FACULTY OF HEALTH AND MEDICAL SCIENCES
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Veterinary Master's thesis

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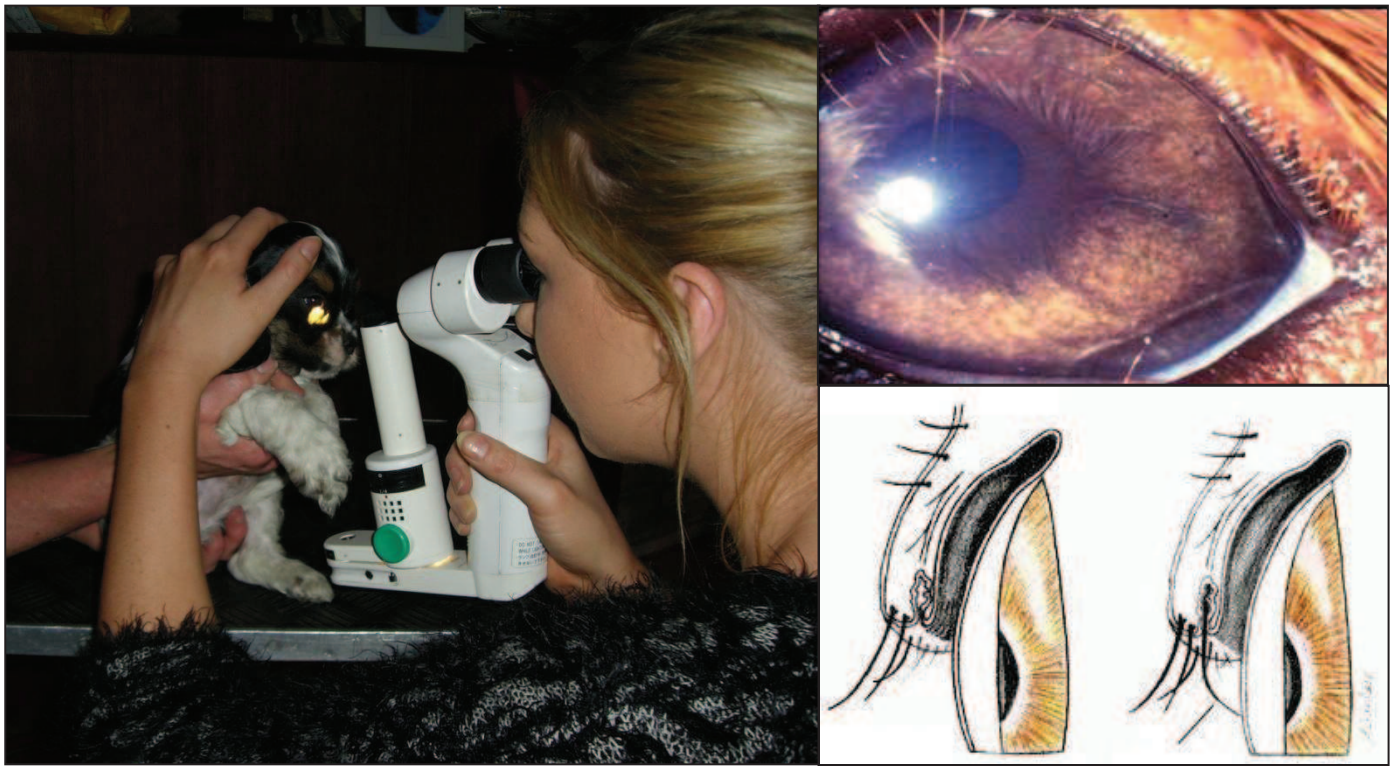
Canine Distichiasis in the Cocker Spaniel

A retrospective study on the occurrence and heredity of the condition in the Danish population



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Canine Distichiasis in the Cocker Spaniel

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Faculty:	Animal Genetics, Bioinformatics and Breeding
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ABSTRACT

Distichiasis has long been recognized as a condition that causes severe discomfort and ophthalmic pain in many species of animals, including dogs and humans. Many breeds are known to be predisposed to the canine distichiasis and the American and English cocker spaniel breeds are known to be some of the most frequently diagnosed, with many affected individuals within the populations. The condition is primarily considered a congenital hereditary disease, but the heredity and exact mode of inheritance has not yet been clarified. Due to the high incidences within the specific breeds, some degree of genetic or environmental influence on the development of the condition has been proposed. The objective of this study was to investigate the heredity and the exact mode of inheritance of canine distichiasis in the cocker spaniel breeds, to determine the prevalence within the two populations, and to find out if specific breeding combinations of parents with different distichiasis status would have a significant effect on the occurrence of distichiasis in the offspring

Existing literature was reviewed in order to describe the properties related to canine distichiasis and the general ophthalmic health in the cocker spaniels in relation to previous research on the subject. Data retrieved from Hundeweb was analyzed in a retrospective cohort study from a population of 1,051 cocker spaniels from the two breeds. The distichiasis status of the parents and their offspring were investigated in order to describe the connection between the occurrences of distichiasis in offspring in relation to the breeding combinations and to determine the prevalence and the potential hereditary predisposition of distichiasis within the two breeds.

