Paws claws and udder things

February 2020

Gribbles VETERINARY

What's inside?

Welcome

Welcome to the first edition of our newsletter for 2020.

This month we have an outstanding article on cystinuria, updates and reminders, and a super interesting *Case of the Month* for you to get your teeth into.

If you have any questions about this issue or have a suggestion for articles to feature, please just get in touch.

Kind regards,

Karen Cooper
Marketing Administrator

Cystinuria

- disease summary and testing options

JENNI DONALD

We have had enquiries about this condition recently and have seen cases in a Miniature Poodle, German Short-haired pointer and a Bulldog. The following is a summary of the disease and the testing options available both here and overseas.

Cystinuria is a hereditary renal transport disorder involving cystine and other dibasic amino acids—ornithine, lysine and arginine (together these are known as COLA). In normal animals, these amino acids are absorbed by the small intestine, freely filtered by the glomerulus and then >99% are reabsorbed by an active process in the proximal convoluted tubule. When tubular resorption is decreased, increased amounts of the amino acids appear in urine, but it is only cystine which causes problems. The low solubility of cystine in acidic urine can result in the formation of cystine crystals and uroliths in the urinary tract, but not all cystinuric animals will form stones. Loss of the COLA amino acids in urine has not been associated with malnutrition or protein deficiency.

Two genes, SLC3A1 and SLC7A9, have been identified as coding for the basic amino acid transporter system and its dysfunction results in cystinuria. SCL3A1 codes for the rBAT protein and SCL7A9, the $b^{0,+}$AT protein and these two proteins combine to form the COLA amino acid transporter.

A severe cystinuria, referred to as **Type I cystinuria**, with autosomal recessive inheritance has been characterized in Newfoundlands with mutations in the SLC3A1 gene. Both males and females are affected but males tend to show more severe effects. In Newfoundlands the disease may appear earlier than in other breeds (some are <1 year of age) and uroliths can be found in the kidney as well as the more typical sites of urethra and bladder. A different mutation in the SLC3A1 gene, which is also an autosomal recessive condition, has been identified in Labrador Retrievers.

Type II cystinuria includes an autosomal dominant form described in Australian Cattle dogs (Type II-A with a deletion in SLC3A1) and a missense mutation in SLC7A9 with autosomal dominance found in Miniature Pinschers (Type II-B) in Europe.

Continued overleaf.

Figure 1 – Cystine crystals in dog urine.

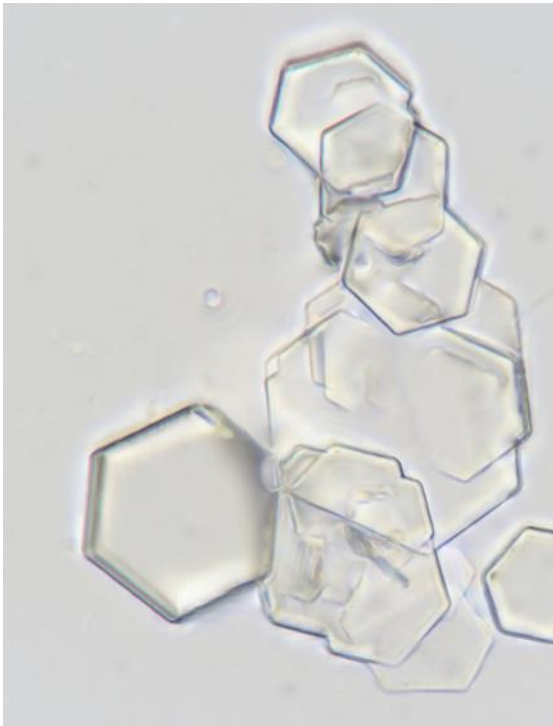


Photo by Lance Wheeler - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=71997490

Cystinuria has also been described in many other dog breeds (at least 70) and in cross-breeds. In many, the disease occurs later in life, is less severe than the breed associated forms and only involves entire males. Surgical, and even medical castration, has been found to resolve excessive cystine and COLA excretion and this condition is now referred to as canine androgen-dependent or **Type III cystinuria**. Most data has come from Mastiffs, French and English Bulldogs, Basset hounds and Irish terriers, but the specific genetic defect is still not known definitively. In Mastiffs there may be a missense mutation in SLC3 A1, but this has not been found in other breeds.

Unlike canine cystine uroliths, which most commonly occur in male dogs, in cats they are seen in equal frequency in males and females, but are rare. At the Minnesota Urolith Centre, cystine uroliths make up <0.2% of stones analysed from cats. They found the mean age of cats at the time of presentation was 3.4 years (range 4 months-12.2 years). About 65% were domestic shorthairs, 20% were Siamese and there was one Main Coon, one Korat and two domestic longhair cats. As in dogs and humans, cystinuria in cats is thought to occur from an inborn error of metabolism.

