystals/LPF and one with 20-30 xanthine-crystals/LPF (figure 2). In two of 10 urine samples 0-1 ammonium urate-crystals/LPF were observed; one urine sample with both xanthine- and ammonium urate-crystals and one with two ammonium urate-crystals observed on the entire glass slide. The minimum dosage of allopurinol and minimum length of treatment resulting in xanthinuria was 16,2 mg/kg/day and 68 days, respectively.



Correlation with dosage and length of treatment

There was no significant correlation found between dosage of allopurinol and amount of xanthine-crystals (p=0,119). While there was no significant correlation between dosage and *amount* of crystals, there was a correlation found between dosage and *incidence* of xanthinuria (p=0,015) (figure 3): the 4 dogs without xanthine received a mean dosage of 12,1 mg/kg/day (standard deviation (std. ± 5,3), while the 6 dogs with xanthine received a mean dosage of 19,2 mg/kg/day (std. ± 1,9). In dogs receiving dosages below 16,2 mg/kg/day no xanthine was observed, while all dogs but one receiving 16,2 mg/kg/day or more did show at least 0-1 xanthine-crystal/LPF. There was no correlation found between length of allopurinol treatment and incidence of xanthinuria (p=1,000). The 4 dogs without xanthinuria had a mean treatment length of 467 days (std. ± 630); the 6 dogs with xanthinuria had a mean treatment length of 198 days (std. ± 114).

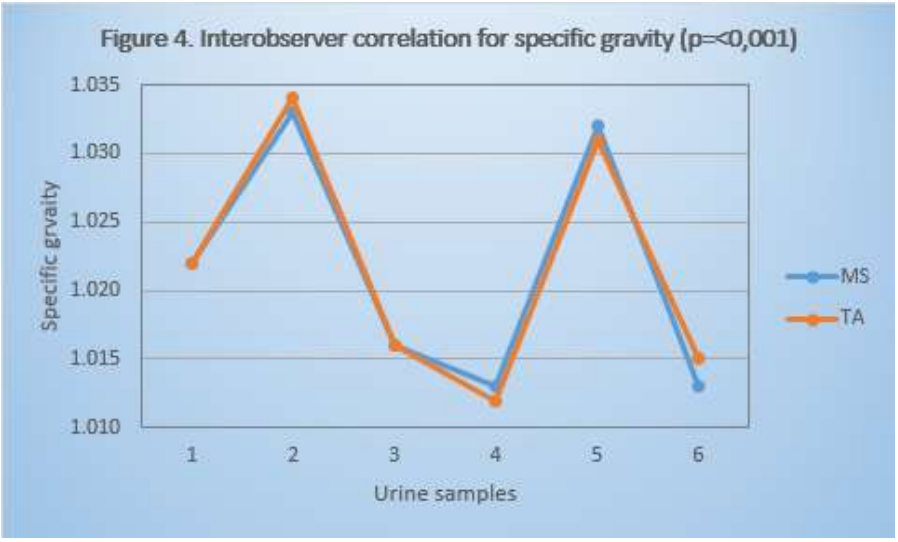


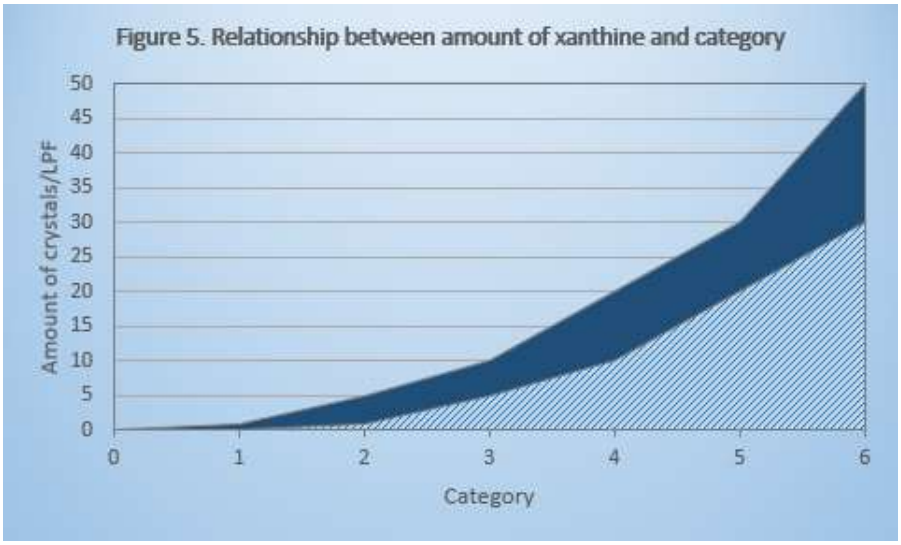
Correlation with specific gravity and pH

There was no correlation found between specific gravity and amount of xanthine-crystals (p=0,359) or incidence of xanthinuria (p=0,392). The 6 dogs with xanthinuria had a mean specific gravity of 1.023 (std. ± 3,3) and the 4 dogs without xanthinuria had a mean specific gravity of 1.031 (std. ± 9,5). One urine sample with a specific gravity of 1.013 showed 1-5 xanthine-crystals per LPF, while the two samples with the highest specific gravities (1.044 and >1.050) presented without xanthinuria. The sample with the highest amount of xanthine-crystals (20-30/LPF) had a specific gravity of 1.022. There was no correlation found between pH and amount of xanthine-crystals (p=0,819) or incidence of xanthinuria (p=0,176). The 6 dogs with xanthinuria had a mean pH of 6,2 (std. ± 0,7) and the 4 dogs without xanthinuria had a mean pH of 6,8 (std. ± 0,6). The two urine samples with the highest amount of xanthine-crystals (10-20/LPF and 20/30/LPF) both had a pH of 6,5, but three urines without xanthinuria also had a pH of 6,5.