The results presented in this thesis demonstrated that canine distichiasis is a hereditary condition that is inherited as an autosomal polygenic trait with threshold properties, and that there is a significant difference in the outcome of distichiasis in the offspring in relation to the breeding combinations of the parents. Offspring descending from two affected parents are two times more likely to be affected, compared to offspring descending from two unaffected parents. The heritability for distichiasis is high (between 0.22 and 0.51) in the cocker spaniel breeds, and the prevalences was found to be 71.93 % in the American cocker spaniel and 48.81 % in the English cocker spaniel. The heredity and mode of inheritance determined in this thesis indicates that selective breeding, predominantly using unaffected individuals, is likely to have a positive effect on the incidences of distichiasis in the future generations of cocker spaniels.

RESUME

Distichiasis har længe været anerkendt som en tilstand der forårsager alvorligt ubehag of oftalmologiske smerter hos mange forskellige arter, herunder også hunde og mennesker. Flere racer er kendt for at være prædisponerede for hundens distichiasis og den amerikanske og engelske cocker spaniel er kendt for at være nogle af de hyppigst diagnosticerede racer med mange afficerede individer indenfor populationerne. Tilstanden bliver primært betegnet som en medfødt arvelig sygdom, men arveligheden og den nøjagtige arvegang er endnu ikke afklaret. På grund af den højre forekomst indenfor bestemte racer, er det blevet foreslået at der er en vis grad af genetisk eller miljømæssig påvirkning mht. udviklingen af tilstanden. Formålet med dette studie var at undersøge arveligheden og den nøjagtige arvegang af hundens distichiasis indenfor de to racer af cocker spaniels, at bestemme prævalensen i de to populationer samt at finde ud af, om specifikke avlskombinationer af forældredyr med forskellig distichiasis status ville have en signifikant indflydelse på forekomsten af distichiasis hos afkommet.

Eksisterende litteratur blev gennemgået for at beskrive forholdene ved distichiasis hos hunde og den generelle øjensundhed hos cocker spanielerne i relation til tidligere undersøgelser på området. Data fra Hundeweb blev behandlet i et retrospektivt kohorte studie som inkluderede 1.051 cocker spaniels fra de to racer. Forældrenes og afkommenes distichiasis status blev undersøgt for at kunne beskrive sammenhængen mellem forekomsten af distichiasis hos afkommene i relation til avlskombinationerne, samt at bestemme prævalensen og den potentielle arvelige prædisposition af distichiasis i de to racer.

Resultaterne præsenteret i dette speciale viste, at distichiasis hos hunde er en arvelig tilstand som nedarves som et autosomal polygent træk med tærskelegenskaber, samt at der er en signifikant forskel på udfaldet af distichiasis hos afkommet i forhold til avlskombinationen af forældrene. Afkom efter to afficerede forældre har to gange større sandsynlighed for at være afficeret i forhold til afkom efter to raske forældre. Arveligheden af distichiasis hos cocker spaniels er høj (mellem 0,22 og 0,51), og prævalenserne var på 71,93 % hos den amerikanske cocker spaniel og 48,81 % hos den engelske cocker spaniel. Den arvelighed og arvegang som er påvist i dette speciale, indikerer at selektiv avl, hvorved man primært anvender raske individer, med stor sandsynlighed vil have en positiv indvirkning på incidensen af distichiasis i fremtidige generationer af cocker spaniels.

PREFACE

This thesis was made as my final project during my education of Veterinary Medicine. It was performed at the Department of Veterinary Clinical and Animal Sciences, Faculty of Animal Genetics, Bioinformatics and Breeding, University of Copenhagen, Denmark.
This thesis is addressed to veterinarians, veterinary students, breeders, researchers and everyone with a special interest in veterinary ophthalmology, breeding regimes and management of hereditary diseases within the canine population.

I would like to thank my main academic advisor, Professor Merete Fredholm, and my additional supervisor, DVM and Special Consultant Helle Friis Proschowsky, for their constructive guidance, kind support and commitment during the project. I would also like to thank DVM and Eye panelist Tommy Hardon at Haslev Dyreklinik for his professional guidance and practical advice in learning the diagnostics of veterinaty ophthalmology. Furthermore, I would like to send a special thanks and gratitude to Charlotte Hansen, owner of Kennel Truly Yours and DVM Søren Nyhuus Rasch at Nyborg Dyrehospital for the opportunity to participate during the ophthalmological examinations; and to Søren for his much appreciated help and time answering questions, loan of equipment and contribution of additional data for the litter study.

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Tanja Petersen (flr662)

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LIST OF ABBREVIATIONS

ACS	American Cocker Spaniel(s)
CI	Confidence interval
D	Deceased
Diag.	Diagnosis or Diagnoses
Dist.	Distichiasis
DKK	Dansk Kennel Klub
DSVO	Dansk Selskab for Veterinær Ophthalmologi
ECS	English Cocker Spaniel(s)
ECVO	European College of Veterinary Ophthalmologists
Exam.	Examination(s)
H&E	Hematoxylin-eosin staining
HEDS	Hereditary Eye Disease Scheme
Incl.	Including
Inf.	Inferior
LNOPPS	The Limit for Number of Offspring Produced by 'Popular Sires' (DK: '*Matadoravls grænse')*
m	Months
mA	Mili ampere
Med.	Medium, degree in the distichiasis grading system
N_2O	Nitrogen
No.	Number
NSAID	Non Steroidal Anti-Inflammatory Drug
OD	Oculus Dexter
Opht.	Ophthalmological
OS	Oculus Sinister
Palp.	Palpebrae
PHTVL/PHPV	Persistent Tunica Vasculosa Lentis / Persistent Hyperplastic Primary Vitreous
PIED	Presumed Inherited Eye Diseases
PPM	Persistent Pupillary Membrane
PRA	Progressive Retinal Atrophy
RD	Retinal Dysplasia
RR	Relative Risk
Sup.	Superior
VCE	Veterinary Care Event
y	Years

