HELP FREE BULL TERRIERS OF KIDNEY DISEASE

The Animal Health Trust got the funding for the research, but need DNA swabs from your Bull Terrier

The sample kits being sent in to the Animal Health Trust has dropped to a trickle. So much effort by a huge number of people *worldwide* went in to raising the funding for the development of the DNA test for the kidney problem – more than £11.000 was raised. However everything is at a standstill now from lack of samples!

So the breed needs YOUR HELP!

Contribute to the research with DNA cheek swabs from your Bull Terrier(s) and encourage your Bull Terriers contacts to do the same.

You can easily swab your dogs yourself – and you need one kit for each dog.

To obtain DNA swab kits - e-mail:

Bryan McLaughlin
Animal Health Trust
bryan.mclaughlin@aht.org.uk
with your name, address - and the number of kits, you need.

Note that you have to replace the form in the DNA kit with a special form, (Attachment 2) which can be downloaded and printed

Genetic inheritance

There are no bull terrier carriers of the kidney disease. They are either affected or clear. When mated, one gene from the sire and one gene from the dam are inherited by the resulting puppy.

Results are:-

Clear gene from sire x clear gene from dam – this bull terrier puppy will never develop or pass on the killer kidney disease

Clear gene from one parent x affected gene from other parent – this bull terrier puppy is born with the gene for kidney disease in their DNA profile and will pass it on if used in a breeding programme

The DNA profile the pup is born with is life long and will not change

Bull terrier kidney disease by Bryan McLaughlin B.Sc.

Input from veterinarians is absolutely pivotal for genetic research into inherited disease seen in any animal. Development of a mutation based DNA test for renal nephropathy in Bull terriers will require a decent volume of samples accompanied by robust clinical information.

If a vet is drawing blood for diagnostic reasons then taking a couple mls extra for research is absolutely fine, but to take blood from healthy bull terrier solely for research purposes requires a special licence issued by the Home Office.

Vets in practice are the most likely to see Bull terriers in their surgery with suspected kidney disease, and at this time it's usual to require a blood sample for analysis, before consideration of a referral. The owner's emotions are typically running high at this time, concerning the health and welfare of their family pet, so it may not be appropriate to broach the subject of research studies. However, permissions for research uses are sometimes incorporated into the consent form that the owner will need to complete when a procedure is being carried out, but alternatively consent can also be given verbally.

Renal nephropathy is considered to follow an autosomal dominant pattern of inheritance with perhaps some incomplete penetrance. The physiological defect is in the structure of the glomerular basement membrane, and kidney failure is generally around 5 years of age, although the severity and rate of progression will vary between individual dogs. Canine genetic researchers at the Animal Health Trust, Newmarket are hoping to acquire a sufficient number of samples from clinically affected cases to enable the determination of a genetic cause, and in turn allow the development of a DNA test which could be used as diagnostic tool by veterinarians, and would also help breeders make informed decisions for their breeding strategies.

Blood samples (in EDTA) accompanied by relevant clinical information should be sent to Bryan McLaughlin at the following address:

Canine genetic research,
Animal Health Trust,
Lanwades Park, Kentford,
Newmarket,
Suffolk,
CB8 7UU.

Swab Sample Collection Procedure

Please follow the instructions below carefully to collect the dog's DNA sample. Five (buccal) cheek swabs are provided in each kit and are intended for use with one individual dog only. The samples should not be taken within one hour after the dog eating to help obtain a clean sample.

1.First label the Swab Envelope clearly with the breed, dogs pet name, age, sex and owners surname as written on the sample submission form.
2.Remove a single swab from its packaging, trying to avoid any or excessive contact with the brush end, ideally but not necessarily, by wearing latex or vinyl gloves to reduce the possibilities of contamination.
3.Hold the dog's head firmly and roll the swab on the inside of the dogs' cheek on each side of the mouth, ensuring the brush is moved across the entire cheek surface. N.B. Cheek cells are not visible to the naked eye but please brush the surface thoroughly to ensure sufficient sample is obtained.
4.Briefly air-dry the swab for a moment and place directly into the Swab Envelope previously labelled at step 1.
5.Repeat steps 2-4 with the remaining four swabs when convenient and seal in the Swab Envelope. All five swabs can be used during the same sampling session, although this isn't necessary as the dog may become impatient. Use of each swab can be at different time intervals.
6.Complete the Sample Submission form. It is imperative that the clinical information given here is accurate otherwise it could adversely affect the outcome of the intended genetic study.
7.Return the sealed Swab Envelope along with the completed Sample Submission form using the return addressed prepaid envelope provided in the kit

Sample Submission Form

Breed: – ***Bull Terrier***

Dogs Pet Name: ..... Age/DOB.....Sex.....

Owners Surname: .....

Veterinary Practice: .....

