OVERVIEW OF BACKCROSS PROJECT: NORMAL URIC ACID IN DALMATIANS

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Categories : Vets

Date : March 7, 2011

Elizabeth Sampson reports on a breeding programme that seeks to remove some of the negative traits found in this breed to form a healthier base for the future

IN 1973, Rober Schaible initiated a breeding programme to correct the genetic defect in Dalmatians that causes them to have a high level of uric acid (HUA) in the urine.

This hyperuricosuria predisposes them to the formation of urinary urate crystals, sludge and stones, which may lead to urinary obstruction and even death. It is arguably the most significant health issue in the breed, but one that can be addressed easily due to its simple autosomal mode of inheritance.

We know from the early work of Trimble and Keeler (1938) that the defect for uric acid metabolism is carried on a simple autosomal recessive gene. They crossed Dalmatians to collies and, through subsequent crosses, determined that the genetic defect in Dalmatians was an autosomal recessive trait.

Dr Schaible made an outcross of an American Kennel Club (AKC)-registered champion pointer sire, C H Sandown's Rapid Transit, to an AKC-registered Dalmatian dam, Lady Godiva. He selected a pointer as being a probable descendent of the closest common ancestor before the Dalmatian breed branched off down its own path. Because of the simple autosomal inheritance, puppies from this mating had one copy of the normal dominant gene for uric acid metabolism and had normal urinary uric acid (NUA) levels. Of course, they had little resemblance to a typical

Dalmatian.

The second generation puppies looked more like purebred Dalmatians and the best of those carrying a copy of the NUA gene were selected for further breeding in the backcross project.

Matings subsequent to the original outcross have been backcrosses to AKC-registered Dalmatians, yielding on average 50 per cent NUA and 50 per cent high uric acid (HUA) progeny. Originally, the NUA and HUA puppies were distinguished by doing a spot urine test to measure uric acid-to-creatinine ratios. Today, DNA analysis is used to identify the specific single gene that has been discovered and is thought to determine normal canine uric acid metabolism. It resides on canine chromosome number three.

This breeding programme is now 14 generations from the original outcross and the offspring have 99.98 per cent AKC Dalmatians in their pedigree, and 99.8 per cent of their DNA is the same as AKC-registered Dalmatians. The NUA puppies have been found to have a 10 times lower urinary uric acid, compared to their HUA litter mates.

Four of these low uric acid descendants have undergone bladder ultrasound and have been found to be free of bladder sludge or aggregate material.

Moreover, they have the phenotype of the typical Dalmatian, with the conformation, spotting, dark eyes and temperament characteristics of the breed. They are, without doubt, true Dalmatians.

Genetics

Dogs have evolved to metabolise proteins, specifically purines, from food by converting uric acid to allantoin in the liver. Allantoin is highly soluble and does not cause precipitation of crystals in the urine. Only humans, great apes and Dalmatians do not make this conversion – uric acid is the end result of their purine metabolism.

In Dalmatians, a genetic defect results in a missing transporter protein that prevents the enzyme uricase (located in the liver) from metabolising uric acid to allantoin. The result is that uric acid is excreted in excess into the urine and, because it is less soluble than allantoin, has a tendency to come out of the solution in the form of urate salts. These salts can form crystals, sludge and stones and, if large enough, can cause obstruction. Indeed, obstructions can result from the accumulation of crystals and sludge alone.

Factors that favour the production of stones are:

• a high concentration of salts in the urine;

• retention of these salts and crystals for periods of time in the urinary tract; and

• an optimal pH that favours the precipitation of salts.

The male dog is particularly at risk from obstruction, as his urethra is longer and narrower than the female's and calculi are most likely to become stuck behind the os penis. This may present as a medical emergency requiring surgical intervention. It is extremely painful and may even result in death. Not all Dalmatians produce stones, but the figures may be higher than originally thought.

Strict breeding for a desired trait has often resulted in making all animals of a particular breed homozygous for a certain health problem. This may be due to the additional effects of the desired gene, or because it is associated with another gene, located nearby, on the same chromosome. The latter is the cause of the defect in uric acid metabolism in Dalmatians. Breeding for better spotting (larger and clearer spots) has inadvertently made the mutant gene for the uric acid defect homozygous because of the close proximity of the genes on the same chromosome. The gene responsible for uric acid metabolism is known as SLC2A9.

In 1940, Keeler attempted to locate Dalmatians with normal uric acid metabolism in the UK and in the US, but was unsuccessful. None have been found since, including in a recent sample of 50 dogs submitted for DNA testing in the UK. It is safe to assume that none exist, apart from those derived from the backcross project.

The mutant gene is also present in other breeds of dogs with hyperuricosuria, notably the English bulldog and the black Russian terrier, but because the healthy dominant gene exists in these breeds, it is easy to eliminate the defective gene using carefully monitored breeding programmes.

In the case of Dalmatians, the only way to reintroduce the healthy gene was through a backcross breeding programme.

The introduction of a congenic strain is the standard way of replacing a mutant defect with a normal gene.

The first step is to make an outcross to an animal with a donor allele. The allele is the alternative form of the DNA sequence of a particular gene. Subsequent generations are backcrosses to the recipient inbred strain. The offspring that have received the donor allele are selected for the next round of backcrossing.

