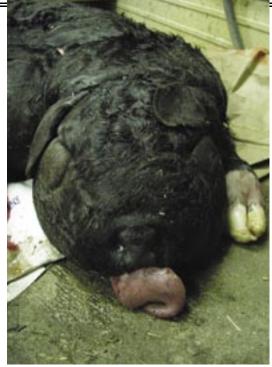# Cowboy Genetics

GENE HUNTING ON HORSEBACK — A TRIP THROUGH THE WILD WORLD OF MOLECULAR GENETICS!

by Lana Kaiser, DVM

I was hoping for a heifer (okay, let's face it, I am always hoping for a heifer, but this was different). When I bred the cow, I did not realize the bull was a tibial hemimeila (TH) carrier. Mid-gestation, the bull tested as a TH carrier so the calf now had a 50% chance of being a TH carrier. The way to clean up the genetic problems we currently face, as Gene McDonald from the American Shorthorn Association says, is to use clean bulls. So I tested the calf and he is a TH carrier and is now a steer. A carrier female can be managed or used as a recipient and, unless she is flushed extensively, she can be easily handled as a single individual. A carrier bull has the potential to impact a large percentage of your calf crop.

The genetic test for TH grants us the ability to make informed decisions regarding our breeding programs. Unfortunately, we do not yet have a test for pulmonary hypoplasia with anasarca (PHA). PHA, like TH, is a recessive trait. For a calf to be born with PHA, it must inherit the defective gene from both parents (Figure 1). Every time a carrier is mated to a carrier there is a 25% chance the calf will be affected and a 50% chance it will be a carrier. This means there is only a 25% chance the calf will both look normal (phenotype) and not have the defective gene (genotype). This risk occurs with every mating, like the toss of a coin. There is a 50% chance you will get heads and a 50% chance you will get tails. Therefore, your risk of having a PHA calf is 25% every time you breed a carrier to a carrier. In a theoretical herd of 100 PHA carrier cows bred to a PHA carrier bull, you would have 25 PHA calves, 50 PHA carriers and 25 normal calves. That would be a heck of a financial loss!

Wouldn't it be nice to know if your animal was a carrier before you bred them? If gene hunting goes well, we may have a test by the time this article is published. How do we find the defective gene responsible for PHA?

Face of a calf born with PHA. The sire was a purebred Maine-Anjou, the dam was a registered Chianina. Note the large amount of swelling of the face and the huge tongue. The ears almost invisible and the eyes appear as slits.

Genetics 201

In a cow, there are 60 chromosomes. There are 29 pairs (that look alike and carry the same type of genetic information) and two sex chromosomes, either XX or XY. Half of each pair is inherited from the dam and half from the sire. The dam can only contribute an X and the sire can contribute either an X or a Y — XX is a female; XY a male. Basically, the bull determines the sex of the calf! *See Figure 2 for a picture of all 60 of the cow chromosomes.*
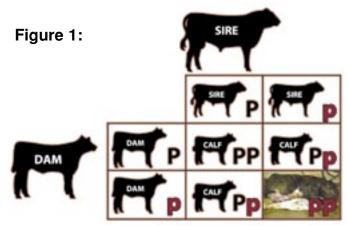
Figure 1:

Figure 1: Mating a PHA carrier bull to a PHA carrier cow. Genotype shows one calf is genetically normal. Two calves will carry the defective gene for PHA and one calf will have PHA. Phenotypically, three calves will look normal at birth but two of the three will be carriers for PHA. Each time you mate two PHA carriers there is a 25% chance of having a PHA calf. Every time you mate a PHA carrier to a PHA carrier you have a 25% risk of having a PHA calf, a 50% risk of having a PHA carrier and a 25% chance of having a genetically normal calf.

The chromosomes are located in the nucleus of the cell. Genes are located on the chromosomes in an orderly, linear fashion. One might imagine them as beads or knots on a string. For example, the genes for horn development and milk yield are on cattle chromosome 1 and are always in the same place on chromosome 1. In the cow, there are about 40,000 genes. See Figure 3 for a diagram of a cow chromosome and the genes located on it.

Genes are made of DNA (deoxyribonucleic acid). DNA is made of two very long chain-like molecules whose individual pieces are called nucleotides. Bases in the nucleotides stick the two chains together. There are four bases (A, C, G and T) and in genetic lingo, a C always pairs with a G and an A always pairs with a T.