Address: .....

..... Phone Number: .....

Clinical History: .....
….....
….....
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Please remove the existing form from the kit and replace with the attached form which is to be used for the bull terrier breed.

Please follow the swab sample collection instructions closely. The swab envelope is already printed and asking for KC. Information. It is Pre-printed by an external company for use in a generic swab collection pack The Animal Health Trust are accepting that the bull terrier breed will be supplying pet information as written in the sample submission form. Should AHT require clinical information from the vet who cared for the dog , the KC name would be useless as your vet would not have a record of it, having treated the dog under his/her pet name. Should a gene be observed on DNA screening which may be considered the causative gene for kidney disease, it is the vet who has treated the dog and ONLY THE VET who will be contacted for further clinical information

Confidentiality is guaranteed

To be certain of success we must all adopt

“The Mastermind Alliance”

which is:-

Two or more persons working in harmony to achieve the same objective.

Persons = The Animal Health Trust and every caring owner of a bull terrier.
Objective = To develop a DNA test to rid the bull terrier breed of kidney disease.

As owners/carers of bull terriers, we are the ones who can provide the DNA material (cheek swabs) to the Animal Health Trust, enabling them to develop the DNA test for kidney disease

With the owners/carers and the Animal Health Trust working in harmony

We will succeed in achieving our objective

Terry Heath,Holloville, Shieldhill Road, Reddingmuirhead,Falkirk FK2 0DU
Tel. 01324 720201 Email:- holloville123@gmail.com

DNA Testing – what is it and why do it?

In December 2004 a $30 million project funded by the National Human Genome Research Institute (NHGRI) in the United States to sequence the entire dog genome was completed and the results made publicly available. The NHGRI made the decision to fund such an expensive project because it recognised the dog as an unrivalled model organism with which to study the genetics of inherited disease. Although the NHGRI's motives for sequencing all the DNA in the dog were primarily human-centric the findings that emerged a little over five years ago have had profound implications for both veterinary and human medical research. Most importantly the pace at which genetic mutations responsible for inherited canine diseases have been discovered has increased dramatically and will continue to do so, as the tools available to dissect the genetic basis of canine inherited traits become increasingly more sophisticated.

Currently DNA tests are available for close to 100 different canine mutations, and over 120 breeds are able to take advantage of at least one DNA test. As pressure to improve the health of purebred dogs continues to intensify dog breeders will come under increasing pressure to make full use of all the DNA tests available to them, so it important that they understand what DNA testing is and what the results mean as well as appreciating the limitations of specific DNA tests.

The Development of DNA Tests

The development of a DNA test for any inherited disease always starts as a research project which, very simply, compares DNA from affected and unaffected dogs to locate and identify the mutation responsible for the condition under investigation.

The research laboratory will collect DNA from dogs that are affected with the condition under investigation (these are referred to as 'cases') and also DNA from dogs that are unaffected (these are the 'controls'). The number of cases and controls that are necessary depend on the condition being looked at; simple recessive conditions that are under the control of a single mutation in a single gene require the smallest number of samples, with as few as 16 cases and the same number of controls often being sufficient for a successful outcome. Complex conditions however, which are those under the control of either multiple genes or the interaction between genes and the environment, require far more samples, with hundreds of cases and controls sometimes being required.

The research laboratory will specify the criteria that define the cases and controls and these will depend on the condition. For example, if the disease under investigation is an inherited eye disorder then it is likely that both the cases and controls will need to have had an eye examination by a veterinary ophthalmologist and owners will need to be able to provide copies of that examination as evidence the dog is either affected or unaffected. If, on the other hand, the condition under investigation is epilepsy the most robust cases will be those dogs that suffer from seizures and that have had a battery of diagnostic tests to exclude other causes of their fits. Suitable controls, however, are merely dogs that have never had a seizure of any type.

The age of suitable controls also depends on the disorder. If the condition is congenital (present at birth) then any dog that is born healthy qualifies as a control. If, however, the condition doesn't typically develop until a dog is four to five years old then the controls will need to be several years older than this and still be free of the condition, to ensure they are in fact unaffected.

The research laboratory will store DNA from cases and controls until they have sufficient to initiate work on the condition. The precise methods used will vary, depending on the condition, the number of samples available and the available funding, but essentially will involve comparing DNA from the cases and the controls to eventually identify the precise mutation in the DNA that is responsible for the condition.

It is worth keeping in mind that DNA is a very complex molecule. A very simple analogy is to think of DNA as beads on a string. There are four different types of bead (A, T, C and G), known as nucleotides, which act as a code for the synthesis of protein and are responsible for determining everything about an animal that is not directly governed by its environment. The canine genome (the complete genetic composition of a dog) consists of 2.4×10^9 nucleotides of DNA. If each nucleotide was 1mm long the canine genome would stretch from Land's End to John O'Groats and back again and a disease-causing mutation can be as small as a single incorrect nucleotide. Locating and identifying the mutations responsible for disease is not, therefore, a trivial task and can take several years from the point at which the case/ control collection is complete and the research is initiated.

