Collie Eye Anomaly

Veterinary Bachelor Thesis, June 2011
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Abstract

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Collie Eye Anomaly is a genetic eye disease that has been known since the middle of the last century. It is a congenital inherited defect and affects several breeds of similar origin. Affected individuals show a wide range of severity, ranging from mild hypoplasia to ocular hemorrhage causing blindness. The prevalence of the causative mutation in the affected breeds is found to be very high in the breeds that are most severely affected, but quite low in others. Possible reasons for this is discussed.

The mutation that causes CEA, a 7799 basepair deletion on the canine chromosome 37 in the NHEJI gene, was discovered in 2007. It was found to be autosomal recessively inherited deletion, and a DNA test is available through Optigen. The test makes it possible to know the genetic status of all animals of the affected breeds and suitable decisions can be taken when choosing breeding animals. It is possible to use this test to make sure that breeders do not produce affected offspring, but for the breeds where the prevalence is highest that goal might take a few more generations to achieve. Still there are some unanswered questions and differing opinions on this anomaly as not all experts agree on inheritance and manifestations.
Preface

This thesis was carried out at the Department of Basic Animal and Veterinary Science at the Faculty of Life Science, University of Copenhagen. The aim of the thesis has been to peer into the literature on the subject of Collie Eye Anomaly as a final part of the bachelor portion of the veterinary degree. It is addressed to veterinarians, veterinary students and other people interested in genetic ocular disease in animals.

I would like to thank my supervisor, Camilla Vibeke Bruun, for her invaluable advice and support throughout the process of writing. I would also like to thank my fiancé Valdimar Ómarsson for his great help, Finn Boserup, Henrik Bartholin, Jennifer Heaven and Elisabet Hrönn Fjóludóttir for their instructions, proof reading and feedback on the thesis and Ellen Bjerkás, Kerry L. Ketring and Osamu YAMATO for the use of their pictures.
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Chapter 1

Introduction

1.1 Background information and problem definition

Collie eye anomaly (CEA) is a congenital inherited ocular disorder in dogs of herding and other related breeds. It is non-progressive and it manifests variably between individuals [12]. It is characterized by regional hypoplasia of the choroid, and coloboma of differing severity between affected individuals. The disease has been known for decades and has been somewhat problematic with regards to breeding. Not only does it cause blindness in a small number of affected individuals, but also due to the "go normal" phenomenon which causes the window to correctly diagnose the anomaly to be fairly short. There are still differing opinions on whether the disease is inherited as a simple autosomal recessive disease with complete penetrance [16] or by a polygenetic mode with incomplete penetrance [18, 19] and also on whether or not the hypoplasia involves the retina or not [17].

The aim of this study is to present the manifestation and current situation of the disease in the affected breeds, to point out how information of genetic status can be used in breeding and to see if the currently available DNA test has had an influence on the prevalence of the mutation.

1.2 Delimitation

This study was conducted as a literature study of books and articles published between 1971 and 2011. It encompasses the breeds known to carry the same mutation that was found to cause CEA. The manifestations are described and the currently available genetic test is presented, as well as how it can be used. Also general anatomical and genetic information is included due to their importance to a full understanding of the complexity of the matter, but are not the main object of this study.
1.2. Delimitation

Introduction
The disease

The disease has been known for years as affecting the Collie breeds, mostly the Rough and Smooth Collie as well as Border Collies, Australian Shepherd and Shetland Sheepdog. It has been somewhat shrouded in mystery when it comes to breeding and genetic background as it varies tremendously between individuals in severity and effect.

2.1 Historical background

Collie eye anomaly was first described in the middle of the twentieth century in Europe[18, 19] but might have been known as early as the nineteenth century [11]. Since then it’s presence has been well known by breeders and researchers and quite a lot of study has gone into understanding the disease as it varies tremendously in severity between affected individuals. But because of this individual difference the true extent of the disease and it’s spread in the affected breeds has never been fully know or it’s significance properly acknowledged. Also, the fact that not all individuals are severely affected with lack, or loss, of vision has led some breeders and breed clubs to consider breeding restrictions to be unnecessary [5]. But even with the long history of CEA it has until recently only been poorly understood, and today we still have some way to go to know everything there is to know about the anomaly.

This anomaly has historically been called posterior scleral ectasia and posterior staphylomas [8] and still today there are differing opinions on the most correct name for this anomaly.