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1. INTRODUCTION

The cocker spaniels are divided into two different breeds, the American and the English cocker spaniels. They are very popular in many countries both as pets and as hunting dogs, and they can be traced back to the mid 19th century (PetMD 2014). The cocker spaniels are predisposed to many presumed inherited eye diseases (PIED) with the most prevalent eye diseases reported by the Danish Kennel Club being distichiasis (47-80 %), cataract and retinal dysplasia (ECVO Definitions 2013, DKK Update 2014). Ophthalmic examination in the cocker spaniels has been recommended since the 1990s, and is now compulsory for those intended for breeding (Proschowsky 2014, personal communication).

Distichiasis has long been recognized as a condition that causes severe discomfort and ophthalmic pain in many species of animals, including dogs and humans (Halliwell 1967, Picó 1957). Forty years ago canine distichiasis had a low prevalence in the general dog population (1:133) (Lawson 1973), and it was suggested that a few breeds might be more commonly affected than others (Halliwell 1967, Bedford 1971, Lawson 1973). Now, it is well known that distichiasis affects many purebred dogs and occasionally some cross bred dogs as well (Lawson 1973, Barnett 1976), and the list of supposedly predisposed breeds now comprises up to 109 different breeds (Miller *et al* 2013). Canine distichiasis is characterized by an additional row of lashes in which the aberrant cilia (distichiae) emerges on the free margin of the eyelids through the meibomian gland orifices (Lawson 1973, DSVO/b 2014). The condition is generally considered a congenital hereditary disease (Bedford 1979), but some authors suggest that the condition could also be acquired due to long term chronic inflammation of the eyelids and conjunctiva (Halliwell 1967, Barnett 1976). Distichiasis is assumed to be inherited as an autosomal dominant trait (Halliwell 1967, Bedford 1973), although the exact mode of inheritance and etiology has not been clarified. Due to the high incidences within specific breeds some degree of genetic and environmental influence has been proposed (Ketteritzsch *et al* 2004).

In 2013 the European College of Veterinary Ophthalmologists (ECVO) introduced a new scheme in order reduce the extent of distichiasis within the predisposed breeds (Hundeweb/b 2014). The scheme is carried out for a 5 years trial period, and includes grading distichiasis into three different degrees according to the severity of the condition. Breeders are advised not to mate two dogs with the same diagnoses, hence the same degree of affection, and cocker spaniels with a severe degree of affection are excluded from breeding (Hundeweb/a 2014, Hundeweb/b 2014).

1.1 THE AIM OF THE STUDY

The objective of this study was to analyze the heredity and the exact mode of inheritance of canine distichiasis within the cocker spaniel breeds, and to determine the prevalence of distichiasis in the two populations. In relation to the high incidences of distichiasis, one hypothesis that is tested is that there is a higher possibility that the offspring will get distichiasis if both of their parents are affected by distichiasis compared to offspring in which one or both parents are unaffected. An analysis of the mode of inheritance of distichiasis would enhance our understanding of the genetic mechanisms underlying the condition, which may aid in the selection of suitable breeding animals and help reduce the extent of the disease.

The following questions are investigated:

- Are there any differences in the occurrence of distichiasis in offspring produced by two dogs that are affected by distichiasis compared to breeding combinations in which one or both parents are unaffected? And if this is the case, can canine distichiasis still be assumed to be inherited in an autosomal dominant matter or may it be polygenic with a complex inheritance?
- Is finding the exact mode of inheritance going to make any difference in preventing distichiasis when the current incidences are so high?

1.2 DELIMITATION

This study only included dogs of the American and English cocker spaniel breeds with a valid ECVO-certificate issued during the 1st of January 2004 – 31st of December 2013. Dogs examined outside this period were not included in the investigation. Ophthalmic examinations that were not issued as an ECVO-certificate were not included, to ascertain that all included dogs had been examined in the same manner.

1.3 METHOD

This study was divided into two sections. Part I - The literature study: Literature review on existing literature and earlier research on canine distichiasis and ophthalmic disease in the American and English cocker spaniels. Part II - The experimental study: A retrospective cohort study on the heredity of canine distichiasis including the apparent prevalence in the cocker spaniels. Part II is divided into 5 sections, including Materials & Methods, Results, Discussion, Conclusion and Perspective.

PART I – THE LITERATURE STUDY

2.1 OPTHALMOLOGICAL DISEASE IN THE COCKER SPANIEL

The cocker spaniels are known to be predisposed to many different conditions, including several ophthalmological diseases (Bedford 1988, Agria Insurance Data 2006, 2011). Since 1993 and 1999, respectively, the American and English cocker spaniels have been screened for presumed inherited eye diseases (PIED), with the most prevalent eye diseases being distichiasis, cataract and retinal dysplasia (RD). Annual screening for PIED in dogs intended for breeding has been compulsory since 2006 (Proschowsky 2014, personal communication, DKK Update 2014). In relation to the general dog population, the cocker spaniels are overrepresented when it comes to ophthalmic diseases of the eye and its adnexa (Lawson 1973, Barnett 1976) and ophthalmic inquiries are a frequent cause of a veterinary care event (VCE) in the two breeds (Agria Insurance Data 2011). Recent counts from Agria Pet Insurance in Sweden demonstrated that the cocker spaniels have an increased risk of having a VCE compared to the average of all other breeds, and are up to > 4 times more likely to be affected by an ophthalmic disease (Agria Insurance Data 2011).

