File 2

Crystalluria in a dog

E. Hooijberg, J. Leidinger and E. Leidinger
Labor inVitro, Vienna, Austria

Interpretation and Diagnosis

The sediment, shown in Figure 1 below, contained small globular yellow-brown crystals occurring together in clumps. The crystals varied in size from about 2um to 10um in diameter and small protrusions were present on some of them. Low numbers of erythrocytes were also present. An initial diagnosis of urate crystalluria was made.

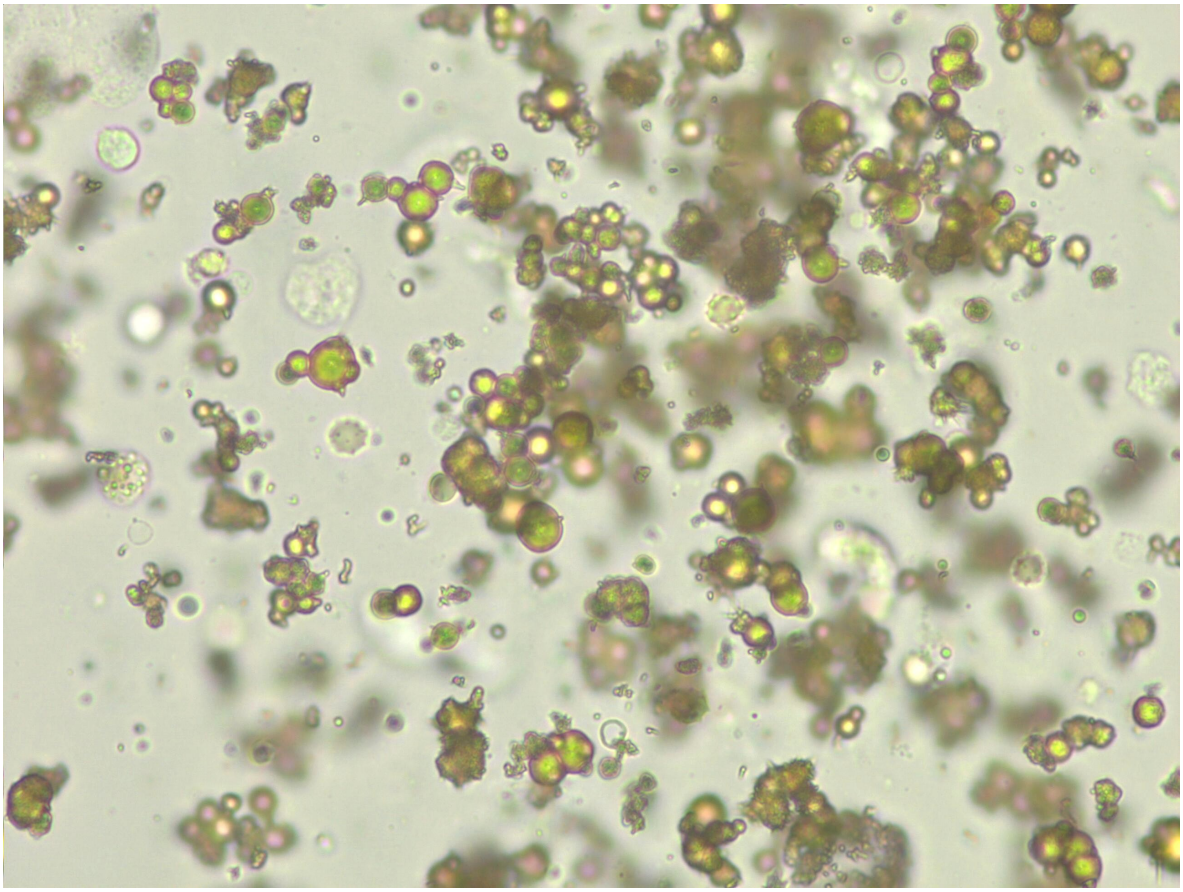

Figure 1: Small yellow-brown spherules in the urine sediment of a German Shepherd Dog. Unstained, x40 objective.

A search of our database revealed that we had 4 months previously received samples from the dog in question. A blood sample for a haemogram and biochemistry and lymph node aspirate for cytological examination were originally submitted.