2.2 Breeds

The anomaly, even though it is named after collies, does not only affect the collie breeds, but they are most commonly affected [16]. In a recent study done at Cornell University on CEA, breeds were grouped together in clusters, using a statistical computer program and genetic material from different breeds, in order to locate the mutation that causes CEA. In their study they found that the breeds most commonly affected by the anomaly were all in one way or another related, and
that they most likely inherited the anomaly from a common ancestor further back. The breeds affected by CEA are the Rough and Smooth Collie, Border Collie, Shetland Sheepdog, Australian Shepherd, Lancashire Heeler, Nova Scotia Duck Tolling Retriever and the long coated variety of the Whippet. The anomaly was also found by chance in a few Boykin Spaniels [16].

The current status of the mutation prevalence in the affected breeds was obtained from Optigen and is depicted below. The percentage includes animals that carry one or two copies of the mutation.

- Rough Collie 72%
- Smooth Collie 62%
- Whippet, Longhaired 60%
- Shetland Sheepdog 52%
- Border Collie 35%
- Lancashire Heeler 30%
- Nova Scotia Duck Tolling Retriever 18%
- Australian Shepherd 15%

2.3 The healthy eye

The eye receives light stimuli from the environment and converts the stimuli to electrical signals that are sent to the brain via the optic nerve where it is translated into the final image. The eyeball is not evenly rounded and the cornea bulges forward.

Figure 2.1: The healthy eye.
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2.3. The healthy eye

The eye is divided into three compartments, the anterior chamber, the posterior chamber and the vitreous layer (figure 2.1). The anterior chamber is between the cornea and iris, the posterior chamber between the iris and the lens and the vitreous chamber is behind the lens surrounded by the retina. Further information about the anatomy of the eye can be found in a wide range of anatomy books, instead we shall focus on the area of the eye of interest with regards to CEA.

The posterior part of the eye, or the fundus, is made of three layers, a fibrous layer (sclera), a vascular layer (choroid) and an inner layer (retina). The sclera (figure 2.2a) is the outer layer and is made of a dense network of collagen fibers and some elastic fibers dispersed therein to help with the internal pressure of the eye. It varies in thickness being thickest at the posterior pole of the eye where the optic nerve enters the eye. The scleral venous plexus, located at the border of the sclera and the corneoscleral junction, drains the aqueous humour regulating the intraocular pressure.

The choroid layer (figure 2.2b) is divided into four sub layers, the supachoroid layer, the vascular layer, the choriocapillary layer and the basal lamina. The supachoroid layer contains a fibril network embedded with pigmented cells, forming a loose connection between the sclera and the choroid. The vascular layer is made of pigmented, lamellar connective tissue and is the thickest part of the choroid. This layer contains the blood vessels that nourish the neuronal layers of the retina. Along with the choriocapillary layer, which these blood vessels send branches to, they form the inner capillary bed of the choroid. This network is responsible for nourishing the outer layer of the retina. Furthermore the canine eye contains an area of reflective layer called the tapetum lucidum (figure 2.2d), which reflects the incoming light thereby enhancing the stimulus and thus helps with night vision.

The retina (figure 2.2c) is the innermost layer of the eyeball and is connected to the optic nerve. It can be divided into the non visual retina and the optic part of the retina. The non visual part consist of two layers, a pigmented outer layer and an unpigmented inner layer. The optic part consist of two parts as well, the outer pigmented layer and inner neuronal layer that includes photoreceptors. The optic part is where visual images are transformed into electric impulses that are transmitted to the visual centers of the brain. The optic disc (figure 2.2e), the area where the optic nerve enters the eye, is completely void of photoreceptors and is commonly referred to as the blind spot [10].
2.4 Clinical manifestation and pathology

CEA involves deformation of the vascular and fibrous layers of the eye. This disease causes four different clinical manifestations listed from mildest to most severe.

- Chorioretinal Hypoplasia
- Coloboma
- Retinal detachment
- Intraocular hemorrhage

[4, 17, 7]

These lesions are usually not symmetrical between the eyes. The mildest form, chorioretinal hypoplasia, is seen in almost all cases of disease. Here the chorioid hasn’t developed normally and gaps lacking in pigment in the chorioid can be seen, usually lateral to the optic disc. Unusually formed vasculature, retinal vascular tortuosity, can also be seen through the unpigmented chorioid and underneath the white sclera though they are not thought to be a part of the anomaly. This malformation is present at birth, and does not change with age[4, 12, 7, 12].

