



Master's thesis

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What consequences can be expected if screening for hip dysplasia in two different canine breeds ceases with regards to HD status?

Comparative retrospective study of the use of HD status in breeding in Labrador retriever and German shepherd dog respectively, and the effect on HD status for each breed.

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Abstract

Canine hip dysplasia (CHD) is an orthopaedic disease, which is multifactorial and partially inherited. It is a biomechanical disease, where abnormal development and laxity of the hip joint lead to decreased congruence, resulting in secondary osteoarthritis and potential lameness. For several decades, attempts at reducing CHD has been made through various breeding programmes based on radiographic evaluation. However, successful breeding based on phenotypic evaluation alone has proven difficult. In latter years, methods of estimating the true breeding values have been implemented in order to enhance breeding results. The purpose of this study was to evaluate the effects of the CHD screening programme in Denmark on HD status of two breeds, Labrador retriever and shorthaired German shepherd dog respectively, and thereby investigate what consequences a cessation of the screening programme can result in. The study was conducted retrospectively, using two types of datasets for each breed in the period 2006-2015, received from the Danish kennel club (DKK). In order to investigate the importance of the screening programme, four sub questions were formulated. It was found that the HD status in both populations have improved during the investigation period. A more accurate selection regarding breeding value of the breeding animals is of major importance, since the HD status of the breeding animals has not changed to the same extent. Conclusively, the results imply that a cessation of the screening programme would result in a decrease in the rate of genetic change.

Resumé

Höftledsdysplasi hos hund (CHD) är en ortopedisk sjukdom som är multifaktoriell och delvis ärftlig. Det är en biomekanisk sjukdom, där onormal utveckling och instabilitet i höftleden leder till minskad kongruens, vilket resulterar i sekundär osteoartrit och potentiell hälta. Under flera decennier har insatser gjorts för att minska CHD genom olika avelsprogram som bygger på radiografisk utvärdering. Emellertid har framgångsrik avel baserad på enbart fenotypisk utvärdering visat sig problematisk. På senare år har metoder för att estimerar de verkliga avelsvärdena införts för att förbättra avelsresultatet. Syftet med denna studie var att utvärdera effekterna av screeningprogrammet för CHD i Danmark avseende HD status för de två raserna, Labrador retriever och Schäfer med hårlagsvariant "normal", och därmed undersöka vilka konsekvenser ett upphörande av screeningprogrammet kan resultera i. Studien genomfördes retrospektivt, med hjälp av två typer av dataregistreringar för varje ras under perioden 2006-2015, som förmedlats från den Danska kennelklubben (DKK). För att undersöka betydelsen av screeningprogrammet, har fyra underfrågor formuleras. Det konstaterades att HD status i båda populationerna har förbättrats under undersökningsperioden. Ackurat selektion av avelsdjur, med avseende på deras sanna avelsvärde, har varit av stor betydelse, eftersom HD status för avelsdjur inte har förändrats i samma utsträckning. Sammanfattningsvis antyder resultatet att ett upphörande av screeningprogrammet skulle resultera i en nedsatt utvecklingstakt av genetisk förändring.

Preface

This study is the result of our veterinary thesis project and was written as part of the Veterinary Medicine master programme at the Department of Veterinary Clinical and Animal Science under the Faculty of Health and Medical Sciences at the University of Copenhagen. This thesis will hopefully provide information of the effects of the screening programme to the Danish kennel club, breeders and pet owners.

We would like to thank our main supervisor professor Fintan McEvoy and our co-supervisor assistant professor Dorte Hald Nielsen. We would also like to thank Ph.D. Helle Friis Proschowsky, our contact at the Danish kennel club, who has provided us with the raw data essential to carry out the study. Finally, we would like to thank veterinarian Susanne Haahr Christensen at Hadsund Dyreklinik for allowance to use the radiographic image on the front page, also known as the “hips of my dreams”.

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List of abbreviations

AM	- Animal Model
BLUP	- Best Linear Unbiased Prediction
CHD	- Canine Hip Dysplasia
CNM	- Centronuclear myopathy
DI	- Distraction index
DKK	- Danish Kennel Club
EBV	- Estimated breeding value
ED	- Elbow dysplasia
EIC	- Exercise induced collapse
GSD	- German shepherd dog
HD	- Hip dysplasia
OCS	- Optimum Contribution Selection

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Introduction

Hip dysplasia is a multifactorial, partially inherited, non-congenital orthopaedic disease (1). Labrador retriever and German shepherd dog are among the breeds most prone to this disease. For about 60 years, attempts of reducing CHD has been made by selective breeding (2). However, phenotypic breeding alone has proven difficult. To enhance the selection of non-diseased dogs, more efficient methods of estimating the true breeding value have been implemented (3) in Denmark in the form of best linear unbiased prediction (BLUP) (4). By using this method, fixed environmental effects can be accounted for, and HD statuses from relatives are also included in the calculation (5). But what would happen if screening for hip dysplasia in Denmark ceased? Our 0-hypothesis is that no difference in the average HD status would be seen in the population of Labrador retriever and shorthaired German shepherd dog in Denmark. The purpose of this project is to evaluate the effects of HD screening for these two breeds in Denmark, regarding the level of disease and to evaluate the importance of following the breeding restrictions set up by the DKK in controlling CHD. However, performing a prospective study was not within the scope of this project, so an indirect, retrospective method based on data received from the Danish kennel club (DKK) was considered more appropriate.

In order to answer the thesis of this project, four sub questions have been formulated. These are stated below:

- 1) On average, which HD status can be expected in an offspring from parents with different combinations of HD statuses (AxA, AxB, AxC, AxD, AxE, BxB, BxC, BxD, BxE, CxC, CxD, CxE, DxD, Dx E)?
- 2) Has the prevalence of the HD statuses (A-E) changed during the last 10 years (2006 – 2015)?
- 3) Has the implementation of the new breeding restrictions and recommendations had any effect on how the breeders choose which individuals to be included in their breeding programmes?
- 4)
 - a) Which parent dogs, with regards to HD status, have breeders chosen during the last ten years for Labrador retriever and German shepherd dog respectively?
 - b) How many of the dogs with unknown HD status are Danish and how many are foreign?

Theoretical background

Canine hip dysplasia - the disease

Canine hip dysplasia (CHD) is a multifactorial, partially inherited, non-congenital disease, which is the most common orthopaedic disease in dogs (1,6). The majority of the affected individuals are of large or giant breeds, with German shepherd dog, Labrador retriever, Golden retriever and Rottweiler constituting the four most common breeds (7). The prevalence varies widely between 1-75%, depending on breed (6). In a summary made 2008, the prevalence of hip dysplasia in German shepherd dog, in four European countries with FCI-standards, was between 13 – 46%. The corresponding prevalence for Labrador retriever being 11 – 32% (8).

CHD is a biomechanical disease, where decreased stability of the hip joint can lead to abnormal development and decreased congruence, in the form of secondary osteoarthritis and clinical signs such as potential lameness (1). Clinical signs of CHD can be present in all age groups, although many dogs diagnosed with CHD show no clinical signs (9). Symptoms associated with CHD range from lameness, shortened stride, hind limb atrophy or other gait abnormalities (10). To assess the presence of CHD, radiographic evaluation of the hip joints is usually performed when the dog is between 12-15 months old (11).

For about 60 years, attempts of reducing CHD has been made by selective breeding, but has proven difficult (7). The prevalence of CHD remains high despite decades of screening programmes (8). During the last decades, a vast amount of selective breeding programmes based on radiographic scoring systems have been applied for several breeds with the purpose of reducing the prevalence of CHD and thereby improving animal welfare (3). In some cases an improvement was achieved (12,13), while in others no improvement or only slow progress was described (14–16). Two of the most problematic factors in controlling CHD, are that the phenotype only correlate with the genotype to some extent, and that the heritability is moderate to low, estimated to around 0,25 for most breeds (1,4). Also, not all dogs in the Danish population are screened. During the last 10 years, 26,4% of German shepherd dogs and 21,2% of Labrador retrievers were screened for CHD in Denmark (lecture 19th of May 2016 by H.F. Proschowsky). Finally, several environmental factors

have been proven to affect the development of CHD, including, but not exclusive to, nutrition, weight, exercise, birth weight and floor covering (1,17).

Heritability of hip dysplasia

The phenotypic trait of an individual is the sum of three components. These include the genetic and environmental components and the residual effects. Environmental effects include fixed effects, such as birth year and sex, whereas residual effects include random environmental effects. There are three types of genetic values; additive, dominance and epistatic. However, dominance and epistasis are considered to contribute very little to the genetic effect, and are therefore included in the random environmental effects. This leaves the additive genetic effect, which is the only component possible to select for. The additive genetic value corresponds to the transmitting ability of the parents, which in turn equals half of the genes that are transmitted to the progeny by each parent. This equation constitutes the linear model used in most predictions of breeding value and can be visualised as (18):

$$\textit{Phenotypic observation} = \textit{environmental effects} + \textit{genetic effects} + \textit{residual effects}.$$

The disease starts to develop at 6 - 7 months of age when the most rapid growth is seen (20). In year 2000, a study was performed including 48 Labrador retrievers, where the prevalence of osteoarthritis in dogs with restricted food intake was compared with dogs without restricted food intake (control group). The study began at 8 weeks of age. The food received by the group of dogs with restricted food intake was 25% less compared to the other group. Prevalence and severity of osteoarthritis in the hips (among other joints), was radiographically evaluated when the dogs were 8 years of age. The prevalence of hip joint lesions in the control group was 15/22 and the severity of the lesions was significantly greater compared to the limit-fed group where the prevalence was only 3/21 (17).

Hip dysplasia is a hereditary disease, but reducing the number of dogs with CHD has been difficult, due to the fact that it has a polygenetic heredity (19). A trait that is polygenetic means that several genes affect the trait. It becomes more difficult to visualize the effects of a specific gene when more genes are involved in a specific trait. This requires that an

individual's performance, and the individual's breeding value is used to evaluate the net effect of the individual's genes that are affecting the specific trait (21).

Hip dysplasia is a threshold trait, i.e. a trait where phenotypes can be divided into different categories (21). By phenotypic evaluation alone, there are often limited possibilities to measure differences between normal dogs. Hence, the hidden differences between normal dogs makes the inclusion of relatives, in addition to individual records, very informative (2).

A trait that is moderately heritable is in the interval 0.2 – 0.4. With a higher heritability, genetic improvement will be faster (21). Phenotypic selection is generally justified for traits with heritability above 0.15 – 0.2 (3). The heritability for CHD is among others, due to breed. When calculating the breeding value for all breeds, the heritability of CHD is set to 0.25 in Denmark, based on the estimated heritability for CHD in German shepherd dog (4). However, estimated heritability for CHD has been described ranging from 0.2 – 0.6 (2,22). Hence, hip dysplasia is considered a trait with moderate heritability, and therefore a favourable outcome is expected, as long as selection is performed (3).

The rate of genetic change per time unit

Factors that affect the genetic trend include the accuracy of selection, the selection intensity, genetic variation and finally the generation interval. The formula, which is frequently called the key equation for genetic change, is set up below (21):

$$\frac{\Delta_{BV}}{t} = \frac{r_{BV,B^V} * i * \sigma_{BV}}{L}$$

or

$$\text{Rate of genetic change per unit of time} = \frac{\text{Accuracy of selection} * \text{Selection intensity} * \text{Genetic variation}}{\text{Generation interval}}$$

The **rate of genetic change** is the rate of change in the mean breeding value in a population due to selection (21).

The ***accuracy of selection*** is a measurement of how close the estimated breeding value is to the true breeding value, or in other words, the accuracy of breeding value prediction. With higher accuracy, the animals selected for breeding will actually be the best parents. Heritability, i.e. the measurement of the strength of the relationship between phenotypic values (HD status in this case), and breeding value, is of great importance. Opposite, with low heritability, the performance record will not be a good measurement of the underlying breeding value. With more advanced techniques for genetic predictions and by using models where increasing amounts of information is incorporated, the accuracy is higher than with phenotypic selection alone, which in turn, is higher than an educated guess from the breeder (21).

The ***selection intensity*** is expressed in standard deviations and is a measurement of which animals the breeders choose when planning a litter. The selection intensity in this case, is higher if only dogs with HD status A or B are selected, rather than choosing random animals for breeding. Naturally, selection intensity is of great importance. If animals were randomly chosen for breeding without regards to HD status, the parents would not be genetically superior to the average, nor would the offspring. Hence, the genetic change would be slow. The selection intensity can mathematically be described as the difference between the mean selection criterion of the parents and the mean selection criterion of all potential parents in a population divided by the standard deviation of the selection criterion. The selection criteria can be an estimated breeding value or a phenotypic value. The standard deviation is the square root of the variance, i.e. the average deviation from the mean (21).

When it comes to threshold traits, i.e. a trait such as hip dysplasia, one has to think of them as having an underlying continuous scale to be able to apply a genetic model. This scale is called the “liability scale”. The sum of the genetic values and the environmental effects for a trait is the individual animal’s liability for the specific trait. The threshold is the breaking point on the liability scale at which an animal displays a certain phenotype above and another below this breaking point. An Estimated Breeding Value (EBV) can be expressed on this underlying liability scale (21).

For threshold traits, where phenotypes are expressed categorically, selection intensities have a tendency to be low. This is because out of the animals with an HD status A, there is

no way to distinguish between these animals, i.e. it is not possible to distinguish which out of these animals are high and low respectively on the liability scale. Choosing an animal from the group with HD status A, really is a random choice, not necessarily the animals with the best hips from a genetic perspective. As more and more dogs in a specific population, for example the population of Labrador retrievers in Denmark, get an HD status A, the lower the selection intensity will become, if selection is based on HD status alone. Choosing to use the EBV instead of HD status will provide a continuously expressed selection criterion, and thereby increase the selection intensity, along with the accuracy of selection as previously described (21).

The **genetic variation** is a measurement of the variability of breeding values within a population for a specific trait. With increasing genetic variation, the best animals are far better than the worst. With decreasing genetic variation, the difference between the best and worst animals is smaller. The greater the genetic variation, the higher the rate of genetic change over time is (21).

The **generation interval** is opposite proportional to the rate of genetic change over time. Hence, a short generation interval leads to a higher rate of genetic change, since there will be more opportunities for selection per unit of time (21).

Methods for evaluation of hip dysplasia

Accurate and standardized evaluations of phenotypic traits, in this case radiographs, are often difficult and prone to subjective results. Several methods for evaluation/grading of hip radiographs are used around the globe (23). The methods of *the Orthopedic Foundation for Animals (OFA)*, *the Fédération Cynologique Internationale (FCI)* and *the British Veterinary Association/Kennel Club (BVA/KC)* evaluate osseous conformation and signs of osteoarthritis, as well as signs of subluxation. The Pennsylvania Hip Improvement program (PennHIP) primarily focus on evaluation of the passive laxity of the hip capsule (8).

