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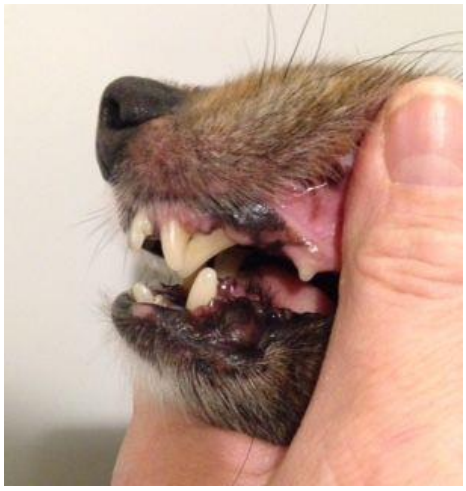
DEPARTMENT OF VETERINARY AND ANIMAL SCIENCES
SECTION OF ANIMAL GENETICS, BIOINFORMATICS AND BREEDING



Veterinary Master's Thesis

Lena Rahbek, wvc118

"Lanced" canines in the Shetland sheepdog, heredity and breeding recommendations

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Submitted on: 6th of July 2018

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Title and subtitle:	“Lanced” canines in the Shetland sheepdog, heredity and breeding recommendations
Topic description:	The aim of this study was to investigate whether “lanced” canines is a hereditary condition in the Shetland sheepdog and to provide further elucidation of the condition itself together with health concerns and treatment options. Furthermore, to propose a breeding recommendation for the Shetland sheepdog regarding “lanced” canines.
Academic advisor:	Professor Merete Fredholm DVM, Ph.D., Dr.Vet.Sci.
Submitted on:	6th of July 2018
Grade:	
Front page pictures:	Left: Anonymous picture of one of the dogs participating in this study. Right: Shetland sheepdog, photo credit to Kirsten Klitsgaard.

Preface

This master's thesis was conducted from February to July 2018 at Copenhagen University, Faculty of Health and Medical Sciences, Department of Veterinary and Animal Sciences, Section of Animal Genetics, Bioinformatics and Breeding in collaboration with the Danish Shetland Sheepdog Club and the Danish Kennel Club.

The aim of this study was to find out if "lanced" canines in the Shetland sheepdog is a hereditary condition and to propose some breeding recommendations in this regard. Furthermore, it was also desirable to get an idea about the possible heritability and mode of inheritance.

The study was proposed by the Danish Shetland Sheepdog Club as a response to requests from multiple breeders and Shetland sheepdog owners wishing to shed some light on the issue in the breed. The club had also noticed that the problem seemed to be more widespread in certain lines. They did not feel that they could take up the investigation themselves since some breeders might not wish for their club to know that they have an issue in their kennel. Therefore, they wanted an "outsider" to conduct the investigation and to make all information anonymous to encourage as many breeders and dog owners as possible to submit data from their dogs to the study.

The process turned out to be more problematic than first expected since it was difficult to get data about dogs with this condition and a lot of time was spend trying to get more dogs included in the study. Furthermore, it was very difficult to find relevant articles about the genetics of tooth disorders in dogs.

A literature search was performed in order to gather information about tooth development, a known tooth disorder, its genetic setup and treatment.

Tolstrup, 6th of july 2018

Lena Rahbek

Acknowledgments

I am very grateful to my academic advisor Professor Merete Fredholm for her guidance and help through the entire process.

I would also like to thank the Danish Shetland Sheepdog Club, especially their health committee for showing interest in the subject and starting up initial data collection. A special thanks to health committee member Tine Wiberg Rasmussen for being my contact person in the Danish Shetland sheepdog club, helping me with further data collection and answering any question I had during the process of writing this thesis.

Furthermore, a special thanks to veterinary specialist Jens Ruhnau from AniCura TandDyreklinikken for giving me a more practical insight into the clinical aspect of odontology and lanced canines and for sharing some of his knowledge and expertise about the subject.

Thanks to the Danish Kennel Club for helping me with further data collection by contacting selected dog owners.

A big thanks to all the breeders and dog owners that provided me with information and pictures of their dogs to use in this study, without your willingness to let your dogs be part of this study I could not have written this thesis.

Also, a big thank you to Dr. James Longden for constructive criticism and proofreading of the thesis.

Abstract

“Lanced canines” is a condition that predominantly affects Shetland sheepdogs, becoming apparent when the permanent upper canines start to erupt. It is characterised by mesioversion of tooth 104 and/or 204 and gives rise to several health issues/concerns among them retained deciduous teeth, traumatic contact with the hard palate, tooth-on-tooth wear and increased risk of periodontal disease. The treatment methods include surgical extraction, corrective regulation with elastic bands and corrective surgery.

Tooth development is a complicated process that includes many stages and developmental processes, all of which are regulated by genes. To date no specific genetic components of “lanced” canines have been discovered however comparable diseases, such as Primary failure of eruption (PFE), have known genetic associations, in this case a mutation is seen in the PTHR1 gene, demonstrating that tooth disorders can indeed be hereditary.

It was the aim of this study to find out if “lanced” canines in the Shetland sheepdog is a hereditary condition and to propose some breeding recommendations in this regard. To do this, 35 dogs known to have “lanced” canines and 25 dogs described as unaffected were included in this study. 4-generation pedigrees were studied and through Singles method 11 whole litters were analysed. This analysis found that we could not reject a null-hypothesis about simple recessive inheritance. However, there were no common ancestors shared between all the 35 affected dogs, which when compared to the results from Singles method would suggest that “lanced” canines is a condition with a high heritability, most likely a threshold trait caused by mutations in several genes. Breeding recommendations must take other diseases/conditions in the Shetland sheepdog breed into consideration. It is therefore recommended to not use any affected individuals in breeding, furthermore to not repeat any mating that has resulted in affected offspring.

The next step should be to try and calculate the prevalence and heritability of the condition in the Danish Shetland sheepdog population and to identify the gene(s) causing “lanced” canines in order to develop a genetic test for the condition. For this, further studies would be required.

Resumé

Lansetænder er en tilstand der hovedsageligt rammer Shetland sheepdogs. Tilstanden bliver først synlig når hjørnetænderne i overmunden begynder at bryde igennem. Lansetænder er karakteriseret ved mesioversion af tand 104 og/eller 204 og giver grobund for adskillige sundhedsproblemer og bekymringer heriblandt tilbageholdte mælketænder, traumatisk kontakt med den hårde gane, tand-mod-tand slid og øget risiko for peridontale sygdomme. Behandlingsmetoder inkluderer tandekstraktion, korrigerende regulering med elastik samt korrigerende operation.
Tændernes udvikling er en kompliceret proces der indeholder mange forskellige stadier og udviklingsprocesser, alt sammen genetisk reguleret. Den genetiske baggrund for lansetænder kendes endnu ikke, men jeg har gennemgået en sammenlignelig sygdom, Primary failure of eruption (PFE) som har en kendt genetisk årsag, en mutation i PTHR1 genet, hvilket demonstrerer at tandsygdomme godt kan være arvelige.
Formålet med dette studie var at finde ud af om lansetænder er en arvelig tilstand og at komme med nogen avlsanbefalinger. I mit studie indgik 35 hunde med lansetænder og 25 hunde beskrevet som værende fri for lansetænder. 4-generations stamtavler blev studeret og ved hjælp af Singles metode blev 11 hele kuld analyseret. Af denne analyse fremgik det at man ikke kan forkaste en nulhypotese om simpel resesiv arvegang. Der var ingen fælles forfædre delt mellem alle de 35 hunde med lansetænder og dette, sammenholdt med resultatet fra Singles metoden ledte til konklusionen at lansetænder er en tilstand med høj heritabilitet, mest sandsynligt en tærskelegenskab forårsaget af mutationer i adskillige gener. Avlsanbefalinger skal sammenholdes med andre sygdomme i shetland sheepdog racen. Det anbefales at man ikke bruger afficerede hunde i avl, samt at man ikke gentager en parring der tidligere har resulteret i afficerede afkom.
Næste skridt må være at finde frem til prævalensen samt heritabiliteten af lansetænder i den danske shetland sheepdog population og at identificere det/de gen/gener samt mutation/er der forårsager lansetænder således at man kan udvikle en genetisk test for lansetænder. For at gøre dette er yderligere studier nødvendige.