Authors differ in their usage of either chorioretinal dysplasia or chorioretinal hypoplasia, and both forms can be found in many books and articles. Dysplasia and hypoplasia are not the same thing, dysplasia being an abnormality in development of tissue and hypoplasia is underdevelopment of tissue [13]. It is interesting that experts seem to differ on this fact as it seems it is not fully understood if this is caused by underdevelopment or abnormal development. Also there are different opinions on the involvement of the retina and the names chorioidal and chorioretinal can be found in different literatures [17].

Coloboma, also called pits or cups, are normally located in the middle of the optic disc, but may in some cases be located besides the disk, or be so big as to involve the whole peripapillary region [12]. Small coloboma do not normally give problems but big ones can give visual problems, retinal detachment and intraocular hemorrhage [4, 7].

Retinal detachment happens in about 4-5%[12] in affected animals and results in reduced vision or even blindness. Retinal detachment can affect either a local area or the entire retina, and is then only attached at the optic disc where the optic nerve leaves the eye. This can both occur spontaneously in adults or be present at birth and the animal is blind in the affected eye.[4] CEA is normally asymptomatic unless there is retinal detachment, which can perhaps account for it’s prevalence [12].

Intraocular bleeding can also occur when the retina detaches, and is not considered a basic part of CEA but a result of the anomaly. Through a rupture of the small vessels of the eye the eyeball can be filled with blood. The blood is unlikely to be cleared once the bleeding has started but it will usually not result in increased ocular pressure and therefore it isn’t painful for the dog [4, 7].
Other defects were originally thought to be a part of CEA, including microphthalmia, corneal opacity and retinal folds, but have later been shown to not be a part of the anomaly [3].

2.5 The “Go Normal” phenomenon

One of the biggest mysteries of Collie eye anomaly is the so called “Go Normal” phenomenon. This is where animals that have been diagnosed clinically affected when they are puppies appear normal when examined as adults. In the puppy eye the fundus appears blue, but at about three months of age the retina changes color to its adult yellow or green appearance. It is therefore possible that this change in color can mask the chorioretinal dysplasia and dogs examined ophthalmoscopically at an adult age appear to be clear of CEA, but when used in breeding they can give affected offspring [3].
2.5. The “Go Normal” phenomenon
Eye examinations

The optimal age to diagnose Collie Eye Anomaly is between 5 and 12 weeks. At three months of age the colour of the retina changes to its adult appearance and this change can mask the mildest manifestation. Eye examinations are minimally invasive to the animal, painless and require no surgical involvement. A normal procedure begins with inducing mydriasis, with for example 1% tropicamide and then examinations are carried out in a dark room by focal illumination and by direct or indirect opthalmoscopy [3]. The examination is relatively inexpensive and takes a short time and litters of puppies can be examined at the same time they have their first set of vaccinations done before they leave the breeder. This is a very useful strategy as the examination then happens at the sixth or seventh week of the puppies life. This is within the optimal time to diagnose CEA. This has also an added benefit of finding clinically affected individuals before the puppies have left the breeder.

3.1 What to look for

Starting with the mildest clinical manifestation, Chorioretinal hypoplasia. Under opthalmoscopy an unpigmented area, usually lateral as seen here, is evident. Due to the lack of pigment the underlying choroidal vessels are evident and between them the white sclera can be seen (figure 3.1). It is also possible that the retina is underdeveloped in this area [4]. In a normal eye the vessels would be very regular and straight but with this anomaly they are very irregular in distribution and shape. This manifestation can be difficult to diagnose correctly in individuals due to the “go normal” phenomenon but is usually quite easy to see in puppies before the age of three months before the retina has changed it’s color to the adult appearance [4].
3.1. What to look for

Eye examinations

Figure 3.1: In right side the area has less pigment, large arrow shows unpigmented area and small arrows show tortuous vessels.
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A coloboma is found in the ocular nerve where it leaves the eye. A coloboma can range in appearance from small dark pits to deep caverns in the optic disc. It is easier to see in real life than from a picture as the 3D of the pit is not fully seen in two dimensions. But a good way to recognize it from an image is to see where the blood vessels fall or plunge into the pit (figure 3.2). Small colobomas usually have little effect on vision, but a large defect can give visual impairment [17, 4].

Figure 3.2: A coloboma in the optic disc (large arrow), small arrows show blood vessels falling into the pit.
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Retinal detachment can appear as focal grayish areas, but with a more extensive retinal detachment it will no longer be seen within the normal retinal plane but will appear as billowing folds (figure 3.3). The detachment can either be just in a
localized area that bulges forward or a total detachment where it is only attached around the optic nerve[17, 4].