Several studies has elucidated the issue of limited interobserver agreement of evaluation and scoring of standard hip-extended radiographs for different screening methods (23–25). In one study, the credibility of the FCI screening method for CHD is found questionable,

based on the restricted agreement of scoring between different evaluators (25). However, observer experience significantly increases interobserver agreement (24).

The Orthopedic Foundation for Animals (OFA) scale is the most commonly used in USA, whereas the Fédération Cynologique Internationale (FCI) scale is one of the most popular in Europe (26), and the method used in Denmark.

Orthopedic Foundation for Animals (OFA):

The screening method of *the Orthopedic Foundation for Animals (OFA)* is used in the USA and Canada and was implemented in 1966. Standard hip-extended radiographs taken on dogs >24 months are evaluated and graded according to a 7-point scoring scheme (excellent, good, fair, borderline, mild CHD, moderate CHD and severe CHD). The primary radiographic criterias considered include hip joint conformation and congruency. Chemical restraint is recommended for muscle relaxation but not mandatory (27).

The British Veterinary Association/Kennel Club (BVA/KC):

In the UK, Ireland and Australia, the BVA/KC model has been used with a numerical scoring system since 1984, where higher scores correspond to a worse hip status. The dogs have to be deeply sedated or anaesthetized, and minimum screening age is 1 year. Standard hip-extended radiographs are evaluated and graded based on 9 different features of the hip joint. These include the Norberg angle (NA), cranial acetabular edge, degree of subluxation, dorsal acetabular edge, cranial effective acetabular rim, acetabular fossa, caudal acetabular edge, femoral head and neck exostosis and femoral head recontouring. Evaluation and scoring of hips is conducted by a group of specialist radiologists or surgeons, each dog is scored by two observers in consensus (28). Only dogs that have a hip score well below the breed average are recommended for breeding. Updates on breed mean score are regularly released by the BVA/KC (23).

The Pennsylvania Hip Improvement Program (PennHIP):

The PennHIP programme was developed in 1983 and became commercially available in 1994. In contrast to the three traditional screening methods, the PennHIP model primarily focus on the passive hip laxity. Hip joint laxity (HJL) is a major risk factor and has been proven to correlate with development of HD later in life (29). In order to obtain optimal

accuracy, it is recommended that the dog is 6 months at the time of screening, but detection of passive joint laxity can be detected in as early as 16 weeks old dogs (30).

The deeply sedated or anaesthetized dog is placed in dorsal recumbency and three radiographic images with different stress projections are taken. Using a custom made distractor a distraction index (DI) can be calculated. DI ranges from 0 to 1, with 0 corresponding to full congruency and 1 representing complete luxation. Finally, a standard extended-hip radiograph is also obtained in order to evaluate signs of osteoarthritis (30). The model is not a “pass or fail” system, as dogs with DI less than 0,3 are considered very unlikely to develop degenerative joint disease (DJD) later in life, whereas dogs with DI above 0,69 are very likely to develop the disease (31).

The Fédération Cynologique Internationale (FCI):

The Fédération Cynologique Internationale (FCI) is a cooperative of different national kennel authorities and was founded in 1911. The FCI screening method is currently applied by 91 national members in Europe, South America, Asia, South Africa and Russia and has been applied for more than 40 years (32). The grading system combines the subjective standard hip-extended radiographic evaluation and the Norberg angle (NA). The NA measurement is given as the angle of the line that connects the femoral head centers, and the line from cranial lateral acetabular margin to the femoral head center (23).

Scoring hip dysplasia using FCI standards

The standard hip-extended radiographs are evaluated according to a scale with 5 grades (A-E), that corresponds to the severity of the disease. Grades are defined descriptively according to the degree of subluxation, size of the NA, shape and depth of the acetabulum and signs of secondary joint disease. Grades A and B are considered nondysplastic while grade C-E are considered dysplastic hips. Each hip is given a score, and the collective HD status equals the hip with the lowest score (33,26).

A specialized veterinarian, approved by the national kennel club and/or the breed club in which the dog is registered, performs the evaluation and grading of the radiographs. However, competence and training varies widely between evaluators, from highly skilled certified radiologists and small animal surgeons to self-trained practitioners. As a result,

quality of evaluation varies accordingly and comparison of final scores between different countries can be problematic (26).

The minimum age for screening is 12 months for the majority of breeds, however for some large and giant breeds, a minimum of 18 months is set. The dog has to be deeply sedated or anaesthetized for complete muscle relaxation. The individual breeding clubs decide whether affected dogs may be used for breeding, not the FCI (23).

A	No Signs of hip dysplasia The femoral head and the acetabulum are congruent. The craniolateral acetabular rim appears sharp and slightly rounded. The joint space is narrow and even. The Norberg angle is about 105°. In excellent hip joints the craniolateral rim encircles the femoral head somewhat more in caudolateral direction.
B	Near normal hip joints The femoral head and the acetabulum are slightly incongruent and the Norberg angle is about 105° or the femoral head and the acetabulum are congruent and the Norberg angle is less than 105°.
C	Mild hip dysplasia The femoral head and the acetabulum are incongruent, the Norberg angle is about 100° and/or there is slight flattening of the craniolateral acetabular rim. No more than slight signs of osteoarthritis on the cranial, caudal or dorsal acetabular edge or on the femoral head and neck may be present.
D	Moderate hip dysplasia There is obvious incongruity between the femoral head and the acetabulum with subluxation. The Norberg angle is more than 90° (only as a reference). Flattening of the craniolateral rim and/or osteoarthritic signs are present.
E	Severe hip dysplasia Marked dysplastic changes of the hip joint, such as luxation or distinct subluxation are present. The Norberg angle is less than 90°. Obvious flattening of the cranial acetabular edge, deformation of the femoral head or other signs of osteoarthritis are noted.

Table 1. Evaluation criterias for each HD status using FCI standards (26).

HD index

The Danish kennel club (DKK) is responsible for registration of the HD results and the hip dysplasia (HD) index. The index calculation model in use underwent a change on the 1st of January 2010. The new model was developed in collaboration with *Videncentret for Landbrug/Dansk Kvæg* (Seges) that has vast experience in calculation of estimated breeding values for production animal breeding. Seges is conducting the analysis and

calculation of the index (Helle Friis Proschowsky, personal communication, September 9, 2016).

The HD index is a number that express the estimated breeding value (EBV) for the individual dog. In the new HD index calculation model, a greater deal of information is incorporated, and it is possible to adjust for several risk factors. The index is calculated using a statistical approach called Best Linear Unbiased Prediction (BLUP) Animal Model (AM) (4).

Best Linear Unbiased Prediction (BLUP) and Estimated Breeding Value (EBV)

EBV usually has superior accuracy, compared to phenotypic evaluation alone, since EBV:s are based on all available information about relatives. Hence, selection of breeding animals based on EBV is more effective. This is because relatives share alleles to a predictable degree. Also, other factors may be weighed in the calculation of EBV, for example the sex and age of the dog (3).

BLUP was developed in the late 1940's and is a methodology of genetic prediction where fixed effects (sex, year of birth etc) can be estimated simultaneously with the breeding value (18).

For many years, BLUP has been successfully used internationally for genetic improvement of livestock. Despite this fact, this methodology has only been implemented to a limited extent in canine breeding. Selection of dogs has traditionally, and to a wide extent continuously, been based on phenotypic selection based on the individual's own characteristics (34).

The fact that BLUP utilizes information from relatives to predict an individual's breeding value leads to increased correlation among EBVs of relatives and thereby the risk of co-selecting related animals (35). As a result, the importance of monitoring and restricting the rate of inbreeding is well known and methods to diminish inbreeding rates have been developed. The Optimum Contribution Selection (OCS), which restricts the rate of inbreeding while maximizing genetic improvement, is well suited for this purpose. However, BLUP has limitations, in some breeds, the proportion or number of HD screened

dogs is too low to calculate an accurate breeding value. Additionally, in very small populations the risk of increased inbreeding makes the use of BLUP inappropriate (34).

Calculation of old HD index

In April 2000, DKK went from using a 10-point HD scale (A1, A2, B1, ...E1 and E2) to the more internationally recognized 5-point HD scale (A, B, ... and E) still used today. In the old HD scale, everything from B2 – E2 was considered affected by HD by various degrees, whereas in the new system, A and B are considered healthy and C-E affected by HD by various degrees. In the old HD index, an animal's breeding value consisted of 3 components; their parents', offsprings' and own performance. The information concerning grandparents, half and full siblings were included in their parents' breeding value. The HD index was expressed in relation to 100, where > 100 was, and still is, considered above breed average. The breeding value of a dog's parents could only be included once, i.e. if the parents' breeding value were updated due to newly registered offspring, the new information could not be utilized for said dog. No consideration was taken with regards to gender or age at the time of HD scoring. The old HD index calculation was also considerably less complex, resulting in a less accurate EBV compared to the formulas used today (36,37).

Calculation of new HD index

The HD index is a number that express the EBV for the individual dog. The index is expressed as in relation to 100, which correspond to the mean breeding value for the entire population. The HD index of a specific dog does not necessarily give the correct information about the dog itself – however, it gives an indication for what to expect from the offspring if the dog is used in breeding. An individual with an index above 100 are expected to produce offspring superior to the average population, with regards to HD status. On the other hand, an individual with an index below 100 is expected to produce offspring inferior to the average population (4).

In order to enable calculation of HD index from HD status, the HD scores – which is given as letters (A-E) – is translated to numbers (A=1, B=2, C=3 etc.). To start with, the mean value of the breed is calculated. The mean is mathematically set to 100. The calculation includes all dogs in the breed that have radiographs evaluated and graded in Denmark, and are between 1 and 11 years. The mean value is updated once a month (38).

Secondly, the individual dog's HD index is calculated by using BLUP. The index is calculated by incorporating information from:

- The individual's own HD status (requires that the dog has been radiographed).
- HD score from all relatives that has been photographed and evaluated.
- The gender of the dog.
- The age of the dog on the date of radiography.
- Place where radiographs were taken (veterinary clinic/country).

Close relatives have a greater effect on the result than more distant relatives do. The HD index is, as the mean HD status of the breed, updated once a month (39).

In some breeds, there is an uneven gender predisposition for developing HD. The index calculation model can therefore include gender as a risk factor in the calculation. The situation where two siblings of different gender, radiographed on the same day, with the same HD status and without any evaluated offspring, have different HD index can consequently occur.

The index calculation model also considers increased age at time of radiographing as a risk factor. The reason for this is that one of the components evaluated on a HD-screening radiograph is signs of osteoarthritis, which is likely to increase in severity with increasing age (38).

Accuracy of HD index

The more evaluated relatives, especially offspring, that are included in the calculation of an individual dog's HD index, the more accurate the index becomes, and thereby the true prognosis of the dog's HD potential. The accuracy of the index is expressed between 0 and 1, increasing number reflects increasing security. The number is, like the index, automatically calculated from some established statistical rules. An accuracy of 1 (or 100%) is never established in real life. The higher the number of the accuracy gets, the more information is required to further increase the accuracy. For instance, an increase from 0,8 to 0,9 necessitate a greater amount of new information than an increase from 0,5 to 0,6 (40).

Breeding restrictions and recommendations in Denmark

The Danish kennel club generally dissuade from using dogs with HD status D or E for breeding. A dog with status D or E may in rare cases be used in breeding, however the breeding partner should be free of HD, i.e. status A or B (41).

There are two types of canine pedigree books in Denmark; "Basis stambøger" and "Basis Plus stambøger". The system with two types of pedigree books was introduced in 2012 as a part of a meeting process between the different breed clubs and DKK (Helle Friis Proschowsky, personal communication, October 11, 2016). A dog that is only bred according to the breeding restrictions is eligible to the "Basis stambog", whereas a dog that also follows the breeding recommendations is eligible to the "Basis Plus stambog" (42). For German shepherd dogs, the new breeding restrictions and recommendations were introduced in May 2012 (Helle Friis Proschowsky, personal communication, October 11, 2016). For Labrador retriever, the breeding recommendations and restrictions were introduced the 1st of January 2013 (43). Before the introduction of the new breeding restrictions and recommendations, it was prohibited to use a dog with HD status D-E for breeding (Dorte Hald Nielsen, personal communication, November 24, 2016).

Different breeds may have different breeding restrictions and recommendations. This fact is relevant to the two breeds investigated in this thesis. As an example, for Labrador retriever, a dog that is bred according to the breeding recommendations is eligible to the following endorsement on the pedigree book; "This puppy is bred according to DKK and Danish Retriever Club's breeding recommendations" (43).

It is the breeder's responsibility to assess, if a dog with hip dysplasia possesses other qualities that justify using the dog for breeding. The breeder may be asked to justify the choice. A dog with status D or E may only be used for breeding once. If the breeder wishes to use the dog for breeding once more, a written evaluation of the first litter must be presented, based on HD photography of at least half the litter (44).

German shepherd dog and Labrador retriever

For both Labrador retriever and German shepherd dog, the dog should be at a minimum 12 months old at the time for radiography (41).

German shepherd dog	Restrictions: - Official HD status registered in DKK for both parents. A dog with HD status D or E may exceptionally be used for breeding, if the breeder assesses that the dog's overall contribution to the breed is positive. In this case, the breeding partner shall be free of HD. - Documentation that the father has normal testicles normally positioned. Recommendations: - Both parents should be "avliskåret" in Schæferhundklubben for Danmark (Danish German shepherd dog club) or another cynological association approved by the FCI. - DNA-profile registered in DKK for both parents.
Labrador retriever	Restrictions: - Official HD status registered in DKK for both parents. A dog with HD status D or E may exceptionally be used for breeding, if the breeder assesses that the dog's overall contribution to the breed is positive. In this case, the breeding partner shall be free of HD. - Official ED-status registered in DKK for both parents. A dog with ED grade 3 may exceptionally be used for breeding, if the breeder assesses that the dog's overall contribution to the breed is positive. In this case, the breeding partner shall be free of ED. - Documentation that the father has normal testicles normally positioned. - At least one of the parents needs to be genetically free of prcd/PRA (progressive rod-cone degeneration/progressive retinal atrophy) in DKK. Recommendations: - HD status A or B registered in DKK for both parents. - ED-status 0 or 1 registered in DKK for both parents. - Eye examination by a member of Den Danske Dyrlægeforenings øjenpanel (The Danish Veterinary Association eye panel) or another veterinarian specialized in ophthalmology. The two parents may not have the same eye disease registered in the DKK. - At least one of the parents needs to be registered free of EIC (exercise-

	induced collapse) in the DKK. - At least one of the parents needs to be registered free of CNM (centronuclear myopathy) in the DKK.
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Table 2. Breeding restrictions and recommendations for Labrador retriever and German shepherd dog (41).