List of abbriviations

CRL – Crown-rump length

DEJ – Dentino-enamel junction

DKK – Dansk Kennel Klub (The Danish Kennel Club)

FCI – Fédération Cynologique Internationale (World Canine Organization)

LC – Lanced canines

PFE – Primary failure of eruption

PTH – Parathyroid hormone

PTHLH - Parathyroid hormone like hormone

PTHR1 - Parathyroid hormone receptor 1

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1. Introduction

1.1 “Lanced” Canine Teeth. Definition, problems and treatment

1.1.1 Definition

“Lanced” canine teeth is a mesioversion of tooth 104 and/or 204. It predominantly affects Shetland Sheepdogs, but a few other breeds such as Italian greyhounds, miniature schnauzers, fox terriers and some cats can also be affected by this condition (Legendre and Stepaniuk 2008). The adult upper canine tooth is displaced forward, pointing towards the nose. The degree of displacement varies a lot. Because of the displacement the upper canine tooth can then be located in front of the lower canine instead of behind it as is normal. Where the normal upper canine tooth is almost perpendicular in relation to the hard palate, a “lanced” canine Tooth is located more parallel to the hard palate.

The deciduous teeth are positioned normally and therefore it does not become evident that the dog has “lanced” canine teeth until the adult canines erupt.

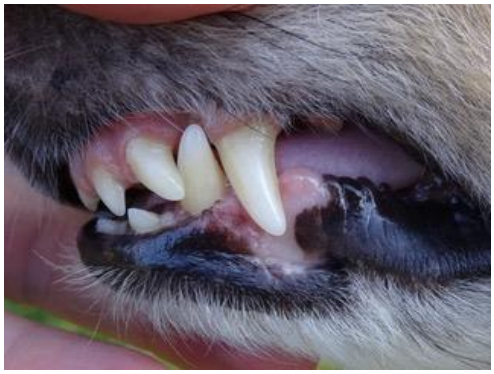


Figure 1: Shetland sheepdog with normally placed upper left canine. (Anonymous picture)

Figure 2: Shetland sheepdog with “lanced” upper left canine. (Anonymous picture)

1.1.2 Health concerns and problems

The abnormal position of the canine tooth can result in several problems (Legendre and Stepaniuk 2008) and can even prevent the mouth from closing completely. If the diastema between the lanced tooth and the lateral incisor is too small it can also prevent the lower canine (304 and 404) from growing into a normal position between those teeth. The lower canine is then forced to deviate from its normal position either labially or lingually. This can cause painful

traumatic contact with the hard palate, irregular contact with the lip and tooth-on tooth wear that can result in concussive pulpitis and possible pulp necrosis. Furthermore, food and calculus can accumulate between the lanced tooth and the adjacent incisor, which can increase the risk of periodontal disease between these two teeth. When the permanent teeth grow out in an abnormal position the normal pressure on the deciduous tooth can also be lacking resulting in retained deciduous teeth.

1.1.3 Treatment options

Optimal treatment strategy is dependent on several factors including the age of the dog, the severity of the disease, the dog's general health status and owner compliance and economy (Jens Ruhnau, personal communication, May 28, 2018). Sometimes the displacement of the canine tooth is only cosmetic and therefore does not require any correction, however if correction is required this can be achieved through surgical or non-surgical means:

- Surgical extraction

Surgical extraction of a maxillary tooth can be performed using a scalpel to sever the epithelial attachment of the maxillary tooth (Tsugawa, Lommer, and Verstraete 2012). Making sure the flap is of sufficient width to allow the suture line to rest over solid bone and not the vacated alveolus. Hereafter the tooth is loosened with a periosteal elevator. An alveolectomy (removal of alveolar bone surrounding the tooth) is performed and the tooth is then loosened even more using a luxator. When the tooth is sufficiently mobile the extraction forceps can be used to deliver the tooth. The flap is then sutured in place.

- Corrective regulation with elastic band (orthodontic movement)

There are different techniques to achieve this, one example as recommended by Legendre and Stepaniuk (Legendre and Stepaniuk 2008) is as follows: Depending on the side of the lanced canine either 108 together with 109 or 208 together with 209 are used as anchor teeth. A lingual button is fixed to the mesiobuccal cusp of the maxillary first molar tooth using an orthodontic bonding agent. Hereafter a maxillary central incisor bracket, with its base slightly bent to better follow the contour of the mesial cusp of the maxillary fourth premolar tooth, is bonded in place. Next a length of orthodontic bracket tie wire is twisted from the lingual button to the distal prongs of the bracket. The twisted end is cut short and pushed in between the distal prongs in such a way that there is no irritation to the patient. Finally an elastic chain is cut at 80 to 75% of its resting length and stretched from the hook to the mesial two prongs of the bracket. The elastic

chain is changed every 2-weeks until the desired position of the target tooth is attained. Then the appliances are removed.

This method can be used on dogs of any age, but the older the dog, the longer it will take. For example, it takes approximately 2-3 months to reposition the tooth in dogs 7-8 months of age but 4-5 months for dogs 1 year of age (Jens Ruhnau, personal communication, May 28, 2018). Before the procedure both the tooth/teeth to be moved and the teeth serving as anchor teeth must be radiographed to ensure that their root systems are mature and healthy enough to withstand orthodontic treatment without undue damage (Legendre and Stepaniuk 2008).

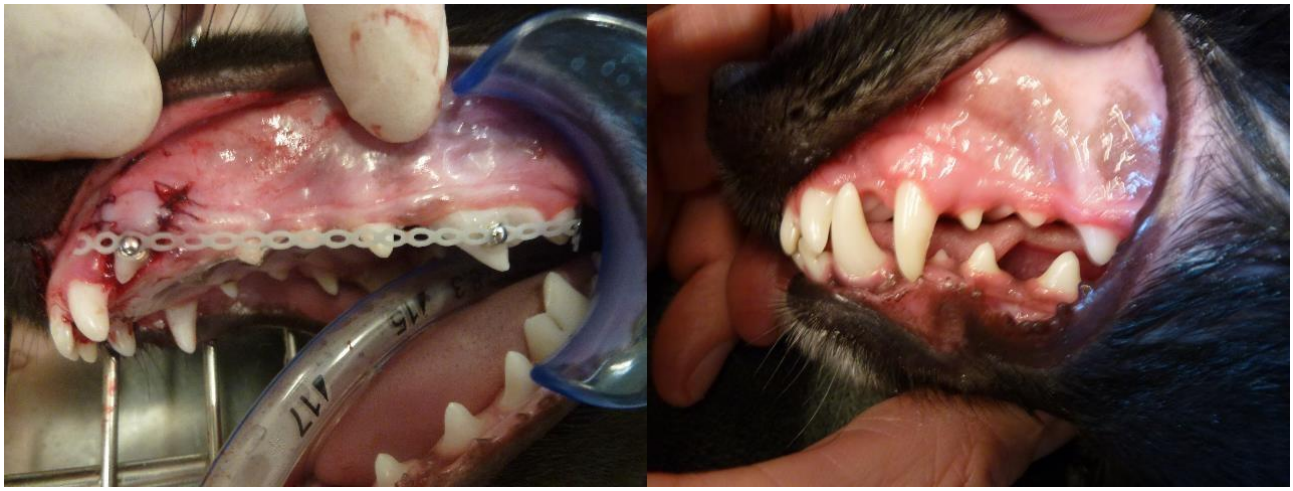


Figure 3: Corrective regulation. On the left dog with a left lanced canine that has just had regulation appliances put on. On the right the same dog after the end of corrective regulation, now with a tooth 204 that is no longer "lanced" (Picture from AniCura TandDyreklinikken).