Figure 3.3: *Retina seen as almost billowing in the eye.*

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3.1. What to look for

Eye examinations
Chapter 4

Genetics

Genes contain the recipe for all life and in them all the information of life is contained.

4.1 Mendelian inheritance

The work of an Austrian monk named Gregor Mendel brought one of the biggest breakthroughs in genetics with his work with pea plants and their traits. Mendel showed that inheritance follows certain laws, that later were named after him, the Mendel’s law of inheritance. Mendel proposed that for each trait, every plant carries two copies of each unit of inheritance. These units are genes, and for each gene every individual carries two copies, two alleles, which influence the trait. Furthermore each gene usually have several allelic variants, which may be dominant, recessive, codominant and incompletely dominant in relation to each other. It is not the object of this article to delve deeply into the basic knowledge of genetics, so the reader is advised to find further knowledge in the copious amount of literature on this subject. Instead a deeper look at what is of importance to Collie Eye Anomaly shall be taken.

As previously stated CEA is an autosomal recessive disease, but what does that in fact mean? Apart from the egg and sperm cells (gametes) that contain only half the number of chromosomes of a complete cell, the nucleus of the dog cell contains 38 pairs of chromosomes and 2 sex chromosomes. A total of 78 chromosomes. These cells are diploid, meaning they contain two copies of all chromosomes. The gametes on the other hand are haploid as they contain only half the number of chromosomes, a total of 38 Chromosomes and one sex chromosome.

Crucially, of the pairs of chromosomes that exist, the pair of sex chromosomes in females are identical (XX) but in males are non identical (XY). The Y chromosome is shorter and thus contains less genetic information. When the gametes combine in fertilisation this leads to a departure in inherited traits between males and females that is not confined to sex alone. A good example of this in humans is the propensity for males rather than females to suffer colour blindness. The other chromosomes are called autosomal chromosomes and traits inherited on an autosomal chromosome usually do not show any difference between males and females and are inherited equally in both sexes [9].
4.2 Mutations

When a sperm and an egg fuse in fertilization they create a new cell, the so called oocyte that is the first cell of the offspring. This cell then duplicates and multiplies and from it all the cell in the offspring stem from. When the chromosomes in the sperm cell and the egg unite the oocyte again has two copies of each chromosome, one derived from the father and one derived from the mother. The offspring therefore has half of its genetic material from each parent and a full number of chromosomes, in the case of dogs 78 [9].

4.2 Mutations

The chromosomes carry the genes, and on one chromosome there are multiple genes all controlling different things in the animal. The genes can be visualized as the recipe from which the animal is made, so a flaw in the recipe can cause major changes in the final product. A simple typo might change salt to saft (a concentrated juice), 2 teaspoons to 23 teaspoons, water to wetar and 200 ml to 20 ml. If a recipe like this is followed to the letter the end product might be nothing like it is supposed to be or the cook might see through them and they would have hardly any effect. All these mistakes are possible in the genetic code in the DNA string, that is in the sequence of its bases.

Mutations can happen either during DNA replication which happens before cell division, or as a cell damage caused by for instance to X-ray or UV light. There are five possible categories of mutation that can happen to the DNA string, a substitution, a deletion, an insertion, an inversion and a reciprocal translocation. A substitution is when a base at a certain location on the string is replaced by one of the three other possible bases. A deletion is when one or more nucleotides are lost from the DNA code and an insertion is the exact opposite, that is when one or more nucleotides are inserted into the string. The last mutation, inversion is when a segment of the DNA string rotates $180^\circ$. A reciprocal translocation is when parts of two non-homologous chromosomes switch places. Recent research showed that the mutation that causes CEA is a deletion, where 7799 bases were lost from the gene. For a mutation to be carried to the next generation it needs to be located in the gametes. Otherwise, if it is only located within the normal cells of the body, it will not be replicated in the sperm and eggs and therefore the mutation will die out with the individual and not cause any further trouble[9].

4.3 Recessive versus dominant mutation

In the simplest form of dominance one allele is dominant over the other. That means that only one copy of the allele is needed for the effect, or phenotype, to be present. An example of this is coat color. A black coat color is dominant over brown, so only one copy of the allele for black is needed for the dog to be black. The dog can then carry either one allele or two for a black coat, but even though the phenotype (in this case a black coat) is evident, the dogs genetic makeup, or genotype is not. If a dog is brown, it inherited an allele for brown color from both it’s parents. The brown coat color is an example of a recessive coat color. For a recessive phenotype to be present the individual needed to inherit two alleles that
are recessive for that phenotype. When it comes to genetic faults that are dominant or recessive with a recessive disorder one copy of the normal allele is enough for the individual to be normal. If both alleles are faulty or mutated neither of them work correctly but if one allele is normal that is enough for things to work normally. When a fault on the other hand is dominant one faulty or mutated allele is enough for things to go awry. In this instance it is not enough to have one allele working normally as it is with a recessive disorder.