Testing for cystinuria

URINALYSIS – Cystine is one of the sulphur-containing amino acids; therefore, the urine may have the characteristic odour of rotten eggs. The sediment exam may show typical flat, colourless hexagonal crystals, which are essentially pathognomonic of cystinuria. (Figure 1). The crystals tend to aggregate in the urine sediment, giving a layered appearance.

IMAGING & ANALYSIS OF UROLITHS – Cystine uroliths have intermediate radiodensity. They are less radiodense than struvites and calcium oxalates, but more so than ammonium urates. In some cases contrast studies and/or ultrasound are needed. They are typically smooth and spherical, and range from <1mm to several centimetres in diameter. Typically dogs present with multiple uroliths. Secondary urinary tract infections are uncommon. Quantitative analysis of cystine uroliths submitted to the Minnesota Urolith Center revealed that most are pure.

URINE NITROPRUSSIDE TEST - The sodium cyanide–nitroprusside test is a rapid, simple and qualitative determination of cysteine concentrations. Cyanide converts cystine to cysteine. Nitroprusside then binds, causing a purple hue in 2-10 minutes. The test detects cysteine levels of higher than 75 mg/g of creatinine. As a qualitative test, it is not as sensitive as the quantitative tests (see COLA tests below) and not all dogs will have detectable cystine in the urine. Ampicillin- and sulphur-containing drugs in the urine may cause false-positive results. The quantitative COLA tests have generally replaced this test, however it is available at the University of Pennsylvania.

COLA QUANTIFICATION - These tests are performed in medical diagnostic laboratories and we have tested a few at LabPLUS, Auckland City Hospital. Concentrations of each of the four amino acids (AA), cystine, ornithine, arginine and lysine are reported as an AA:creatinine ratio, expressed as µmol of the AA/mmol creatinine. We do not have locally validated reference intervals, but references from literature can be used. At least 2mL of urine is required for testing (more is probably better) and freezing the sample is recommended if it cannot be delivered to the laboratory immediately. The cost is approximately $70 +GST, but check with your local laboratory for a current price.

This test can detect Type I and Type II cystinuric animals but not all dogs with Type III. If a positive result is obtained in dogs with Type III disease, then this test can be used following castration and for monitoring afterwards. The recommendation is to test before castration and then re-check 3-4 months after castration.

GENETIC TESTING - Testing for recessive (Type I) cystinuria is available at various laboratories overseas for Newfoundland/ Landseers and Labrador Retrievers. As it is a recessive trait, the mutation test not only detects affected dogs but also the asymptomatic carriers.

For Type II disease there are no carriers as it is autosomal dominant. An animal will therefore test clear, heterozygous affected (one copy) or homozygous affected (two copies). Tests are available overseas for Australian cattle dog and Miniature Pinscher.

Testing for canine androgen-dependent (Type III) cystinuria is more complicated. The causative mutation has not been identified but a linked marker is available that identifies risk alleles in Mastiffs and related breeds, but not Irish Terriers or Scottish Deerhounds. This test is unlikely to be applicable to other breeds and mixed breeds.

The WSAVA Hereditary Disease Committee have established a web-based database of genetic tests available for hereditary diseases in dogs and cats worldwide. This is useful resource can be checked to find which laboratories do the specific tests.

Please note: Gribbles Veterinary is no longer referring genetic testing overseas. You will need to arrange this directly with the service providers.

Did you know . . .

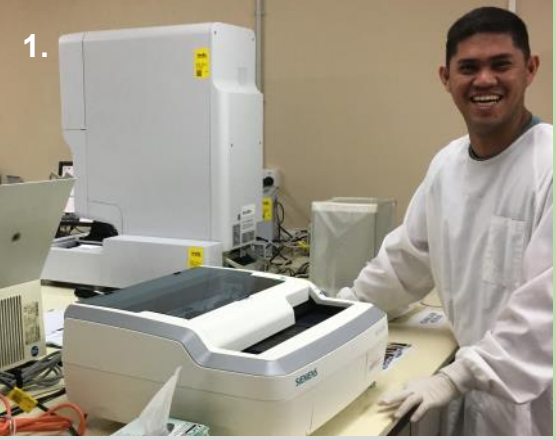
. . . that we have an outstanding range of resources available free-of-charge on our website? There is also no longer any need to log in to access this information.