Evidently, the major difference relevant to this thesis is that, for Labrador retriever, the breeding recommendations include that “both parents shall before mating have HD status A or B registered in Denmark”. This however, is not a recommendation for German shepherd dog (41). Consequently, there are different requirements for the two breeds to be eligible with the “Basis Plus stambog” with regard to HD status.

Foreign dogs

When a foreign dog is used for breeding in Denmark, the HD status of the individual dog and its relatives should be registered in DKK. The HD index of the foreign dog is then calculated and the information will contribute to the calculation of the index of the offspring. A foreign dog is thus included in the system in the same way as a DKK registered Danish dog. The fact that it is a foreign dog is however registered as a factor in the calculation of the index. It is hereby possible to adjust the index if the HD status from foreign evaluation does not give the expected result in the offspring. It is possible to register as many foreign relatives as one wish, against a fee. The risk that only dogs with good HD-statuses are registered exist and thereby falsely improving the HD index of some dogs. The recommendation from DKK is therefore - if one wish to register foreign relatives - to include information from all (45).

Since the 1st of January 2011 it is possible to have Danish dogs (i.e. dogs with a Danish registration number) radiographed and evaluated, with regard to HD status, in other FCI countries and have the results registered in the DKK. Since 1st of March 2012 the possibility of sending radiographs taken in Denmark for evaluation in another country is approved by DKK, provided that the country concerned accepts this rule. In case of re-evaluation, the same country has to perform the new radiographic evaluation (46).

As previously mentioned, in most breeds, there is some adjustment in the index calculation of dogs with foreign evaluation of HD status and this will also apply to dogs radiographed in Denmark but evaluated in another country. Radiographs taken in another country can normally not be evaluated in Denmark, with a few exceptions (46).

Material and methods

The background material has been retrieved from different databases, such as PubMed, Web of Science and Google Scholar.

For the statistical calculations, we have used a quantitative method, i.e. the data has been quantified categorically or numerically, to mainly perform hypothesis testing. We have used an abductive reasoning in our discussion and conclusion, since most likely more questions need to be formulated to help answer the main question in this thesis. The questions stated are chosen since these hopefully may give an indication of what would happen to the average hip dysplasia status if the screening ceased in Denmark.

Data was received from the Danish kennel club, spanning from 2006 - 2015 for Labrador retriever and German shepherd dog (note that only short-haired GSD was included in the study, i.e. long-haired rejected). One type of data (litter lists) contains Excel sheets where every DKK registered offspring and the parents of litters are presented, including the parents' HD status. The results is listed in 10 separate Excel-sheets (one per year) for each breed. The number of litters is 3313 for Labrador retriever and 3462 for German shepherd dog. The other type of data contains Excel sheets where HD scores and HD status can be visualized for individuals screened in the time period stated above, and HD status of their parents. These results are listed in 10 separate Excel-sheets (one sheet per year) for each breed and consist of 6112 and 6343 original registrations for Labrador and GSD respectively. To get a clean data set, we have used filters and commandos in excel (see Appendix E) and to perform the calculations, Excel and the statistics programme PSPP were used.

Since four different subquestions have been formulated to answer the main question, different materials and calculation methods have been used respectively. These are described under each question and/or appendix.

The hypothesis testing was done using a 5% significance level.

Results

Question 1

On average, which HD status can be expected in an offspring from parents with different combinations of HD statuses (AxA, AxB, AxC, AxD, AxE, BxB, BxC, BxD, BxE, CxC, CxD, CxE, DxD, DxE)?

Method

The Chi-square test was used to see if there was a significant correlation between non-diseased parents (HD status A-B) and non-diseased offspring (HD status A-B). In addition, Chi-square tests were performed to investigate the relation of HD status of offspring from non-diseased parent groups (AxA vs BxB and AxA vs AxB).

The material consisted of the result of all Danish Labrador retrievers and German shepherd dogs screened for HD in Denmark, in Excel sheets, one for each year from 2006-2015.

The data was separated by the parents' respective HD status combinations (AxA, AxB, AxC etc), to calculate the prevalence of HD status in the offspring, based on the parents HD status. The datasets were reviewed for missing results, duplicates and other errors for both offspring and parents. Dogs with missing HD scores for left and right hip were included as long as a collective HD status of the dog and its parents were included. No consideration has been taken to the age of the parents or offspring, at the time of radiography. Foreign dogs were not excluded. The calculations were done using Excel and PSPP.

The 0-hypothesis was that no significant changes were seen in the prevalence of different HD statuses in offspring from non-diseased parents (AxA, AxB, BxB) compared to the matings containing one or two parents with HD status C-E (AxC, AxD, AxE, BxC, BxD, BxE, CxC, CxD, CxE, DxD, DxE, ExE).

Results

By using Chi-square test to investigate the correlation between diseased vs. non-diseased parents and diseased vs. non-diseased offspring, the p-value was calculated to $<0,001$ in both Labrador retriever and German shepherd dog respectively, with a confidence limit of 95%, meaning that our 0-hypothesis can be rejected at a 5% level. The alternative hypothesis is accepted, i.e. there is a clear difference in HD status (diseased vs. non-diseased) in offspring based on the parents' HD status (diseased vs. non-diseased).

Labrador retriever		HD status offspring	
		Diseased	Non-diseased
HD status parents	Diseased	41	107
	Non-diseased	634	4629
p-value	$<0,001$		
Attributable fraction	56,5%		
Relative risk	2,3		

German shepherd dog		HD status offspring	
		Diseased	Non-diseased
HD status parents	Diseased	159	313
	Non-diseased	1300	4104
p-value	$<0,001$		
Attributable fraction	28,6%		
Relative risk	1,4		

Q1.1. Chi-square test comparing the relationship between parents' HD status, and HD status of their offspring.

For German shepherd dog, there is a significant correlation between parents with HD status BxB producing offspring with HD status C, D and E. For Labrador retriever no significant correlation was found.

Labrador retriever		HD status offspring	
		Diseased	Non-diseased
HD status parents	BxB	25	139
	AxA	362	3169
p-value	0,056		
Attributable fraction	32,7%		
Relative risk	1,5		

German shepherd dog		HD status offspring	
		Diseased	Non-diseased
HD status parents	BxB	157	346
	AxA	466	1981
p-value	<0,001		
Attributable fraction	39,0%		
Relative risk	1,6		

Q1.2. Chi-square tests comparing the relationship between non-diseased parents (AxA and BxB) and the HD status of offspring (diseased vs. non-diseased).

For both breeds, a there is a significant correlation between parents with HD status AxA producing offspring with HD status A.

Labrador retriever		HD status offspring	
		B	A
HD status parents	BxB	37	102
	AxA	486	2683
p-value	0,001		
Attributable fraction	42,4%		
Relative risk	1,7		

Labrador retriever		HD status offspring	
		B	A
HD status parents	AxB	257	1064
	AxA	486	2683
p-value	0,001		
Attributable fraction	21,2%		
Relative risk	1,3		

German shepherd dog		HD status offspring	
		B	A
HD status parents	BxB	158	188
	AxA	610	1371
p-value	<0,001		
Attributable fraction	32,6%		
Relative risk	1,5		

German shepherd dog		HD status offspring	
		B	A
HD status parents	AxB	724	1053
	AxA	610	1371
p-value	<0,001		
Attributable fraction	24,4%		
Relative risk	1,3		

Q1.3. Chi-square tests comparing the relationship between combinations of non-diseased parents with HD status of non-diseased offspring.

See Appendix A for explicit calculations and tables.

Question 2

Has the prevalence of the HD statuses (A-E) changed during the last 10 years (2006 – 2015)?

Method

The data consisted of results of all HD screenings from 2006 – 2015 for Labrador retriever and German shepherd dog.

First of all, the datasets were reviewed for missing results, duplicates and other forms of errors in the data. Dogs with missing HD scores for left and right hip were included as long as a collective HD status of the dog was registered. On the other hand, dogs that did not have an HD status were excluded from the datasets.

Secondly, foreign dogs and dogs younger than 1 year or older than 2 years of age at the time of radiography were excluded from the study. This rejection was performed to isolate the Danish population of each breed and to avoid confounding the prevalence of different HD statuses of a certain year with results that in fact should have been affecting an earlier year. To give an accurate and comparable prevalence for each year, the sample had to be as uniform as possible. For instance, a dog born in 2006 should be registered, and reflect the prevalence of a certain status, in 2007 or 2008, not, for example, in 2012 where the prevalence reflects the different HD statuses for dogs born in 2010 or 2011.

In addition, age has a documented effect on HD score and comparisons of HD status consequently require all dogs to be screened at the same age (2,47). However, consideration should be taken when evaluating the hips of an older dog, where secondary changes are likely to be present, due to old age (lecture by Dorte Hald Nielsen, May 19th 2016).

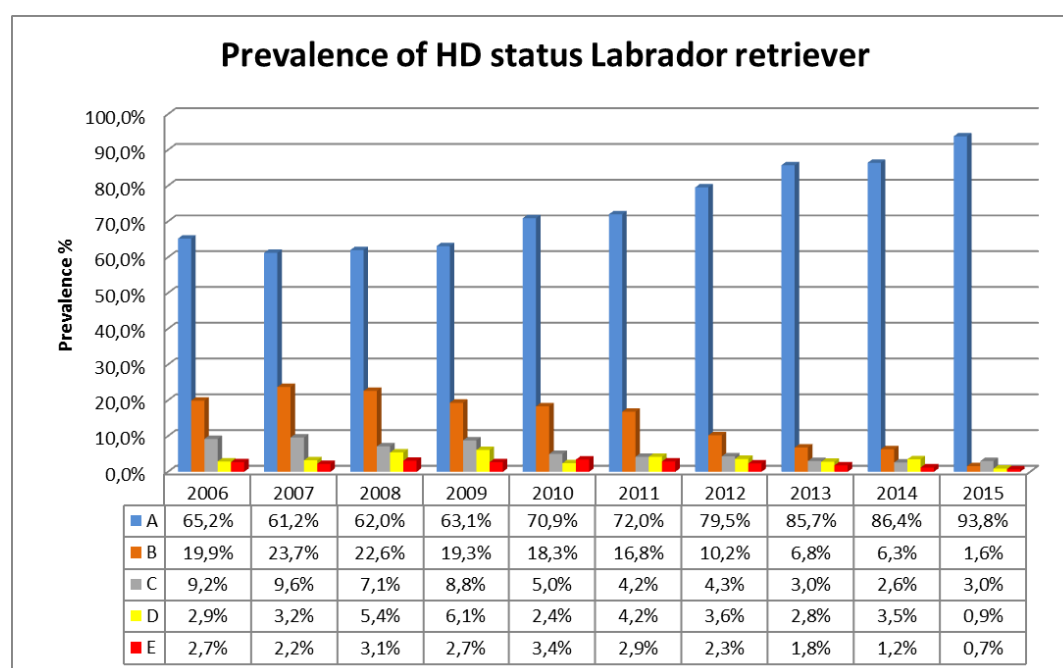
The selection was done using the dogs' registration numbers. When calculating the prevalence for 2010 for instance, only dogs with a Danish registration number displaying that the dogs were born in 2008 or 2009 were included. Dogs with a registration number dated later than the specific year of screening, eg. DKxxxxxx/2014 in the recordings of 2010, were excluded from the data before further analysis. These dogs are typically dogs imported to Denmark, hence the registration number does not reflect the year of birth but

the year of registration (Helle Friis Proschowsky, personal communication, October 11, 2016). Since the true age of these dogs could not be deduced from the information in the dataset, including these dogs would increase the risk of bias in the result. After revision, the dataset consisted of 4576 Labrador retrievers and 5178 German shepherd dogs.

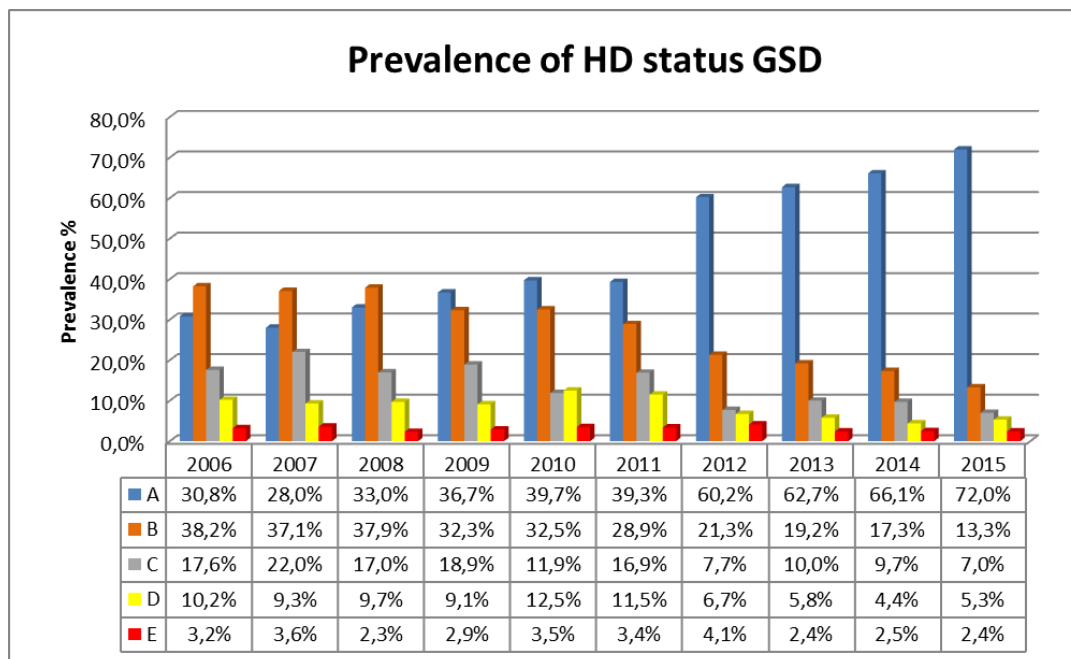
For each registration year, the prevalence of each different HD status was calculated. To test whether there was a significant difference in the proportion of non-diseased (HD status A and B) and diseased (HD status C, D and E) dogs between the different years in the investigation period, a contingency table was constructed. Moreover, to investigate the changes in prevalence of perfectly healthy hips (i.e. status A) the differences in proportion of status A between the different years were evaluated using the same procedure. The result enables testing of the 0-hypothesis; no difference in proportion of non-diseased and diseased between the different years 2006 – 2015.

To localize the potential differences between specific years, further calculations using the Marascuilo procedure was done using Excel (48).

Results



Q2.1. Trends for each specific HD status for Labrador retriever in the period 2006 - 2015.