The blood sample revealed a non-regenerative anaemia (Hct 29%, reference interval 37-55%), leukocytosis with a neutrophilia (WBC 29.1 $x10^9$/l, reference interval 6.0-15.0, neutrophils (segmented)14.7$x10^9$/l, reference interval 3.3-12.0), and a mild hyperproteinaemia (7.7g/dl, reference interval 5.7-7.5). An immunofluorescent antibody test for *Leishmania infantum* revealed a high titre of 1:1280 (borderline titre 1:80). The aspirate from an enlarged

submandibular lymph node was examined cytologically and *Leishmania* organisms were found (Figure 2).

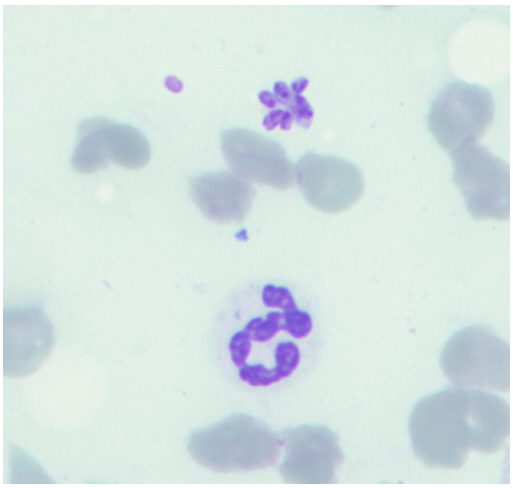

Figure 2: Submandibular lymph node aspirate with *Leishmania* organisms.

A discussion with the submitting veterinarian revealed that the dog originally came from Greece and had been living in Austria for several years. At the time of blood sampling the dog had a dermatopathy typical of leishmaniasis as well as a lymphadenomegaly. After the diagnosis was confirmed from the lymph node aspirate, the dog was started on an initial treatment course of meglumine antimoniate (100mg/kg im sid) for 15 days, after which allopurinol treatment, at a dose of 600mg per day, was begun. The dog improved clinically and a urine sample was submitted to our laboratory for a control urinalysis after 3.5 months of allopurinol treatment.

In light of this new information the diagnosis was revised and a provisional diagnosis of xanthine crystalluria made. A second urine sample was submitted 2 weeks later. The sediment findings were similar to the first sample and the small, spherical yellow-brown crystals were still present. The remaining urine was centrifuged, the supernatant removed and the sediment left to dessicate. This sample was then sent to the Urolith Analysis Centre at the University of Bonn in Germany for further analysis.

An infrared spectroscopic examination confirmed the crystals to be 100% xanthine.

Discussion

Xanthine crystals form in the urine due to deficiency or inhibition of the xanthine oxidase (XO) enzyme. XO catalyses the oxidation of hypoxanthine, an intermediate product in the purine degradation pathway, to xanthine, and the further oxidation of xanthine to uric acid (Figure 3). Uric acid is further oxidised to allantoin which is excreted in the urine. These reactions take place in hepatocytes. Xanthine, hypoxanthine, allantoin and uric acid are all excreted in urine (1).

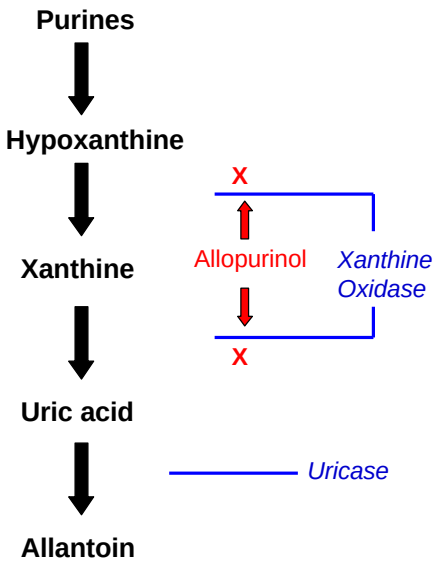

Figure 3: Purine metabolism. Allopurinol inhibits the xanthine oxidase enzyme.

A XO deficiency or inhibition results in a build-up of xanthine and hypoxanthine which in turn leads to high levels of xanthine and hypoxanthine in the urine. The presence of xanthinuria together with a highly concentrated urine leads to a xanthine oversaturation, xanthine crystalluria and eventually xanthine urolithiasis.