2.1.1 THE AMERICAN COCKER SPANIEL

The American cocker spaniel (ACS) is predisposed to a number of different diseases, including several PIED (American Kennel Club 2013, Williams *et al* 1979). The relative risk (RR) that an ACS will have a VCE compared to the average of all other breeds has increased from 1.2 to 1.3 between 1995-2006 and 2006-2011 (Agria Insurance Data 2006, 2011). This increase either indicates that the ACS breed has become unhealthier compared to other breeds, or that the general dog population have had less VCEs during 2006-2011 than during 1995-2006. **Figure 1** demonstrates the general causes of VCEs in the ACS and the relative risk of the specific VCEs in relation to the average of all other breeds. The ACS are more than 4 times more likely to have a VCE related to an ophthalmic condition compared other breeds (Agria Insurance Data 2011). A survey on the incidences of PIED registered in *Hundeweb* during a 5 years period from the Danish population of cocker spaniels was performed by the Danish Kennel Club. 114 ACS were included based on 209 ophthalmic examinations. Distichiasis was the most prevalent condition with an incidence of 80 %. Other frequently diagnosed conditions included cataract (non-congenital) (6%), RD (6 %), corneal dysplasia (6 %), ectropion (3 %) and PHTVL/PHPV (1 %) (Proschowsky 2014,

personal communication). In a survey from USA on 146 ACS, distichiasis was demonstrated in 74%, whereas cataract (12%) and RD (12%) were the second most prevalent (Williams *et al* 1979).

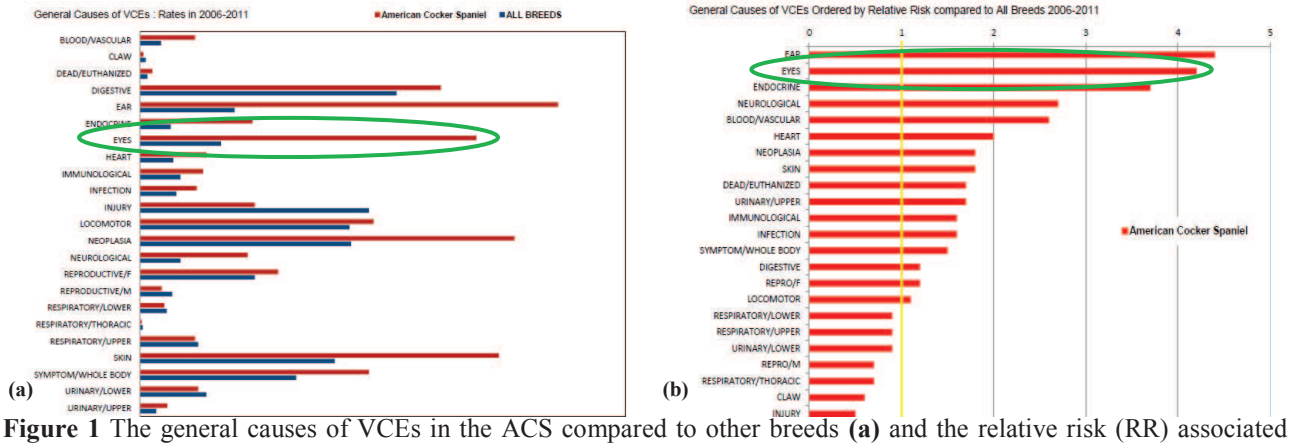

Figure 1 The general causes of VCEs in the ACS compared to other breeds **(a)** and the relative risk (RR) associated with the VCEs **(b)**. Note the high proportion of ophthalmic inquiries in the ACS compared to other breeds. Red bars to the right of the yellow line illustrate conditions in which the ACS is more likely to be affected compared to the average of all other breeds (Modified figure from Agria Insurance Data 2011).

2.1.2 THE ENGLISH COCKER SPANIEL

The English cocker spaniel (ECS) is considered a fairly healthy breed (Spanielklubben 2009). The relative risk (RR) that an ECS will have a VCE compared to the average of all other breeds has was reduced from 1.2 to 1.1 between 1995-2006 and 2006-2011 (Agria Insurance Data 2006, 2011). That the RR is close to 1 indicates that the ECS is almost comparable to the average of the general dog population. **Figure 2** demonstrates the general causes of VCEs in the ECS and the relative risk of the specific VCEs in relation to the average of all other breeds. From the above mentioned survey, a total of 462 ECS were included based on 768 ophthalmic examinations. Distichiasis was the most prevalent ophthalmic disease with an incidence of 47 %. Other ophthalmic conditions, including RD, cataract (congenital and non-congenital), ectropion, entropion, corneal dystrophy, PPM, PRA and PTHVL/PHPV had incidences less than 1 %.