Another thing of interest for dominant and recessive disorders is penetrance. Penetrance describes how many individuals of a population, in this case dogs of certain breeds, show the expected phenotype. If a disorder has complete penetrance all individuals will show the phenotype but if a disorder has incomplete penetrance only a certain percentage of those that carry the mutation will show the phenotype [9, 14].

But genetics are not all of this simplest form as can be seen in the genetic eye disease Progressive Retinal Atrophy or PRA. PRA is a group of diseases that all affect the photo receptor cells in the retina. The degeneration of the photo receptor cells is gradual and the dogs are born with normal vision but it deteriorates with age until they are completely blind. But it is not the same mutation that causes PRA in different dog breeds, in some it is inherited as an autosomal recessive disorder, in others as an autosomal dominant and in a few it is inherited as X-linked on the sex chromosomes. Some breeds can have more than one mutation with in the breed so even though there are now available genetic tests for some of the mutations, for those breeds genetic testing is not enough to be certain of the genotype of the dog. Also the fact that the manifestation is not present at birth but the cells degenerate with age brings a risk of offspring inheriting the disease from sires and dams that themselves have not begun to show symptoms [4, 7].
4.3. Recessive versus dominant mutation
5.1 Causative mutation

A group of researchers at Cornell University and Fred Hutchinson Cancer Research Center took on the task of researching the genetic defect that causes Collie Eye Anomaly. Their work showed that the disease is caused by an autosomal recessive mutation on chromosome number 37. Their work further showed that both the mild and severe form stem from the same mutation in all tested cases and that all affected individuals were homozygous for the same mutant gene [16]. The reason for the difference in severity is not fully understood. It is though likely that there are other genes acting as modifiers that influence CEA gene expression [15].

The researchers used the three breeds most commonly affected to map the location of the genetic mutation, the Collie, Border Collie, and Australian Shepard. These breeds, along with the Shetland Sheepdog (which is also affected by the disease) are thought to come from the same ancestral source and to have acquired the same mutation from a common ancestor [16].

These researchers were the first to locate the mutation responsible for CEA. Their work led to the discovery of a 7799 basepair deletion on the canine chromosome 37 in the NHEJI gene. All affected dogs tested were homozygous for this deletion, that is they have two copies of the diseased allele. The same deletion was found in all the aforementioned dog breeds, further establishing that the CEA mutation arose as a single disease allele in a common ancestor of herding breeds. Furthermore this research showed that both the mild and the severe form of CEA are a result of the same deletion [16].

5.2 DNA test

The test itself is a PCR test, which stands for polymerase chain reaction. The PCR test is a fast and relatively inexpensive test that amplifies the desired DNA strand in only a few hours. This reaction uses two primers, a left primer and a right primer, which are carefully selected with regards to the fragment that shall be amplified. These primers are short strands of DNA complimentary to the DNA strand on either side of the target region. The genetic material, the primers, a
solution of the four deoxynucleotides (Adenine, Guanine, Cytosine and Thymine) and a \textit{Taq} DNA polymerase are put into test tubes and put into a machine called a thermal cycler. This machine repeatedly runs a cycle of three temperatures levels.

In the first step, the initializing step in the first cycle but the denaturation step in subsequent cycles, the blend is heated to a temperature of over $90^\circ C$, usually about $94^\circ C$ and this temperature is held for 5 minutes. Here the DNA is denatured by the breaking of hydrogen bonds between the bases yielding two separate single strands of DNA. The next step is the annealing step. Here the temperature is lowered to 50 - 60$^\circ C$ and held for two minutes. In this step the primers base pair to the complementary site on the DNA strand on each side of the target area. The specificity of the temperature is related to the length and ratio of GC:AT in the primer used. The third step is the extension/elongation step where the polymerase attaches to the primer and polymerization along the strand starts. In this step the temperature is raised to $72^\circ C$ and held there for two minutes. At the end of this step the cycle is over and the amount of the target DNA has doubled.