Interobserver bias

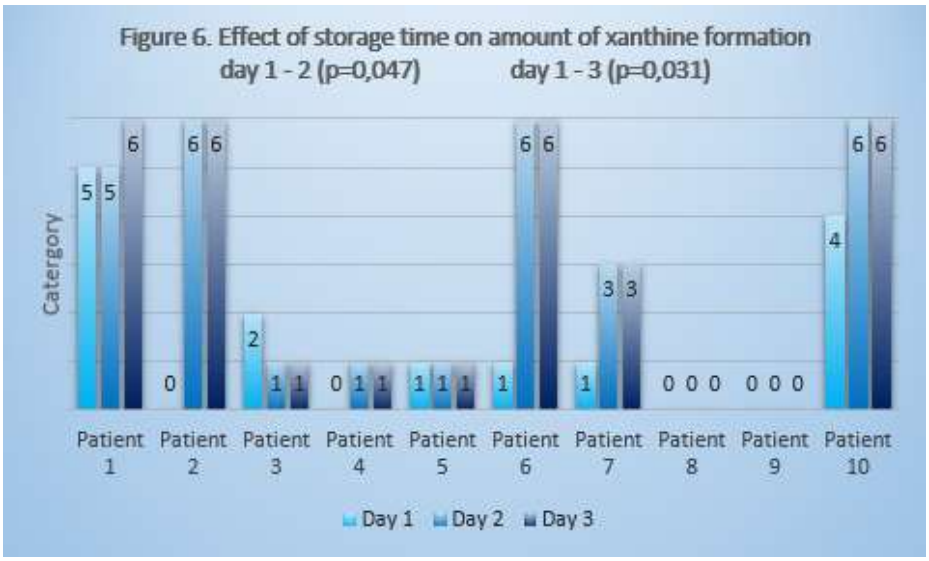
Seven out of 10 urinalyses were performed by two observers. Six out of 7 urinalyses were performed using the same urine samples equally divided into two; the 7th urinalysis was performed on different samples of the same dog. A strong interobserver correlation of 0,994 was found for specific gravity for the 6 urinalyses performed on identical samples (p=<0,001) (figure 4). An interobserver correlation of 0,912 for pH was found for the 6 urinalyses performed on identical samples (p=0,011). When xanthinuria was seen by the UVDL it was noted as present (without further quantification), therefore inter-observer correlation for amount of xanthine-crystals was impossible. Incidence of xanthinuria between observers was not significantly different (p=0,157); MS described xanthinuria in 6/7 samples while AT described xanthinuria in 4/7 samples, with the same outcome per patient in 5/7. The two samples with different outcomes were both from identical samples and MS quantified the amount of xanthine as 0-1 and 1-5 crystals/LPF.





Effect of storage time on crystal formation

All 10 urine samples were included. Strong correlations were found for both specific gravity and pH between day 1, 2 and 3 (p=<0,001 for all). Correlations for specific gravity were: day 1 – 2 = 1.000; day 1 – 3 = 0,999; day 2 – 3 = 0,999. Correlations for pH were: day 1 – 2 = 0,974; day 1 – 3 = 0,976; day 2 – 3 = 0,979. Amount of xanthine-crystals was converted into ordinal variables by creating categories that have an exponential relationship with the amount of crystals (figure 5). The mean category on day 1 was 1,40 (std. ± 1,776); on day 2 was 2,90 (std. ± 2,601) and on day 3 was 3,00 (std. ± 2,708). Differences between day 1 – 2 (p=0,047) and day 1 – 3 (p=0,031) were significant (figure 6). A strong correlation of 0,981 for amount of xanthine-crystals was found between day 2 – 3 (p=<0,001).



Retrospective study

Animal data

A total of 57 dogs with CanL met all inclusion criteria. These 57 dogs had reports for a total of 207 urinalyses including 155 urinalyses during allopurinol treatment. Median number of urine samples per dog was 2 (range 1 – 10). Thirty-nine out of 57 dogs were defined as cross breed including three dogs defined as cross breed (German) shepherd or cross breed Labrador retriever. The other 18 dogs were defined as Boxer (N=3), Podenco (N=3) and one of each: Leonberger, Groenendael, Australian shepherd, German shepherd, Portuguese water dog, Welsh Springer spaniel, Breton spaniel, spaniel type unknown, German pointer, Jack Russel terrier, Fox terrier and pug. Population contained 30 males (19 neutered; 11 intact) and 27 females (25 spayed; 2 intact).