DNA Tests

Once a mutation has been identified a DNA test can be developed and offered to the public. Worldwide there are now many facilities offering canine DNA tests. The process of DNA testing involves the submission of a sample of a dog's DNA to an appropriate testing laboratory. The DNA can often be submitted as a simple cheek swab that an owner can take themselves, although some tests/laboratories may require a blood sample. The testing laboratory analyses the DNA for the presence or absence of the relevant mutation and will report back, usually within a few weeks, with the result (the dog's 'genotype'). The results will inform the owner whether the dog being tested has zero, one or two copies of the mutation being tested for.

DNA tests for disease-associated mutations - what do the test results mean?

It is worth considering what the results of a DNA test mean. It is important to remember that clinically similar conditions can be caused by different mutations. For example, forms of progressive retinal atrophy are known to affect many different breeds; currently around a dozen different mutations have been identified that cause PRA in specific breeds whereas the causal mutation for many more breeds have yet to be identified. Although clinically affected dogs of the same breed will usually share the same causal mutation it is possible for genetically distinct forms of the same disease to exist within the same breed. It is important for owners and vets to appreciate that most DNA tests only assay for a single, specific mutation, and not for any other mutations that cause clinically similar conditions. For example, mutations in the gene *HSF4* have been associated with hereditary cataract (HC) in the Staffordshire Bull Terrier, the French Bulldog, the Boston Terrier and the Australian Shepherd and DNA tests are currently offered to these four breeds (www.aht.org.uk) (Mellersh et al., 2006; Mellersh et al., 2007; Mellersh et al., 2009). But HC is known to affect many more breeds and *HSF4* has been excluded from involvement in most, meaning different mutations (as yet unidentified) are responsible for the condition in these other breeds. A clear DNA test result is not, therefore, an absolute guarantee that a dog will never develop a clinically similar disease to that being tested for, although dogs that are clear of specific mutations can be considered at very low risk of developing disease. Because many clinically similar inherited ocular conditions are known to have different genetic causes a DNA test can not, and should not, replace a clinical eye examination that has the ability to diagnose multiple defects and also detect newly emerging conditions.

Owners and veterinarians should not become complacent when a DNA test becomes available for a specific condition in a particular breed, as other, genetically distinct conditions, may well emerge. An example of a breed that is affected by genetically distinct forms of a similar condition is the Irish setter. A DNA test for the early onset form of PRA known as rcd1 has been available to the Irish setter since the early 1990's. Levels of testing within the breed have been extremely high, and rcd1 has been all but eliminated as a result. However, a new form of PRA, that has a much later age of onset (rcd4), has now emerged, and because the age of onset is beyond breeding age (and therefore the age at which even breeding dogs have routine eye examinations) the mutation is now estimated to be fairly widespread throughout the breed (Downs et al, submitted for publication).

Most of the DNA tests currently available are for mutations responsible for 'simple' or single gene diseases. This means that the disease is a result of a single mutation; no other genes or environmental factors are involved. For these diseases the results of DNA tests are easy to interpret and an individual dog's risk of developing the condition can be estimated with a very high level of certainty from the DNA test results. Many simple inherited conditions have a recessive mode of inheritance. Recessive diseases are the result of mutations that cause the loss-of-function of a biologically important gene, as opposed to dominant conditions which usually result from mutations that cause an inappropriate gain-of-function of a gene. Every dog has two copies of each gene, one inherited from the dam and one from the sire, and carriers that have inherited a single copy of the normal gene from one parent and a single copy of a mutant gene from the other parent

usually have sufficient functional protein to remain clinically healthy. It is only when a dog inherits a faulty gene from both parents that it becomes clinically affected. Consequently, if a mutation is recessive then dogs with zero or one copy of the mutation will remain clinically free of the disease, although heterozygous carriers will pass the mutation onto around half of their offspring. Dogs with two copies of the mutation (homozygotes) will almost certainly develop the disease during their lifetime, although they might be clinically clear at the time of testing. If a mutation is dominant dogs with one or two copies of the mutation will develop the condition (unless there is evidence of incomplete penetrance), whereas dogs that are clear of the mutation will remain healthy. Examples of single-gene DNA tests that are available include those for L-2-Hydroxyglutaric aciduria and hereditary cataract in the Staffordshire Bull terrier, canine leukocyte adhesion deficiency (CLAD) in the Irish and Irish red and white setters and progressive rod-cone degeneration (prcd) in multiple breeds.