Since the gene responsible for uric acid metabolism is passed on by simple autosomal recessive inheritance, the likely percentage of offspring with one or two copies of the normal gene can be predicted.

The gene for normal uric acid metabolism is dominant U and the abnormal gene is recessive u. Only one copy of the normal gene is required for a dog to have normal levels of urinary uric acid (Table 1).

In the first outcross carried out by Dr Schaible, the puppies were all Uu, as expected, and, therefore, all had normal uric acid levels (Figure 1). Subsequent backcrosses have produced, on average, 50 per cent NUA and 50 per cent HUA puppies (Figure 2). Since 2008, there have been seven Dalmatians that are homozygous for the dominant gene and are, therefore, UU – as confirmed by DNA testing. These are the result of the mating seen in Figure 3.

Identification

Until recently, the only method of differentiating between NUA and HUA Dalmatian puppies was by doing a 24-hour urinary uric acid-to-creatinine excretion ratio (UUA:CR).

This is subject to fluctuations due to different rates of digestion, absorption and metabolism of the diets. However, it has still been found that the UUA level from NUA and HUA Dalmatians falls into two distinct classes, making it clear whether a puppy carries a copy of the normal gene.

A more basic screening can be carried out in the home. Urine is collected – preferably a concentrated early morning sample – and is refrigerated (Figure 4). A sample from a HUA dog will go cloudy due to the precipitation of urate crystal in the urine.

Urine fridge test

In Figure 4, the sample on the right has become opaque and indicates that it is from an HUA Dalmatian. While the fridge test will provide a basic indication as to which puppies in a litter have a copy of the dominant gene, a more specific and conclusive result is now obtained through DNA testing.

As mentioned earlier, the mutation underlying the disorder is in a gene called SLC2A9, and this can be tested from a buccal swab at the Animal Health Trust in Newmarket. The advantage of DNA testing is that it can be used to distinguish between a carrier and a homozygous normal condition. To date, there has been a perfect correlation between the DNA test and the UUA:CR test.

Health

Not all Dalmatians go on to produce urate stones, with their associated medical problems. A large part of this may be due to awareness of the condition by breeders who pass information on to future owners about how best to manage the condition. The recommended methods of minimising the risk of urinary stone formation are:

• adequate hydration (some evidence suggests that bottled, distilled or filtered water can be beneficial. Techniques such as adding splashes of milk to the water to encourage drinking and floating the food in water are often used);

• encourage the animal to perform frequent urination;

• limit the animal's purine intake (food tables that show the purine levels in different foods are readily available. High-purine foods, such as brewer's yeast and offal – especially liver, kidneys and heart – should be avoided);

• use pH indicators to monitor urinary acidity and aim for pH neutral; and

• in the event of crystal or stone formation, special prescription diets may be required, as well as the use of allopurinol, although the latter increases the risk of struvite stone formation. However, it is likely that the incidence of urinary stones is higher than reported figures suggest.

Other aspects of health have not been neglected in this project. Dogs have been chosen very carefully for breeding by using only those with bilateral hearing, brown eyes and good hip scores. Inbreeding has been avoided and only Dalmatians with good temperaments have been selected.

The other important aspect of this programme is that it is carried out with care and extreme patience. It is important to avoid genetic bottlenecks and make sure there is as much genetic diversity as possible. This is why close breedings have been avoided and why only eight Dalmatians carrying two copies of the healthy gene have been produced. No UU x UU matings have been performed as, at present, these dogs are too closely related. The aim is to spread the dominant gene throughout the Dalmatian population in a responsible way, so that we improve the health of the breed for many generations to come.

NUA Dalmatians in Europe

A working group led by Julie Evans, a highly respected breeder in north Wales, has been set up to help make the public aware of this ongoing project. More information can be found on its website (www.nuadalseurope.co.uk).

Mrs Evans has imported two NUA bitches from the US, and the registration of these dogs and their progeny was approved by The Kennel Club in January 2010, subject to assessment by two independent judges. Both girls carry one copy of the dominant gene.

Fiona BIMBS BISS Grand Champion Fiacre's First and Foremost (Uu) is three years old and has kindly been lent by Carol Chase Healy of Fiacre Dalmatians. She will have a litter of puppies sired by a Dalmatian from the UK. If this is successful, she will return to the US next year.

Sally Stocklore Sally Forth (Uu) is 15 months old and is the result of a mating between a Uu dam and a Uu sire. She has been imported by Mrs Evans and will eventually be bred to a UK Dalmatian. Approximately half of her puppies should have one copy of the NUA gene.

Wendy Stocklore Forrest Windsong (Uu) is two years old and was imported into France by veterinary surgeon Dominique Vincent. She has been entered into the French Kennel Club register and will be mated during her next season.

Selia Champion Fiacre N Aberdeen Seeing Is Believing (UU; is one of the small group of Dalmatians that carries two copies of the NUA gene. She is 15 months old and has excellent spotting.

It is hoped that with the introduction of this healthy gene into the European Dalmatian population and, indeed, worldwide, a significant health issue can be removed from this breed. If this is done in a careful, patient and responsible manner, it is an achievable aim.

References

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