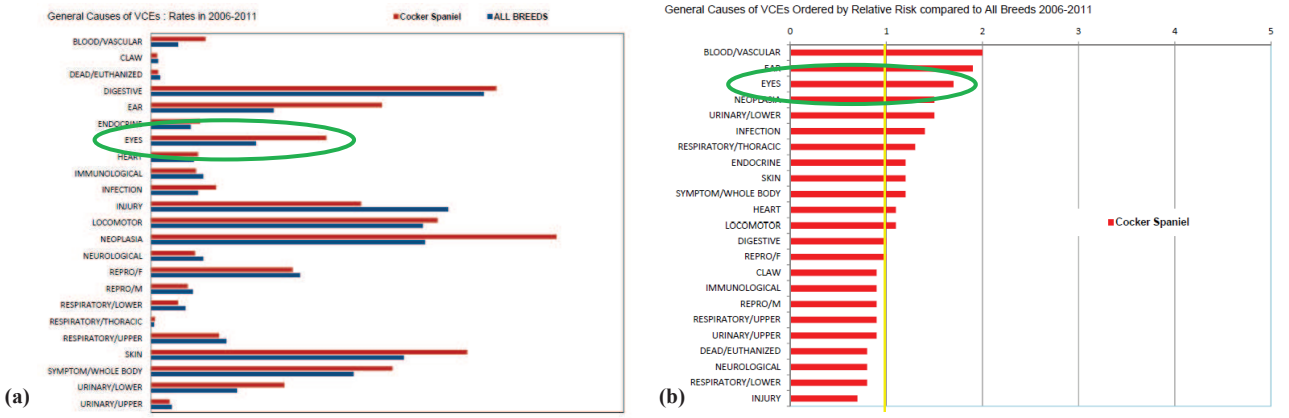

Figure 2 The general causes of VCEs in the ECS compared to other breeds **(a)** and the relative risk (RR) associated with the VCEs **(b)**. Note the high proportion of ophthalmic inquiries in the ECS compared to other breeds. Red bars to the right of the yellow line illustrate conditions in which the ECS is more likely to be affected compared to the average of all other breeds (Modified figure from Agria Insurance Data 2011).

2.2 CLASSIFICATION AND PROPERTIES OF CANINE DISTICHIASIS

The term *Distichiasis* originates from the Greek words *di* and *stichos*, meaning two and rows, respectively (Lawson 1973). Thus, as the name implies distichiasis is characterized by an additional row of lashes which erupts on the free margins of either the upper or lower eyelids, or both in conjunction (Lawson 1973, Raymond-Letron *et al* 2012). Even though both lids may be affected (Lawson 1973), the upper lids seem to be the most common site for the adventitious cilia in the dog (Halliwell 1967, Bedford 1988). In a review study based on a series of 19 cases of dogs referred to the School of Veterinary Medicine, University of Cambridge, England, during a period of approximately ten years, K.C. Barnett (1976) found that in all cases only the upper eyelid was involved, and that the adventitious cilia never occurred in both the right and left eye in the same individual. This is not universally applicable, however, as the extent of the condition varies greatly among the affected dogs, and may involve one or both eyes as well as one or both lids (Lawson 1973, Bedford 1979).

Distichiasis is primarily considered a congenital hereditary ocular disease and is usually detected in young and adolescent dogs, with clinical signs being manifest from 2 to 6 month of age (Barnett, 1976, Bedford 1979, Williams *et al* 1979). Referral patients to the School of Veterinary Medicine, University of Cambridge were between 7 weeks to 8 years of age when presented with clinical symptoms (Barnett 1976). Furthermore, in a survey on the ocular findings in the American cocker spaniel comprising 146 dogs, Williams *et al* (1979) found that clinical signs appeared in dogs ranging from 6 month to 7 years of age.

It has been proposed that distichiasis may also be an acquired condition, and that the adventitious cilia may arise later in life due to long term chronic inflammation of the eyelids and conjunctiva and from corneal irritation (Halliwell 1967, Barnett 1976), although this could not be confirmed on histological examination of affected tarsal glands, as they showed no signs of dystrophic, atrophic or inflammatory changes (Raymond-Letron *et al* 2012). Acquired distichiasis seems to be more familiar in man, where it occurs secondary to ophthalmic diseases or physical or chemical trauma to the eyelids (Anderson & Harvey 1981).

The adventitious cilia (distichia) originate from abnormally located hair follicles in the tarsal plate tissue. The hairs usually grow from or in between the meibomian glands and emerge through the meibomian gland orifices on the free margin of the eyelids, or less frequently through the glands of Moll or glands of Zeis, which are the routes of least resistance (Bedford 1988, Raymond-Letron *et*

al 2012). The free margin of the eyelids, the *margo-intermarginalis*, is characterized by a narrow, hairless area which lies in between the irregular row of lashes on the upper lid or the hair coat of the skin on the lower lid and the inside of the palpebrae (Lawson 1973). Cilia are only present on the upper eyelid in the dog, why the definition of a second row of lashes often is more applicable in man (Lawson 1973). One or several cilia may use the same orifice as a common route of protrusion, and the affected lid may bear only a few cilia or, on occasion, the entire length of the eyelid is occupied, suggesting the existence of a double row of lashes in the dog as well (Bedford 1973, Lawson 1973) (**Figure 3**). The free margin on each eyelid contains 20 to 25 meibomian glands. Halliwell (1967) suggested that the meibomian glands are more developed in the upper eyelids and that this might be the reason, that the upper lids are the more prevalent site of affection. However, this could not be confirmed by other authors (Lawson 1973). The main function of the meibomian glands, which are modified sebaceous glands, is secretion of the viscous oily part of the precorneal tear film (meibom). The fluid protects the globe and serves as a dam, preventing overflow of lacrimal secretions onto the periorbital hairs (Halliwell 1967).