Figure A

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GGCATCCCCGTGGAGGAGCTT
|||||||||||||||||||||
CCGTAGGGGCACCTCCTCGAA
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Figure B

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GGCATCCCCGAGGAGGAGCTT
|||||||||||||||||||||
CCGTAGGGGCTCCTCCTCGAA
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Figure A is the normal DNA sequence for a region of the gene defective in ovine hereditary chondrodysplasia or Spider Lamb Syndrome (SLS). Figure B is the same sequence but the T/A base pair in the normal sequence is an A/T base pair in the sequence that causes SLS. See Figure 4 for a diagram of a DNA strand.

In the cow genome, there are over three billion bases. Each gene controls a function. For the gene to work properly, the bases must be lined up in a specific way. A change as simple as a one base for another can result in disease, as seen in the previous sequence.

If you look at the entire chromosome, you will see lots of DNA. Some of the pieces of DNA are what may be called "real genes." They code for something specific, like coat color, polled, milk production, etc. Some of the pieces are called "markers." Markers are like

Figure 2

Figure 2: Photograph of chromosomes of the cow. There are 60 chromosomes, half inherited from the sire and half from the dam. The chromosomes are arranged in pairs (one from each parent), 1 through 29 and the X and Y. For example, the gene for coat color is located on chromosome 1 - each parent contributes one gene for coat color.
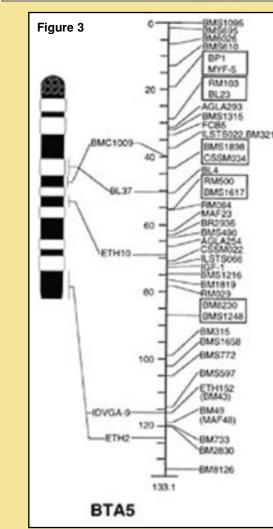

Figure 3

BTA5

Figure 3: Schematic of bovine chromosome 5. On the right are listed the genes that have been identified. The chromosome can be thought of as a road - identified genes have a particular "street number" on the road. New genes can be identified, but like new houses being built on a road, they have to fit between known genes. The markers are like street signs, always in the same place and telling you where you are on the chromosome.

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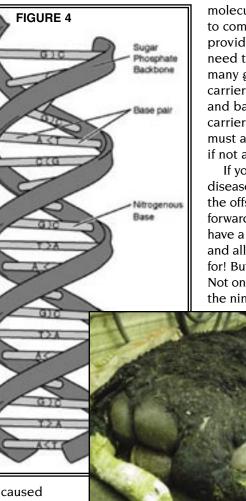
Gene hunting

Molecular genetics is a complicated field relying heavily on computer programs and high tech machines and techniques. However, you also need pedigrees, samples and in the case of livestock, people willing to take time to help obtain samples and pedigrees. Molecular genetics has its own lingo and just about everything has two or three different names! There are a couple different ways to find the gene in question depending on the genetic problem, mode of inheritance and information in the literature. Three basic ways to find the gene are using the karyotype, candidate gene and genetic mapping.

Karyotype

This technique looks at the chromosomes and compares a "normal" karyotype to one from the defective animal. In a normal karyotype, all the chromosomes are present and in pairs (except for the unpaired XY or XX) and they are not missing any pieces or have abnormal shapes. Figure 2 shows a normal bovine karyotype. If there is an abnormality, you know right away which chromosome it is on because that chromosome does not look right. A translocation (of one part of a chromosome to another) in Simmentals is responsible for reduced fertility. In the case of PHA, there is no problem with the karyotype so we have to look elsewhere to find our genetic problem.

Candidate gene

Looking for a candidate gene means you are looking for a gene known to cause a similar problem in another species. Let's look at TH (tibial hemimelia). Phenotypically, there is a short or absent tibia so you would look for the same defect in other species. How would you find it? You would spend hours and hours searching through many computer databases looking for similar defects. Let's say you find a mouse defect with a short tibia. You would then look for a gene in your cow similar to the mouse gene

FIGURE 4

Sugar Phosphate Backbone

Base pair

Nitrogenous Base

that caused the problem. Unfortunately, there is not a candidate gene for PHA so we have to look elsewhere to find our genetic defect.