Q2.2. Trends for each specific HD status for German shepherd dog in the period 2006 - 2015.

Using the Chi-square test, the result of the contingency table rejects the 0-hypothesis ($P < 0,0001$) of equal proportions of “non-diseased” and “diseased” between different years in the investigation period for both Labrador retriever and German shepherd dog. The same result was found for proportions of “status A” and “status B-E” in the same time period. Consequently, the risk to reject the 0-hypothesis while it is true is $< 0,01\%$.

The results of the Marascuilo procedure are visualized below.

See Appendix B for explicit calculations and tables.

Marascuilo procedure: Proportion A				Marascuilo procedure: Proportion A+B			
Contrast	Value	Critical value	Significant	Contrast	Value	Critical value	Significant
p(2006) - p(2007)	0,040	0,130	No	p(2006) - p(2007)	0,002	0,096	No
p(2006) - p(2008)	0,033	0,121	No	p(2006) - p(2008)	0,006	0,090	No
p(2006) - p(2009)	0,021	0,126	No	p(2006) - p(2009)	0,027	0,097	No
p(2006) - p(2010)	0,057	0,120	No	p(2006) - p(2010)	0,041	0,086	No
p(2006) - p(2011)	0,067	0,120	No	p(2006) - p(2011)	0,036	0,087	No
p(2006) - p(2012)	0,143	0,113	Yes	p(2006) - p(2012)	0,046	0,085	No
p(2006) - p(2013)	0,205	0,110	Yes	p(2006) - p(2013)	0,073	0,083	No
p(2006) - p(2014)	0,212	0,108	Yes	p(2006) - p(2014)	0,076	0,081	No
p(2006) - p(2015)	0,286	0,096	Yes	p(2006) - p(2015)	0,103	0,075	Yes
p(2007) - p(2008)	0,007	0,132	No	p(2007) - p(2008)	0,004	0,098	No
p(2007) - p(2009)	0,019	0,137	No	p(2007) - p(2009)	0,025	0,104	No
p(2007) - p(2010)	0,097	0,132	No	p(2007) - p(2010)	0,043	0,094	No
p(2007) - p(2011)	0,107	0,132	No	p(2007) - p(2011)	0,038	0,095	No
p(2007) - p(2012)	0,183	0,126	Yes	p(2007) - p(2012)	0,048	0,093	No
p(2007) - p(2013)	0,244	0,123	Yes	p(2007) - p(2013)	0,075	0,091	No
p(2007) - p(2014)	0,252	0,121	Yes	p(2007) - p(2014)	0,078	0,089	No
p(2007) - p(2015)	0,326	0,110	Yes	p(2007) - p(2015)	0,105	0,084	Yes
p(2008) - p(2009)	0,012	0,128	No	p(2008) - p(2009)	0,020	0,099	No
p(2008) - p(2010)	0,090	0,123	No	p(2008) - p(2010)	0,047	0,088	No
p(2008) - p(2011)	0,100	0,123	No	p(2008) - p(2011)	0,042	0,089	No
p(2008) - p(2012)	0,176	0,116	Yes	p(2008) - p(2012)	0,053	0,087	No
p(2008) - p(2013)	0,237	0,113	Yes	p(2008) - p(2013)	0,079	0,085	No
p(2008) - p(2014)	0,245	0,111	Yes	p(2008) - p(2014)	0,082	0,083	No
p(2008) - p(2015)	0,319	0,099	Yes	p(2008) - p(2015)	0,109	0,077	Yes
p(2009) - p(2010)	0,078	0,128	No	p(2009) - p(2010)	0,068	0,095	No
p(2009) - p(2011)	0,088	0,128	No	p(2009) - p(2011)	0,063	0,096	No
p(2009) - p(2012)	0,164	0,121	Yes	p(2009) - p(2012)	0,073	0,094	No
p(2009) - p(2013)	0,225	0,119	Yes	p(2009) - p(2013)	0,100	0,092	Yes
p(2009) - p(2014)	0,233	0,116	Yes	p(2009) - p(2014)	0,103	0,090	Yes
p(2009) - p(2015)	0,307	0,105	Yes	p(2009) - p(2015)	0,130	0,085	Yes
p(2010) - p(2011)	0,011	0,123	No	p(2010) - p(2011)	0,005	0,085	No
p(2010) - p(2012)	0,086	0,116	No	p(2010) - p(2012)	0,005	0,083	No
p(2010) - p(2013)	0,148	0,113	Yes	p(2010) - p(2013)	0,032	0,080	No
p(2010) - p(2014)	0,155	0,110	Yes	p(2010) - p(2014)	0,035	0,078	No
p(2010) - p(2015)	0,229	0,099	Yes	p(2010) - p(2015)	0,062	0,072	No
p(2011) - p(2012)	0,076	0,116	No	p(2011) - p(2012)	0,010	0,084	No
p(2011) - p(2013)	0,137	0,113	Yes	p(2011) - p(2013)	0,037	0,082	No
p(2011) - p(2014)	0,145	0,110	Yes	p(2011) - p(2014)	0,040	0,080	No
p(2011) - p(2015)	0,219	0,099	Yes	p(2011) - p(2015)	0,067	0,074	No
p(2012) - p(2013)	0,061	0,105	No	p(2012) - p(2013)	0,027	0,079	No
p(2012) - p(2014)	0,069	0,102	No	p(2012) - p(2014)	0,030	0,077	No
p(2012) - p(2015)	0,143	0,090	Yes	p(2012) - p(2015)	0,057	0,071	No
p(2013) - p(2014)	0,008	0,099	No	p(2013) - p(2014)	0,003	0,075	No
p(2013) - p(2015)	0,082	0,086	No	p(2013) - p(2015)	0,030	0,068	No
p(2014) - p(2015)	0,074	0,083	No	p(2014) - p(2015)	0,027	0,066	No

Q2.3. Marascuilo procedure for Labrador retriever visualizing between which years statistical significant changes in proportion of “status A”, and “status A + B” respectively are present.

Marascuilo procedure: Proportion A				Marascuilo procedure: Proportion A+B			
Contrast	Value	Critical value	Significant	Contrast	Value	Critical value	Significant
p(2006) - p(2007)	0,028	0,112	No	p(2006) - p(2007)	0,039	0,116	No
p(2006) - p(2008)	0,022	0,113	No	p(2006) - p(2008)	0,019	0,111	No
p(2006) - p(2009)	0,059	0,112	No	p(2006) - p(2009)	0,000	0,110	No
p(2006) - p(2010)	0,089	0,118	No	p(2006) - p(2010)	0,031	0,113	No
p(2006) - p(2011)	0,085	0,117	No	p(2006) - p(2011)	0,009	0,114	No
p(2006) - p(2012)	0,294	0,120	Yes	p(2006) - p(2012)	0,125	0,106	Yes
p(2006) - p(2013)	0,319	0,118	Yes	p(2006) - p(2013)	0,128	0,106	Yes
p(2006) - p(2014)	0,353	0,122	Yes	p(2006) - p(2014)	0,144	0,107	Yes
p(2006) - p(2015)	0,412	0,120	Yes	p(2006) - p(2015)	0,162	0,106	Yes
p(2007) - p(2008)	0,050	0,115	No	p(2007) - p(2008)	0,058	0,116	No
p(2007) - p(2009)	0,087	0,113	No	p(2007) - p(2009)	0,039	0,115	No
p(2007) - p(2010)	0,117	0,120	No	p(2007) - p(2010)	0,070	0,118	No
p(2007) - p(2011)	0,113	0,118	No	p(2007) - p(2011)	0,030	0,119	No
p(2007) - p(2012)	0,321	0,121	Yes	p(2007) - p(2012)	0,164	0,112	Yes
p(2007) - p(2013)	0,346	0,120	Yes	p(2007) - p(2013)	0,167	0,111	Yes
p(2007) - p(2014)	0,381	0,123	Yes	p(2007) - p(2014)	0,183	0,113	Yes
p(2007) - p(2015)	0,440	0,121	Yes	p(2007) - p(2015)	0,201	0,111	Yes
p(2008) - p(2009)	0,037	0,115	No	p(2008) - p(2009)	0,019	0,110	No
p(2008) - p(2010)	0,067	0,121	No	p(2008) - p(2010)	0,012	0,114	No
p(2008) - p(2011)	0,063	0,119	No	p(2008) - p(2011)	0,028	0,115	No
p(2008) - p(2012)	0,271	0,122	Yes	p(2008) - p(2012)	0,106	0,107	No
p(2008) - p(2013)	0,296	0,121	Yes	p(2008) - p(2013)	0,109	0,106	Yes
p(2008) - p(2014)	0,331	0,124	Yes	p(2008) - p(2014)	0,125	0,108	Yes
p(2008) - p(2015)	0,389	0,122	Yes	p(2008) - p(2015)	0,143	0,107	Yes
p(2009) - p(2010)	0,030	0,120	No	p(2009) - p(2010)	0,032	0,112	No
p(2009) - p(2011)	0,026	0,118	No	p(2009) - p(2011)	0,008	0,113	No
p(2009) - p(2012)	0,235	0,121	Yes	p(2009) - p(2012)	0,125	0,105	Yes
p(2009) - p(2013)	0,260	0,120	Yes	p(2009) - p(2013)	0,128	0,105	Yes
p(2009) - p(2014)	0,294	0,123	Yes	p(2009) - p(2014)	0,144	0,106	Yes
p(2009) - p(2015)	0,353	0,121	Yes	p(2009) - p(2015)	0,163	0,105	Yes
p(2010) - p(2011)	0,004	0,124	No	p(2010) - p(2011)	0,040	0,116	No
p(2010) - p(2012)	0,205	0,127	Yes	p(2010) - p(2012)	0,093	0,109	No
p(2010) - p(2013)	0,230	0,126	Yes	p(2010) - p(2013)	0,097	0,108	No
p(2010) - p(2014)	0,264	0,129	Yes	p(2010) - p(2014)	0,112	0,110	Yes
p(2010) - p(2015)	0,323	0,127	Yes	p(2010) - p(2015)	0,131	0,108	Yes
p(2011) - p(2012)	0,209	0,126	Yes	p(2011) - p(2012)	0,133	0,110	Yes
p(2011) - p(2013)	0,234	0,124	Yes	p(2011) - p(2013)	0,137	0,109	Yes
p(2011) - p(2014)	0,268	0,127	Yes	p(2011) - p(2014)	0,153	0,111	Yes
p(2011) - p(2015)	0,327	0,126	Yes	p(2011) - p(2015)	0,171	0,109	Yes
p(2012) - p(2013)	0,025	0,127	No	p(2012) - p(2013)	0,003	0,101	No
p(2012) - p(2014)	0,060	0,130	No	p(2012) - p(2014)	0,019	0,103	No
p(2012) - p(2015)	0,118	0,128	No	p(2012) - p(2015)	0,038	0,102	No
p(2013) - p(2014)	0,035	0,129	No	p(2013) - p(2014)	0,016	0,102	No
p(2013) - p(2015)	0,093	0,127	No	p(2013) - p(2015)	0,034	0,101	No
p(2014) - p(2015)	0,059	0,130	No	p(2014) - p(2015)	0,019	0,103	No

Q2.4. Marascuilo procedure for German shepherd dog visualizing between which years statistical significant changes in proportion of “status A”, and “status A + B” respectively are present.

Question 3

Has the implementation of the new breeding restrictions and recommendations had any effect on how the breeders choose which individuals to be included in their breeding programmes?

The new breeding restrictions and recommendations were introduced in May 2012 for German shepherd dog (Helle Friis Proschowsky, personal communication, October 11, 2016) and the 1st of January 2013 for Labrador retriever (43). The breaking point of the investigation was set to the 1st of January 2013. Although no breeding recommendations regarding HD status were introduced for German shepherd dog at this point, the same comparison was performed for this breed.

Method

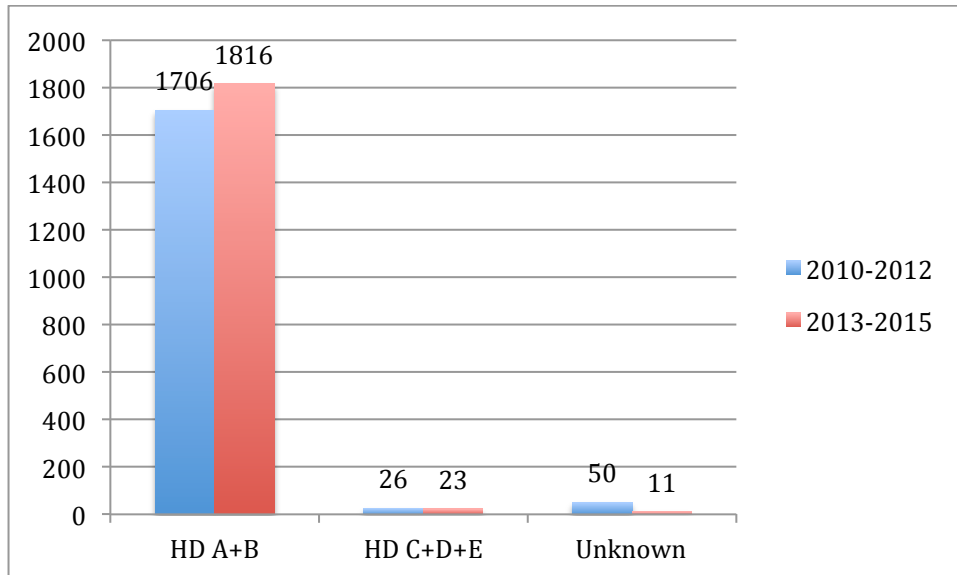
Excel sheets of litter lists for Labrador retriever and German shepherd dog were used from the year 2010 to 2015 (i.e. six years). The excel sheets were reviewed for missing values and other errors. Parents with unknown HD status were included in the diagram and categorized as "unknown". Each mating was isolated to ensure that the number of offspring within each litter would not be a cause of miscalculation (i.e. one specific mating counted several times depending of the number of offspring in each litter). The ensuing calculation was done using Excel and PSPP, including Chi-square test.

The exposed group consisted of the parents of litters born before the new breeding restrictions and recommendations were introduced (years 2010 – 2012), and the non-exposed group consisted of parents of litters born afterwards (years 2013 – 2015). The diseased group was considered parents with HD status C - E, and the non-diseased group was considered parents with HD status A - B. The 0-hypothesis was that no significant change in prevalence was seen in the diseased group (status C – E) after the new breeding restrictions and recommendations were introduced (2013 – 2015).