Figure 3 Canine distichiasis. Many cilia are emerging from the meibomian gland orifices on the upper eyelid and lower eyelids in this dog, suggesting the existence of a double row of lashes (Bedford 1973).

2.2.1 DEVELOPMENT AND HISTOLOGICAL FEATURES

Raymond-Letron *et al* (2012) demonstrated that the adventitious cilia of distichiasis originates from aberrant hair follicle bulbs within the tarsus in close vicinity to or in direct contact with the meibomian (tarsal) glands, and not within the glands themselves as earlier assumed (Lawson 1973, Bedford 1971). Until recently it was assumed that the distichiae arose from metaplastic tarsal glands, thus from abnormal differentiation of the tarsal glands or from germinal follicles located

within or close to the tarsal glands, which causes the glands to take on hair-bearing function (Bedford 1973, ECVO Manual 2012).

The study conducted by Raymond-Letron *et al* (2012) was the first of its kind to examine the histopathological features of canine distichiasis. Specimens were collected from 20 dogs diagnosed with distichiasis by surgically removing strips of tarsoconjunctival tissue containing both hair and root of the selected distichiae. As a control they used full thickness eyelid tissue specimens from 11 euthanized dogs without distichiasis. A total of 239 slides was stained with hematoxylin-eosin (H&E) and evaluated under microscope (Raymond-Letron *et al* 2012). On histological section affected glands are seemingly normal, even though the meibomian glands aren't normally associated with hairs of any kind (Raymond-Letron *et al* 2012). The distichiatic hair follicles connect to the meibomian gland with what seems to be a pilosebaceous entity comparable with that of the skin. One to multiple segments of hair follicles occurs in the tarsus, most of which are located in the connective tissue surrounding the glands. Hair shafts are found inside the lumen of the central excretory duct of the meibomian glands from which they emerge (Raymond-Letron *et al* 2012) (**Figure 4**). Infrequently the gland orifices appear stenotic and normal secretion may be difficult (Lawson 1973).

During embryogenesis, the eyelids, cilia, epidermis and the conjunctival epithelium develop from the surface ectoderm. The meibomian glands develop when surface ectoderm proliferates from the eyelid margin into the tarsal plate. The tarsal plate, dermis and other deeper structures of the eyelid develops from the neural crest (Raymond-Letron *et al* 2012). Raymond-Letron *et al* (2012) demonstrated that the development of the aberrant cilia might be associated with abnormal hair follicle morphogenesis. While not yet confirmed, it was assumed that the ectopic hair follicles theoretically might result from abnormal expression of factors that either activate placode promoters or on the contrary inhibits placode repressors (Raymond-Letron *et al* 2012).

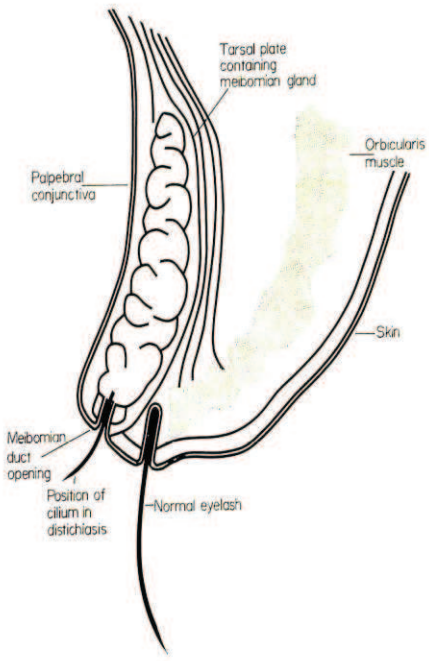

Figure 4 Aberrant cilia emerging from the meibomian gland orifice. Note the hair shaft within the gland (Bedford 1971).

2.2.2 BREED PREDISPOSITIONS

Many breeds are known to be affected by distichiasis. Especially purebred dogs are affected, but the condition is occasionally seen in crossbred dogs as well (Lawson 1973). The American and English cocker spaniel breeds are known to be some of the most frequently diagnosed, with many affected individuals within the populations (Bedford 1973, Lawson 1973, Barnett 1976). In two of the earliest studies on canine distichiasis, Lawson (1973) and Barnett (1976) both demonstrated that out of all breeds included, the cocker spaniels comprised over one fourth of all cases. In the first study, 58 dogs admitted to the University of Glasgow Veterinary Hospital were included, comprising 52 dogs from 15 different breeds and 6 crossbred dogs. Fifteen of 58 cases were cocker spaniels (26 %) (Lawson 1973). The second study comprised 84 dogs including 80 purebred dogs from 21 different breeds and 4 crossbred dogs, of which 22 dogs were cocker spaniels (26 %) (Barnett 1976). Other breeds that are predisposed to distichiasis includes the Pug, Tibetan terrier, Welsh springer spaniel, Cardigan welsh corgi, Golden retriever, Pekingese and many more(American Kennel Club 2013, Maggs *et al* 2013). Earlier records often referred to the same few breed regarding predisposition (Halliwell 1967, Bedford 1971, Lawson 1973), however, the list of supposedly predisposed breeds now comprises up to 109 different breeds (Miller *et al* 2013).

