Antagene's HS-test and HSIMS Tool

By Birgitte Damsgaard

In 2002 I attended my first international Berner health symposium in Lenzburg, Switzerland as a member of the breeding committee of the Danish Bernese Mountain Dog Club (DBSK). After that I have attended all the following symposia, and I have followed the development of the DNA risk test for Histiocytic Sarcoma (HS), a cancer which kills far too many of our Bernese Mountain Dogs. In 2006 I moved to Sweden where I breed BMDs with the kennel prefix Bernerbandens. That same year I lost my first BMD to HS.

I am a biologist with a Master of Science from the University of Copenhagen, and I have been teaching biology for the Advanced levels for over 30 years. So, this topic is of both private and professional interest to me.

What is cancer and how does my dog get it?
The body has billions of cells. They are all made from repeated copying and divisions from just one single stem cell, the fertilized egg. Therefore, all these cells have the same genetic information. As the body grows and develops the cells will specialize into various functions, muscle cells, brain cells, blood cells etc., but they all still carry the original information. That is why a DNA test from an individual dog will give the same result no matter which cells are used for the test.

In the adult body every cell has its own specific job to do. If the genes in one cell are altered (mutation), the result can cause the cell to stop doing its job and it may start to divide out of control instead. This is the first step of a developing cancer. Because there are many control mechanisms to put out of order before you have a fully developed malignant cancer, it takes more than one mutation. Mutations may arise spontaneously, or they can be induced by a host of environmental factors such as smoking, radiation, chemicals etc. That is why many cancers are the result of the sum of a lifetime of different influences, and why the risk of cancers increases with age.

Most mutations take place in somatic cells like skin or bone. These mutations only matter to the individual dog in which they arise. But if a mutation occurs in one of the stem cells that are going to develop into an egg or a sperm cell, the mutation will be carried on to the puppy that develops from that cell. This puppy will have the mutation in all his cells, including future egg or sperm cells, and will therefore pass it on to his own offspring. Now, the mutation has become hereditary. Some of these mutations may be beneficial, most have no effect, and some can be detrimental.
If a dog has inherited a large number of the mutations that make cells divide out of control, it doesn't take very many additional mutations to develop cancer. You could say that he is born with a high risk of getting cancer. This may explain why some heavy smokers live well into their 80's, or why some people who never smoke die young from lung cancer.

What is so special about HS?

Some time in the early days of the breed some mutations arose in the European BMD population that gave a high risk of an otherwise very rare type of cancer called Histiocytic Sarcoma (HS). What is special about this type of cancer is that it affects the histiocytes, a type of white blood cells whose job it is to creep around between other cells and eat bacteria. As histiocytes are located all over the body, a cancer that originates from these cells can develop in all kinds of organs at the same time. That is why HS is a particularly aggressive type of cancer. It will often be present in multiple organs that will begin to fail at the same time. So far there is no good treatment or cure, and the time from the day your dog gets diagnosed with HS till he is dead will typically be 2-6 weeks.

In the beginning there may have been just one dog with the HS mutations, and he was probably healthy because some of the mutations that cause HS may be recessive and would have to be present from both parents to cause HS. This dog must have been well used for breeding and because it was a numerically

small breed, and with the use of line breeding and popular sires, the mutations became widespread throughout the breed. Some of the early breed health surveys reported death from HS to impact about 17% of the breed. It is now widely accepted that this disease is 250 times more frequent among BMDs than most other breeds.

DNA, mutations, and DNA-testing
For each of a dog's 39 pairs of chromosomes (38 autosomal pairs, and one sex XY pair), one copy is inherited from the dam from her egg cell, and one from the sire from his sperm cell. DNA is the chemical substance that build the genes. DNA are long chains of a smaller unit that comes in 4 varieties. We call these A, T, C and G, abbreviations of the names of the units. The sequence of these 4 units make up the recipes to make all the proteins produced by a cell, and proteins are what make every process in a cell happen. If there is an error in the sequence of the units, the right proteins will not be made, and the cell will not be able to function correctly. This is what we call a mutation.

If your dog has inherited a mutation from just one of his parents, the intact gene from the other parent will often enable the cell to make the right protein and do its job anyway. We call this a recessive mutation. But if he got the mutation from both parents, the cell has no way of making that protein, and it has lost one of its functions. That could mean loss of the ability to prevent untimely divisions.