Results Labrador retriever

Using Chi-square test, the p-value was calculated to 0,618 (see appendix C table 4). Hence, we found the 0-hypothesis to be true, i.e. there was no significant change in the use of parent dogs with status A-B and C-E after the introduction of the new breeding restrictions and recommendations. No dogs with HD status D or E were used in breeding in the period

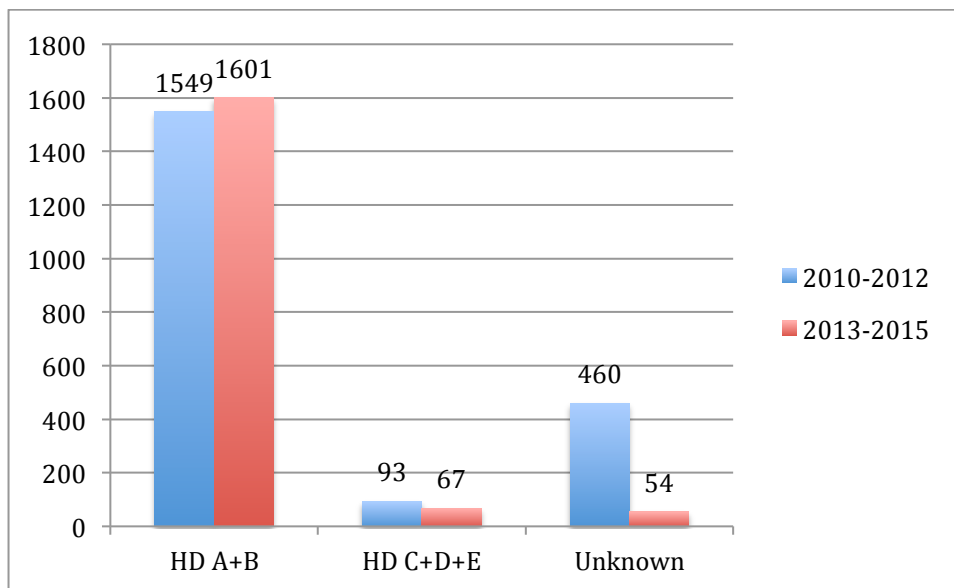
2010 – 2012. In the period 2013 – 2015, two dogs with status D were used. For Labrador retriever, the amount of dogs with unknown HD status has decreased from 2,8% in the period 2010 - 2012 to 0,6% in the period 2013 - 2015.



Q3.1. Fractions of Labrador retrievers with HD status A+B, HD status C-E and unknown status used for breeding in the years 2010 – 2012 and 2013 – 2015 respectively.

Results German shepherd dog

Although German shepherd dog has not been subdued to the same breeding restrictions and recommendations as Labrador retriever, there has been a significant difference (p-value 0,033) in the proportion of diseased and non-diseased dogs used for breeding in the period 2010 – 2012 compared to 2013 – 2015. Note that the new breeding recommendations and restrictions were introduced in May 2012 for German shepherd dog as well as the large amount of dogs with unknown HD status. No dogs with HD status D and E were used in breeding in the period 2010 – 2015. The amount of dogs with unknown HD status has decreased from 21,9% in the period 2010 - 2012 to 3,1% in the period 2013 - 2015.



Q3.2. Fractions of German shepherd dogs with HD status A+B, HD status C-E and unknown status used for breeding in the years 2010 – 2012 and 2013 – 2015 respectively.

See Appendix C for explicit calculations and tables.

Question 4

- a) Which parent dogs, with regards to HD status, have breeders chosen during the last ten years for Labrador retriever and German shepherd dog respectively?
- b) How many of the dogs with unknown HD status are Danish and how many are foreign?

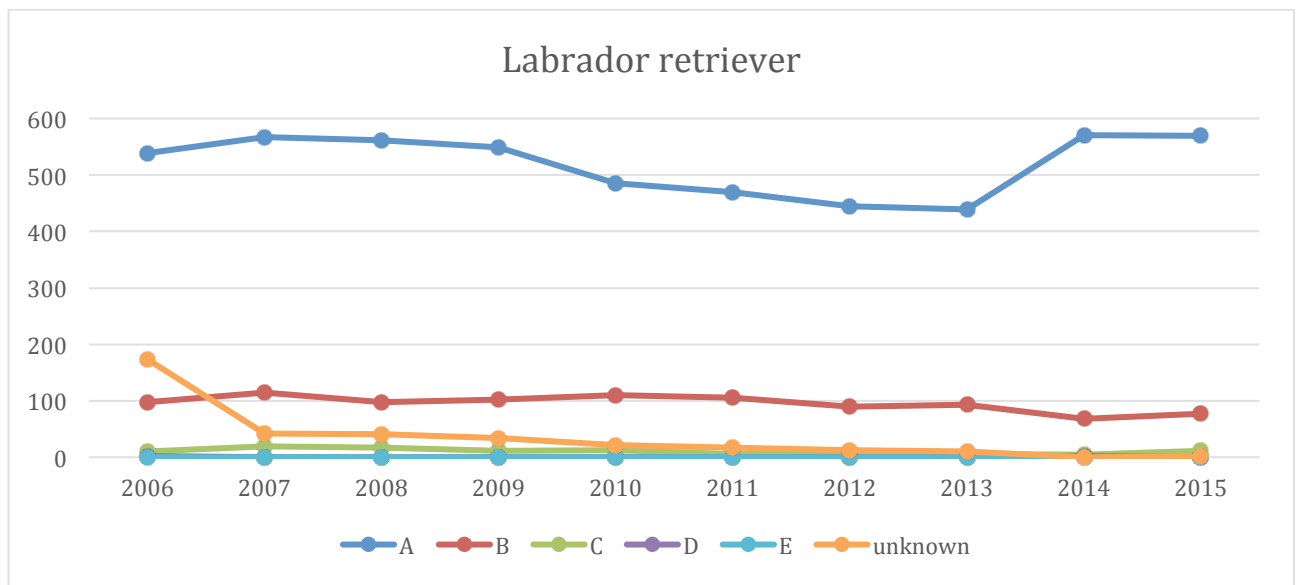
Method

a) The litter lists were reviewed for missing values and other errors. In the diagrams, parents with unknown HD status were included and categorized as "unknown". The data was reduced so only one line for each mating was included. The number of parents with each HD status for each year was recorded, calculated and put into tables and graphs using Excel. Secondly, to investigate if there was a possible, significant difference in which parent dogs the breeders have chosen in different years with regards to HD status (A vs B-E and AB vs C-E) a contingency table was constructed. To identify between which years a potential difference is present, further analysis using the Marascuilo procedure was performed in Excel. There was a large amount of dogs with unknown HD status for German shepherd dog, and a larger amount of dogs with unknown status in 2006 for Labrador retriever.

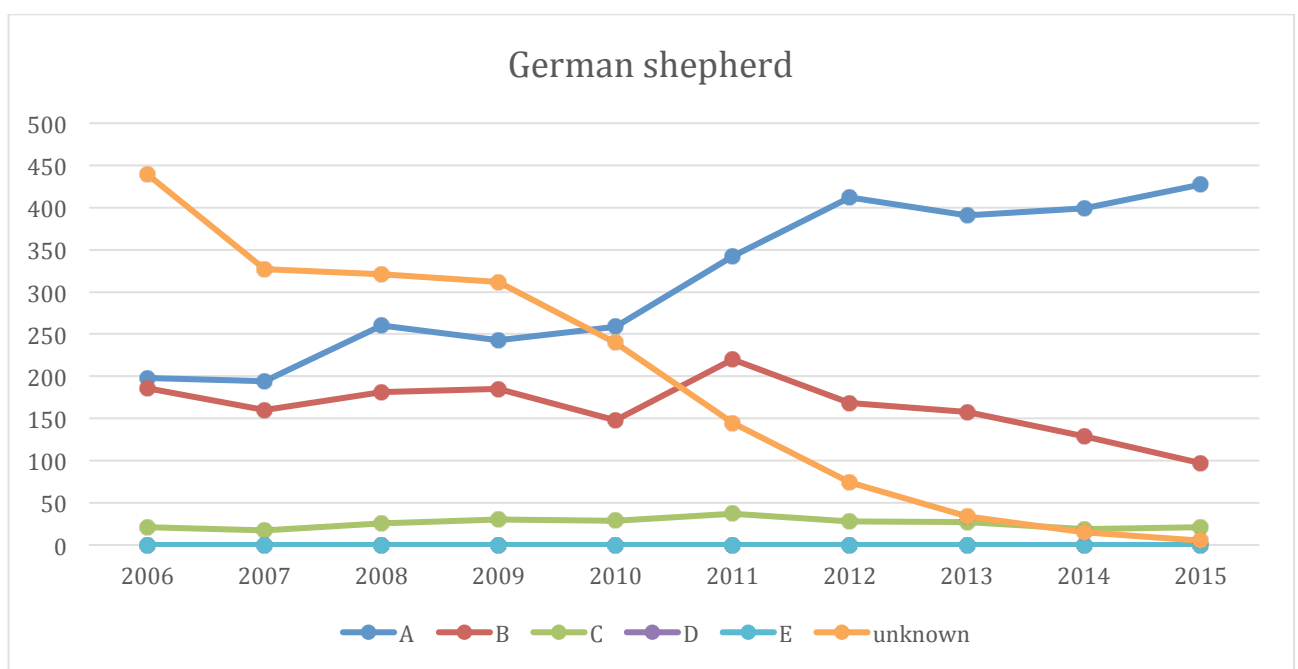
b) Out of the parents with HD status "unknown", we have investigated the distribution of dogs registered as Danish and foreign dogs looking at the registration number, as well as the distribution between males and females. Excel was used to draw the diagrams.

Results

- a) The diagrams below show the number of Labrador retrievers and German shepherd dogs respectively, with different HD statuses, used for breeding for each year (2006 – 2015).



Q4.1 Diagram visualizing the HD status of the parent dogs used for breeding in Labrador retrievers in the years 2006-2015.

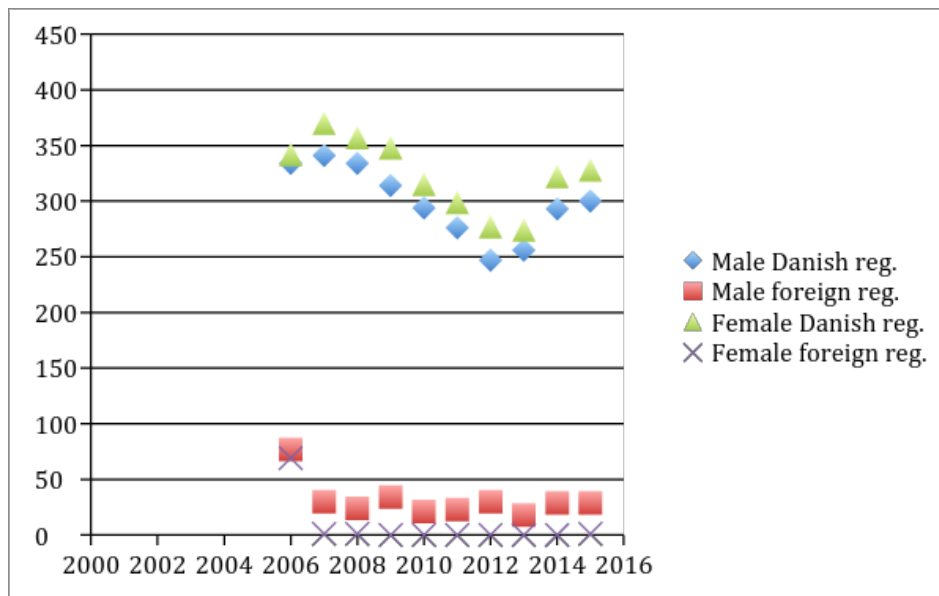


Q4.2 Diagram visualizing the HD status of the parent dogs used for breeding in German shepherd dogs in the years 2006-2015.

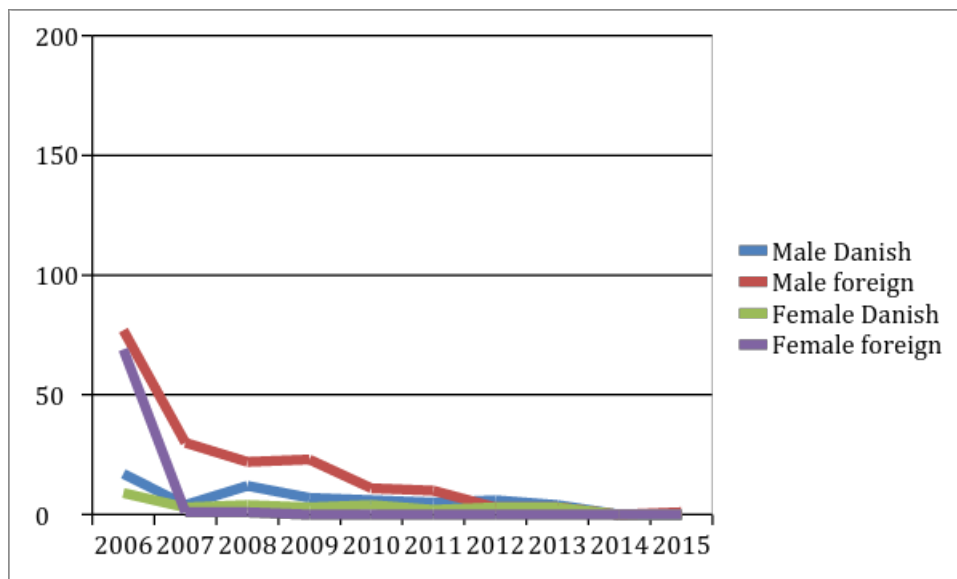
For Labrador retriever, no significant difference was present in the distribution of dogs with HD status A+B used in different years by the breeders. However, there was a significant difference ($p=0,001$) in the distribution of HD status A used in different years. The Marascuilo procedure showed that a significant difference was present between the years 2010 and 2014.

The same conditions are found for German shepherd dog; no significant difference was present in the distribution of dogs with HD status A+B. However, there was a significant difference ($p < 0,001$) in the distribution of HD status A used in different years. The Marascuilo procedure in combination with the distribution table (Q4.2) showed that a significant increase in dogs with HD status A in the latter years was present.