Congenital xanthinuria has been reported in a family of Cavalier King Charles Spaniels and in a few unrelated Dachshunds (2, 3, 4). The mode of inheritance in the affected CKCS dogs was consistent with an autosomal recessive pattern. In all these dogs, formation of xanthine calculi had already taken place by the time of the diagnosis and stones were found in the kidneys, ureter and bladder.

Allopurinol is a structural isomer of hypoxanthine and competitively inhibits the xanthine oxidase enzyme, thus causing a decreased production of uric acid and increase in the levels of xanthine and hypoxanthine (Figure 3) (5). In animals, allopurinol is used to manage cases of ammonium urate urolithiasis, as it leads to a decrease in uric acid/urate levels and may even aid in urate urolith dissolution. Allopurinol is also used to treat leishmaniasis: the *Leishmania* organism falsely incorporates allopurinol into its RNA, instead of hypoxanthine which leads to impaired protein synthesis and a decreased ability to reproduce (5).

During a literature search on the subject, no reports on the prevalence of xanthine crystalluria in dogs could be found, however several studies mentioning xanthine urolithiasis were found (6,7,8,9). Data in these reports from institutions analysing canine uroliths report 0.05% to 0.3% of all canine stones to be xanthine. Dalmations were the breed most commonly affected, due to their genetic predisposition to form urate stones and the treatment thereof with allopurinol.

Definitive diagnosis of xanthine urolithiasis is by infrared spectroscopic analysis of submitted stones. Examination of the urine sediment may reveal the presence of brown or yellow brown spherical crystals which cannot be distinguished from ammonium and amorphous urates (6, 9). This is illustrated in Figure 4.

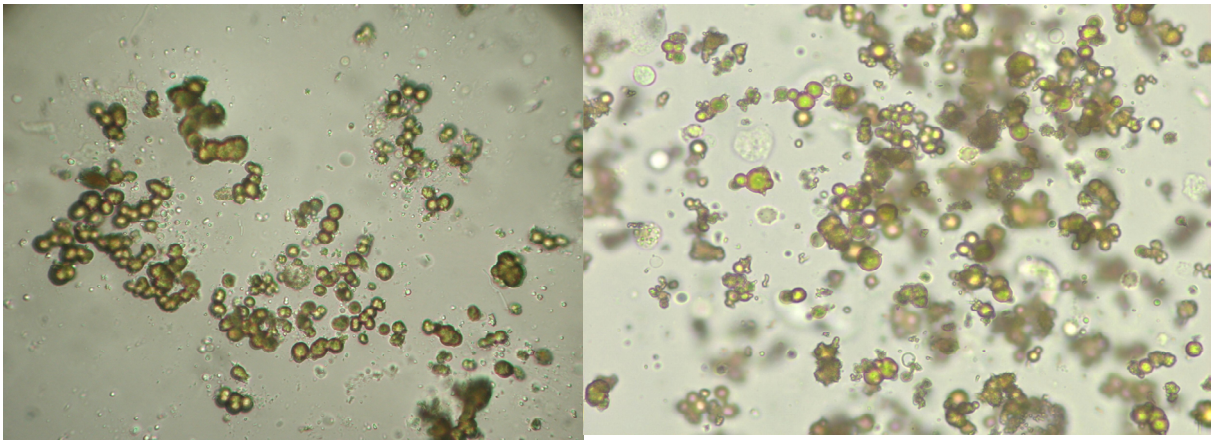

Figure 4: Comparison of ammonium biurate (left) and xanthine crystals (right) in canine urine.

A history of allopurinol treatment or the presence of these crystals in a young CKCS or Dachshund should raise the suspicion of a xanthine crystalluria. Other reported urinalysis findings in dogs with xanthine uroliths are a urine specific gravity of more than 1,030 and a urine pH of more than 6.8 (9).

The presence of xanthine crystalluria may signify the presence of xanthoine uroliths.The radiolucency of xanthine stones is similar to that of urate – often radiolucent or only slightly radiodense – and double contrast cystography and positive contrast urography are recommended to locate stones in the urogenital tract (6).

Xanthine stones are small (usually less than 1cm in diameter), very numerous, have a smooth surface and are grey-brown, yellow or green colour (6, 9).

