URIC ACID STONES and DALMATIAN-POINTER OUTCROSSING/BACKCROSSING ISSUES

The first discovery that Dalmatians excrete higher levels of uric acid than other breeds of dog was made by Benedict in 1916. He compared four Dalmatians with a dog of 'doubtful breeding'. All four Dalmatians excreted significantly higher concentrations than the dog of doubtful breeding.

In 1918, Onslow crossed a Dalmatian bitch with a ' large white terrier dog'. The resultant two offspring, together with the terrier sire, were determined to produce significantly lower urinary uric acid concentrations than that for the Dalmatian dam. Onslow concluded that the ability to destroy uric acid was a dominant characteristic in the terrier and the offspring.

So where does uric acid come from?
It arises from the metabolism of purines, a group of chemicals referred to as 'bases'. They are important constituents of DNA, and hence found in the cells of all organisms, including meats and vegetables. Accordingly, diets generally would include purines to one degree or another.

In the normal metabolic pathway of mammals, purines are converted in a number of stages to uric acid, and then to allantoin, which is excreted without problem. Dalmatians share with humans and higher apes an inability to make this last step in the process, and instead stop at the point of producing uric acid. It is assumed that all Dalmatians suffer this problem. Although it can be argued that the scientific basis for this assumption is not entirely conclusive, the probability is that it is true.

So why is it a problem?
Quite simply, uric acid has a relatively low solubility. If the concentration of uric acid in bladder urine rises too high, then the uric acid will tend to come out of solution, and precipitate as a solid. This solid may, on occasions, aggregate and build up to a urate stone. However, it is important to recognise and distinguish the fact that whilst all Dalmatians excrete higher concentrations of uric acid, not all form stones.

So why don't all Dalmatians form stones?
Firstly, it is quite evident that males are more likely to present with urate stone disease than bitches due to their anatomical difference. Males have a bone called the os-penis which restricts the urethra or its ability to dilate, so any stone which might form cannot be dispelled so easily.

But even with males, only a small minority present with urate stones despite the fact that they all excrete high concentrations of uric acid. The simple truth is that the real reasons are not understood. It is thought that certain environmental factors might have an influence, for example frequency of urination, water intake and volume of urine produced, and diet – all of which seem perfectly logical - but these alone are unlikely to be the sole causes. In order to investigate this hitherto inexplicable phenomenon, the Dalmatian Club of America has commissioned very recently a study where the DNA sequence of 15 pairs of siblings will be analysed and compared. The siblings will be selected on the basis that one has been diagnosed with urate stone disease, whilst the other has not. The results will be interesting.

One thing that we can say with confidence is that there is no evidence that urate stone disease occurs in some lines, but not others. It is simply chance that some breeders have never had experience of stone disease, whereas others have.

So how can urate stone disease be addressed. Before considering the question, it is important to understand how prevalent the condition is, and what is the mode of inheritance of the problem.

Arguably the most objective survey of UK Dalmatian health is that contained in the KC/BSAVA Health Survey conducted in 2004. This was carried out amongst Breed Club members, and 210 owners responded, giving a percentage of returned surveys for Dalmatians of 30.6% against an all-breed average of 24%. These returns represented 452 live dogs and 199 deaths.

Table 3 from the Health Survey lists the most common causes of disease, first by organ system and then by category.

Table 3. Disease conditions by organ system/category for Dalmatians.

		All conditions		
Disease condition		N	%	Most common specific conditions in descending order
1	Dermatologic	48	16.0	Superficial folliculitis; mites (sarcoptes>demodex); dermatitis; recurrent pyoderma
2	Reproductive	44	14.7	False pregnancy; infertility; pyometra; irregular heat cycles; cryptorchid
3	Musculoskeletal	34	11.3	Arthritis (forelimb=spine>unspecified or other); spondylitis; lameness (forelimb>other)
4	Urologic	32	10.7	Incontinence (unspecified>after spay); cystouroliths (urate); cystitis
5	Neurologic	22	7.3	Seizures; deafness; IVDD
6	Gastrointestinal	20	6.7	Colitis; diarrhoea; vomiting; GDV
7	Immune mediated	15	5.0	Food hypersensitivity; flea allergy; atopy
8	Cardiac	14	4.7	Heart murmur (unspecified>grade 3=without clinical signs); heart rhythm disorder
9	Ocular	14	4.7	Cataracts; blocked tear duct; epiphora
10	Benign neoplasia	13	4.3	Lipoma; unspecified
11	Aural	11	3.7	Otitis externa
12	Respiratory	6	2.0	Kennel cough; coughing
13	Cancer	5	1.7	Type unspecified (skin=mammary>testicular)
14	Unknown	5	1.7	Undiagnosed illness
15	Dental	4	1.3	Dental disease; retained puppy teeth
16	Uncode and other	4	1.3	Unspecified; polydipsia (undiagnosed)
17	Endocrine	3	1.0	Hypothyroidism
18	Behaviour	2	0.7	Aggression; unspecified
19	Trauma	2	0.7	Hindlimb; spine
20	Anal gland	1	0.3	Anal sacculitis
21	Hepatic	1	0.3	Portosystemic shunt (PSS)
	Total	300	100.0	