- Corrective surgery

A third, novel, method that has not yet been described in the litterature (Jens Ruhnau, personal communication, May 28, 2018) is to move the tooth's position by changing the way the root is positioned, similar to the method described above. But with this method it is all done in just 1 surgery where force is applied in order to "break" the root so that when the root continues to grow it will grow at a crooked angle. For this surgery to be effective it is necessary that the tooth is still root-open, i.e. that the root is still developing and maturing. In practise this means that this kind of surgery must be performed preferably around 6 months of age, but no older than 8 months, when the "lanced" canine has just started to erupt. Corrective surgery is therefore not an option for dogs with a fully developed "lanced" canine. After that regulation will have to be done by the methods described above instead. It is recommended that the dog return to the clinic a few months after to check if the surgery was a success.

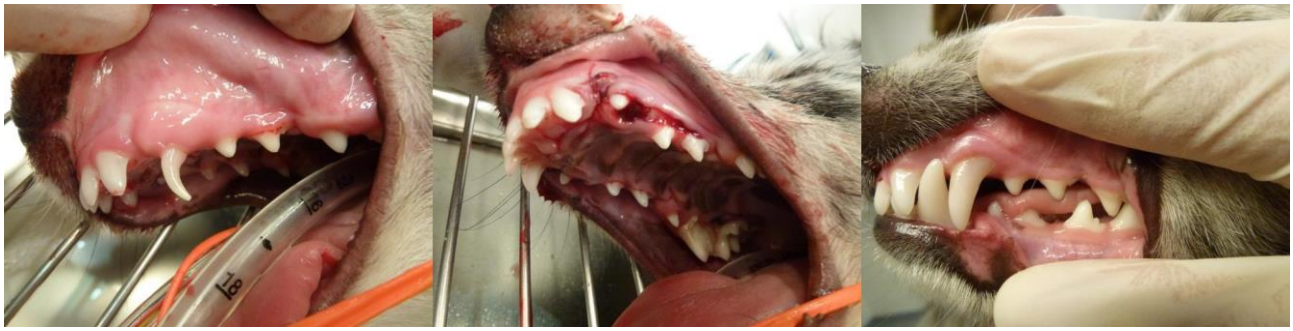


Figure 4: Corrective surgery. Left picture shows a dog with a retained deciduous upper left canine and a "lanced" canine about to erupt. Middle picture shows the same dog after having the retained deciduous tooth removed and corrective surgery performed. Right picture shows the same dog 4 months later now with a normally placed left upper canine.

- Methods comparison

As corrective surgery or corrective regulation can save the tooth these are generally considered the methods of choice (Legendre and Stepaniuk 2008). However surgical tooth extraction is a more readily available treatment compared to corrective regulation and corrective surgery since these procedures require technical expertise by the veterinarian and more advanced equipment. In addition, both tooth extraction and corrective surgery can be performed in only one visit to the veterinary clinic, whereas regulation with the elastic chain requires multiple office visits together with an almost daily oral hygiene routine for the dog undergoing treatment. This also makes regulation using the elastic chain more expensive compared to the other two methods, at roughly double the price (Jens Ruhnau, personal communication, May 28, 2018). Given that corrective surgery is only applicable before the "lanced" canine has fully developed it is generally considered optimal to perform corrective surgery in dogs up to 8 months old, after which corrective regulation should be used (Jens Ruhnau, personal communication, May 28, 2018).

1.2 Tooth development

The deciduous dentition develops during the embryonic and foetal stages of a dogs gestation period, whereas the permanent dentition develops during the foetal and neonatal stages of development (Tutt 2006). A dogs gestation period is approximately 61 days (Pretzer 2008) with the embryonic stage lasting until day 35, then the foetal stage begins and last until birth. The neonatal stage last from birth until approximately 3 weeks of age (Bartges et al. 2012).
During this time tooth development progresses through several stages termed initiation, bud, cap and bell (Tutt 2006).

1.2.1 Stages

- Initiation stage (12-16 mm CRL ((Williams and Evans 1978))

In order for the initiation to begin, interaction between different embryological tissue is necessary. This interaction, also known as induction, occurs between mesenchymal tissue and ectodermal tissue (Tutt 2006).
The oral epithelium is derived from ectoderm. The ectoderm is separated from the underlying mesenchyme by the basement membrane. Firstly, a narrow band of thickened epithelium (primary epithelial band) forms on the developing mandible and maxilla (Teaford, Ferguson, and Meredith Smith 2000). These bands specify the areas of epithelium from which teeth are capable of forming, later on giving rise to the dental lamina when it grows down into the mesenchyme (Tutt 2006)

- Bud stage (27 mm CRL / 32 days (Williams and Evans 1978))

The dental lamina that has now been formed proliferates into the mesenchyme where it forms buds. It is from these buds that the teeth will develop later on. All teeth develop from ectoderm and mesoderm (Tutt 2006).

- Cap stage (33 mm CRL / 35 days (Williams and Evans 1978))

Parts of the tooth bud undergo differential growth which leads to a cap shape. During this stage the shape of the tooth is determined by the process of morphogenesis. The enamel organ, which is of ectodermal origin, develops during this stage deep within the tooth bud and will later on produce enamel that will cover the surface of the crown. The dental papilla, giving rise to dentin and pulp, is formed by mesenchymal tissue. The dental sac, from which the periodontium will develop, is formed from the mesenchyme surrounding the enamel organ (Tutt 2006).

- Bell stage (72 mm CRL / 42 days (Williams and Evans 1978))

During this stage proliferation, morphogenesis and differentiation continues. The cells of the enamel organ differentiate into four distinct layers: Inner enamel (dental) epithelium which differentiate into first pre-ameloblasts and, later on, ameloblasts that produce enamel, stratum intermedium that supports enamel production, stellate reticulum, that also supports enamel production, and outer enamel (dental) epithelium that protects the enamel organ during amelogenesis.

The dental papilla differentiates into two layers: The outermost layer that will differentiate into odontoblasts and produce dentin. The inner layer develops into the tooth pulp.

The dental sac differentiates into its separate tissues (gingiva, alveolus, periodontal ligament and cementum) at a later stage (Tutt 2006).

1.2.2 Development

Further development occurs when the odontoblasts start to produce pre-dentine and the basement membrane starts to disintegrate. Upon contact with pre-dentin the pre-ameloblasts develops into ameloblasts. The ameloblasts start amelogenesis where they secrete enamel matrix onto the disintegrating basement membrane, thereby forming the dentino-enamel junction (DEJ). The ameloblasts produce primary dentin until apexogenesis is complete, hereafter they produce secondary dentin throughout the lifespan of the tooth (Tutt 2006).

Root development starts once the crown is fully formed and begins to erupt into the mouth. The root is formed by the cervical loop, that is a portion of the original enamel organ. The cervical loop grows down into the dental sac enclosing more of the dental papilla forming Hertwig's root sheath which determines the shape of the root / roots. Production of pre-dentine begins and the root continues to develop until the apex is formed. When the basement membrane and Hertwig's root sheath disintegrates, thereby exposing undifferentiated cells from the dental sac to the root dentine, these cells become cementoblasts. The cementoblasts secrete cementoid that undergoes mineralisation into cementum.

During the development of the root and the crown, mesenchyme from the surrounding dental sac starts to form the periodontal ligament and the alveolus. This ligament is designed to withstand rotational force and other forces applied to the tooth to keep it within the alveolus.

1.2.6 Gene expression and tooth development

- Initiation and tooth bud formation

As mentioned above the tooth development process starts with the formation of the primary epithelial band and then tooth buds forming at specific places in the dental lamina. This process is controlled, at least in part, by the Msx-1 and Msx-2 homeobox genes, which are both expressed in epithelial and mesenchymal cells during tooth development (Teaford, Ferguson, and Meredith Smith 2000). Early expressions domains of Msx-1 and Msx-2 prior to tooth bud formation suggest a role for these genes in initiating the primary epithelial band a hypothesis that was further supported by in vivo experiments in which targeted mutation of the Msx-1 gene resulted in the development of all teeth being arrested in the early bud stage (Satokata and Maas 1994).