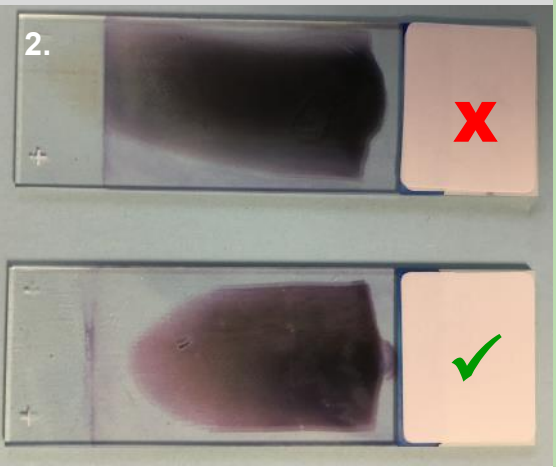
Our Vet Handbook is a fantastic resource for detailed information on most tests we offer, sample collection guides, recommendations for diagnostic work-ups, and guidance on result interpretation. You can search and read it all online, download a section or a copy of the entire handbook for offline access.

We also have a series of "How to" guides that provide step-by-step instructions for some basic lab procedures. These will help in your clinic laboratories, as well as when sending samples to our laboratories.

Plus of course, there are all our information sheets for tests, sample handling, POC analysers and so much more!

So don't miss out or mess up, check them out today!





Better staining

KATHRYN JENKINS

Joining our Auckland and Christchurch laboratories, Gribbles Veterinary Palmerston North has recently added a new Siemens Hematek 3000 semi-automated staining machine to the laboratory's diagnostic suite. This enables fast, consistent, high quality staining of up to 60 slides per hour, and stains both blood films and cytology preparations.

When an EDTA blood sample is submitted for a CBC, it is beneficial (and no extra charge) to send us a freshly made and air dried blood film from the same patient. This enables us to differentiate storage artefact from real changes (especially neutrophil band formation and toxic change), and allows accurate evaluation of cell morphology. This also applies to fluid cytology (e.g. joints and effusions).

Automated stainers do not stain the top 10mm of the glass slide (see photograph 2). A blood film or cytology smear extending to the top end of the slide would not be stained by this method. Shorter smears are therefore recommended.

Photographs left:

1. *Adrian Alpes, Haematology Scientist loving the new staining machine.*
2. *Top blood smear - too long and the top of the film has not been stained, missing the crucial monolayer for evaluation. Bottom blood smear - much shorter and fully stained.*

Case of the month

CATHY HARVEY

Clinical history:

Three-week-old puppies developed clear nasal and ocular discharge, but were still relatively bright and eating. The puppies were from the first litter of a dam that had one vaccination at 6 months of age. Two of the puppies faded and died over the weekend, and the other two were presented for veterinary examination, with one dying on arrival at the clinic. The surviving puppy was dyspnoeic and crying, with pale mucous membranes, a temperature of 36.8°C and a very mild watery discharge from the nose.

Post-mortem of the dead puppy revealed petechial haemorrhages in the small intestines and kidneys, and possibly liver (see photograph 1). Some of the lung lobes were dark red and consolidated.

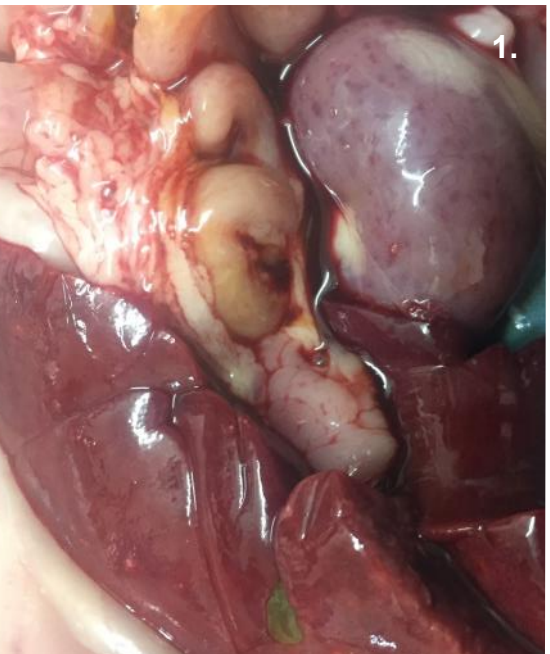
Laboratory testing:

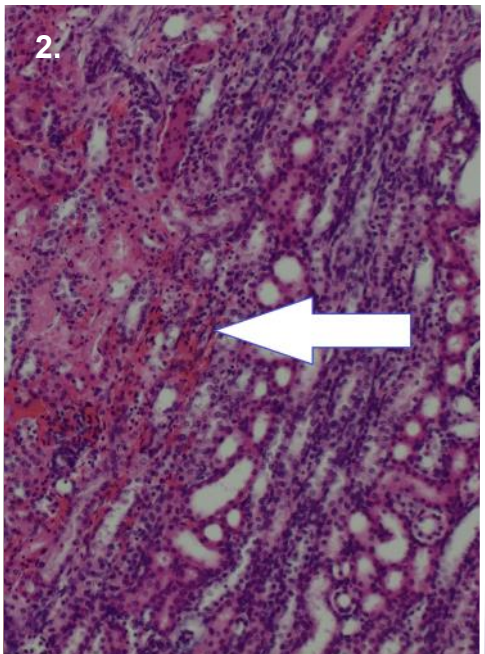
Multiple fixed tissues were submitted to the laboratory. Histopathology showed multifocal areas of acute necrosis and haemorrhage (see photograph 2), and intranuclear eosinophilic viral inclusions (see photograph 3) within the epithelial cells of the kidney, liver, lung and intestines.

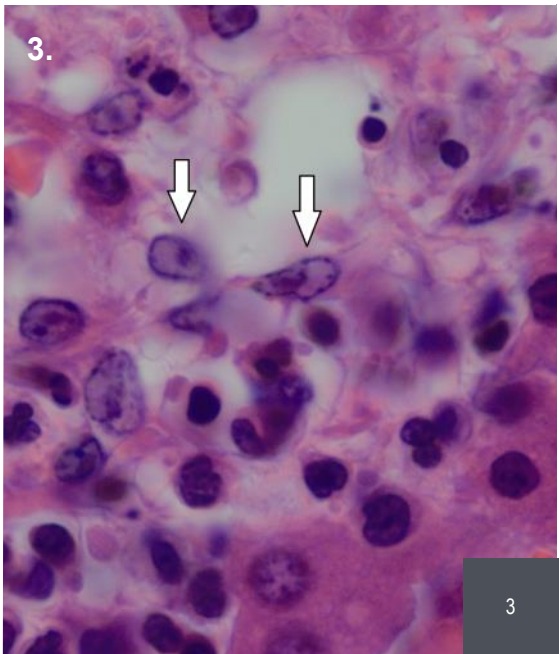
See page 4 for diagnosis and discussion.

Photographs below:

1. *Gross kidney and liver haemorrhage.*
2. *Histopathology showing kidney haemorrhage and necrosis. H&E stain, 100x magnification.*
3. *Histopathology of liver with viral inclusions in the hepatocyte nuclei. H&E stain, 1000x magnification.*







Snippets

- **Waitangi day** - all of our laboratories will be closed on Thursday February 6.
- **Facial eczema spore counts** - a reminder to send us through your data by 2pm each Thursday so we can include it in our national report each week. Find a form and counting instructions, as well as a link to sign up to receive the report and/or weekly reminders on our website here.
- **Include ice packs with samples please** - now that the warmer weather is here, it is especially important to include ice-packs with your samples when sending via overnight courier. Most sample types are adversely affected by heat and can be rendered unsuitable for testing if they are not kept cool during transport. We will return your ice-packs to you along with your Bio-bottle shipping containers. So please remember to ***"chill-out"*** when packing up your samples for transport!

Case of the month

CONTINUED FROM PAGE 4

Diagnosis: These are pathognomonic lesions for canine herpes virus-1 infection.

Discussion: Canid herpesvirus-1 (CaHV-1) infection in puppies causes disseminated focal necrosis and haemorrhages, with amphophilic intranuclear inclusions bodies in epithelial cells of parenchymal organs, including the liver, kidney and lung (as in this case).

Manifestation of disease is dependent on the route of exposure and age of the host.

In adults or older puppies, CaHV-1 may produce mild upper respiratory infection, and inapparent infections are common. Infection of the neonate is possible by various routes. It may be transmitted from external lesions of genital infection to the pup as it traverses the birth canal, from initial maternal infection, or by recrudescence of existing latent virus.

CaHV-1 infection is regularly fatal for new born puppies. Resistance to the disease is sharply age-related: pups exposed after 2 weeks but up to 8 weeks of age will not develop severe illness. The disease has an incubation period of 3 to 7 days, after which the affected puppies rapidly sicken and die, usually within 2 days. CaHV-1 can cause abortion, stillbirth and infertility, depending on the time of gestation and inoculation of bitches with the virus.

Reference: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, Sixth Edn. 2015.

Many thanks to Nena Nepia from The Vet Centre Maungaturoto, for allowing us to use this as a case report, and for submitting the great history, photos and gross description of the necropsy and multiple well fixed tissues for histopathology.

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