Median age at time of diagnosis was 3,72 years old (range 0,58 – 12,15) and median weight was 17,3 kg (range 5,3 – 45,0). All dogs but one either originated from or travelled to a foreign country, with the majority of 49 dogs originating from: Spain (N=33), Greece (N=7), Portugal (N=4), Italy (N=3),Turkey (N=1) and unknown (N=1). The remaining 7 dogs travelled to one or more of the following countries: Spain (N=5), France (N=3), Italy (N=1), Swiss (N=1) and Austria (N=1). The one exception neither originating from nor travelling to a foreign country was known to lick the wounds of its Leishmania-positive Greece housemate.

Table 2. Baseline characteristics of dogs in the retrospective study. Descriptive analysis is described as mean ± standard deviation or median (minimum – maximum). MI = male intact, MN = male neutered, FI = female intact, FS = female spayed, ref = reference interval, NA = not applicable.

	Retrospective population	Normal distribution
Number of dogs	57	NA
Urinalyses during allopurinol treatment/total urinalyses	155/207	NA
Urine samples per dog	2 (1 – 11)	No (p<0,001)
Breed (purebred/crossbreed)	18/39	NA
Sex: male/female (MN/MI) (FS/FI)	30/27 (19/11) (25/2)	NA
Age at diagnosis (years)	3,72 (0,58 – 12,15)	No (p<0,001)
Weight (kg)	17,3 (5,3 – 45,0)	No (p=0,003)
Country of origin: Spain/Greece/Portugal/Italy/Turkey/NA	33/7/4/3/1/1	NA
DAT titre	5120 (320 – 81920)	No (p<0,001)
ELISA titre (ref. >12 = pos.)	61,8 (15,6 – 81,1)	Yes (p=0,495)
IFT titre (ref. >320 = pos.)	>3200	NA
Amastigotes in lymph nodes: Yes/no/NA	26/16/15	NA
Allopurinol dosage (mg/kg/day)	20 (10 – 40)	No (p<0,001)
Length of treatment (days)	217 (6 – 2021)	No (p<0,001)
Specific gravity	1.025 (1.006 – >1.050)	No (p<0,001)
pH	6,5 (5,0 – 9,0)	No (p<0,001)

All 57 dogs had clinical signs of CanL at time of diagnosis. Signs included: lethargy, emaciation, stiff gait or lameness, polyuria/polydipsia, exfoliative dermatitis, lymphadenopathy, polyarthritis, glomerulonephritis, anaemia, neutropenia, thrombocytopenia, hypoalbuminemia, hypergammaglobulinaemia, uraemia, increased blood creatinine, increased liver values (ALT) and proteinuria.

On 54/57 dogs serology was performed with 46/54 titres obtained at the UVDL using DAT, 7/54 obtained at IDEXX Laboratory using ELISA and 1/54 obtained at unknown using IFA (titre >1/3200). DAT titres ranged from 1/320 to 1/81.920 with 30/46 dogs with a titre of 1/5120. ELISA titres ranged from 15,5 to 81,1 with a median of 61,8. In two of the three dogs without titres amastigotes were directly observed to confirm diagnosis. In the last dog serology was performed by the referring veterinarian and described as positive, however the level of the titre and the immunoassay used remains unknown. In 42/57 dogs cytology was performed, with observations of *Leishmania* amastigotes in 26 dogs and absence of amastigotes described in 16 dogs.

Median dosage of allopurinol was 20 mg/kg/day (range 10 – 40). Exact calculations of dosage were impossible since weight slightly shifted during allopurinol treatment. Forty-seven out of 57 dogs received a dosage of approximately 20 mg/kg/day. Six out of 57 dogs received a dosage of approximately 15 mg/kg/day because of reduced glomerular filtration. Two out of 57 dogs received a dosage of approximately 10 mg/kg/day and the last 2 dogs received a dosage of approximately 40 mg/kg/day. Median length of treatment for all 155 urinalyses performed during allopurinol treatment was 217 days (range 6 – 2021). Eleven out of 57 dogs had urinalyses performed after allopurinol treatment was discontinued. Median length of time between last allopurinol dose and urinalysis was 230 days (range 63 – 1126). All data was abnormally distributed except for ELISA titres. All baseline characteristics and normality tests are summarized in table 2.