2.3 MODE OF INHERITANCE

Canine distichiasis in the cocker spaniels is assumed to be inherited as an autosomal dominant trait with an irregular penetrance (Halliwell 1967, Bedford 1973, Long 1991). Nevertheless, the condition seems to be inherited in a somewhat complicated manner and the exact etiology, heredity and mode of inheritance has not yet been clarified (Bedford 1973). Canine distichiasis show many similarities to distichiasis in man, although the etiology might not be the same (Halliwell 1967). Early researcher in human medicine suggested that distichiasis was a heterologous developmental anomaly in which the aberrant cilia completely replaced the meibomian glands whereas others believed it was connected to atavism (Scheie & Albert 1966, Anderson & Harvey 1981). Distichiasis in man is rare and is also assumed to be inherited as an autosomal dominant trait (Picó 1957). From an analysis of the systematic and environmental influences and the additive genetic variation of PIED in the Tibetan terriers registered in the International Kennel Club in Germany, Ketteritzsch *et al* (2004) demonstrated that distichiasis was considered to be genetically influenced, with a heritability of 0.043. The heritability indicates that the condition may be inherited as a polygenic threshold trait, although this has not been confirmed in other breed.

2.4 PATHOGENESIS AND CLINICAL SIGNS

Clinical signs associated with distichiasis manifest in different ways depending on the size, rigidity and amount of distichiae present (Bedford 1979), although the severity of manifestation is not directly proportional to the amount of cilia present (Bedford 1979). Distichiasis usually becomes apparent at an early age, when the adventitious cilia emerge on the eyelid margins and impinge on the cornea, but they may occur at any age of life (Halliwell 1967, Lawson 1973). **Figure 5** illustrates the pathogenesis of distichiasis.

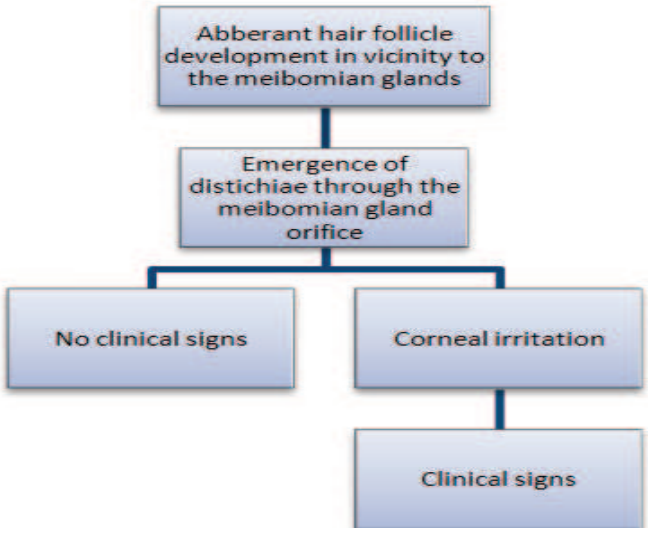

Figure 5 Pathogenesis of canine distichiasis. The presence of distichia does not always cause clinical signs, and the condition may be clinically insignificant in some dogs.

2.4.1 CLINICAL SIGNS

The clinical appearance of canine distichiasis varies greatly among the affected individuals, and though most aberrant cilia in contact with the corneal surface is presumed to cause corneal irritation, distichiasis may be of no clinical significance in some dogs (Lawson 1973, Bedford 1988). Distichiasis is only clinically significant when it causes corneal irritation (Bedford 1979). Size, texture and amount of cilia are crucial to the outcome of the condition. Soft and long distichia in small amounts may produce mild clinical signs if any, whereas small and rigid distichia, as well as large amounts, or many distichiae located in clusters almost inevitably cause corneal irritation (Lawson 1973, Bedford 1988). Duration of clinical signs varies from a few days to years (Lawson 1973), or may be present from puppyhood and cause lifelong discomfort (Long 1991).

The most characteristic clinical signs of canine distichiasis are corneal irritation and excessive lacrimation (Lawson 1973). Corneal irritation occurs when the aberrant cilia impinge on the corneal surface and cause trigeminal irritation leading to excessive lacrimation, mild conjunctivitis and blepharospasm (Halliwell 1967, Bedford 1979, Bedford 1999). Excessive lacrimation, or epiphora, is presented as a constant moistness around the eyes and tear streaking at the temporal or nasal canthus, which is the most typical complaint from the owner, together with rubbing and scratching of the eyes (Lawson 1973). In the more advanced cases, clinical signs are more pronounced, especially when trauma to the cornea is induced (Halliwell 1967). Clinical signs associated with moderate to severe cases of distichiasis include photophobia, swelling and hyperemia of the nictating membrane and superficial punctate keratitis (Halliwell 1967, Lawson 1973). If left untreated, the cornea may become ulcerated, and the affected part of the cornea often appears cloudy due to edema and leukocyte adhering to the endothelium which might affect vision (Halliwell 1967, Bedford 1979, Long 1991). Corneal ulceration and superficial punctate keratitis is very painful and markedly increase blepharospasm, which then might worsen the condition if distichiae are not removed (Halliwell 1967)

2.5 DIAGNOSIS

Distichiasis is often suspected based on the appearance of specific clinical signs compared with the information obtained from the owner regarding the clinical history of the dog (**Figure 6a**). In order to detect distichiasis, a thorough ophthalmic examination of the eye and its adnexa is required. The diagnosis is made by identifying one or multiple cilia emerging from the meibomian gland orifices, or by observing lashes that are in contact with the cornea or the conjunctival lining of the eye (Lawson 1973, VCA Animal Hospital 2014) (**Figure 6b-c**).