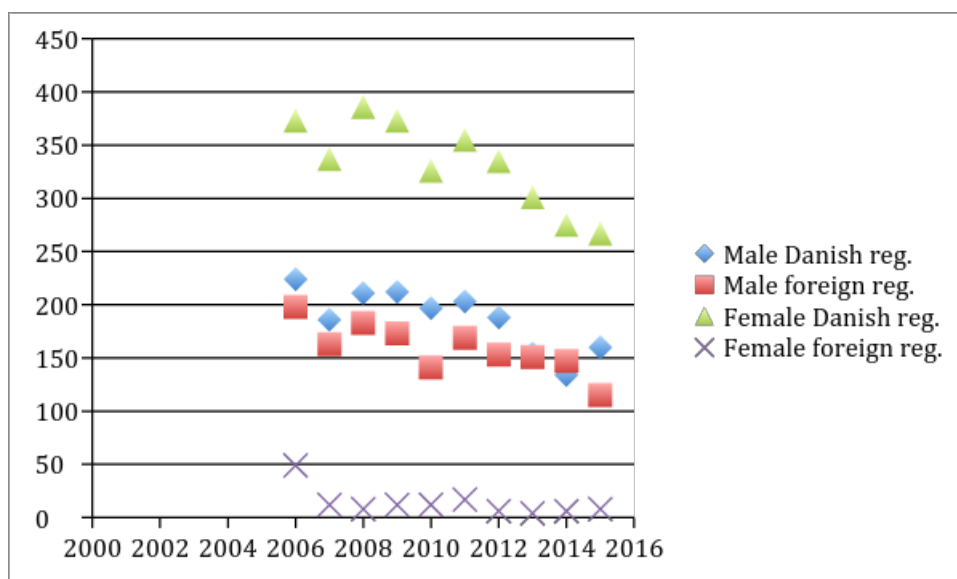
- b) The diagrams below visualize the distribution of dogs with unknown HD status divided into Danish males and females and foreign males and females for Labrador retriever and German shepherd respectively.



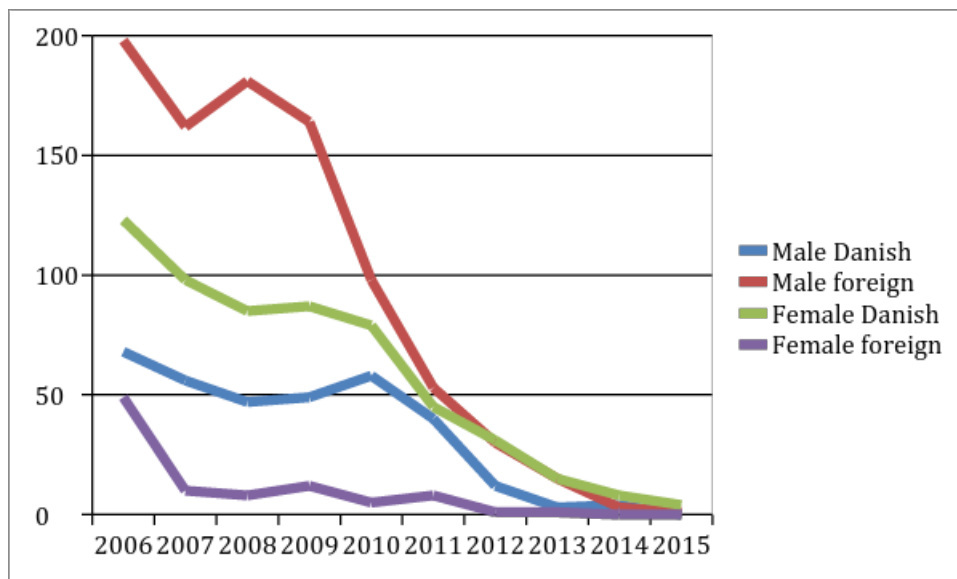
Q4.3. Labrador retriever – distribution of Danish and foreign dogs (known and unknown status) divided into males and females.



Q4.4. Distribution of dogs with unknown HD status divided into Danish males and females and foreign males and females for Labrador retriever.



Q4.5. German shepherd dog – distribution of Danish and foreign dogs (known and unknown status) divided into males and females.



Q4.6. Distribution of dogs with unknown HD status divided into Danish males and females and foreign males and females for German shepherd dog.

For Labrador retriever, the amount of dogs with unknown HD status has decreased from 20,9% in 2006 to 0,2% in 2015. For German shepherd, the decrease is more substantial, from 51,9% in 2006 to 0,9% in 2015.

See Appendix D for explicit calculations and tables.

Discussion

Question 1

There is a risk of bias due to the fact that the age of the individual dogs was not an exclusion criterion, meaning that increasing age can negatively affect a dogs HD score. However, when evaluating the hips of an older dog, consideration should be taken since secondary changes may be due to old age (lecture by Dorte Hald Nielsen, May 19th 2016). Also, foreign dogs were not excluded and there is a possibility that radiologists from different countries may interpret the radiographs differently.

For both breeds, there is a clear correlation between parents' HD status and the status of the offspring, i.e. non-diseased parents are significantly more probable to produce non-diseased offspring.

Within the group of non-diseased parents of German shepherd dogs, there is a definitive advantage in choosing parent dogs with HD status AxA, due to the fact that BxB matings are significantly more likely to produce diseased offspring. For Labrador retriever, the same correlation could not be proven.

Another advantage of choosing dogs for breeding with HD status AxA is that this mating combination will give a significantly higher probability of producing offspring with HD status A for both breeds. For offspring with HD status B in Labrador retrievers, 42,4% is attributable to the fact that they have parents with HD status BxB. For German shepherd dog, the attributable fraction is 32,6% under the same circumstances.

Question 2

The prevalence of dogs with HD status A has markedly increased in the Danish population of both Labrador retriever and German shepherd dog. For Labrador retrievers born in 2013-2014, the prevalence of dogs with HD status A is close to 94%, which is a marked improvement compared to the prevalence (65,2%) for dogs born in 2004-2005. The prevalence of dogs with HD status A in German Shepherd dogs born in 2013-2014 was 72,1%, and hence not as high as for Labrador retriever. On the other hand, the prevalence

of status A for dogs born in 2004-2005 was markedly lower in the German shepherd dog compared to the Labrador retriever in the same period.

The results of the Marascuilo procedure show that there is a statistically significant difference in the proportion of HD status A and B in the period 2006 – 2015 for both breeds. Since the 0-hypothesis is rejected, the alternative hypothesis is accepted, i.e. there is a difference in proportions of HD statuses A and B between the different registration years.

Interestingly, at first glance at the HD status diagrams, the improvement of HD status seems to be greatest in the Labrador retrievers compared to the German shepherd dogs. However, the result of the statistical analysis indicates that the most significant changes and the “number of significant differences” between specific years are greater for German shepherd dog. Even though the outcome is more successful in the Labrador retrievers regarding non-diseased HD status, the statistical evidence for improvement in the German shepherd dog has been more evident than for the Labrador retrievers during the investigation period. The result seems paradoxical due to the fact that the breeding recommendations are stricter with regards to HD status for Labrador retriever.

According to the result from the Marascuilo procedure (Q2.3 and Q2.4) there are several significant differences between years *before 2010* and years *after 2010* in both breeds. Furthermore, there are several significant differences in-between years *after 2010*, but none in-between years in the time period *before 2010*. A reasonable explanation to this could be the implementation of the new HD index calculation model in 2010, which has led to further improvement in HD status in general, and enhancing the rate of improvements in particular. There is a clearly marked improvement of the trend of HD status A, starting from 2010-2011 for Labrador retriever and 2011-2012 for German shepherd dog, with the unfavorable HD statuses declining accordingly. These results are in correlation with conclusions in numerous studies; incorporation of accurate breeding values is required to optimize reduction in unwanted traits like CHD (49,12,50).

Since the registration number does not reflect the year of birth but the year of registration for imported dogs, there is a theoretical risk that an imported dog with a Danish

registration number matching the period counted in a specific HD screening year, are included in the analysis. Consequently, there is a risk of including dogs of older age.

Question 3

For Labrador retriever, there was no significant decrease in dogs with HD status C-E used for breeding after the introduction of the new breeding recommendations and restrictions. It follows that the breeders of Labrador retriever either has always been very much aware of HD status of the dogs chosen for mating, or that the introduction of the breeding recommendations in 2013 has not had a great impact. Most likely, the previous is true, which can be visualized in diagram Q3.1.

However, it is also of importance to remember that HD status is not the only requirement in the breeding recommendations. Other requirements include ED status for both parents, eye examination for both parents, genetic freedom of EIC for at least one of the parents and finally that at least one of the parents shall be genetically free from CNM.

The breeding restrictions for German shepherd dog did not, in essence, change with the implementation of the new breeding recommendations and restrictions in May 2012. However, a change in how breeders choose parent dogs is seen. There is a greater amount of dogs with unknown status in the period 2010 - 2012 compared to the latter period. Assuming that all dogs with an unknown HD status in the two periods compared had in fact a status A or B, the difference between these periods is no longer significant (p-value 0,46) when looking at prevalence of HD status A-B and C-E (see appendix C table 6). The larger amount of dogs with known HD status in the latter years may be attributed to the fact that breeders have become more prone to choose dogs for breeding with known HD status in the latter years. What is more, breeders may, to a larger extent, encourage the buyers of puppies to screen these dogs for HD, since their result will affect the HD index of the parents. Finally, with the new HD index in 2010, it was decided that foreign dogs should have an HD index calculated, where calculations are based on the individual's HD status, in addition to the relatives' HD status.

Performing the opposite calculation for Labrador retriever, i.e. assuming that all dogs with unknown status in fact have HD status C-E, the p-value is calculated to <0,001, showing a

significant decrease in the amount of dogs with HD status C-E used for breeding after the introduction of the new breeding restrictions and recommendations (see appendix C table 5).

Question 4

Due to the vast amount of dogs with unknown HD status, especially for German shepherd dog, it is problematic to draw any substantiated conclusions from the statistical analysis.

For Labrador retriever, despite a statistically significant difference between the proportions of parent dogs with HD status A used in two different years, one can hardly argue for a marked improvement in HD status among dogs used in breeding, since an unequal distribution of the status “unknown” may equalize the significant difference.

However, there is a clear difference between how breeders of the two breeds have chosen dogs with regards to country of origin, when planning a litter in the time period investigated. For Labrador retrievers, breeders tend to choose both males and females with a Danish registration number. Opposite, breeders of German shepherd dog tend to use foreign males to a greater extent. For both breeds, females have, to a great extent, a Danish registration number. Since there might be interobserver differences in interpretation of radiographs, and thereby differences in interpreting radiographs between countries, there is a risk of reduced interobserver agreement in the HD status given to the population of German shepherd dogs in particular.

Conclusion

The conclusion is done using the rate of genetic change per time unit since this is a measurement of the effectiveness of selection (21). The Danish kennel club is continuously working to increase both physical and mental health, often in collaboration with the club of the specific breed (51). For the two breeds included in this thesis, this work includes promoting the use of dogs without hip dysplasia for breeding, which is identified by the screening programmes. Therefore, if screening programmes ceased, animals would likely be randomly chosen, as long as no other means to determine the HD score was implemented. In the conclusion, the investigation is done by looking at the effects that screening has resulted in for the last ten years, assuming that the opposite would imply if no screening had been performed. It was not, however, within the scope of this project to investigate the genetic variation and the generation interval; factors that are included in the formula for rate of genetic change per time unit.

Use of the formula to assess the effects of the screening for HD

Direct measurement of the rate of genetic change per time unit

The results from question 2 shows that there has been a genetic change in the last ten years (2006- 2015) in the Danish population of both Labrador retriever and German shepherd dog. This was confirmed by a statistically significant difference in the proportion of different HD statuses in this period of time. Hence, it is clear that a genetical change has taken place in this period for both breeds.

There are several significant differences between years in the beginning of the the investigation period compared to later years for both breeds, which in combination with the HD status diagrams, Q2.1. and Q2.2., visualizes the improvement in HD status. Furthermore, regarding the prevalence of HD status A, there are several significant improvements in between years *after 2010* in both breeds, but none in between years in the time period *before 2010*. These findings imply that the improvement have been more rapid in recent years, which most reasonably is attributed to the new way of calculating HD index.

Accuracy of selection

In question 1, an investigation regarding which HD status could be expected in an offspring from parents with different combinations of HD statuses was performed. The results showed that there is a clear difference in HD status (diseased vs. non-diseased) in offspring based on the choice of parents' HD status (diseased vs. non-diseased). Since parents with HD status A are significantly more probable to produce offspring with HD status A, the phenotypic value correlates with the true breeding value.

In theory, using BLUP to calculate EBV, has a higher **accuracy** than phenotypic selection alone, i.e. the estimated breeding value is a better approximation of the true breeding value. Labrador retriever and German shepherd dog are breeds where HD index is used, which should give a higher **rate of genetic change** than phenotypic selection alone. One key factor of the calculation of HD index is, of course, the individual's own HD status. The fact that hip dysplasia has a heritability high enough to justify phenotypic selection, is visualized by the significant relationship between the offsprings HD status and the HD status of the parents, however it is established that BLUP gives an even higher **accuracy of selection**.

Conclusively, the **rate of genetic change** per time unit should increase by today's methods of choosing parent animals for the two breeds investigated.

Selection intensity

The results of question 3 and 4 can be used to assess the selection intensity.

In question 3, the results showed that with the implementation of the breeding restrictions and recommendations in 2013 for Labrador retriever, there has been no significant change in how breeders choose breeding animals when planning a litter with regards to diseased and non-diseased animals. For German shepherd dog however, there was a significant difference, although the only difference regarding the new and the old breeding programme (before May 2012) was that the use of animals with HD status D-E were prohibited in breeding.

Realistically, this should have a negative impact on the average HD status. However, during the period 2010 - 2015, no German shepherd dogs with HD status D or E were used in breeding. Consequently, a more likely cause of the of the significant difference in the proportion of diseased and non-diseased dogs in the period 2010 - 2012 compared to 2013 - 2015 is that the amount of dogs with unknown status has decreased. According to this result,

the new breeding recommendations and restrictions has not altered the *selection intensity* significantly and consequently not the *rate of genetic change* either.

The use of HD index instead of HD status however, will give an increased selection intensity due to the fact that HD index is expressed in a continuous scale, as opposed to HD status which is expressed as categorical data as a threshold trait.

In question 4, the large amount of dogs with unknown status (primarily in the GSD) in the earlier years is a potential bias. Therefore, no conclusion can be drawn to whether breeders to a larger extent have chosen breeding animals without hip dysplasia in the latter years or not.

Genetic variation and Generation interval

Assuming that the genetic variation is reduced due to higher selection intensity, the rate of genetic change must decrease somewhat. This has no negative impact on the proportion of dogs without hip dysplasia. Genetic change can still take place. Reduced genetic variation simply means that the genetic change will slow down simultaneously as the proportion of dogs with HD status A and B increases along with dogs with HD status C-E decreasing.

In this thesis, we have not investigated whether the generation interval has decreased. To increase the rate of genetic change, this can perhaps be incorporated in the breeding programmes. However, from a philosophical standpoint, the breeding of dogs, in contrast to livestock, has other objectives such as a family pet, rather than the wish to increase productivity, which usually is the case for livestock.