Gene mapping

This technique requires multiple generations of animals and the use of molecular genetics. Basically you look for the defective gene and the marker that are inherited together from an "index" animal (the "bad apple" or the animal who originally contributed the mutation to the population). The defective gene and the marker are "linked." You become suspicious that you have found the defective gene because you identify them in the index animal, in animals known to be carriers (because they have had a PHA calf) and in the PHA calves, but NOT in "normal" animals.

How does this work? Let's do it in two parts, pedigree and

Rear of a calf born with PHA. T*he huge bilateral swelling is fluid, not muscle. T*he scrotum is the size of a two-year-old bull. U*nfortunately it is completely filled with fluid.*

molecular genetics. We will then try to combine them. For a pedigree to provide us with the information, we need two things, information from many generations including known carriers for the defective gene, and based on PHA calves, known carriers for the defective gene. We must also have samples from most, if not all, animals in the pedigree.

If you have mice with a recessive disease, you can breed them, breed the offspring, cross them, back breed, forward breed and in short time you have a multi-generational pedigree and all the samples you could ask for! But cattle are a little different. Not only do cows not have litters, but the nine month gestation time, the possibility of sending animals to slaughter (and being lost forever for testing) and the cattle being all over the country, make it difficult to get all the information and the samples you need. That is why many people work with mice, but not Dr. Jon Beever, he likes cows and a challenge!

Look at the sample pedigree (Figure 5). We flush Bessie to Fred and have four ET calves and one calf has PHA. We know both Fred and Bessie are PHA carriers. We keep breeding our cattle and find Mike, a direct son of Fred, is the grandsire of a PHA calf. This means Mike's son, John is a PHA carrier and the dam of the PHA calf is also a carrier. We do not know if John got the defective gene from his sire, Mike, or his dam, but since Mike's full sib had PHA, it is suspicious Mike is a carrier and John received the defective gene from Mike. Down the road, Mike is bred to Sally and has a PHA calf. By definition, Sally is a PHA carrier. Interestingly, Sally
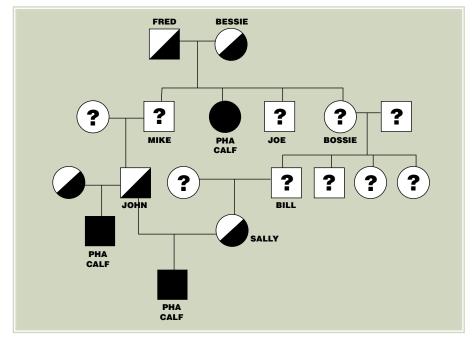
Figure 5: Sample PHA pedigree of the bull "Fred" and the cow "Bessie." In this pedigree, squares represent bulls and circles cows. Filled in symbols show the animal was born dead with PHA. When the animal is a carrier, half the symbol is filled in. Bessie was flushed to Fred and four embryo calves resulted. One calf had PHA, therefore we know both Fred and Bessie are carriers. Three other ET calves, Mike, Joe and Bossie look normal but we do not know if they are carriers or not. Other matings occur and more calves are born. In the fourth generation, a PHA calf is again sired by a son of Mike. Interestingly, this fifth generation PHA calf has Mike's flush mate, Bossie on the dams side of the pedigree implicating Bossie as a carrier as well as Bossie's third generation son, Bill. When you have a PHA calf, you can make some assumptions about the status of the parents of the affected calf.

is a great granddaughter of Fred and Bessie again suggesting Sally received the defective gene from her sire's side of the family. In order to analyze pedigrees, we need lots of information on lots of generations and based on the data we have, we can make assumptions about inheritance of the defective genes.

Mapping

Looking for a defective gene depends upon a couple of "genetic laws." The idea here is when cells split (to form embryos and eventually baby bovines), some parts of the DNA always travel together. In normal populations, genetic traits and markers will occur in all possible combinations with the frequency of combinations determined by the frequencies of the individual genes. If a mutation in a gene causes a disease in a particular subpopulation, it almost always occurs with a particular marker. Basically, you are on a treasure hunt to find the gene that is always linked to the same marker and

whenever you find that gene-marker combination, you have a calf with PHA (two copies of the gene-marker combo) or a carrier for PHA (one copy of the gene-marker combo).