Incidence of xanthinuria

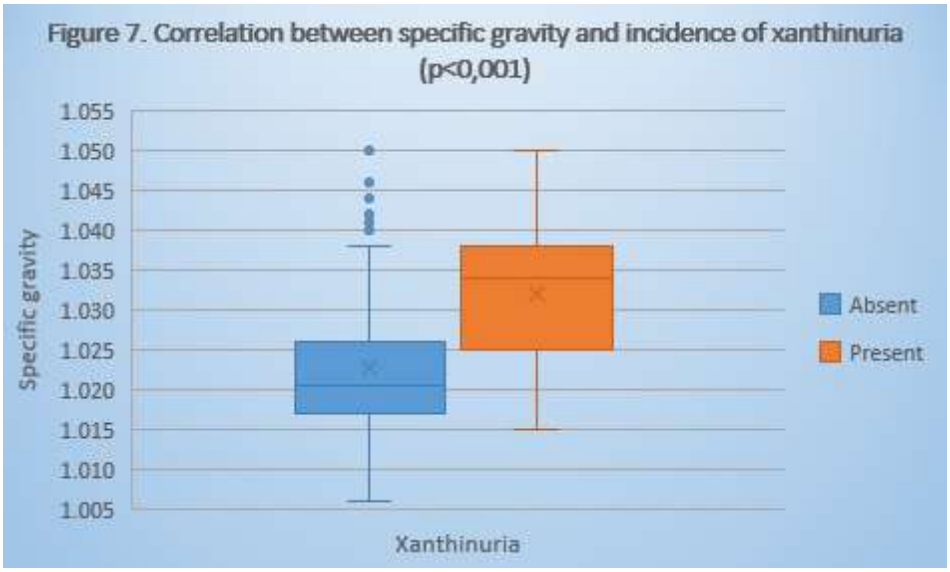
Thirty-three out of 57 dogs (58%) presented with xanthinuria at least once. Minimum dosage of allopurinol and length of treatment resulting in xanthinuria was 15 mg/kg/day and 13 days, respectively. There was a wide variety in incidence of xanthinuria per dog in between its samples. For example, one dog with 9 urinalyses (6 during allopurinol treatment) only presented with xanthinuria once, while another dog with 9 urinalyses (7 during allopurinol treatment) presented with xanthinuria in all samples taken during allopurinol treatment. Xanthinuria was present in 59/155 urine samples taken during allopurinol treatment. All 52 urine samples taken before or after allopurinol treatment presented without xanthinuria. Two dogs presenting with xanthinuria during allopurinol treatment had normalized urinalyses on day 225 and 230 after treatment was discontinued.

Correlation with dosage and length of treatment

There was no correlation found between dosage of allopurinol and incidence of xanthinuria (p=0,300); the 24 dogs without xanthinuria received a mean dosage of 19,50 mg/kg/day (std. ± 5,209), while the 33 dogs with xanthinuria received a mean dosage of 20,15 mg/kg/day (std. ± 3,850). Four out of 6 dogs (67%) receiving a dosage of 15 mg/kg/day due to reduced glomerular filtration presented with xanthinuria. Thirty out of 49 dogs (61%) receiving a dosage of 20 mg/kg/day presented with xanthinuria. One out of 2 dogs (50%) receiving a dosage of 40 mg/kg/day presented with xanthinuria.

There was no significant correlation found between length of allopurinol treatment and incidence of xanthinuria (p=0,064); the 96 urine samples without xanthinuria belonged to dogs with a mean treatment length of 468,70 days (std. ± 457,512), while the 59 urine samples with xanthinuria belonged to dogs with a mean treatment length of 326,75 days (std. ± 402,051). Both extremes in length of treatment resulting in xanthinuria were observed. Five out of 33 dogs presented with xanthinuria after only 23 days of allopurinol treatment or less. Two of these dogs were on their second

treatment due to relapse of clinical CanL, but the other 3 presented with xanthinuria after only 13, 16 or 18 days of allopurinol treatment. Three out of 33 dogs presented with xanthinuria after 82 days of allopurinol treatment or more, while previous urinalysis at day 28, 40 or 48 of treatment did not show xanthinuria. One dog without xanthinuria on day 103 of treatment did show xanthinuria on day 193 and another dog without xanthinuria on day 132 of treatment did show xanthinuria on day 405.



Correlation with specific gravity and pH

A strong correlation was found between specific gravity and incidence of xanthinuria (p<0,001) (figure 7); the 96 urine samples without xanthinuria had a mean specific gravity of 1.022,88 (std. ± 9,726), while the 59 urine samples with xanthinuria had a mean specific gravity of 1.032,15 (std. ± 8,986). This strong correlation was also found in the 6 dogs receiving 15 mg/kg/day (p<0,001): the 18 urine samples without xanthinuria had a mean specific gravity of 1.016,89 (std. ± 4,626), while the 5 urine samples with xanthinuria had a mean specific gravity of 1.033,20 (std. ± 4,087). There was no correlation found between pH and incidence of xanthinuria (p=0,516); the 96 urine samples without xanthinuria had a mean pH of 6,760 (std. ± 0,8795), while the 59 urine samples with xanthinuria had a mean pH of 6,703 (std. ± 0,9055). There was also no correlation found between pH and incidence of xanthinuria when only the dogs receiving 15 mg/kg/day were taken into account (p=0,445).

Incidence of xanthine-uroliths

Fifteen out of 57 dogs (26%) had reports of abdominal ultrasounds during allopurinol treatment. Uroliths were reported in 12/15 dogs (80%) with ultrasound reports (table 3). All 12 dogs presented with nephrolithiasis; 5 dogs also presented with urolithiasis and 4 dogs also presented with debris in the bladder. Five out of 12 dogs (42%) with uroliths developed clinical signs: three dogs experienced (recurrent) cystitis due to urolithiasis, one dog experienced an urethra obstruction a few months after confirmed xanthine-uroliths were removed from the bladder and one dog experienced polyuria/ polydipsia, uraemia and azotaemia due to extensive nephrolithiasis. Four out of 12 dogs (33%) with uroliths never presented with xanthinuria, while all four dogs had reports of at least two urinalyses during allopurinol treatment.