When developing a DNA test, biochemical methods are used to record the sequence of the units (A, T, C, and G) in a specific area of the DNA. It is possible to find out if the dog has two normal genes, two mutated genes, or one normal and one mutated gene.

Test development and validating
In 2000 the Swiss Bernese Mountain Dog club (KBS) arranged the first international health symposium, where clubs from all over the world got together to discuss the health of the BMD. In the following period there was a symposium almost every year, and the discussions soon concentrated on the short lifespan and high cancer incidence of the breed.

The 2005 symposium was attended by the French geneticist Dr. Catherine André, who told us that she and her co-workers at the University of Rennes were researching the genetics behind HS, a cancer also found in humans. Because of the high incidence of HS among BMDs, the breed is a good model for research purposes. We – and especially the French club - started to collect samples from BMDs who were divided into two groups: Affected dogs who had HS or had died from it, and healthy dogs who had reached an age of at least 10 years. The first group, sadly, being the largest. From this material Dr. André's group has identified 9 areas of the dog's DNA where the affected dogs differed from the healthy ones.

When there are 9 areas in which a dog can be either normal (N) or mutated (M), there are 3 possible combinations (NN, MM or NM) for each area. That adds up to 3^9 (=19683) possible combinations that a dog can have. The 9 genetic markers are given different weights and values and then a score from 0 to 1 is determined, where a dog having MM in all 9 areas gets a 0, a dog with all NNs gets a 1, and everybody else gets a number 0.xxx depending on the mutations.

From the first examinations of 256 affected and 165 healthy BMDs the research team got a result that can be summarized like this: (The chart has been reconstructed from the original boxplot, it does NOT show the actual data).
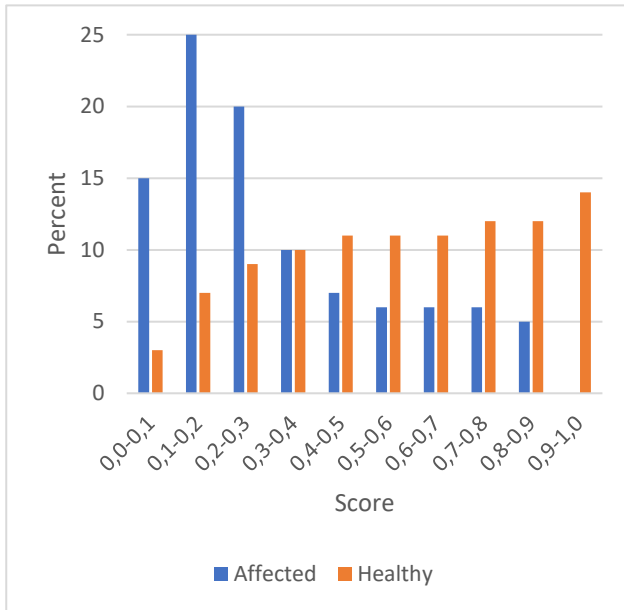
We can see that affected dogs with high scores and healthy dogs with low scores do exist, but the there is a clear trend that show more low score dogs are affected and more high score dogs are healthy. This was tested and found to be statistically significant.

Now the dogs were divided into 3 risk groups:
A – dogs with a score of 0.55 and over. That was 47% of the healthy dogs and 10 % of the affected.
So, over 4 times the chance of being healthy.
B - dogs with a score between 0.2 and 0.55. That was 43% of the healthy dogs and 50% of the affected.
So, approximately the same risk of being healthy or affected.
C - dogs with a score below 0.2. That was 10 % of the healthy dogs and 40% of the affected.
So, 4 times the risk of being affected.

This preliminary research and test development were for the most part done with hundreds of samples from French BMDs in collaboration between the research team and the French club, AFBS. In the following years, hundreds of more validation samples were supplied from many other countries, and the test has now been validated for BMDs world-wide.

Ordering the HS test and using the result
The test is performed by the French lab Antagene. You will find a step by step description of the procedure on this website http://bernese.co.uk/health/using-the-histio-test/ .
You can test a dog older than 3 months of age by supplying a blood sample
New-born puppies can also be tested. In this case you send in a cheek swab. If the puppy has not yet been chipped the result will be registered as unofficial.
(A third possibility is letting your vet do the cheek swab on a puppy that has been chipped but is not yet 3 months old. This will be an official result.)
The results will be shown as A – four times less risk of developing HS, B – equally likely to develop HS or not, or C – four times greater risk of developing HS.