It is apparent that diseases of the urologic system are the fourth most common, and account for 10.7% of total incidences of disease. Note that cystouroliths (urate) are the second most prevalent condition within this group, meaning that they account for about 4% of the overall total reported diseases, or a maximum of about 12 reported occurrences. By simple extrapolation, this equates to a level of incidence of urate stone disease of about 2.7% of the survey population. This correlates extremely well with the survey conducted by WAFDAL in Norway and the Netherlands, which recorded an incidence of 2.2%, where it was concluded that urate stone disease 'is not a major problem'. A Dalmatian Club of America survey in 2003, conducted by a professional survey company, arrived at an incidence of 3%, so there is a widespread concurrence of data in this regard. This low level of incidence may indeed be the principal reason that some breeders have never experienced urate stone disease.

When one considers the results for causes of death, taken from Table 1 in the Health Survey, the urologic system slips to seventh on the list, but more noticeably perhaps, there were no specific incidences recorded for death due to urate stone disease.

Table 1. Causes of death by organ system/category for Dalmatians.

Cause of death		N	%	Most common specific causes in descending order
1	Old age	54	27.1	Old age & old age combinations
2	Cancer	38	19.1	Liver; unspecified; lymphoma; spine; leukaemia
3	Neurologic	18	9.0	Seizures; IVDD; spondylitis; spinal disease unspecified
4	Cardiac	13	6.5	Attack; failure; cardiomyopathy
5	Gastrointestinal	12	6.0	Gastric dilatation/volvulus (GDV); pancreatitis
6	Other	11	5.5	Weakness or paresis; unspecified
7	Urologic	10	5.0	Kidney failure chronic > acute; incontinence
8	Cerebral vascular	8	4.0	Stroke or cerebral vascular accident
9	Combinations	5	2.5	Senility; incontinence; arthritis; multiple organ failure
10	Hepatic	5	2.5	Liver failure chronic > acute
11	Musculoskeletal	5	2.5	Arthritis
12	Unknown	5	2.5	
13	Perioperative	3	1.5	
14	Trauma	3	1.5	Road traffic accident
15	Behaviour	2	1.0	Aggression
16	Poisoning	2	1.0	
17	Collapse	1	0.5	
18	Died	1	0.5	Natural causes
19	Internal bleeding	1	0.5	
20	Senility	1	0.5	
21	Sudden death	1	0.5	
	Total	199	100.0	

The underlying genetic cause of the uric acid disorder in the Dalmatian has been identified recently by researchers in the USA as a mutant gene referred to as SLC2A9. It is easier to refer to it by the lower case letter '**u**', and the corresponding dominant 'wild' or 'normal' gene as upper case '**U**'. It appears that the mutation is inherited in a simple recessive way. All animals carry two copies of each gene, inheriting one randomly from the pair borne in turn by each parent. In the Dalmatian, it is believed that only the recessive mutant form occurs, i.e. the relevant gene pair in all Dalmatians is '**uu**', meaning all Dalmatians are '**affected**' and unable to fully metabolise uric acid. They will all excrete relatively high concentrations of uric acid. If a dog carries a single copy of the dominant '**U**' gene, i.e. the gene pair '**Uu**' then the animal will excrete relatively low concentrations of uric acid, the dominant gene masking the effect of the recessive mutant gene. However, it will be a '**carrier**' of the mutant gene. Finally, if a dog only carries the normal gene, i.e. the gene pair '**UU**', then it will be '**clear**'. It will also excrete relatively low concentrations of uric acid. The various permutations of matings involving these possible gene pairings is what determines the statistical genetic inheritance of any offspring.