Discussion

Research questions

This research investigated the adverse effects of allopurinol treatment in dogs with CanL: the incidence of xanthinuria and its consequence; xanthine-urolith formation in the urinary tract. This research consisted of two independent studies: a prospective and a retrospective study with an incidence of xanthinuria of 60% and 58%, respectively. More importantly, in the retrospective study following dogs in time xanthine-uroliths were discovered in 80% of the dogs with ultrasound reports and these uroliths were causing clinical signs in 43% of those dogs.

Incidence of xanthinuria in the prospective study was 60% (6/10 dogs). Segarra et al. (2017) described an incidence of xanthinuria of 41,3% (12/29 dogs) in a prospective study. The present study might have overestimated the incidence of xanthinuria, since MS was inexperienced and highly motivated to find xanthine-crystals. Segarra et al. (2017) performed three serial urinalyses on each dog at 0, 30 and 180 days of allopurinol treatment, resulting in two urinalyses per dog during treatment. Therefore, the incidence described by Segarra et al. (2017) is presumably more accurate.

Incidence of xanthinuria in the retrospective study was 58% (33/57 dogs). Torres et al. (2016) described an incidence

Table 3. Summary of dogs with xanthine-uroliths present on abdominal ultrasound. *Number of urinalyses presenting with xanthinuria; **Number of urinalyses during allopurinol treatment; ***Polyuria/polydipsia.

	Xanthinuria* / Urinalyses**	Location(s) of uroliths on ultrasound	Clinical signs
Patient 1	0 / 3	Nephrolithiasis + urolithiasis	Yes (urethra obstruction)
Patient 2	2 / 6	Nephrolithiasis + debris in the bladder	No
Patient 3	0 / 2	Nephrolithiasis	No
Patient 4	0 / 4	Nephrolithiasis	Yes (PU/PD*** + uraemia + azotaemia)
Patient 5	1 / 7	Nephrolithiasis + urolithiasis	Yes (recurrent cystitis)
Patient 6	2 / 4	Nephrolithiasis + urolithiasis	Yes (recurrent cystitis)
Patient 7	7 / 7	Nephrolithiasis + urolithiasis	No
Patient 8	2 / 2	Nephrolithiasis + urolithiasis	No
Patient 9	0 / 4	Nephrolithiasis + debris in the bladder	No
Patient 10	1 / 1	Nephrolithiasis + debris in the bladder	No
Patient 11	1 / 1	Nephrolithiasis + debris in the bladder	No
Patient 12	3 / 8	Nephrolithiasis + urolithiasis	Yes (recurrent cystitis)

of xanthinuria of only 13,1% (42/320) in a retrospective study about the adverse urinary effects of allopurinol in dogs with CanL. Torres et al. (2016) mainly focused on the 42 dogs with xanthinuria and how many of them developed xanthine-uroliths and clinical signs of urinary obstruction and dysuria. The other 278 dogs in the study were only used to calculate the incidence of xanthinuria. Both present study and Torres et al. (2016) had no influence in the level of experience of the observer performing urinalyses in recognizing xanthine-crystals. Present study has therefore assumed all ammonium urate-crystals reported to be xanthine-crystals, which lead to a much higher incidence of xanthinuria. Torres et al. (2016) has made no statement about the possibility that xanthine-crystals might have been reported as ammonium urate crystals, which could have led to a substantial under-estimation. Therefore, incidence in the present study is presumably more accurate. After considering all ifs and buts, the exact incidence of xanthinuria is presumably 45 – 55%.

The prospective study including 10 dogs found a significant correlation between dosage of allopurinol and incidence of xanthinuria. This correlation was expected to be found since Daudon et al. (2017) described a high daily dose of drug as a risk factor for nephrolithiasis in men. Despite the significant correlation between dosage of allopurinol and *incidence* of xanthinuria, the same correlation was not found between dosage of allopurinol and *amount* of xanthine-crystals. However, one dog in this study mistakenly received 40 mg/kg/day for 45 days approximately 150 days prior to sampling and this dog presented with the highest amount of xanthine-crystals during urinalysis (20-30 crystals/LPF). This might be a coincidence, but it could be that a correlation was left undiscovered due to small sample size.

This correlation between dosage of allopurinol and incidence of xanthinuria was not found in the retrospective study including 57 dogs. In this case, the prospective study was more reliable, since all dogs received one urinalysis at a certain point in time and the exact dosage was calculated based on current weight. Some dogs received inadequate dosages which could have led to a bias, but the Shapiro-Wilk test revealed a normal distribution. In the retrospective study, most dogs had multiple reports of urinalyses and had received allopurinol treatment for a long period of time with weight shifts throughout this time. Calculating the exact dosage of allopurinol per urinalysis was therefore impossible.

In the prospective study, no xanthinuria was observed in dogs receiving dosages below 16,2 mg/kg/day, while all dogs but one receiving 16,2 mg/kg/day or more did present with xanthinuria. This might indicate a threshold in dosage of allopurinol for xanthinuria to appear, although current sample size is too small for definite conclusions. Interestingly, the one dog not presenting with xanthinuria despite receiving a dosage of 19 mg/kg/day is the same dog who has been receiving daily allopurinol treatment for almost 4 years. This might indicate some dogs are not susceptible for developing xanthinuria.