It has also been suggested that Msx-1 is required for a signalling pathway from bud mesenchyme to dental epithelium in tooth histogenesis by regulating the expression of signalling molecules, possibly Bmp-4 (Teaford, Ferguson, and Meredith Smith 2000).

Tooth development in Msx-1/Msx-2 double mutants is reported as being arrested earlier than the tooth bud stage and initiation may not occur at all when both genes are absent.

- Tooth positioning and shape

The 'odontogenic homeobox code' is a molecular model for the patterning of the dentition, based on the expression of several homeobox genes in neural crest-derived ectomesenchyme (Sharpe 1995). The expression of homeobox genes such as Dlx-1, Dlx-2, Msx-1 and Msx-2 are proposed to specify the development of tooth germs into either molars or incisors. So different genes regulate the formation of teeth at different positions. For example, Dlx-1/ -2 -/- embryos have normal upper and lower incisors and lower molars, but do not develop any upper molars (Qiu et al. 1997; Thomas et al. 1997). Further supporting the specificity of gene regulation between the different teeth is the fact that null mutation of the activin-βA gene (activin is a growth factor-like signalling molecule) in transgenic mice makes them develop no incisor teeth and no mandibular molars, but the development of maxillary molars is always normal (Matzuk et al. 1995).

- Regulation of tooth shape

As described above the enamel knot is a part of the enamel organ that differentiates into different cells that all play a role in the formation of the tooth shape and roots.

It has been proposed that Msx-2 expression provides a molecular link between tooth initiation

and shape (Mackenzie, Ferguson, and Sharpe 1992). Several more genes have been shown to be restricted to enamel knot epithelial cells in the tooth bud, for example the expression of the genes for the secreted factors Shh, Bmp-4, Bmp-7 and Fgf-4 are all localised in enamel knot cells. It has also been proposed that the enamel knot acts as a signalling centre to direct secondary knot formation which controls local epithelial cell proliferation rates (Vaahtokari et al. 1996).

1.3 Genetics, heredity and teeth

The genetic basis for “lanced” canines is currently un-known, with no specific inherited component yet identified. Therefore, the following literature review will instead focus on another tooth disease that is known to have an inherited genetic component in order to demonstrate how tooth disorders can indeed be genetically inherited.

2. Primary failure of eruption (PFE) – a literature review

As there are not any well-known tooth diseases that only effect the position of the tooth in the same way as occurs with “lanced” canines primary failure of eruption (PFE) will instead be used as an example of a comparable disease effecting the teeth.

2.1 Background

PFE is defined as incomplete tooth eruption despite the presence of a clear eruption pathway where the alveolar bone above the crown has been reabsorbed (Hanisch et al. 2018; Stellzig-Eisenhauer et al. 2010). It involves the partial or complete non-eruption of a non-ankylosed tooth due to a disturbance in the eruption mechanism (Stellzig-Eisenhauer et al. 2010). PFE is a rather rare disease with a prevalence of 0.06% (Hanisch et al. 2018) and it can affect both primary and permanent teeth, but only posterior teeth are affected (Hanisch et al. 2018; Stellzig-Eisenhauer et al. 2010). Teeth posterior to the most anterior affected tooth are usually affected as well and the condition is usually asymmetrical (Hanisch et al. 2018; Stellzig-Eisenhauer et al. 2010). PFE can be classified into 3 subtypes (Frazier-Bowers et al. 2007; Stellzig-Eisenhauer et al. 2010); in type I all affected teeth have a similar lack of eruption potential. In type II a tooth distal to the most mesial affected tooth has a greater, but still inadequate eruption potential. In type III patients both forms appear in the various quadrants.

2.2 Clinical impact, health concerns and treatment

The clinical impact of PFE is often very severe. Affected teeth often present dilacerations resulting in a severe lateral open bite (Stellzig-Eisenhauer et al. 2010) that results in, for example, eating dysfunction with chewing difficulties.
Treatment options for affected teeth are quite limited (Frazier-Bowers et al. 2007; Hanisch et al. 2018; Stellzig-Eisenhauer et al. 2010), for example extrusion never succeeds because the teeth become ankylosed as soon as orthodontic force is applied. In very mild cases the teeth can be treated conservatively by restoring them with onlays and crowns. Otherwise treatment options are limited to extraction or surgical removal of the affected teeth followed by implantation or installation of a removable prothesis, the latter often being the only reasonable solution.

2.3 Inheritance

It has previously been shown that non-syndromic PFE has an autosomal-dominant mode of inheritance with complete penetrance and variable expressivity (Frazier-Bowers et al. 2007; Stellzig-Eisenhauer et al. 2010).

2.4 Molecular genetic analysis

In order to identify the genetic cause of PFE parametric linkage analysis with a dominant model has been performed (Stellzig-Eisenhauer et al. 2010). This analysis identified two regions with a maximum LOD score of 2.41. Usually an LOD score > 3 is considered an indicator for linkage, however, this was not achievable due to the given family structure in their cohort. The first identified region was excluded since no disease-associated mutations were found following sequencing of exons from two patients and an unaffected control person.
The second identified region was located on chromosome 3 in interval p14.3-p24.3. This section of the chromosome contains 301 genes. In order to narrow down the number of potential candidate genes factors such as expression in the bone or bone-associated tissue, functional considerations on the encoding protein and a known role in disease processes were considered. This analysis identified the gene encoding parathyroid hormone receptor 1 (PTHR1) as a potential candidate for the gene responsible for PFE. Additionally, it is known that PTHR1 binds parathyroid hormone (PTH) and parathyroid hormone-like hormone (PTHLH) with equal affinity. PTHLH expression is restricted to the epithelial layer during tooth development, while PTHR1 expression is observed in the adjacent dental mesenchyme and alveolar bone. This would indicate a role for PTHLH and/or PTHR1 in the signal transduction pathways regulating epithelial-mesenchymal interactions during the development of epithelial organs such as teeth.

Validation of this hypothesis was undertaken by analysing all 14 PTHR1-coding exons and their respective intron-exon boundaries using direct sequencing (Stellzig-Eisenhauer et al. 2010). In each of their four multiplex pedigrees (Families grouped into ZD1, ZD2, ZD3 and ZD11) they identified one of three distinct heterozygous mutations (ZD1 and ZD3: c.1050-3C>G; ZD2: c.543+1G>A; ZD11: c.463G>T). These mutations were only observed in study participants that were affected by PFE (Stellzig-Eisenhauer et al. 2010).
It would therefore seem likely that the autosomal dominant PTHR1 mutation observed in families with non-syndromic PFE most likely led to premature proteolytic degradation of the

precursor protein and that this strongly suggested that haplo-insufficiency of the receptor was the cause of non-syndromic PFE (Stellzig-Eisenhauer et al. 2010).

2.5 Conclusion

PFE has been shown to have an autosomal dominant mode of inheritance and mutations in the PTHR1 gene are associated with non-syndromic PFE.

3. Materials and methods

3.1 Segregation analysis

3.1.1 Data collection for segregation analysis

- Complete selection

There are many ways of collecting data for segregation analysis. However one such method is to choose a random sample within the population or through parents with no regard to the phenotype of the children (Mi 1967). This way of sampling is defined as complete selection and may include all possible mating types (affected x affected, affected x normal, normal x affected and normal x normal) and all types of s,r combinations (childless, nonsegregating and segregating families) s being sibship size and r being number of affected siblings.

- Incomplete selection

In contrast to complete selection incomplete selection includes sampling through the affected children (Mi 1967). In this way only simplex and multiplex families will be included in the sample and this will introduce a bias that can result in the proportion of affected individuals being over estimated. For example, when dealing with a recessive disease it could be possible for litters from two carriers to not include any affected offspring. These offspring should still be included in the statistical calculations, but when using incomplete selection, they are not.