If one **affected** '**uu**' dog is mated to another **affected** '**uu**' dog, then obviously all (100%) of the offspring must also be **affected** and have a gene pairing '**uu**'. Each offspring can only take a '**u**' copy from each parent.

		Affected Parent B	
		u	**u**
Affected Parent A	**u**	**uu**	**uu**
	u	**uu**	**uu**

If a **carrier** '**Uu**' dog is mated to an **affected** '**uu**' dog, then the statistical gene pairings of the offspring would be 50% **carriers** ('**Uu**') and 50% **affected** ('**uu**'). In this example, each offspring will take a '**u**' copy from the affected Parent B, and either the normal '**U**' or the mutant '**u**' from the carrier Parent A.

		Affected Parent B	
		u	**u**
Carrier Parent A	**U**	**Uu**	**Uu**
	u	**uu**	**uu**

Next consider the mating of two **carriers** ('**Uu**'). Here three gene pair combinations are possible, resulting in (statistically, of course) 25 % '**clear**' ('**UU**'), 50% '**carriers**' ('**Uu**'), and 25% '**affected**' ('**uu**').

		Carrier Parent B	
		U	**u**
Carrier Parent A	**U**	**UU**	**Uu**
	u	**Uu**	**uu**

The mating of a **clear** ('**UU**') dog to an **affected** (**'uu**') dog will produce 100% **carriers** as the only combination possible is '**Uu**'.

		Affected Parent B	
		u	**u**
Clear Parent A	**U**	**Uu**	**Uu**
	U	**Uu**	**Uu**

Crossing a **clear** (‘**UU**’) to a **carrier** (‘**Uu**’) will result in 50% **clear** and 50% **carrier**, ……

		Carrier Parent B	
		U	**u**
Clear Parent A	**U**	**UU**	**Uu**
	U	**UU**	**Uu**

…….and last but not least, the mating of two **clear** (‘**UU**’) dogs can only produce **clear** (‘**UU**’) offspring.

		Clear Parent B	
		U	**U**
Clear Parent A	**U**	**UU**	**UU**
	U	**UU**	**UU**

You may have noticed that the only way in which a mutant recessive gene can be eliminated from the gene pool is by only mating **clear** to **clear**.

This simple pattern of inheritance can be applied to any so-called autosomal recessive trait – if the **U** and **u** had been substituted by **B** and **b**, we would have just discussed the perhaps more familiar inheritance of spotting colour in the Dalmatian

The identification of the mutant uric acid gene was accompanied by a DNA test allowing identification of the genetic status in an individual dog. Some 250 Dalmatians have been tested in this manner, and all have been shown to be **affected**, i.e. ‘**uu**’. This is the most significant evidence put forward to date, and suggests that the probability is quite high that all Dalmatians carry only the mutant gene. The sample number is, as yet, small, but as it increases and if it continues to show the same result then this probability will progressively increase. This is actually a very meaningful investigation, because unequivocal evidence that all Dalmatians are indeed **affected** is a prerequisite to any backcross project.

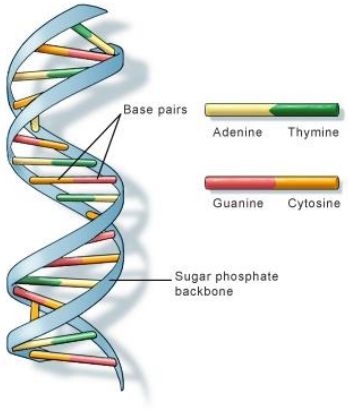
It has been pointed out by some that we do not know for certain that there are no Dalmatians carrying a copy of the normal gene, and that screening as many Dalmatians as possible with the new DNA test should be a priority. If it does transpire that there are Dalmatians with a copy of the normal gene, it is most likely that the number will be small.

The genetic inheritance patterns for the possible matings which have been described, form the basis for the Pointer-Dalmatian backcross project. This started in 1973, when a Pointer was crossed with a Dalmatian to produce Pointer-Dalmatian hybrids. Obviously, the DNA make up of these hybrids would be 50% Pointer origin and 50% Dalmatian, whilst their genetic status would be as **carriers**, i.e. '**Uu**'.