Both the prospective and retrospective study found no correlation between length of treatment and incidence of xanthinuria. This was somewhat unexpected since Daudon et al. (2017) described long-standing treatment as a risk factor for nephrolithiasis in men. However, incidence of xanthinuria and urolith formation is not necessarily the same. In men, allopurinol is mainly converted into oxipurinol, a metabolite which also inhibits xanthine oxidase. Oxipurinol is eliminated unchanged in urine, but is then largely recovered via tubular resorption (Accord Healthcare, 2020). This could explain the lack of correlation between incidence of xanthinuria and length of treatment: the tubular resorption leads to a high plasma concentration and a low urine concentration of oxipurinol. Therefore xanthine oxidase is largely inhibited inside the kidneys (leading to accumulation of xanthine and possibly uroliths), but this inhibition of xanthine oxidase is less substantial inside the rest of the urinary tract. If the same mechanisms can be extrapolated to dogs, this could explain why xanthinuria is not always present in dogs with CanL despite long-standing allopurinol treatment. Of course in combination with differences in urine composition because of, for example, water intake.

Both the prospective and retrospective study found no correlation between urine pH and incidence of xanthinuria. This was somewhat unexpected since Daudon et al. (2017) described an abnormally low or high urine pH as a risk factor for nephrolithiasis in men. According to Daudon and Frochet (2015) formation of xanthine-crystals is only moderately dependent on urine pH. This article also states the average pH which results in xanthinuria is 5,6 with a range of 4,8 to 7,2. Present studies had a median pH of 6,5 (range 5,3 – 7,7) and 6,5 (range 5,0 – 9,0). Both statements by Daudon and Frochet (2015) could explain the lack of correlation in the present studies: urine pH only moderately influences xanthine-crystal formation and most urine pH's found in the prospective and retrospective study were within the range when xanthine-crystals are formed.

The retrospective study including 155 urine samples found a strong correlation between specific gravity and incidence of xanthinuria. This correlation was expected since Daudon et al. (2017) described low urine output as a risk factor in urolith formation in men. Low urine output is usually the result of dehydration, which means urine will be highly concentrated expressing itself in a high specific gravity. That a high xanthine-concentration in urine would lead to more formation of xanthine-crystals makes sense, because with more xanthine-molecules in a small volume these molecules are more likely to interact with each other and less likely to be interrupted by water-molecules. Similar to the theory that refrigeration facilitates crystal-formation because water molecules are less likely to flow and disrupt the attraction between ions that are capable of forming crystals (Albasan et al., 2003).

This correlation between specific gravity and incidence of xanthinuria was not found in the prospective study including 10 urine samples. In this case, the retrospective study is more reliable because of the much larger sample size. Because the data of the retrospective study was not normally distributed, nonparametric tests were used and revealed a strong correlation ($p<0,001$). The prospective study was too small to reveal this correlation: post-hoc power of the prospective study is less than 16% ($\alpha=0,05$; $\nu=9$; $p=0,329$; Lenth, 2007). Because of the strong correlation between specific gravity and incidence of xanthinuria, stimulation of the water intake of dogs with CanL receiving allopurinol treatment is recommended.

In the prospective study, an interobserver bias for incidence of xanthinuria was not significant, but MS observed xanthinuria in 2 samples where TA did not. MS was inexperienced in urinalyses and spent about 30-45 minutes investigating one glass slide with a high motivation to find xanthinuria; TA has years of experience in urinalyses and spent approximately 5-10 minutes per glass slide with no preference whether or not to find xanthinuria. However, MS consulted TA on each possible xanthinuria (either by direct observation or by a picture taken through the microscope) and TA has confirmed all samples labelled as xanthinuria by MS. MS performed urinalysis within 3 hours after sample collection and samples were stored at room temperature in the

meantime; TA performed urinalyses within 24 hours after collection and samples were stored in a refrigerator in the meantime. Three options remain: (1) MS has wrongfully accused two dogs of presenting with xanthinuria, (2) MS has been more thorough in searching for xanthinuria, although one xanthine-crystal on an entire glass slide might be irrelevant or (3) xanthinuria dissolved during refrigeration. Option 1 is unlikely since TA confirmed all cases; option 2 is definitely possible; option 3 is unlikely since refrigeration is known to facilitate crystal formation because water molecules are less likely to flow and disrupt the attraction between ions that are capable of forming crystals (Albasan et al., 2003).

The prospective study found a significant effect of storage time on xanthinuria. A storage time of 24 hours or more may inaccurately indicate the amount and even presence of xanthine-crystals. Six out of 10 urine samples increased in amount of xanthine after 24 hours or more: two of them increased by one category, two increased by two categories and the last two samples increased by 5 or 6 categories. Intra-observer bias could result in small differences, but current data requires another explanation. Three theories might explain this phenomenon: (1) some urine samples were supersaturated with xanthine and something (temperature decrease (body to room), being centrifuged and remerged, etc.) caused a chain reaction resulting in in vitro formation of xanthine-crystals; (2) some urine samples were supersaturated with hypoxanthine (which is highly soluble); xanthine oxidase was also present in small amounts and this enzyme facilitated conversion from hypoxanthine into xanthine resulting in in vitro formation of xanthine-crystals (Pais et al., 2006); (3) some urine samples contained bacteria able to produce urease and a growth of these bacteria resulted in high concentrations of ammonia (Albasan et al., 2003).