3.1.2 Singles method

When data is obtained through incomplete selection as described above a correction for the bias that this mode of data collection presents is required. This correction can be performed using Singles method (Nicholas 2003), the object of which is to test if the observed disease frequency (p) variates from the expected frequency (p_0) by a given form of inheritance. We can test if $p = p_0$ by calculating the test value Z^2. If the test value is greater than the critical value of the chi-square distribution, we reject the null hypothesis (NIST/SEMATECH 2010). When using the significance level $\alpha = 0.05$ and 1 degree of freedom the critical value of the chi-square distribution is 3.841 (NIST/SEMATECH 2010).

The test value Z^2 can be calculated as follows (Nicholas 2003):

$$Z^2 = \frac{(p\hat{} - p_0)^2}{Est.var(p\hat{})}$$

Where $p\hat{}$ is an estimate of the true p and can be calculated using the following formula:

$$p\hat{} = \frac{A-A_1}{T-A_1}$$

And the variance-estimate of $p\hat{}$ can be calculated as follows:

$$\text{Est.var}(p\hat{}) = \frac{(T-A)}{(T-A_1)^3}\left\{A - A_1 + 2A_2\ \frac{(T-A)}{(T-A_1)}\right\}$$

A: Total number of affected offspring
A_1: Number of litters with only 1 affected offspring
A_2: Number of litters with 2 affected offspring
T: Total number of offspring

3.2 My data

The data used in this study included 35 dogs, 13 males and 22 females, known to have "lanced" canines and 25 dogs described as being free of "lanced" canines. All the unaffected dogs included in this study had at least one affected sibling. The full status of offspring in a whole litter were known for 11 litters.

3.2.1 Data collection and potential issues

- Collecting of data

The initial data, the 35 dogs with "lanced" canines, were collected in different ways. Some through the Danish Shetland Sheepdog Club as a response to posts regarding the disease, some through my master's thesis advisor and some directly to me. The ones directly to me were through FB posts by the Danish Shetland Sheepdog Club, e-mail correspondence with breeders, personal meeting with a breeder and through personal friends.
The status of most of the dogs were confirmed through either photographs or the dog visiting a veterinary professional prior to this study. However, in the case of 3 of the dogs I only have the owner/breeder's assessment of the dog being affected.
The status of the 25 unaffected siblings included in this study where collected by incomplete selection through affected siblings. The data was obtained through e-mail correspondence with breeders, personal meeting with a breeder and a questionnaire sent out to owners of dogs from the same litter as an affected dog. The questionnaire included a description of "lanced" canines and photos (see appendix A).

- Potential issues

Overall there are two potential issues with this method of data collection. The first is that the status of some of the dogs included in this study, mainly within the group described as being unaffected, is an assessment from the owner or breeder themselves. Thus, even though “lanced” canines is a fairly simple condition to asses, some dogs who only exhibit mild symptoms of “lanced” canines might accidently be classified as free of “lanced” canines since the owner or breeder was not trained in assessing the dogs teeth. Also, the fact that some breeders and dog owners still believe that wrongly positioned canines is always a result of retained deciduous teeth could classify an affected dog as free of “lanced” canines. This could result in some dogs being placed in the wrong category (affected / not affected). If this is the case the overall prevalence of the condition in this dataset could be underestimated.

Another issue, regarding the segregation analysis, is that incomplete selection was used. As described earlier this introduces a bias that can result in the proportion of affected individuals being over estimated.

4. Results

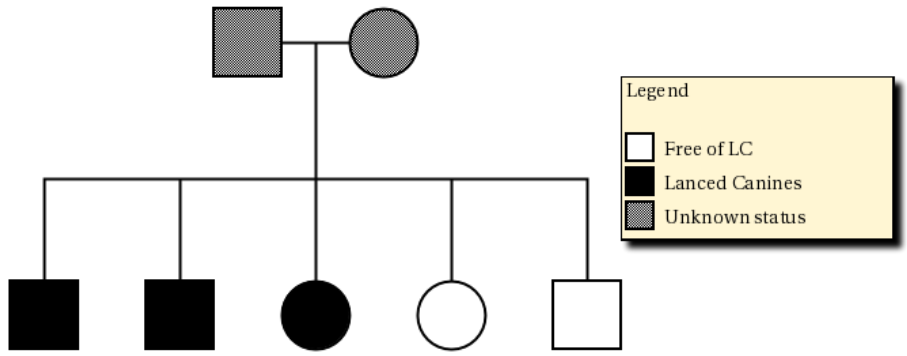
4.1 Overview

Looking at the initial data (appendix B) it can be seen that although some dogs do have common ancestors, this is mainly the case when there are dogs directly related to each other for example from the same breeder, siblings or mother and offspring. Some have a few common ancestors although they are not directly related through the same parents, but there is no dog that connects all of the data (or even just a significant portion of it). The maximum number of dogs sharing a common ancestor (MD2) is 8 dogs, and 3 of the affected dogs have no connection to any of the other dogs, at least not in the 4 generations included in this study.

4.2 Singles method

The composition of the 11 litters included in this study can be found in Appendix C with litter A illustrated below as an example.

Litter A



In summary the dataset was composed of the following litters:

Litter	Number of affected offspring	Littersize
A	3	5
B	2	5
C	2	5
D	1	5
E	2	3
F	1	3
G	1	5
H	1	4
I	1	4
J	1	1
K	1	1
Total	**16**	**41**

Number of litters with 1 affected offspring	7
Number of litters with 2 affected offspring	3

From this the test value Z^2 could be calculated as follows:

$$p\hat{} = \frac{A-A_1}{T-A_1} = \frac{16-7}{41-7} = 0.265$$

$$\text{Est.var}(p\hat{}) = \frac{(T-A)}{(T-A_1)^3}\left\{A - A_1 + 2A_2\ \frac{(T-A)}{(T-A_1)}\right\} = \frac{(41-16)}{(41-7)^3}\left\{(16-7) + (2 \cdot 3)\left(\frac{(41-16)}{(41-7)}\right)\right\} = 0.008531$$

$$Z^2 = \frac{(p\hat{}-p_0)^2}{Est.var(p\hat{})} = \frac{(0.265-0{,}25)^2}{0.008531} = \underline{\mathbf{0.0264}}$$

As the critical value of chi-square distribution with 1 degree of freedom and a significance level of 5% is 3.84 the calculated Z^2 value shows that there is no significant difference between the observed segregation frequency and the expected segregation frequency (0.25). Therefore, we cannot reject the null hypothesis about simple recessive inheritance.

5. Discussion

5.1 Heredity and “lanced” canines

The result from applying Singles method indicates that a simple recessive mode of inheritance cannot be ruled out. But considering the lack of shared ancestors between the dogs in this study, and the fact that some dogs have no hereditary link to the other dogs in this study, a simple recessive mode of inheritance seems unlikely. Although what is clear from the analysis of this dataset using Singles method is that lanced canines is a condition with a high heritability. This, together with the fact that the condition has highly variable expressivity and possibly variable penetrance, would suggest that “lanced” canines is likely a threshold trait where several genes influence the development of the condition.

5.2 Breeding restrictions and recommendations in the shetland sheepdog

The Danish Kennel Club (DKK) has a set of breeding restrictions that must be met in order to have the puppies registered in DKK. There is also a set of breeding recommendations that the breeders can decide for themselves if they want to follow. But the type of pedigree the puppy will receive depends on whether the breeder follows only the restrictions (basic pedigree) or both the restrictions and recommendations (Basic plus pedigree). It is decided by the Danish Shetland Sheepdog Club what these restrictions and recommendations are.