Some diseases are more complex, and result from mutations in multiple genes or the interaction between genes and the environment. Individual mutations might increase a dog's risk of developing the associated condition, but cannot predict with certainty whether a dog will become clinically affected. One such example is the DNA test for a mutation associated with Hyperuricosuria (HUU) in the Russian Black Terrier, the Bulldog, the Large Munsterlander and the Dalmatian. The mutation increases an individual dog's risk of developing urinary calculi (stones) which may then require surgery, although some dogs that carry two copies of the mutation remain clinically free of the condition. It is suspected that additional mutations and/or environmental factors exist that modify the effects of the HUU mutation and explain why some dogs remain healthy (Bannasch et al., 2008).

Why test for disease-associated mutations?

DNA tests can play a critically important role in the control and eventual elimination of inherited diseases. Recessive diseases are notoriously difficult for the dog breeder to eliminate, because of the existence of clinically healthy carriers within the population that can only be detected retrospectively, once they have produced affected offspring or one of their parents has been diagnosed as affected. The problem is confounded for late-onset conditions where affected animals may be innocently bred with before they are themselves diagnosed and this problem is applicable to dominant as well as recessive diseases.

The availability of a DNA test is often the only way in which a recessive condition or a late-onset dominant condition can be reliably eliminated from a breed. Breeders should have their breeding stock tested prior to mating and make sensible breeding choices, based on the genotype of their dog, that minimise the risk of producing affected offspring. Disease mutations can be very common within specific breeds and once a DNA test becomes available the instinct of many breeders is to only breed from clear dogs. This practise will obviously eliminate the disease mutation from the breed very rapidly, but may do so at the expense of genetic diversity if large numbers of dogs are instantly removed from the gene pool. High levels of inbreeding and loss of genetic variation are well-documented to have detrimental effects on the health and

fertility of animals. For common recessive mutations it is therefore advisable for breeders to continue breeding with carriers, at least for the first generation following DNA test development. Provided all carriers are paired with DNA-tested, clear mates only clear and carrier puppies will be born; no clinically affected dogs will be produced and breeders can select a clear dog to breed on from the resulting litters. Table 1 details the outcomes of mating dogs with different genotypes (with respect to a recessive mutation) and whether they can result in clinically affected offspring.

Table 1

Combination of Dogs	Outcome	Possibility of clinically affected offspring?
Clear X Clear	All puppies will be clear	No
Clear X Carrier	50% of puppies will be clear 50% of puppies will be carriers	No
Clear x Affected	All puppies will be carriers	No
Carrier x Carrier	25% of puppies will be clear 25% of puppies will be affected 50% of puppies will be carriers	Yes
Carrier x Affected	50% of puppies will be affected 50% of puppies will be carriers	Yes
Affected x Affected	All puppies will be affected	Yes

For dominant mutations the situation is different. All offspring that inherit a disease-associated dominant mutation will develop clinical signs at some stage during their lives so breeding with animals that carry such mutations is harder to justify.

How accurate are DNA tests?

All laboratories offering DNA tests should provide clear and detailed information about the test. They should make it clear whether the DNA test is a mutation-based or a linkage test, and if it's a linkage test what the estimated error-rate is. As described above mutation-based tests examine a dog's DNA for the presence or absence of the precise mutation that causes a particular disease. The vast majority of current DNA tests are mutation-based tests and within the limits of human error a well-designed mutation-based test is 100% accurate. Linkage-based tests, in contrast, do not detect the disease-causing mutation itself, but instead analyse DNA markers that are known to be located very close to the mutation. Linkage-based tests can be

inaccurate in a small percent of dogs tested, because of the potential for genetic recombination to occur between the mutation and the markers being analysed.

The DNA testing facility should also explain whether the mutation being tested for is recessive or dominant, and the associated risks to dogs with different genotypes of developing clinical disease. If the mutation is incompletely penetrant (i.e. not all dogs with the mutation develop disease) then that should be documented. The laboratory should also give details of any genetically distinct forms of the same disease that are known to exist within the breed so owners can appreciate their dog's risk of developing a disease even if it is receives a clear DNA test result. It is also useful if the service provider can communicate the frequency of a specific mutation within a breed as a whole or at least from a specific geographic location, so that owners and breeders can use their judgement regarding the need to test their dogs. One way in which the validity of a DNA test can be judged is its publication in a peer-reviewed journal and it would seem prudent for owners and vets to remain sceptical about tests that remain unpublished 12 months after their launch dates.

Summary

Rapid advances in current technology mean that the number of DNA tests available to the dog will steadily increase in the coming years. If used wisely DNA tests can be very powerful tools with which to control and eliminate inherited disease and improve the genetic health of dogs. As the media continue to focus attention on the health of purebred dogs it should be the responsibility of anyone who breeds, cares for or treats dogs to understand the benefits and uses, as well as the limitations, of DNA tests.

References

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