Sounds simple right? Of course you are looking at thousands of pieces of DNA for the gene-marker combination. There are computer programs and databases to help with the search. Once you find the gene marker combination you think is the dirty do'er, there is a formula to determine how likely it is this combo is the result of chance versus the problem gene. This is called a lod score. The higher the lod score the better. A lod score of three tells you there is one chance out of 1,000 the gene and the marker

are not linked. A lod score of eight basically tells you the gene and the marker are linked, one chance out of 100 million they aren't. This means chances are you have found the defective gene.

Now what? You have located the gene but still must find the specific mutation causing PHA. You have narrowed the possibilities from one in three billion DNA base pairs to roughly one in 50,000. The complete DNA sequence for the gene is determined in a normal, carrier and affected animal. The DNA sequences are compared and mutations are consistent with each animals expected genotype (i.e., known PHA negative is homozygous normal, known carrier is heterozygous and the PHA affected individual is homozygous for the opposite allele as the normal individual) are documented and examined further. Each mutation you examine is another panel of DNA samples from different types of animals. All the calves with PHA should have two copies of the defective gene; all carriers should have one normal gene and one defective gene; and non-carriers should have two normal genes.

How do you make a test? First, you need samples and pedigrees as discussed above. Next, you need to validate the test. You need lots of samples for validation. You need samples from the affected calf (two defective genes), sire and dam of the calf (one normal and one defective gene) and a number of "normal" samples (no defective gene). You run all these samples to

Calf born by C-section with PHA. Note the massive swelling of the entire body.

	FRED	BESSIE	MIKE	PHA1	JOE	BOSSIE
PHA	XXXX	XXXX	XXXX	XXXXX		XXXX
NORMAL	XXX	XXX	XXX		XXXXX	XXXX

Figure 6: Schematic representation of what the PHA test will look like once the PHA gene is identified. This is a gel testing for PHA in cattle from our sample pedigree. Each lane is the sample for one individual (Fred, Bessie, Mike, etc.). PHA indicates the gene for PHA and Normal indicates the normal gene. An animal with one band in PHA and one band in Normal is a PHA carrier (Fred, Bessie, Mike, Bossie). An animal with one band in the Normal region has two normal genes. Once the gene is identified, we can use molecular techniques to identify PHA carriers, normals (non carriers) and PHA affected calves.

Figure 7: Photograph demonstrating the DNA-based test for tibial hemimelia (TH). Using molecular genetic techniques, the DNA from each of ten individuals was used to determine their TH status. Each lane (1 through 10) represents one animal. The band closer to the top of the gel (labeled TH) is the gene for TH; the band closer to the bottom of the gel is the normal gene. Each animal inherits two genes; if both genes are normal (or both genes are TH), only one band appears because the bands overlap. If the animal is a carrier, two bands appear - one TH and one normal. Animals in lanes 1, 6 and 9 are homozygous normal. Animals in lanes 2, 4 and 8 are homozygous for the TH mutuation, indicating that the samples were taken from TH affected calves. Animals in lanes 3, 5, 7 and 10 possess both genes indicating they are heterozygous or carriers of the TH mutation.

validate the test. If you get the results you expect with the validation, you have the test and you have the gene! This is exactly what happened with TH and now we have a test to identify TH carriers as well as those who are TH free (do not carry the defective gene). See Figure 6 for a schematic of the test for our pedigree and Figure 7 for a picture of the TH test.

What do we need to develop a genetic test? First, we must have a dedicated and somewhat possessed molecular geneticist who is interested in livestock genetic disease, especially cattle. It is our good fortune to work with Dr. Jon Beever. Next, we need a dedicated and somewhat possessed cattle veterinarian who has the foresight to know when things are not quite right and follow up on them. Again, we are fortunate that Dr. Chuck Hannon is in the field. Finally, we need breeders who care about the breed and their cattle that have submitted samples and will continue to submit samples.

Genetic diseases occur in all species and all breeds. They are more likely to occur when humans make breeding decisions based on desired phenotype. In essence, we have created the problem, now we have a responsibility to fix it. Let's provide Dr. Beever with samples and once the test is available, use it to breed the best Maine-Anjou cattle we can.

For more information on PHA or if you think you have a PHA calf, contact:

Dr. Beever (217) 333-4194 jbeever@uiuc.edu or

Dr. Chuck Hannon (219) 863-0528 chuck@liljasper.com or

Dr. Kaiser (517) 282-7899 kaiser@msu.edu

We need your samples.

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