5.2.1 Restrictions

- Collie eye anomaly (CEA)

For Shetland sheepdogs the restriction states: “*Puppies cannot be registered in DKK, unless both parents, before mating, have been examined for CEA by a member of the Danish Veterinary Association eye panel or by another veterinary ophthalmologist approved by the DKK. The optimal time for examination is in the pups 7.-9. weeks of life. The CEA attest applies for life”* (Dansk Kennel Klub)

- Colour breeding

Here the restrictions state: "*Two Shetland sheepdogs of the colour blue merle are not allowed to be mated. A Shetland sheepdog of the colour blue merle is not allowed to be mated with a zobel*" (Dansk Kennel Klub)

5.2.2 Recommendations

- Progressive Retinal Atrophy (PRA)

Here the recommendations states: "*Before mating both parents must have an eye examination by a member of the Danish Veterinary Association eye panel or by another veterinary ophthalmologist approved by the DKK and found free of any signs of PRA. The earliest examination time is when the dog is 12 months old. Parent animals desired to be used in breeding after the age of 5 years, must have a new eye examination."* (Dansk Kennel Klub)

- Collie eye anomaly (CEA)

Here the recommendation states: *"At least one of the parent animals has to be registered as free of CEA in DKK by examination before mating. If there, in addition to the eye examination result, is also a registered DNA status on the dog this must also indicate that the dog is CEA free."* (Dansk Kennel Klub)

- Show results

Here the recommendation states: *"Both parents must be rewarded with at least 'Very Good' at an FCI/DKK acknowledged show before mating"* (Dansk Kennel Klub)

5.3 "Lanced" canines and breeding

Our results indicate that "lanced" canines is a condition with high heritability and it should therefore be recommended to avoid using any affected individuals in breeding. In principle using any dog for breeding that has previously produced offspring with "lanced" canines, or has affected siblings, should be avoided, regardless of whether the dog is free from "lanced" canines itself. Luckily "lanced" canines can be diagnosed clinically before the dog reaches breeding age, so it is possible to know the disease status before mating. But since "lanced" canines seems to be widespread in the population, excluding a high number of dogs from the breeding pool could lead to other effects of inbreeding depression (Lyons 2010). There are also other diseases that need to be considered when choosing breeding dogs. For example, as described earlier in the

breeding restrictions and recommendations for the Shetland sheepdog, the eye diseases CEA and PRA. With breeding it will always be a compromise between desired traits and undesired traits. It could therefore be argued that given that "lanced" canines is neither a fatal nor disabling disease once it is treated it should not be as big a factor in choosing breeding material compared to more serious diseases. Nevertheless, "lanced" canines can be a big problem for the individually affected dogs and an economic burden for the owners of these dogs. So breeders should strive towards reducing the number of affected individuals and eventually, slowly eradicating the disease altogether.

5.4 Genetic testing

5.4.1 Genetic testing in general

Genetic testing in dogs is of growing importance in veterinary medicine. More and more mutations that have an impact on canine health or appearance are being discovered, and genetic tests for these mutations developed. Genetic testing gives veterinarians an important tool for the diagnosis of diseases. Some non-genetic components, for example toxins and infections, and some environmental influences, such as diet and exercise, can produce a phenotype that looks just like an inherited characteristic or disease. In these cases, genetic testing can assist the veterinarians in finding out more about the underlying cause of the disease. Genetic testing can also help breeders in choosing their breeding materials and which combinations of dogs to breed, in order to prevent or even eradicate diseases (Lyons 2010)

5.4.2 Why is finding a possible genetic test a good idea?

Even though "lanced" canines do indeed become evident before the dog reaches breeding age, and therefore there is no risk of breeding with a dog that will later turn out to be affected, it does present complications that the disease itself is not clinically evident in puppies before weaning. This means that puppies can be bought as future breeders and when the permanent canines erupt and "lanced" canines becomes evident the breeder now has a dog they cannot use as intended. Given that many of the smaller kennels do not simply have the option to buy a new dog, there is both a personal and economic cost. On a personal level it might mean that breeders will have to rehouse the intended breeding dog, since it can no longer be used in breeding, and they might not have enough space nor the time for both a new dog and the dog with "lanced" canines. Economically there is of course the direct cost to having the "lanced" canines treated, but there is also the more hidden cost of lost time with regard to when the kennel can expect puppies, the

expenses already used on the dog that is now unfit to breed with, such as food, veterinary bills, training costs etc and the expense of buying in a new dog for the kennel's breeding programme. Thus if a genetic test could be found, then puppies could be tested before weaning and only puppies free of the condition would be sold as future breeders.

6. Conclusion

6.1 My study

“Lanced” canines seems to be a widespread condition amongst Shetland sheepdogs. It has a high heritability and the development is most likely caused by mutations in several genes. The data presented in this study points towards it being a threshold trait with both variable penetrance and variable expressivity. However more studies are needed to try and find the genes responsible for the condition and make genetic testing possible.

6.2 Breeding recommendations regarding “lanced” canines

It is recommended to avoid the use of any affected individuals in breeding. Furthermore, it is recommended to never repeat a mating that has previously produced offspring with “lanced” canines regardless of whether the parent animals are free of “lanced” canines.

7. Perspectives

It would be interesting to find the prevalence for “lanced” canines in the Danish Shetland sheepdog population, this could be done by using the following equation:

$$Prevalence = \frac{Number\ of\ dogs\ in\ the\ sample\ with\ lanced\ canines}{Total\ number\ of\ dogs\ in\ the\ sample}$$

The question would then be how many dogs would we need to sample in such a study? Given that the objective in a study designed to estimate the prevalence of a given condition in a geographic area is to sample sufficient population in order to get adequate number of subjects correctly classified as having the condition of interest or not (Arya, Antonisamy, and Kumar 2012). It would therefore require a large number of randomly selected Shetland sheepdogs to participate, something that might prove a bit difficult.

It would also be very interesting to get an estimate of the heritability to get an idea about how much of the variation in “lanced” canines in the population is due to genetic variation. In other words, to find out how big a role the genetic differences in the population play compared to environment and random chance. The heritability is expressed as $H^2 = \frac{V_g}{V_p}$

H^2 being the heritability estimate, V_g the variation in genotype and V_p the variation in phenotype. (Meaney and Taylor 2018; Wray and Visscher 2008)
The way you calculate heritability is in short, that you compare how often a disease/condition occurs in families compared to how often it occurs in the population. So, in order to do this, it is needed to find the prevalence in the population as discussed above.

Knowing that “lanced” canines is a hereditary disease the next step would be to identify the gene(s) causing “lanced” canines in order to then develop a genetic test for the condition. This could for example be done by whole genome sequencing of a dog with “lanced” canines that could then be compared to a reference dog’s genome to find the sequence variants unique to the affected dog. Only the sequences that are predicted to result in an altered gene product are of interest. To narrow it down further one could see if any of the mutated genes would also likely alter the function of the gene product. One could also see if any of the sequences were located in genes already associated with dental anomalies. If multiple whole genome sequencing results from dogs with “lanced” canines are available these could also be compared and all mutant sequences that are shared among dogs with “lanced” canines, but not with reference dogs free of “lanced” canines, could be of interest for further analysis.

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Appendix

Appendix A – Dog owner questionnaire

Denne mail er udsendt fra DKK for at hjælpe Dansk Shetland Sheepdog Klub og specialestuderende Lena Rahbek med et forskningsprojekt.

Kære hundeejer,

På Dansk Shetland Sheepdog Klubs opfordring er der etableret et veterinært specialeprojekt på KU/SUND, som har til formål at undersøge arvegangen for Lansetænder (fejlstillede hjørnetænder i overmunden – se fotos nederst). Der er, på frivillig basis, indsamlet oplysninger om forekomsten af denne tanddefekt i den danske population, og i den forbindelse har det vist sig, at en kuldsøskende til din hund født DD/MM-YYYY har/har haft Lansetænder. For at finde arvegangen skal der så vidt muligt indsamles oplysninger fra hele kuld. Det vil derfor være en stor hjælp for projektet, hvis du vil oplyse din hunds status i forhold til Lansetænder. Oplysningerne vil blive anonymiseret og behandlet fortroligt.