With subsequent steps, the amount of the target DNA grows exponentially so within 20 repetitions there are over a million copies of the desired DNA. The final step is at $72^\circ C$ for 5 to 15 minutes to make sure that all single-stranded DNA is fully extended. After that the temperature is lowered and the example is ready. \cite{9, 14}

The times and temperatures cited here are not stated as a rule but rather as guidelines as different machines, primers, polymerase and length of DNA strand that is to be copied may have influence on optimal temperature and time for each step. This is not meant to give the reader a precise protocol for a PCR reaction, but rather an overview over how the cycle works and what happens in each step.

The final step in the test is the gel electrophoresis. Electrophoresis is a procedure where charged molecules move in an electric field, in this case a gel. A negative and a positive electrode are set on each side of the gel, and as the DNA molecule is negatively charged the fragments move toward the positive electrode. Charged molecules of different sizes move with different speeds through the gel. The PCR product is placed into indentations in one side of the gel called wells. The gel is then set into a buffered aqueous solution and an electric field is set up with wires, connected to a power supply, at each end. The electric field causes the charged molecules to move toward the electrode with the opposite charge. The gel is then photographed to show the final result. \cite{9, 14}
In figure 5.1 a product of a CEA test can be seen. Here the wild-type allele, denoted with W, is the healthy allele and the mutant allele, denoted with M, is the CEA carrying allele. For this test two pairs of primers are used, one pair to multiply the healthy allele and one pair to multiply the mutated allele. Both pairs contain the same forward primer, that is the primer that sets the starting point, but the pairs then contain a different reverse primer. The reverse primer for the wild-type allele is set within the deletion and the DNA fragment is then 636 basepairs long. The reverse primer for the mutant allele is located on the other side if the deletion and gives a DNA fragment that is 941 basepairs long. When the product of these two PCR reactions are set side by side on a gel and the gel is electrophorised it gives a very easy way to determine if the tested individual is genetically normal, a carrier or affected.

With this test it is now possible to test all individuals of these affected breeds to find their genetic status. This will eliminate the doubt when it comes to discriminating true affected from false positives, as false positive can arise from clinical confusion between for example an albinotic or subalbinotic fundus and choroiretinal dysplasia. The subalbinotic fundus is commonly found in merles, a colour found in most of the affected dog breeds. Also, although very rare, a dog that is a carrier may be clinically diagnosed as affected (or “go normal”) and this would alter its possibility and or use in breeding [15].

The test now brings the opportunity to test the breeding stock for the anomaly which is of great help to find the affected animals that “went normal”. These individuals are of course able to give the defect on to their offspring. With the preva-
5.2. DNA test

ience of the allele, in particular in the Collies, the capacity to pre-select breeding pairs is a major step forward in attempts to ameliorate the problem.
The study of genetics is not just about improved husbandry, but it is one of it’s most practical applications. The knowledge that Collie Eye Anomaly is an autosomal recessive disorder gives us a powerful tool to use when it comes to breeding dogs, and in fact any animal we decide to breed.

6.1 Previous breeding practices

Early recommendations to breeders was to advise them to breed only mildly affected dogs, that is to say those that only had chorioretinal hypoplasia. Dogs with more severe manifestations, like coloboma, were to be avoided and kept out of the gene pool. [8, 19] Seeing as both the hypoplasia and coloboma are inherited with the same deletion [16] this was rather disastrous to the breeds that are most affected.

The high prevalence of the allele with the Rough and Smooth Collies and the Shetland Sheepdogs compared to Border Collies and Australian Sheepdog is interesting. Even though it is known that these breeds arose from similar breeding stock there is a possibility that the breeders selection had some influence on the prevalence of the anomaly. In Barnett’s and Stade’s article (1979) on CEA in the Shetland Sheepdog they found an interesting family line of Shetland Sheepdogs that had been linebred for several generations. This particular line had kept an old type appearance, having large circular eyes and a domed scull, and they had apparently no signs of CEA in any of the tested dogs, or indeed of any other ocular anomaly [2].

This does in fact pose an interesting question, is there any correlation between the preferred head and eye shape and the prevalence of CEA in the affected breeds. Each breed has a breed standard where the outer appearance of the perfect dog is described in detail and dogs are judged according to this standard in dog shows. For the Rough and Smooth Collies and the Shetland Sheepdog their standard calls flat sculls and a head shape resembling a blunt wedge and medium sized eyes of almond shape (figure 6.1b). For the Border Collie and Australian Sheepdog their standards call for wider and more domed sculls and somewhat larger eyes (figure 6.1a) [6].
6.2. Possible genetic combinations

It is possible that the preferred head and eye shape phenotype is associated with the CEA disease status. Even though it is an interesting question, it requires some amount of research and will not be addressed here.