At the first backcross level, the **carrier** hybrid was mated to an **affected** Dalmatian and so would have given rise to offspring which on average would have been 50% **carrier** and 50% **affected**. They would have a DNA make up, statistically, of 25% Pointer, 75% Dalmatian. At the time, of course, there was no DNA test available, so the offspring were categorised as low or high uric acid respectively by a spot uric acid test. At each subsequent mating, the backcross mated to another purebred Dalmatian was selected as a low uric acid producer, i.e subsequent matings continued as **carrier** to **affected**. Presumably some consideration was given to spotting appearance in selecting which low uric acid backcross to use in subsequent matings. Of course, whilst this practice introduces the normal gene into the gene pool, it has a relatively small effect at this stage as it continues to produce equal numbers of **affected** offspring. Eventually, when the gene pool of **carriers** is sufficiently diverse, then **carrier**-to-**carrier** matings can be contemplated which would produce a proportion of **clear** offspring. Being realistic, and certainly not negative, this is likely to take a very long time indeed, perhaps several decades to produce a diverse gene pool of **carriers** and dare I say a century or more to produce a substantial nucleus of **clear** dogs. It would also rely upon a widespread acceptance of backcrosses by Members in their breeding programmes.

This backcrossing has now reached its 14th generation, I understand, and the dogs concerned are said by simple calculation to have 99.8% Dalmatian DNA. So what does the residual 0.2% Pointer DNA mean? It has been claimed several times that the Pointer-Dalmatian backcrosses differ from purebred Dalmatians only in the uric acid gene. But this is certainly not so.

DNA is made up of two long chains of sugar phosphate molecules held in a famous double helix conformation by bases attached to each sugar-phosphate.



The bases form two specific 'base-pair' interactions across the two chains, which is what holds them together. There are 4 bases, adenine, guanine, cytosine and thymine, which pair as shown. Incidentally, adenine and guanine are purines. The order of the bases along each chain is what is termed the 'DNA sequence', whilst segments of this sequence are referred to as 'genes'.

The canine genome is made up of about 2.4 billion base pairs, and has been fully sequenced and identified to contain about 20,000 genes. Accordingly, the 0.2% Pointer DNA corresponds to about 40 Pointer genes, not 1! Whilst there might be a measurable error bar on this simple calculation, it is the best estimate which we have.

It must be said, however, that no-one knows which Pointer genes these represent (with the exception being the uric acid gene), and therefore whether they are beneficial, deleterious, or neutral.

Are there alternative approaches, and are they acceptable? This is not an easy question to answer. One has to take account of the incidence level for the disease, the prognosis, whether medication regimes are deemed acceptable, whether diet alternatives are practical and acceptable and so on.

It is well known that medication with allopurinol prevents uric acid formation. This is because allopurinol inhibits the enzyme which converts xanthine to uric acid in the metabolic process. It does not appear to have any particular side effects. A risk of forming xanthine stones instead is a consideration, but the relatively few cases which have been reported seem to be associated with the administration of extremely high doses of allopurinol.

What of diet? Until recently, it has been argued that prescription diets are too expensive, or have very low protein levels. One well known prescription diet does indeed exhibit a low protein content of only 11%, but an alternative prescription diet contains 18%. Most recently, an OTC breed-specific low purine diet has been introduced specifically for the Dalmatian which contains 22% protein. These diets, whether prescription or not, share with the backcross project the principal of 'remove the uric acid, remove the problem'.

In the time available, I hope that I have given you all a basic outline of the background and issues involved, in order that you can discuss the subject objectively.

So, in summary:

- All Dalmatians excrete high concentrations of uric acid which predisposes them to the risk of urate stone disease;
- The incidence level in the UK is about 2.7%, with a nil or extremely low rate of morbidity;
- The backcross project provides an option to introduce the normal uric acid gene; but
- Its introduction is likely to be slow and its effects likely to be limited;
- Diets, including non-prescription ones, are available to manage/prevent the disease;
- Medication is available, should it be required.

The issue is not an easy one for which to find the perfect solution.. It could expressed in terms of obtaining the appropriate balance between breed purity and health and welfare and its management. Certainly, much is still not understood, and research continues aimed at a better understanding of what triggers urate stone disease.

Dr John Stevenson
Breed Health Coordinator
25 October 2009