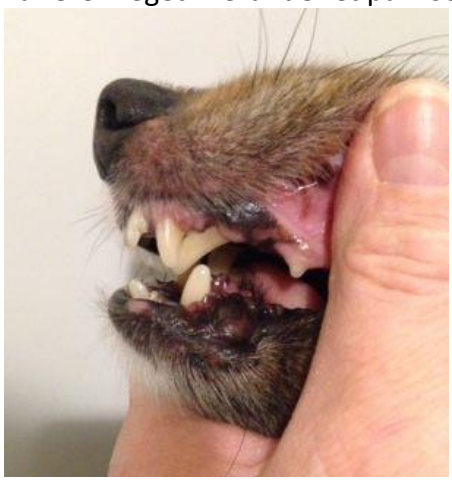
Du svarer ved at besvare denne mail og krydse af i nedenstående skema:

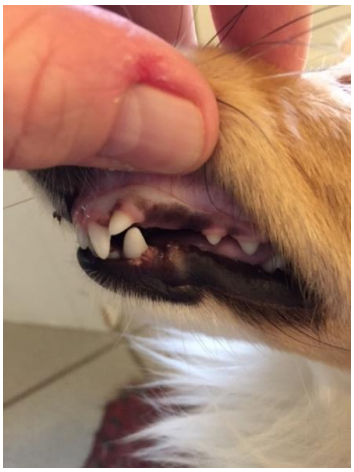
Min hund har/har haft "Lansetænder"	
Min hund har ikke/har ikke haft "Lansetænder"	
Ved ikke*	
Andet (beskriv f.eks. om hunden har andre tandfejl)	

*Hvis du er i tvivl, er du velkommen til at sende billeder af hundens hjørnetænder i begge sider. Så kan vi hjælpe med at vurdere dem.

Hvad er en lansetand?

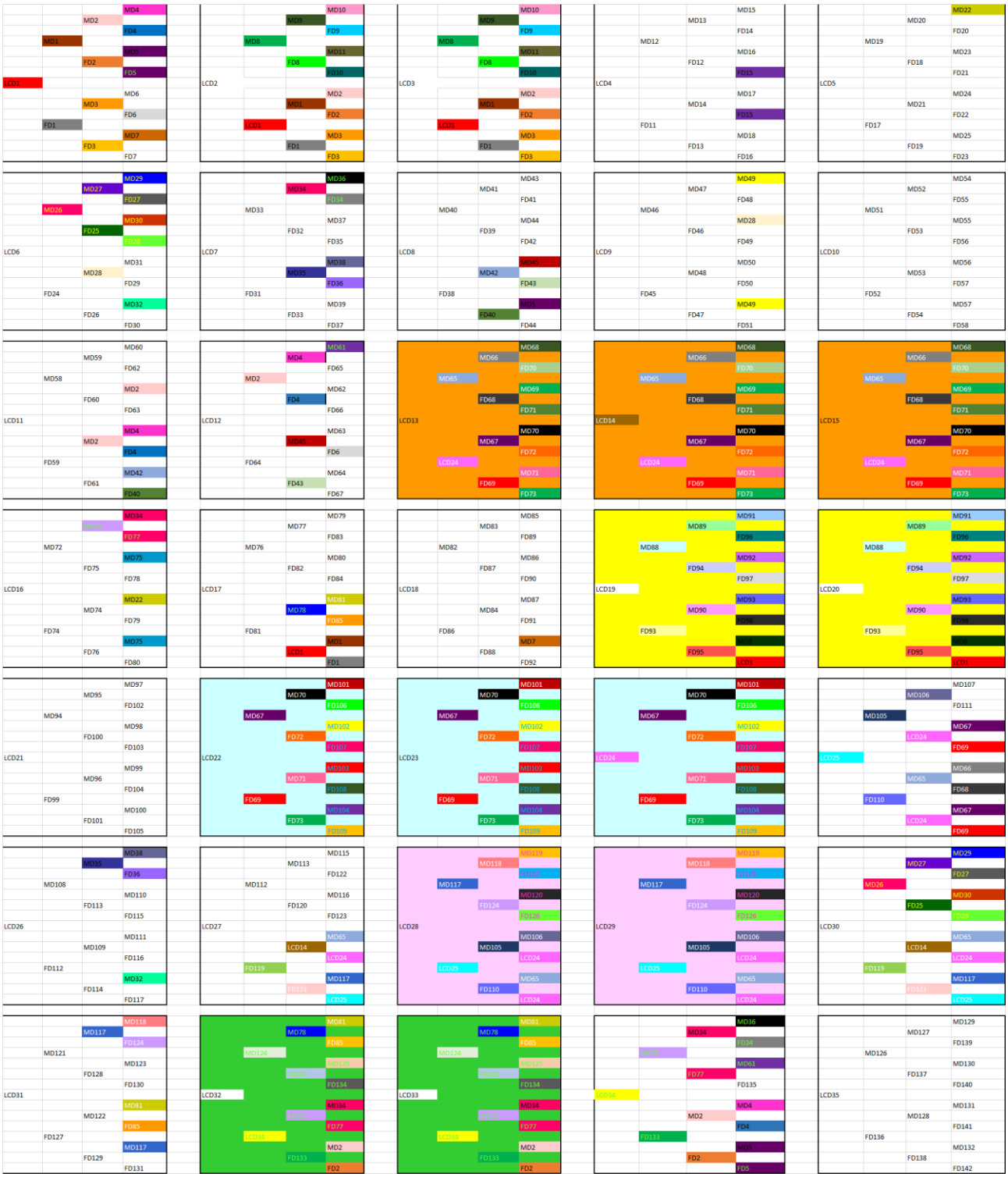
En lansetand er en tandstillingsfejl hvor hjørnetanden i overmunden peger fremad. Graden kan variere meget. Herunder et par fotos af en lansetand:





Med venlig hilsen/Kind regards,
DANSK KENNEL KLUB

Appendix B – Initial data overview

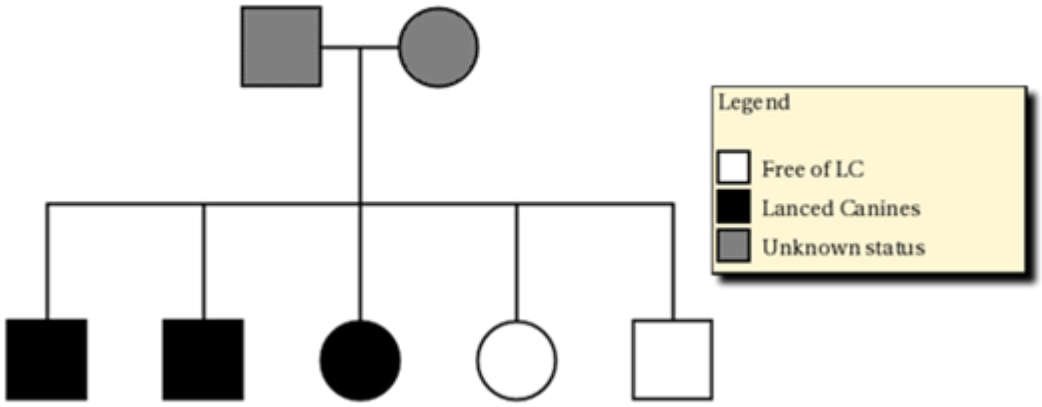


Same colour box&text = same dog

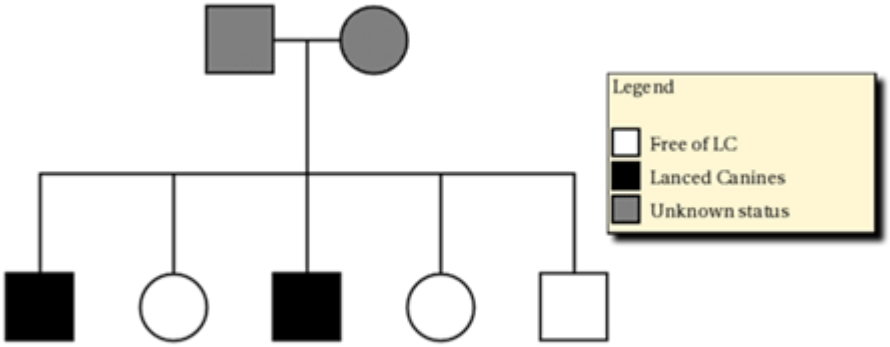
Same colour full pedigree = dogs from the same litter