6.2 Possible genetic combinations

The existence of a genetic test does make breeding of the affected breeds easier, as the knowledge of the genetic status of the breeding animals gives breeders a chance to use the information and choose accordingly which dogs to mate. The fact that this is a recessive disorder means that both normal and carriers can be used, which gives a much wider gene pool to work with rather than if it was only possible to use genetically normal animals, as with dominant disorders. But how can this information be used?

With the genetic status of the breeding animals known it is possible to find the odds of the genetic status of each offspring. For each mating a Punnet square can be drawn to predict the outcome of a particular cross or breeding. The Punnet square is a visual summary of a cross between two individuals (Figure 6.2) and it is used to determine the probability of an offspring having a particular genotype, it summarizes every possible combination of one paternal allele and one maternal allele for each gene in the mating. In this particular instance it is the CEA deletion that is of interest [9]. Historically a dominant allele is designated with a capital letter, in this case the letter A was chosen, and a recessive allele is designated with a lower case letter, a.
6.2. Possible genetic combinations

(a) A mating between two dogs who are both homozygous normal (AA) for CEA.

(b) A mating between a homozygous normal (AA) dog and a heterozygous (Aa) carrier dog. This mating has a 50% chance for each offspring that it is normal.

Figure 6.2

Figure 6.2a represents a mating between two homozygous normal animals, both carrying two copies of the dominant allele A and neither carrying the defect, and therefore the only possible combination in the offspring is 100% normal. This mating will only produce normal offspring. Figure 6.2b on the other hand shows a mating between a homozygous normal individual (AA) and a heterozygous carrier (Aa). This combination can produce both genetically normal offspring and carriers with a 50% chance for each phenotype in every offspring. The rule of probability says that in a litter of four offspring this combination would produce 2 homozygous normal animals and two heterozygous carrier animals. But probability is not set in stone so a litter of all normal animals and a litter of all carrier animals are equally likely.

(a) A mating between a homozygous normal (AA) dog and a homozygous affected (aa) dog. This mating produces 100% heterozygous carriers.

(b) A mating between two heterozygous (Aa) carriers, this mating has the possibility to produce all genetic states.

Figure 6.3

Figure 6.3a shows the outcome of a mating between a homozygous normal individual and a homozygous affected individual. All offspring of this mating will be carriers but even though one parent is affected it will not produce affected individuals. Figure 6.3b depicts the first combination that has a chance of producing homozygous affected individuals. When two heterozygous carriers are mated the
6.3 Frequency of Collie Eye Anomaly

Probability is 25% of a homozygous normal individual, 50% of heterozygous carrier and a 25% of a homozygous affected individual. Even though it is not given that a mating like this will produce any affected individuals it is not advisable as dogs usually produce a number of puppies in a litter so there is a chance that an affected individual will be born from such a mating.

(a) A mating between a heterozygous (Aa) carrier and a homozygous affected (aa) individual. This combination has a 50% chance of producing an affected individual.

(b) A mating between two homozygous (aa) affected individuals. This mating will only produce affected offspring.

Figure 6.4

Figure 6.4a shows the mating between a heterozygous carrier and a homozygous affected individual. This mating results in a 50% chance of an affected offspring and a litter of four offspring could have two carriers and two affected. The last possible mating, depicted in figure 6.4b shows a mating between two homozygous affected individuals. This mating can only produce affected offspring.

6.3 Frequency of Collie Eye Anomaly

Many studies have reported on the frequency of affected animals in certain countries. Usually they look at the Rough Collie and statistical data can be found for the frequency in the Rough Collie in many countries. In Wallin-Haakonsons and Hedhammars article on the influence of selective breeding on the prevalence of chorioretinal dysplasia and coloboma (2000) they gather together the prevalence of CEA in Rough Collies in Europe from different articles. The number of affected dogs varies a bit between countries and articles from 64% in the United Kingdom to 31% in Finland [19]. Also they noted that matings where both parents had colobomas resulted in smaller litter size. Knowing that this is a recessive disease that means that the number of carriers does not come into these statistics and the true prevalence is drastically higher. The prevalence of CEA has in other articles been reported to be between 79.9% to 97% [2] in the Rough Collie.
Chapter 7

Discussion

The knowledge of an animal’s genetic status can be used as guidelines in breeding as it is quite easy with a recessive disorder to breed so as not to get affected animals. But with an anomaly as CEA it is not as detrimental to get affected animals as with other disorders, because affected animals are usually not severely affected. This lack of severity might have something to do with how wide spread the defect allele is in the affected populations. But with the test and knowledge that it gives breeders, breeding for genetically normal individuals is now possible. It should always be one of the deciding factors when choosing breeding animals that there is least chance of breeding affected animals. Therefore it is advisable that in each mating at least one of the individuals is genetically normal.