Initially the recommendation was to simply avoid mating two high risk dogs (C) to each other. The French research team is explicitly warning against excluding the C-dogs from the breeding programme as this would result in the elimination of more than 30% of the breeding population. And that is what gave us the problem in the first place.

If you test your new-born puppies the result can be used to help you choose the puppy you are going to keep for future breeding. The question here is what to do with the C-puppies. What do you tell the puppy buyers? Is it OK, ethically, to sell a puppy that you know is in the high-risk group? Well, you could argue that the puppy does not have a greater risk than it had before the testing, and that the general risk of a BMD developing HS is at least 17% anyway. But it is obviously something you would have to think about before choosing to test your litter.

HSIMS – Finding the best match
Now that more and more dogs are being tested, it turns out that some A-A combinations produced some C-puppies, and that a C-B combination sometimes could give a better result than a C-A combination. How could that be?
Well. When we are combining 9 DNA areas with 3 possible combinations each (NN, MM, NM) from 2 dogs you get up to 262144 possible outcomes in the puppies. There are still "only" 19683 possible unique

combinations, but the frequency of these combinations among the 262144 will vary depending on the genetic setup of the two parents. Depending on the actual configuration of Ns and Ms in the two parents it is possible to calculate and score all these combinations, and predict the chances for each puppy to have an A, B, or C result from any given combination of dam and sire.

Thus, it is possible to determine which A-male would be the best match for the C-female you want to breed. But because the calculations involved are so complex, a tool has been developed to do the job: HSIMS (Histicytic Sarcoma Index Mate Selection). HSIMS is accessed from the client section of the Antagene website. You can use the tool free of charge if your own dog has been tested.

Instructions for using the HSIMS tool can be found here: http://bernese.co.uk/health/using-the-histio-test/ and here: http://www.bmdca.org/health/Antagene_histio_pretest.php .

Here is an example: 3 females from the same family (female 1 is the full sister of the mothers of females 2 and 3) combined with 5 different males.

Puppy index	Female 1 (index A)			Female 2 (index A)			Female 3 (index C)		
	A	B	C	A	B	C	A	B	C
Male 1	83%	16%	1%	88%	11%	1%	52%	39%	9%
Male 2	11%	78%	11%	31%	60%	9%	0%	13%	87%
Male 3	50%	50%	0%	63%	31%	6%	25%	25%	50%
Male 4	56%	44%	0%	71%	27%	2%	25%	44%	31%
Male 5	75%	25%	0%	83%	16%	1%	34%	53%	13%

You can see that two related females with the same index can produce quite different results by the same males. You also see that even though breeding a C-dog will generally produce more high-risk puppies, it is still possible to find a good match for a C-dog.

Now that we have this tool, the actual index of the male you are considering for your female is no longer necessary. But we need to have all the tested dogs made available in the HSIMS app. This is easily done by the dog's owner by logging in to the Antagene client section (client.antagene.com) and ticking off the dogs that you want to activate. The dog's actual status will not be shown to other users.

Conclusion

Dog breeding has always been based on selecting the traits we like and trying to eliminate the more undesirable ones.

At first breeding stock was selected from visible traits like conformation, movement, and behaviour. Then came X-rays and other health tests. And now we have DNA testing for a lot of different diseases, some more relevant to BMDs than others.

One needs to be careful not to be tempted to select too harshly for just one or a few of these factors, as the worst that can happen is a reduction in the genetic variation for the breed overall. By line breeding our predecessors have managed to "stabilize the type" of the BMD. But what this really means is that variation was minimized, and this also led to breed specific diseases becoming more frequent. The same can happen again if we were to exclude, say, the 30% of BMDs that have HS index C from being bred.

Gene testing is a fantastic tool, but it must be used with care. The greatest danger will always be having a high degree of inbreeding, which is associated with shorter lifespans, and increase of cancer and autoimmune issues, fertility problems, and reduced litter sizes.

This test gives us our first data driven ability to breed away from HS. Research is still ongoing to develop a direct test for the genes that specifically cause the disease. But until that is available, the HS risk test and HSIMS tool provide the best method to reduce histiocytic sarcoma in our breed.

Links:
Antagene: https://www.antagene.com/en

Client section: https://client.antagene.com/
Step by step guide: http://bernese.co.uk/health/using-the-histio-test/
HSIMS tool guide: http://www.bmdca.org/health/Antagene_histio_pretest.php