Theory 1 would indicate high amounts of xanthine were already present in vivo, while theory 2 would indicate xanthine was formed in vitro. If theory 1 is correct and xanthine-crystals are able to form and dissolve in vitro, the same could be possible in vivo, meaning optic sediment analysis might be inaccurate in predicting xanthine-urolith formation. If theory 2 is correct, xanthine itself is not supersaturated in vivo and optic sediment analysis is in fact accurate in predicting urolith formation. In order to discriminate between theory 1 and 2, further research is necessary involving quantitative analysis of hypoxanthine- and xanthine-concentration in urine samples followed in time. Theory 3 relied on the thought that high concentrations of ammonia could lead to formation of ammonium urate, but the truth is ammonia increases pH and forms ammonium phosphate (struvite) crystals (Trinchieri, 2014), so this theory can be rejected since struvite crystals do not resemble xanthine-crystals and pH remained fairly stable. Because storage time significantly affected the incidence and amount of xanthine-crystals, xanthinuria present in stored samples should be re-evaluated in fresh urine.

The retrospective study was originally designed to investigate the incidence of xanthinuria and xanthine-uroliths, because knowing the odds of xanthinuria turning into xanthine-uroliths is much more valuable than incidence of xanthinuria alone. There used to be an additional inclusion criteria for this study: at least one abdominal ultrasound performed during allopurinol treatment. This inclusion criteria was deleted because dogs presenting with xanthinuria rarely had an ultrasound examination performed to investigate possible xanthine-uroliths: 70% of dogs (22/33) who presented with xanthinuria at least once never had an abdominal ultrasound performed. Maintaining the original inclusion criteria would have led to a small sample size of only 15 dogs instead of 57.

Although abdominal ultrasounds were only performed on a few dogs, the present study found 80% of dogs (12/15) had developed xanthine-uroliths. This high incidence might be the result of an inclusion bias, since most dogs with ultrasound reports might have had a specific reason for the ultrasound. However, in a retrospective study performed by Torres et al. (2016) about the adverse urinary effects of allopurinol in dogs with CanL there was a similar incidence found with 79% of dogs (33/42) with xanthinuria which had developed xanthine-uroliths on ultrasound. Even more importantly, the present study found these xanthine-uroliths caused clinical signs in 42% of the dogs (5/12). Comparably, Torres et al. (2017) found 45% of dogs (19/42) presented with clinical signs of urinary obstruction or dysuria: 17 dogs with xanthine-uroliths present on abdominal ultrasound and 2 dogs with clinical signs due to severe xanthinuria. Therefore the present study strongly encourages clinicians who treat dogs with CanL to start monitoring xanthine-uroliths with abdominal ultrasounds when dogs present with xanthinuria because of serious concern for patient wellbeing.

Another notable fact about the dogs with xanthine-uroliths on abdominal ultrasounds, 33% of the dogs (4/12) never presented with xanthinuria, despite having reports of at least two urinalyses during allopurinol treatment. This lack of xanthinuria while xanthine-uroliths are present, is likely to be the result of the tubular resorption of oxipurinol (Accord Healthcare, 2020), as previously described. This could indicate there is a poor correlation between the incidence of xanthinuria and the formation of xanthine-uroliths. Another factor possibly contributing to the lack of xanthinuria observed in these dogs was the level of experience of the observer performing urinalyses in recognizing xanthine-crystals (explanation of this statement will follow later). In order to investigate a possible correlation between xanthinuria and xanthine-uroliths, further research is needed. This research would preferably be a prospective study involving newly diagnosed dogs with CanL undergoing abdominal ultrasounds in addition to urinalyses at fixed time intervals, for example at day 0, 60 and 180 of allopurinol treatment.

One dog with clinical signs caused by xanthine-uroliths experienced polyuria/polydipsia, uraemia and azotaemia due to the consequences of extensive nephrolithiasis. These clinical signs could have been caused by CanL itself, but this was deemed unlikely since values of creatinine and urea had been within normal limits during previous blood analyses and all clinical signs (exfoliative dermatitis, lymphadenopathy, hypergammaglobulinaemia and proteinuria) had disappeared or stabilized and not recurred by the time this renal failure was discovered. This dog had a moderate to severe proteinuria (glomerulonephritis) at the time of diagnosis which quickly stabilized into a mild proteinuria after allopurinol treatment was initiated. The proteinuria always remained mild thereafter, but the creatinine- and urea-values had increased after 3 years of allopurinol treatment without an increase in gammaglobulines or any other indication the CanL had recurred.