An ideal mating would of course be between two normal individuals, but with the low numbers of genetically clear individuals in the Rough and Smooth Collies for example, excluding all affected animals from breeding would most likely result in a whole other batch of problems. This effect is called a genetic bottleneck and would likely result in a huge loss of valuable genetic material. Such loss of diversity might be so devastating for these breeds that they might not survive it. A dog is not just eyes but a whole, constructed of countless parts, all equally valuable. Therefore it might very well be valuable to use an affected individual in breeding, that is itself not adversely affected by CEA, if its other qualities are such that they outweigh the presence of CEA. Since the offspring can be tested as soon as they are born with the DNA test, affected animals can be sold as pets. Just the knowledge that not all affected individuals are severely affected should not be enough to justify a mating that might result in an affected offspring being produced.

Even though this disease has been known for a very long time little progress was made for a long time in eradicating it from the affected breeds. The advice given to breeders [8, 19] may likely just have added fuel to the fire since both chorioretinal hypoplasia and coloboma are inherited with the same deletion [16]. Therefore only discarding animals with colobomas from breeding did not really help and might have caused CEA to become so widespread, especially with the Collies and the Shetland Sheepdogs.

The possibility that breeders have been unintentionally selecting for CEA along with a certain head type with the Collies and Shetland Sheepdog might pose a dilemma as finding genetically normal individuals of said breeds that fit the desired breed standard might be difficult. This could on one hand push breeders to use affected
animals without much regards to the chance of breeding affected individuals, or it could on the other hand create a situation where certain stud dogs will be overused because they are genetically normal for CEA. Those stud dogs, usually referred to as matadors, might bring their own set of genetic trouble that then would become to common in the breeds. Breeders of these breeds have a job for the future to make sure to find the middle ground in their efforts to reduce the prevalence of the CEA deletion in their respective breed populations.

It would be pleasant to be able to say that with the presence of the DNA test all the troubles of breeding are solved. But breeders should not let their guard down completely. There is always a possibility, although very small, that a new mutation arises in the same gene that might cause a similar or the same defect. Other genetic disorders have more than one mutation or genotype that all causes the same phenotype. The fact that other ocular disorders occur in these breeds, with PRA in the forefront, require that breeding dogs undergo eye examinations before breeding. This is of benefit to individuals that are registered as genetically free by default, who come from a mating of two normal individuals or two individuals who themselves are genetically free by default.

If it happened that a new mutation would arise it is more likely that it would be caught before the individual is be used for breeding and the genetic mutation is spread further. It is also advantageous when it comes to choosing individuals for breeding that this anomaly is present at birth and does not deteriorate with age unless intraocular hemorrhage or retinal detachment occurs. Therefore continued eye examinations for breeding animals and also perhaps a spot check for the DNA status of the individuals that are genetically normal by default every couple of generations should be advised.

For the Border Collies, Lancashire Heelers, Australian Shepherds and Nova Scotia Duck Tolling Retrievers the job is more simple as with the existence of the current DNA test affected individuals can be removed from breeding without a great loss of genetic material. With the prevalence in these breed ranging from 15 - 35% the numbers of homozygous affected individuals are low enough so that CEA is hopefully not creating a big problems for these breeds. But the knowledge that CEA can theoretically be eradicated in these breeds with correct breeding practices, eye examinations and genetic testing is very positive.
Collie eye anomaly has for a long time posed a challenge to breeders of the affected breeds. A big diversity of clinical manifestations along with the “go normal” phenomenon, as well as the limited number of adversely affected individuals, posed a problem with defining the mode of inheritance. Without it breeders did not have any clear guidelines for breeding unaffected animals and the prevalence in the Rough and Smooth Collies and the Shetland Sheepdog are very high. The existence of the genetic test offered by Optigen is an invaluable tool for breeders, who can today know the genetic status of their breeding animals and all the offspring they breed. But still not all questions about this anomaly are answered as it is quite likely that there are other modifier genes that influence the diversity of phenotypic manifestations seen with this anomaly. Even with what is known today there is still some way to go before all that is important about CEA is known, and there is ample room for future research into the subject.
Bibliography


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