Appendix C – Litter compositions litters A till K

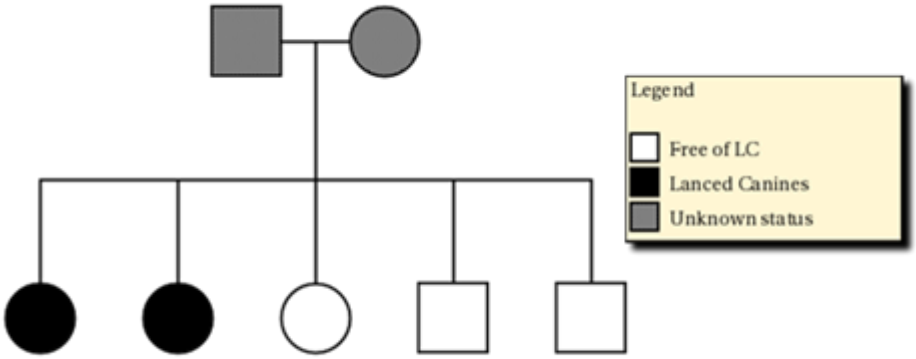
Litter A



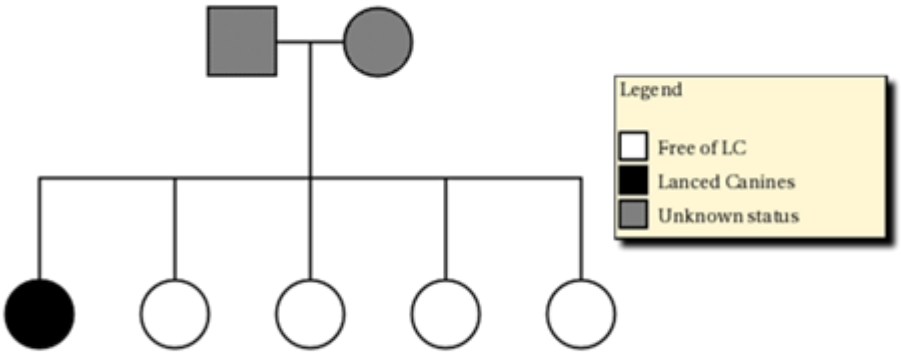
Litter B



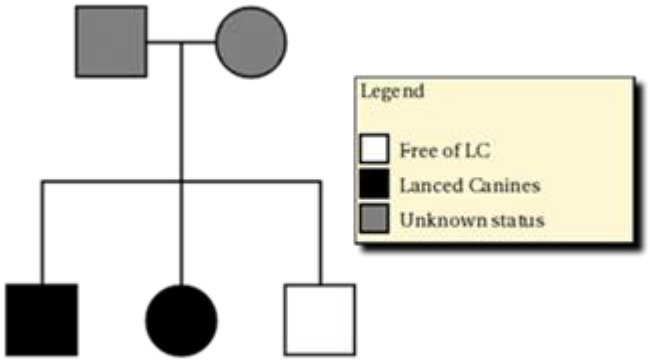
Litter C



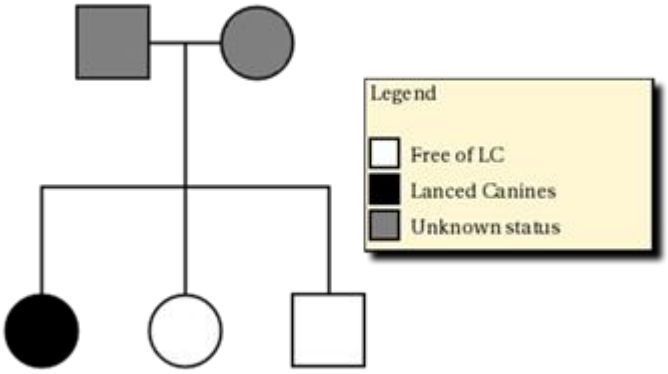
Litter D



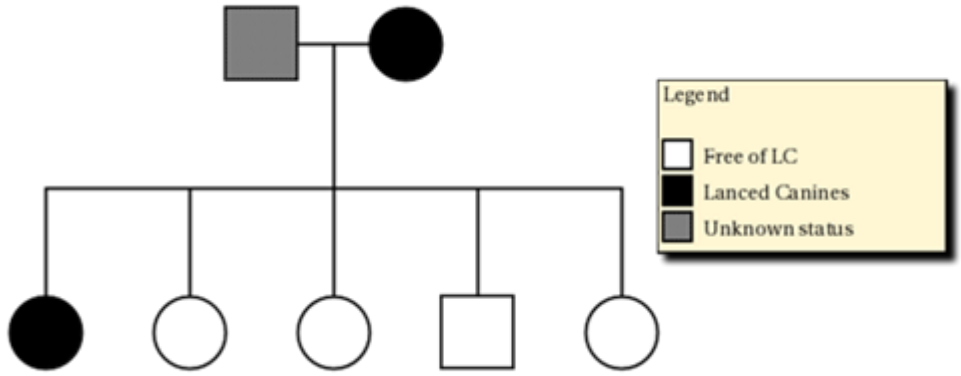
Litter E



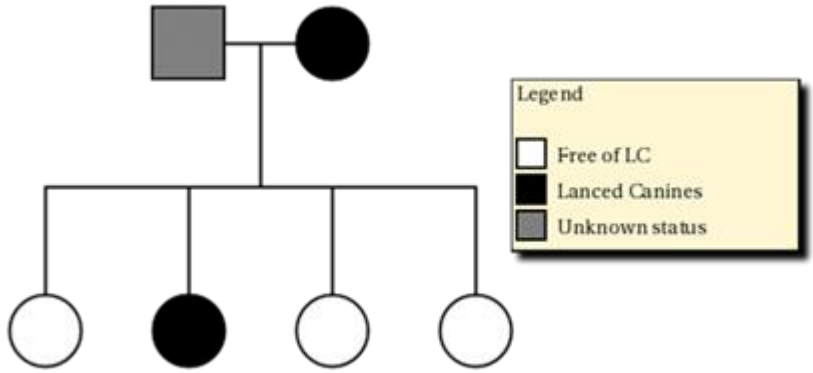
Litter F



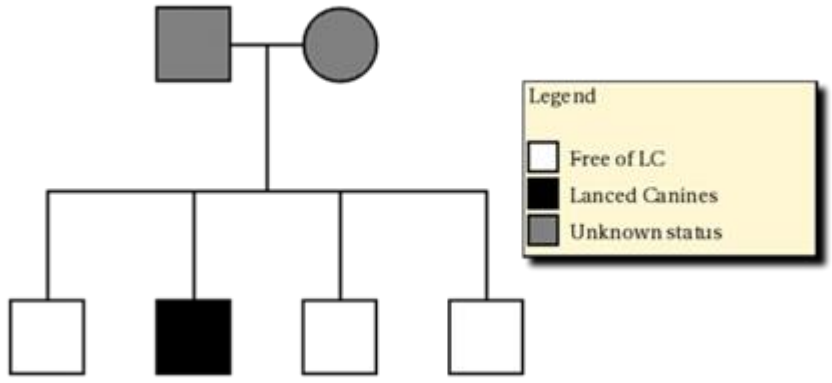
Litter G



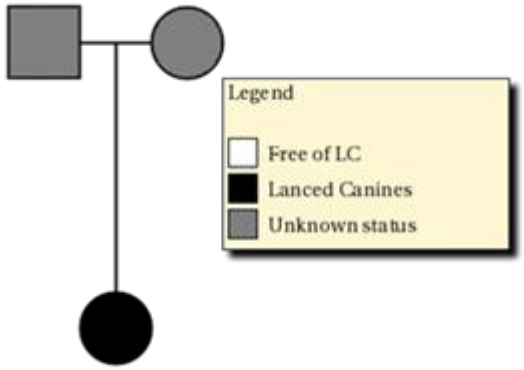
Litter H



Litter I



Litter J



Litter K