The prospective study contained several weaknesses. First of all, sample size is relatively small and power-analysis prior to the study concluded a sample size of 30 dogs would lead to a power of 85%. Ideally sample size would have been larger, but unfortunately only 10 suitable dogs visited the UCCA during data gathering (which has been extended by 3 weeks to include 3 additional dogs). Most dogs were infected with other vector borne diseases besides CanL and received

treatment for these diseases. Most dogs also received intensified treatment for CanL. Both could lead to alterations in urine composition. Two dogs were included despite not showing clinical signs of CanL and receiving inadequate dosages of allopurinol, leading to larger variations of baseline characteristics. Despite these differences, all dogs were included in order to obtain a larger sample size and the highest possible power.

The retrospective study also contained several weaknesses, mostly due to its retrospective nature which made any standardisation impossible. There were no fixed time intervals between urinalyses, urinalyses were performed by an unknown number of observers with unknown level of experience in recognizing xanthinuria and xanthinuria was not quantified. Exact calculations of dosage of allopurinol at every urine sample was impossible, because exact weights were not reported at every urinalysis.

Notable issues

The prospective and retrospective study contained 60% (6/10) and 67% (38/57) of dogs originating from Spain, respectively. These high numbers of dogs could be the result of either a higher prevalence of CanL in Spain or a higher level of imports from Spain to the Netherlands. The latter would mean these studies contained a biased population. Ninety-eight out of 225 dogs (43,6%) imported from Mediterranean countries originated from Spain (unpublished data). In 217/225 dogs DAT titres were obtained with 32/217 positive titres (14,7%). Twenty-one out of 32 titres (65,6%) belonged to dogs originating from Spain. So while the overall prevalence of CanL was 14,7%, the prevalence in dogs originating from Spain was 21,6% with 21/97 positive titres (unpublished data). Therefore, the truth is likely a combination of both: relatively more dogs are imported from Spain and Spain has a higher prevalence of CanL.

Two dogs included in the prospective study were treated with allopurinol based on a borderline positive IFA titre performed in Spain. Three scenarios might lead to this borderline positive titre: (1) test outcome was a false positive, (2) the dogs were infected with *Leishmania* but experienced subclinical infections or (3) the dogs displayed mild clinical symptoms of CanL which were not thoroughly investigated and/or reported. When considering possibility 1 and 2, these dogs were wrongfully treated with allopurinol. But when possibility 3 is considered, the allopurinol treatment could in fact be beneficial for these dogs (although both dogs received inadequate dosages of 6,7 and 13,3 mg/kg/day). Both dogs tested negative with their DAT titres (<40) at the UVDL, which took place approximately 2 months after allopurinol treatment was started. Four out of 32 dogs (12,5%) with a positive DAT titre presented with a decreased DAT titre after 9-12 months (unpublished data). All four dogs presented with clinical signs of CanL during the first DAT titre and all four dogs were treated with 20 mg/kg/day allopurinol. Titres decreased from 1280 to 160; 640 to <40; 20480 to <40; 1280 to 80. In conclusion, all three scenarios remain possible, but scenario 1 and 2 are more likely since both dogs had no clinical signs of CanL after two months of treatment and dosages of allopurinol were inadequate.

In the retrospective study, 30/46 dogs (65%) had a DAT titre of 1/5120. The reason for this high number is simple: for a long time this was the highest dilution made by the UDVL. Only after the publication of a research paper by another student from Project Leishmania DAT titres were diluted to 1/81.920 or even more is necessary. This additional dilution started around March 2020 and a few older samples were tested again with additional dilution.

As mentioned earlier, all ammonium urate-crystals noted in the retrospective study were assumed to be xanthine-crystals. This assumption was made because xanthine was quite unknown by employees of the UVDL back in the day and ammonium urate and xanthine appear very similar under the microscope. This possibly let to a small overestimation, since ammonium urate-crystals were occasionally seen in urine samples from the prospective study. These few ammonium urate-crystals might be the result of high protein, meat-based diets (Bartges et al., 1995). In the prospective study, one dog presenting with 0-1 ammonium urate-crystals/LPF did receive a high protein, meat-based diet; the diet of the other dog presenting with both 0-1 xanthine- and ammonium urate-crystals/LPF was unknown.

In the retrospective study, there was an increase in reported incidence of xanthinuria over time, which is likely related to the increased awareness of xanthine-crystals. Xanthinuria was first mentioned in a urinalysis report of June 2018, urinalysis reports between 2008 and May 2018 only mentioned ammonium urate-crystals. In addition to this finding, the prospective dogs which were also included in this study contributed to a higher incidence of xanthinuria in UVDL reports. This might indicate the presence of MS at the UVDL led to even more awareness of xanthinuria. Therefore, xanthinuria might have been underreported previously at the UVDL.

Conclusion

An incidence of xanthinuria of 58-60% was found in this research, although an incidence of 45-55% is presumably more accurate. Because of the strong correlation between specific gravity and incidence of xanthinuria, stimulation of water intake is recommended. Because storage time significantly affected the incidence and amount of xanthine, xanthinuria present in stored samples should be re-evaluated in fresh urine. Because 80% of dogs with ultrasound reports presented with xanthine-uroliths and 43% of dogs with xanthine-uroliths developed clinical signs due to these uroliths, this study strongly encourages clinicians to perform an abdominal ultrasound if xanthinuria is detected.

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Conflict of interest

The author of this article has no financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of this master thesis.

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