Infrared spectroscopy is a quantitative technique which works on the principle that when infrared waves are passed through a sample, some waves are absorbed and some transmitted through the sample. This results in a pattern unique to the sample which can be compared to reference spectra for identification (10). It is particularly used to differentiate ammonium urate, sodium urate, uric acid and xanthine (11). Polarized microscopy, also known as optical crystallography, is not a recommended method for the diagnosis of xanthine uroliths as xanthine crystals cannot easily be differentiated from urate crystals by this method (10). As in this case, not only uroliths but also a dried sediment of urine crystals can be sent for spectroscopic analysis.

The treatment of xanthine cystalluria involves firstly the feeding of a purine-restricted diet, preferably a moist canned diet in order to increase water consumption. Several commercial diets are available, for example Hills Prescription Diet u/d. In fact, allopurinol treatment for dogs with urate stone problems should only be given after a purine-restricted, high-moisture nonacidifying diet alone has proved to be unsuccessful, particularly in dogs with renal disease. Allopurinol is excreted via the kidneys and a decreased renal function will lead to a build-up of the drug and thus the formation of more xanthine. In dogs receiving allopurinol treatment for urate stones or leishmaniasis the dose should be decreased.

This case demonstrates the importance of a clinical history and signalment in the diagnosis of xanthine crystalluria and xanthine urolithiasis. Xanthine crystals can be easily confused with ammonium urate crystals, differentiation using infrared spectroscopy should be performed.

Dogs receiving allopurinol treatment should always be placed on a low-purine diet and the allopurinol dose should be adjusted accordingly if renal disease is present.

Additional comments

1. The proteinuria was ++ with a normal USG and a pH of 7; therefore an SSA test was used to confirm the proteinuria. A UP/C was done the next day (pyrogallol red method) and it was 1.49. Three weeks later UP/C increased to 7.54, USG still was 1.038. So we'd argue that there was a renal (partly due to tubular damage, because we found granular casts in the last urinary sample from this patient) damage probably due to the persistent Leishmania infection. Further renal damage could be caused by treatment with allopurinol.
Unfortunately we didn't receive a serum sample at this time, so TP concentration is unknown but it could have been quite elevated as often seen with chronic leishmaniasis.

2. Blood was +++ by dipstick in the first urine analysis (that's the one reported). At the urine analysis 3 weeks later blood was + by dipstick with max 1 RBC, in the last urine sample received 7 weeks after the first one blood was + again, there where no intact RBC detectable in the sediment. So there are several possibilities:

A. the first urine sample was voided urine and arrived in our lab the next day, so a proportion of the RBCs could have been lysed at the time of the analysis for unknown reasons.
B. or part of the +++ blood dipstick reaction could have been false positive e.g., by a cleaning agent used to rinse the urine container (we sometimes receive urine samples in glass containers previously used for food).
Anyway, we can conclude that there was a mild persistent haematuria for unknown reasons over the period in which we received samples from the dog which did not increase parallel to the proteinuria.

References

1) Stryer L: Biochemistry (3rd ed). 1988, WH Freeman, New York

2) van Zuilen CD *et a:l* Xanthinuria in a family of Cavalier King Charles spaniels. Vet Q. 1997;19:172-174

3) Flegel T *et al:* Xanthine urolithiasis in a dachshund. Vet Rec. 1998;143:420-423

4) Kucera J *et al:* Bilateral xanthine nephrolithiasis in a dog. J Small Anim Pract 1997;38:302-305

5) Swiss Institute for Veterinary Pharmacology and Toxicology: http://www.vetpharm.uzh.ch/reloader.htm?perldocs/toxsygry.htm?inhalt_c.htm Accessed on 18/2/2010

6) Osborne CA *et al:* Drug-induced urolithiasis. Vet Clin North Am Small Anim Pract. 2009; 39: 55-64

7) Houston DM *et al*. Canine urolithiasis: a look at over 16 000 urolith submissions to the Canadian Veterinary Urolith Centre from February 1998 to April 2003. Can Vet J. 2004; 45:225-230

8) Hesse A *et al*: Urolithiasis: Therapie und Prophylaxe. Kleintier Konkret 2002; 5:9-13

9) Hesse A and Neiger R: Harnsteine bei Kleintiere. 2008, Enke, Stuttgart; pp87-94 and 104-107

10) Osborne CA *et al:* Canine Uroliths: Frequently Asked Questions and Their Answers. Vet Clin North Am Small Anim Pract. 2009; 39: 161-182

11) Moore, A: quantitative analysis of urinary calculi in cats and dogs. Vet Focus 2007; 17:22-27