Final conclusion

To summarize, the improvement in HD status that have been seen in both breeds during the investigated time period, can not be fully explained by increased selection intensity in latter years. The generation interval has most likely been relatively constant during the same time. In addition, the genetic variation, with regard to HD status, should have decreased as a consequence of the improvement in HD status. This fact leaves the accuracy of selection as the most significant element in the equation of genetic change. The increase

in accuracy of selection can most likely be attributed to the implementation of the new HD index in 2010, based on the more significant and rapid improvement in HD status post 2010.

In conclusion, if screening programmes ceased, animals would likely be randomly chosen, as long as no other means to determine the HD status was implemented, such as lameness evaluation. Consequently the accuracy of selection would decrease, meaning that the opposite would be true with regards to genetic change of HD status seen in the period 2006-2015.

New tools of diagnosing hip dysplasia may be on the way. In 2015, an article regarding the possibility of developing a diagnostic genetic tool for early diagnosis of hip dysplasia in Labrador retrievers was published. 775 Labrador retrievers were included in the study, and for each dog, a radiograph of the hips and a blood sample was taken. A mathematical model, including 7 SNPs, was developed using logistic regression and showed good accuracy. The authors of the article state that genomic screening might contribute to the reduction of canine hip dysplasia in the future, as well as detecting HD at an early age (52).

Another area of potential increase in the rate of genetic change could be that HD index is included in the breeding recommendations, where breeders should use dogs for breeding where the mean HD index of the parents is >100 .

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Appendices

Appendix A

Calculations question 1

Labrador	AxA	AxB	BxB	Tot
A+B	3169	1321	139	4629
C+D+E	362	247	25	634
Total	3531	1568	164	5263

Labrador	AxC	AxD	AxE	BxC	BxD	BxE	CxC	CxD	CxE	DxD	DxE	ExE	Tot
A+B	85	1	0	18	0	0	3	0	0	0	0	0	107
C+D+E	29	0	0	10	0	0	2	0	0	0	0	0	41
Total	114	1	0	28	0	0	5	0	0	0	0	0	148

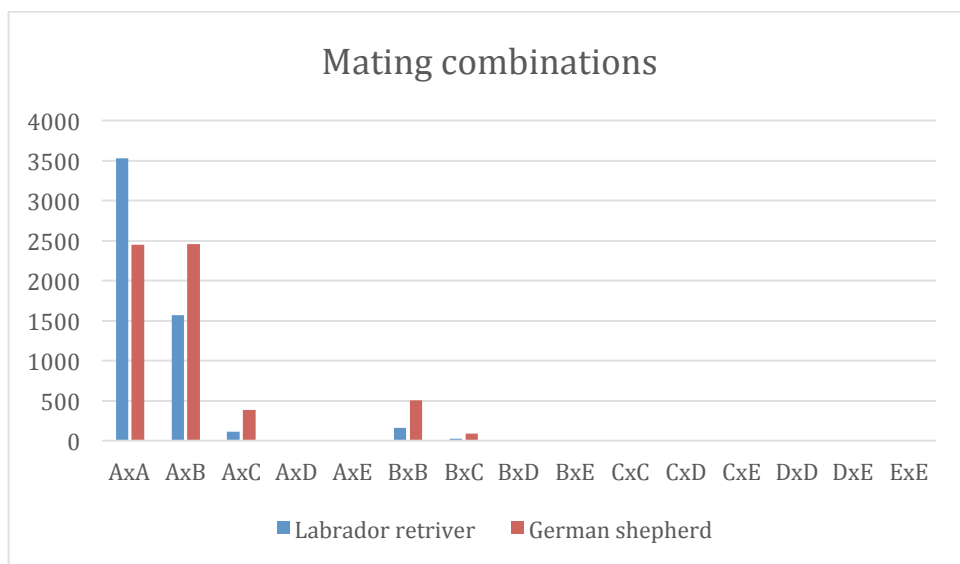
GSD	AxA	AxB	BxB	Tot
A+B	1981	1777	346	4104
C+D+E	466	677	157	1300
Total	2447	2454	503	5404

GSD	AxC	AxD	AxE	BxC	BxD	BxE	CxC	CxD	CxE	DxD	DxE	ExE	Tot
A+B	258	0	0	55	0	0	0	0	0	0	0	0	313
C+D+E	123	0	0	36	0	0	0	0	0	0	0	0	159
Total	381	0	0	91	0	0	0	0	0	0	0	0	472

AA.1. Tables displaying the numbers used for Chi-square tests in Q1.1., Q1.2.

	Labrador retriever n=5 411	Labrador retriever %	German shepherd dog n= 5 876	German shepherd dog %
AxA	3 531	65,3	2 447	41,6
AxB	1 568	29	2 454	41,8
AxC	114	2,1	381	6,5
AxD	1	0,02	0	0
AxE	0	0	0	0
BxB	164	3	503	8,6
BxC	28	0,5	91	1,5
BxD	0	0	0	0
BxE	0	0	0	0
CxC	5	0,09	0	0
CxD	0	0	0	0
CxE	0	0	0	0
DxD	0	0	0	0
DxE	0	0	0	0
ExE	0	0	0	0

AA.2. Table displaying the parents' HD status combinations for each scored offspring.



AA.3. Diagram displaying the parents' HD status combinations for each scored offspring.

Labrador	AxA	AxB	AxC	AxD	AxE	BxB	BxC	BxD	BxE	CxC	CxD	CxE	DxD	DxE	ExE
A	2683	1064	61	1	0	102	11	0	0	2	0	0	0	0	0
B	486	257	24	0	0	37	7	0	0	1	0	0	0	0	0
C	187	113	12	0	0	11	6	0	0	0	0	0	0	0	0
D	111	85	10	0	0	9	2	0	0	2	0	0	0	0	0
E	64	49	7	0	0	5	2	0	0	0	0	0	0	0	0
Total	3531	1568	114	1	0	164	28	0	0	5	0	0	0	0	0

Labrador %	AxA	AxB	AxC	AxD	AxE	BxB	BxC	BxD	BxE	CxC	CxD	CxE	DxD	DxE	ExE
A	76	68	53,5	100	0	62,2	39,3	0	0	40	0	0	0	0	0
B	13,8	16,4	21	0	0	22,6	25	0	0	20	0	0	0	0	0
C	5,3	7,2	10,5	0	0	6,7	21,4	0	0	0	0	0	0	0	0
D	3,1	5,4	8,8	0	0	5,5	7,1	0	0	40	0	0	0	0	0
E	1,8	3,1	6,1	0	0	3	7,1	0	0	0	0	0	0	0	0

AA.4. Tables displaying the numbers used for Chi-square tests in Q1.3. for Labrador retriever

GSD	AxA	AxB	AxC	AxD	AxE	BxB	BxC	BxD	BxE	CxC	CxD	CxE	DxD	DxE	ExE
A	1371	1053	149	0	0	188	32	0	0	0	0	0	0	0	0
B	610	724	109	0	0	158	23	0	0	0	0	0	0	0	0
C	265	368	67	0	0	95	17	0	0	0	0	0	0	0	0
D	142	231	40	0	0	50	13	0	0	0	0	0	0	0	0
E	59	78	16	0	0	12	6	0	0	0	0	0	0	0	0
n	2447	2545	381	0	0	503	91	0	0	0	0	0	0	0	0

GSD %	AxA	AxB	AxC	AxD	AxE	BxB	BxC	BxD	BxE	CxC	CxD	CxE	DxD	DxE	ExE
A	56	43	38,6	0	0	37,4	35,2	0	0	0	0	0	0	0	0
B	25	29,5	28,5	0	0	31,4	25,3	0	0	0	0	0	0	0	0
C	10,8	15	17,6	0	0	18,9	18,7	0	0	0	0	0	0	0	0
D	5,8	9,4	10,5	0	0	9,9	14,3	0	0	0	0	0	0	0	0
E	2,4	3,2	4,2	0	0	2,4	6,6	0	0	0	0	0	0	0	0

AA.5. Tables displaying the numbers used for Chi-square tests in Q1.3. for German shepherd dog.

Appendix B

Calculations question 2.

Year	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	Tot
A	360	248	324	281	329	326	373	341	370	412	3364
B	110	96	118	86	85	76	48	27	27	7	680
C	51	39	37	39	23	19	20	12	11	13	264
D	16	13	28	27	11	19	17	11	15	4	161
E	15	9	16	12	16	13	11	7	5	3	107
Tot	552	405	523	445	464	453	469	398	428	439	4576

AB.1. Absolute number of Danish Labrador retrievers between one and two years old at time of radiography, at each specific registration year.

Year	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	Tot
A	182	148	183	225	204	211	296	314	287	298	2348
B	226	196	210	198	167	155	105	96	75	55	1483
C	104	116	94	116	61	91	38	50	42	29	741
D	60	49	54	56	64	62	33	29	19	22	448
E	19	19	13	18	18	18	20	12	11	10	158
Tot	591	528	554	613	514	537	492	501	434	414	5178

AB.2. Absolute number of Danish German shepherd dogs between one and two years old at time of radiography, at each specific registration year.

Labrador retriever

Year	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	Total
A + B	470	344	442	367	414	402	421	368	397	419	4044
C + D + E	82	61	81	78	50	51	48	30	31	20	532
Total	552	405	523	445	464	453	469	398	428	439	4576

AB.3. Number of non-diseased and diseased Labrador retrievers over the investigation period 2006-2015.

Assuming the null-hypothesis is true; an estimate of the single overall proportion of diseased dogs can be calculated by pooling the results of all the samples;

$$\frac{(82 + 61 + 81 + 78 + 50 + 51 + 48 + 30 + 31 + 20)}{(552 + 405 + 523 + 445 + 464 + 453 + 469 + 398 + 428 + 439)} = \frac{532}{4576} = 0,116$$

The proportion of non-diseased dogs can consequently be calculated as $1 - 0,116 = 0,884$.

By multiplying these two proportions with the sample sizes of each year, the expected proportions of diseased and non-diseased dogs are given;

Year	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	Total
A + B	488	358	462	393	410	400	415	352	378	388	4044
C + D + E	64	47	61	52	54	53	55	46	50	51	532
Total	552	405	523	445	464	453	469	398	428	439	4576

AB.4. Expected numbers of diseased and non-diseased dogs in Labrador retriever

To test the null hypothesis of equality of all proportions against the alternative hypothesis that not all 10 proportions are equal, the observed and expected values from the tables above are included in the Chi-Square test statistic: Where f_o is the observed proportion in a given cell of a $2 \times n$ contingency table, and f_c is the expected proportion in a given cell if the null-hypothesis were true. The result is displayed below:

f_o	f_c	$(f_o - f_c)$	$(f_o - f_c)^2$	$(f_o - f_c)^2 / f_c$
82	64	18	324	5,0625
61	47	14	196	4,170212766
81	61	20	400	6,557377049
78	52	26	676	13
50	54	-4	16	0,296296296
51	53	-2	4	0,075471698
48	55	-7	49	0,890909091
30	46	-16	256	5,565217391
31	50	-19	361	7,22
20	51	-31	961	18,84313725
470	488	-18	324	0,663934426
344	358	-14	196	0,547486034
442	462	-20	400	0,865800866
367	393	-26	676	1,720101781
414	410	4	16	0,03902439
402	400	2	4	0,01
421	415	6	36	0,086746988
368	352	16	256	0,727272727
397	378	19	361	0,955026455
419	388	31	961	2,476804124
				69,77331934

AB.5. Contingency table Labrador retriever.

According to the Chi-Square distribution table the critical value at 0,05 level of significance is 16.92 at 9 degrees of freedom $((R - 1)(C - 1) = (2 - 1)(10 - 1) = 9$ degrees of freedom (R =rows, C =columns)). The test statistic (69,77) clearly exceeds the critical value and the null hypothesis can consequently be rejected ($P < 0,0001$).

The exact same procedure is done for proportion of HD status A, distribution is shown in table below;

Year	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	Total
A	360	248	324	281	329	326	373	341	370	412	3364
B+C+D+E	192	157	199	164	135	127	96	57	58	27	1212
Total	552	405	523	445	464	453	469	398	428	439	4576

AB.6. Observed numbers of status A and status B plus diseased dogs in Labrador retriever

Resulting in a observed Chi-square value of 282,53 (P<0,0001)

German Shepherd dog

Year	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	Total
A + B	408	344	393	423	371	366	401	410	362	353	3831
C + D + E	183	184	161	190	143	171	91	91	72	61	1347
Total	591	528	554	613	514	537	492	501	434	414	5178

AB.7. Observed number of non-diseased and diseased German shepherds dogs.

Expected values;

$$\frac{3831}{5178} = 0,740$$

$$\frac{1347}{5178} = 0,260$$

Year	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	Total
A + B	437	391	410	454	380	397	364	371	321	306	3832
C + D + E	154	137	144	159	134	140	128	130	113	108	1346
Total	591	528	554	613	514	537	492	501	434	414	5178

AB.8. Expected numbers of diseased and non-diseased dogs in German shepherd dog

f_o	f_c	$(f_o - f_c)$	$(f_o - f_c)^2$	$(f_o - f_c)^2 / f_c$
183	154	29	841	5,461038961
184	137	47	2209	16,12408759
161	144	17	289	2,006944444
190	159	31	961	6,044025157
143	134	9	81	0,604477612
171	140	31	961	6,864285714
91	128	-37	1369	10,6953125
91	130	-39	1521	11,7
72	113	-41	1681	14,87610619
61	108	-47	2209	20,4537037
408	437	-29	841	1,924485126
344	391	-47	2209	5,649616368
393	410	-17	289	0,704878049
423	454	-31	961	2,116740088
371	380	-9	81	0,213157895
366	397	-31	961	2,420654912
401	364	37	1369	3,760989011
410	371	39	1521	4,099730458
362	321	41	1681	5,236760125
353	306	47	2209	7,218954248
				128,1759482

AB.9. Contingency table German shepherd dog.

According to the Chi-Square distribution table the critical value at 0,05 level of significance is 16.92 at 9 degrees of freedom. The test statistic (128,18) clearly exceeds the critical value and the null hypothesis can consequently be rejected ($P < 0,0001$).

The exact same procedure is done for proportion of HD status A, distribution is shown in table below;

Year	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	Total
A	182	148	183	225	204	211	296	314	287	298	2348
B+C+D+E	409	380	371	388	310	326	196	187	147	116	2830
Total	591	528	554	613	514	537	492	501	434	414	5178

AB.10. Observed numbers of status A and status B plus diseased dogs in German shepherd dog

Resulting in a observed Chi-square value of 479,74 ($P < 0,0001$)

The rejection of the null hypothesis only concludes that not all proportions between the different years are equal. It gives no information about where the difference is situated. In order to identify the difference in prevalence, the Marascuillo procedure enables simultaneous testing of the differences of all pairs of proportions over the time period.

Pairs of proportions is given by $k(k-1)/2$. In this case 10 years; $10(10-1)/2 = 45$ possible pairwise comparisons, and 45 critical ranges to compute for each breed respectively.

To compute the critical (r_{ij}) values at a given level of significance ($P=0,05$) the following equation have been used (48):

$$r_{ij} = \sqrt{X_{1-\alpha, k-1}^2} \sqrt{\frac{p_i(1-p_i)}{n_i} + \frac{p_j(1-p_j)}{n_j}}$$

$\sqrt{X_{1-\alpha, k-1}^2}$ = Square root of Chi square result

Example 2.1: Labrador retriever, non-diseased proportion 2006 compared to 2007:

$$P_{2006} = 0,85145$$

$$P_{2007} = 0,84938$$

$$P_{2006} - P_{2007} = 0,00207$$

$$r_{2006-2007} = \text{sqrt}(16,92 * \text{sqrt}(0,000229 + 0,000316))$$

$$0,09603$$

$$\Rightarrow 4,11339 * 0,023346 =$$

The absolute difference between years 2006 and 2007 does not exceed the critical value, hence the difference in proportion between the two years are not statistically significant.

Appendix C

Calculations question 3.

Unknown			Known + unknown	
	2010-2012	2013-2015	Tot 2010-2012	Tot 2013-2015
Danish unknown	26	7	1782	1850
Foreign unknown	24	4		
Total	50	11		

Unknown 2010-2012	2,8%	
Unknown 2013-2015	0,6%	

AC.1. Distribution of Labrador retriever with known and unknown status in the litter lists 2010 – 2015.

Unknown			Known + unknown	
	2010-2012	2013-2015	Tot 2010-2012	Tot 2013-2015
Danish unknown	265	34	2102	1722
Foreign unknown	195	20		
Total	460	54		

Unknown 2010-2012	21,9%	
Unknown 2013-2015	3,1%	

AC.2. Distribution of German shepherd dogs with known and unknown status in the litter lists 2010 – 2015.

Labrador retriever			
Period	HD A+B	HD C+D+E	Unknown
2010-2012	1706	26	50
2013-2015	1816	23	11

German shepherd dog			
Period	HD A+B	HD C+D+E	Unknown
2010-2012	1549	93	460
2013-2015	1601	67	54

AC.3. Contracted from litter lists. Labrador retriever (top) and German shepherd dog (bottom) in the periods 2010 – 2012 and 2013 – 2015.

Labrador retriever		HD status parents	
		C + D + E	A + B
Year	2010-2012	26	1706
	2013-2015	23	1816
p-value		0,618	

German shepherd dog		HD status parents	
		C + D + E	A + B
Year	2010-2012	93	1549
	2013-2015	67	1601
p-value		0,033	

AC.4. Chi-square test for Labrador retriever (top) and German shepherd dog (bottom) visualizing exposed + (year 2010 – 2012), exposed – (year 2013 – 2015), diseased + (HD status parents C + D + E) and diseased – (HD status parents A + B). Contracted from litter lists. Dogs with unknown status rejected.

Labrador retriever		HD status parents	
		C + D + E	A + B
Year	2010-2012	26 + 50	1706
	2013-2015	23 + 11	1816
p-value		<0,001	

AC.5. Chi-square test for Labrador retriever visualizing exposed + (year 2010 – 2012), exposed – (year 2013 – 2015), diseased + (HD status parents C + D + E + unknown (red text)) and diseased – (HD status parents A + B). Contracted from litter lists.

German shepherd dog		HD status parents	
		C + D + E	A + B
Year	2010-2012	93	1549 + 460
	2013-2015	67	1601 + 54
p-value		0,46	

AC.6. Chi-square test for German shepherd dog visualizing exposed + (year 2010 – 2012), exposed – (year 2013 – 2015), diseased + (HD status parents C + D + E) and diseased – (HD status parents A + B + unknown (red text)). Contracted from litter lists.

Appendix D

Calculations question 4.

Labrador retriever	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
A	539	567	561	549	486	469	445	439	570	569
B	98	114	97	102	110	106	90	93	68	77
C	10	19	17	11	13	6	7	6	4	11
D	2	0	0	0	0	0	0	0	2	0
E	0	0	0	0	0	0	0	0	0	0
unknown	173	42	41	34	21	17	12	10	0	1
Total:	822	742	716	696	630	598	554	548	644	658

Labrador retriever %	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
A	65,6	76,4	78,4	78,9	77,1	78,4	80,3	80,1	88,5	86,5
B	11,9	15,4	13,5	14,7	17,5	17,7	16,2	17	10,6	11,7
C	1,2	2,6	2,4	1,6	2	1	1,3	1	0,6	1,6
D	0,2	0	0	0	0	0	0	0	0,3	0
E	0	0	0	0	0	0	0	0	0	0
unknown	21	5,7	5,7	4,9	3,3	2,8	2,2	1,8	0	0,2

GSD	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
A	198	194	260	243	259	342	412	391	399	427
B	186	160	181	185	148	220	168	158	129	97
C	21	17	26	30	29	37	28	27	19	21
D	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0
unknown	439	327	321	312	240	145	74	34	15	5
Total:	844	698	788	770	676	744	682	610	562	550

GSD %	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
A	23,5	27,8	33	31,6	38,3	46	60	64,1	71	77,6
B	22	22,9	23	24	21,9	29,6	24,6	25,9	23	17,6
C	2,5	2,4	3,3	3,9	4,3	5	4	4,4	3,4	3,8
D	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0

AD.1. Number of parents (absolute numbers and proportions) used during 2006-2015, divided by HD status, derived from Litter lists.

Chi-square test:	
Chi-square (Observed value)	13,042
Chi-square (Critical value)	16,919
DF	9
p-value	0,161
alpha	0,05

AD.2. Proportion of dogs with HD status A + B for Labrador retriever, 2006-2015.

Chi-square test:	
Chi-square (Observed value)	29,520
Chi-square (Critical value)	16,919
DF	9
p-value	0,001
alpha	0,05

AD.3. Proportion of dogs with HD status A for Labrador retriever, 2006-2015.

Marascuilo procedure:			
Proportion HD status A Labrador 2006 - 2015			
Contrast	Value	Critical value	Significant
p(2006) - p(2007)	0,021	0,086	No
p(2006) - p(2008)	0,001	0,085	No
p(2006) - p(2009)	0,001	0,085	No
p(2006) - p(2010)	0,032	0,090	No
p(2006) - p(2011)	0,023	0,091	No
p(2006) - p(2012)	0,009	0,091	No
p(2006) - p(2013)	0,015	0,092	No
p(2006) - p(2014)	0,055	0,080	No
p(2006) - p(2015)	0,036	0,082	No
p(2007) - p(2008)	0,021	0,085	No
p(2007) - p(2009)	0,019	0,086	No
p(2007) - p(2010)	0,012	0,091	No
p(2007) - p(2011)	0,003	0,091	No
p(2007) - p(2012)	0,011	0,091	No
p(2007) - p(2013)	0,006	0,092	No
p(2007) - p(2014)	0,075	0,080	No
p(2007) - p(2015)	0,056	0,082	No
p(2008) - p(2009)	0,002	0,084	No
p(2008) - p(2010)	0,033	0,089	No
p(2008) - p(2011)	0,024	0,090	No
p(2008) - p(2012)	0,010	0,090	No
p(2008) - p(2013)	0,015	0,091	No
p(2008) - p(2014)	0,054	0,079	No
p(2008) - p(2015)	0,035	0,081	No
p(2009) - p(2010)	0,031	0,090	No
p(2009) - p(2011)	0,022	0,090	No
p(2009) - p(2012)	0,008	0,091	No
p(2009) - p(2013)	0,013	0,091	No
p(2009) - p(2014)	0,056	0,079	No
p(2009) - p(2015)	0,037	0,081	No
p(2010) - p(2011)	0,009	0,095	No
p(2010) - p(2012)	0,023	0,095	No
p(2010) - p(2013)	0,018	0,096	No
p(2010) - p(2014)	0,087	0,085	Yes
p(2010) - p(2015)	0,068	0,086	No
p(2011) - p(2012)	0,014	0,095	No
p(2011) - p(2013)	0,009	0,096	No
p(2011) - p(2014)	0,078	0,085	No
p(2011) - p(2015)	0,059	0,087	No
p(2012) - p(2013)	0,005	0,096	No
p(2012) - p(2014)	0,064	0,085	No
p(2012) - p(2015)	0,045	0,087	No
p(2013) - p(2014)	0,069	0,086	No
p(2013) - p(2015)	0,050	0,088	No
p(2014) - p(2015)	0,019	0,075	No

AD.4. Differences in proportion of dogs with HD status A for Labrador retriever 2006-2015.

Chi-square test:	
Chi-square (Observed value)	14,150
Chi-square (Critical value)	16,919
DF	9
p-value	0,117
alpha	0,05

AD.5. Differences in proportion of dogs with HD status A + B for German shepherd dog, 2006-2015.

Chi-square test:	
Chi-square (Observed value)	233,871
Chi-square (Critical value)	16,919
DF	9
p-value	< 0,0001
alpha	0,05

AD.6. Differences in proportion of dogs with HD status A for German shepherd dog, 2006-2015.

Marascuilo procedure:			
Proportion HD status A GSD 2006-2015			
Contrast	Value	Critical value	Significant
p(2006) - p(2007)	0,034	0,105	No
p(2006) - p(2008)	0,068	0,102	No
p(2006) - p(2009)	0,042	0,102	No
p(2006) - p(2010)	0,105	0,105	Yes
p(2006) - p(2011)	0,082	0,103	No
p(2006) - p(2012)	0,188	0,102	Yes
p(2006) - p(2013)	0,189	0,105	Yes
p(2006) - p(2014)	0,240	0,105	Yes
p(2006) - p(2015)	0,294	0,101	Yes
p(2007) - p(2008)	0,034	0,107	No
p(2007) - p(2009)	0,008	0,107	No
p(2007) - p(2010)	0,072	0,110	No
p(2007) - p(2011)	0,048	0,108	No
p(2007) - p(2012)	0,154	0,107	Yes
p(2007) - p(2013)	0,156	0,110	Yes
p(2007) - p(2014)	0,207	0,109	Yes
p(2007) - p(2015)	0,261	0,106	Yes
p(2008) - p(2009)	0,026	0,104	No
p(2008) - p(2010)	0,038	0,106	No
p(2008) - p(2011)	0,014	0,104	No
p(2008) - p(2012)	0,120	0,104	Yes
p(2008) - p(2013)	0,122	0,107	Yes
p(2008) - p(2014)	0,172	0,106	Yes
p(2008) - p(2015)	0,227	0,103	Yes
p(2009) - p(2010)	0,064	0,107	No
p(2009) - p(2011)	0,040	0,105	No
p(2009) - p(2012)	0,146	0,104	Yes
p(2009) - p(2013)	0,148	0,107	Yes
p(2009) - p(2014)	0,198	0,107	Yes
p(2009) - p(2015)	0,252	0,103	Yes
p(2010) - p(2011)	0,023	0,108	No
p(2010) - p(2012)	0,083	0,107	No
p(2010) - p(2013)	0,084	0,110	No
p(2010) - p(2014)	0,135	0,109	Yes
p(2010) - p(2015)	0,189	0,106	Yes
p(2011) - p(2012)	0,106	0,105	Yes
p(2011) - p(2013)	0,107	0,108	No
p(2011) - p(2014)	0,158	0,107	Yes
p(2011) - p(2015)	0,212	0,104	Yes
p(2012) - p(2013)	0,001	0,107	No
p(2012) - p(2014)	0,052	0,107	No
p(2012) - p(2015)	0,106	0,103	Yes
p(2013) - p(2014)	0,051	0,109	No
p(2013) - p(2015)	0,105	0,106	No
p(2014) - p(2015)	0,054	0,106	No

AD.7. Differences in proportion of dogs with HD status A for German shepherd dog 2006-2015.

Unknown status only	Male Danish	Male foreign	Female Danish	Female foreign	Total unknown male	Total unknown female	% unknown
2006	17	77	9	69	94	78	20,9%
2007	4	30	3	1	34	4	5,1%
2008	12	22	4	1	34	5	5,4%
2009	7	23	3	0	30	3	4,7%
2010	6	11	4	0	17	4	3,3%
2011	5	10	2	0	15	2	2,8%
2012	6	3	3	0	9	3	2,2%
2013	4	3	3	0	7	3	1,8%
2014	0	0	0	0	0	0	0,0%
2015	0	1	0	0	1	0	0,2%

AD.8. Distribution of Labrador retrievers with unknown status in the litter lists 2006 –

2015, divided into dogs with Danish and foreign registration number in addition to male and female.

Unknown status only	Male Danish	Male foreign	Female Danish	Female foreign	Total unknown male	Total unknown female	% unknown
2006	68	198	123	49	266	172	51,9%
2007	56	162	98	10	218	108	46,7%
2008	47	181	85	8	228	93	40,7%
2009	49	164	87	12	213	99	40,5%
2010	58	98	79	5	156	84	35,5%
2011	40	53	45	8	93	53	19,6%
2012	12	30	31	1	42	32	10,9%
2013	3	15	15	1	18	16	5,6%
2014	4	3	8	0	7	8	2,7%
2015	0	1	4	0	1	4	0,9%

AD.9. Distribution of German shepherd dogs with unknown status in the litter lists 2006 –

2015, divided into dogs with Danish and foreign registration number in addition to male and female.

Appendix E

Method for isolation of HD status in dataset containing list of litters:

The datasets (one sheet per year and breed) of all DKK registered offspring contained information about offspring and parent dogs. However, all registered information about the parent dogs (result of HD status, eye examination, DNA-test etc.) was gathered in a “health”-line for each specific dog, and were not written in a specific order. In order to isolate the HD status in the “health-line”, the following commandos were used in Excel:

1, To find the HD status localization in the “health”-line for each dog, which were written as “HD bedømmelse: Status X” (X=A/B/C/D/E), the following command was used in a new column;

=FIND("HD bedømmelse:");[@FarSunnhed])

[@FarSunnhed] specifies the column to isolate from. In this case the “health”-line of male dogs.

The result is given as a number, corresponding to the number of characters in the “health”-line before the term “HD bedømmelse:” appears in the line.

2, The following command was then used in a new column;

=[@[FS Stage1]]+16

where the result from step 1 is given in the column “FS Stage1” and 16 corresponds to the number of characters in “HD bedømmelse:”.

3, Finally, the term “Status X” is isolated in a third new column using the following command;

=MID([@FarSunnhed];[@[FS Stage2]];8)

The command “=MID” displays the characters found in one line, where starting position and length is specified.

[@[FS Stage2]] is the result from step 2 and specifies the starting position.

8 is the specified length of the term one wishes to display, (Status X = 8 characters).

The exact same procedure was applied to the mothers of litters (their results were listed in column MorSunnhed).