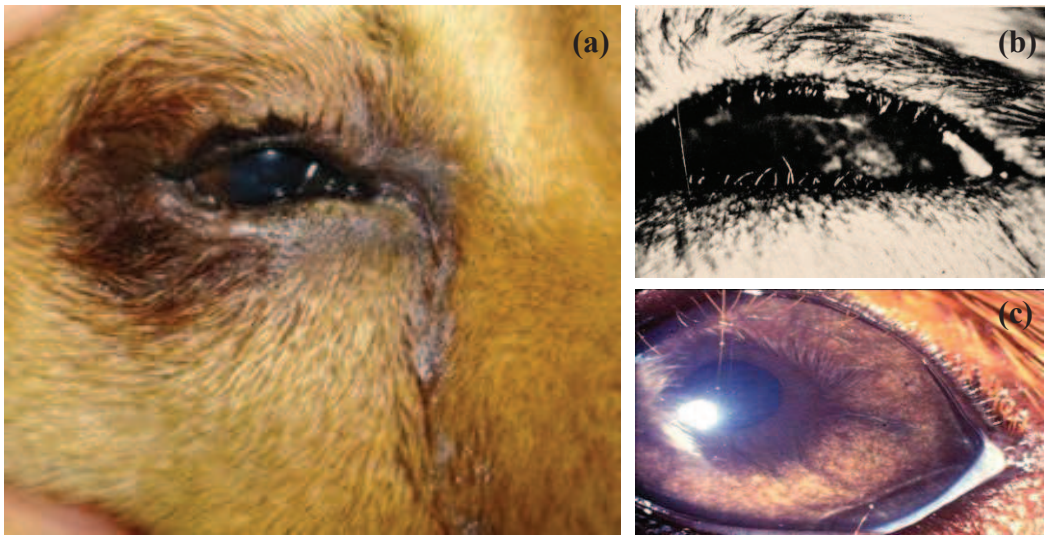

Figure 6 Typical clinical presentation in a dog with distichiasis (UFAW 2014) **(a)**. Distichiae may be short and rigid (Bedford 1973) **(b)** or long and soft (Infovets.com 2014) **(c)**.

2.5.1 THE OPHTHALMOLOGICAL EXAMINATION OF THE EYELIDS

Distichiasis may be diagnosed using only the naked eye when cilia are long or located in clusters (Bedford 1999). In most cases, however, the distichiae are small, has a fine texture and lack pigmentation, which often makes them hard to detect (Halliwell 1967). The preferred method of choice is examination using a high magnification handheld slit lamp, or biomicroscope, with a focal illumination, or an ophthalmoscope (Halliwell 1967, Lawson 1973).

The eyelid is slightly elevated and the free margin is systematically examined using high magnification. Distichiae may be more visible when seen in profile against the bulbar conjunctivae or the sclera (Lawson 1973, Long 1991). Massage and digital palpation of the eyelid may express small amounts of secretions from the meibomian glands making the cilia more visible and aid in determining the exact point of emergence (Halliwell 1967). Where clinical signs of corneal irritation are present, fluorescein staining of the cornea and Schirmers tear test are recommended to assess the extent of any accompanying corneal injury and to rule out other causes of excessive lacrimation (Martin 2010, VCA Animal Hospital 2014). The Schirmers tear test measures the basal and reflex tear secretion rate from the lacrimal glands, and is a useful tool to estimate corneal and conjunctival irritation. The secretion rate is affected by sensory nerve endings of the trigeminal nerve, which is activated when corneal irritation occurs (Bedford 1999, Hamor *et al* 2000). The strip is placed in the middle of the lower eyelid in contact with the conjunctiva. The normal values for dogs are 15-25 mm/min (Martin 2010). Fluorescein is very useful in detecting corneal epithelial defects. It is not in itself a diagnostic tool for identifying distichiasis, but it is used as a measure of corneal irritation and lesions in the corneal surface. It is easy to perform by applying a moist fluorescein strip to the conjunctiva. Where the cornea is intact the aqueous fluorescein is not retained, as the liquid cannot penetrate the lipophilic epithelium of the cornea. When damage to the corneal surface occurs, either by loss of epithelium or by widened intercellular junctions, the fluorescein penetrates the cornea and is retained within the stroma or the epithelium and becomes visibly detectible as a bright green stain (Martin 2010).

Some dogs require topical anesthetics or sedatives to allow a thorough examination of the eye and its adnexa; however, this is rarely the case (Martin 2010, VCA Animal Hospital 2014).

2.5.2 DIFFERENTIAL DIAGNOSES

When diagnosing distichiasis it is important to determine the exact point of emergence, to distinguish it from other ophthalmic diseases caused by adventitious cilia, as illustrated in **Figure 7**. The three most relevant differential diagnoses are ectopic cilia, trichiasis and pseudo-distichiasis, all of which have the same clinical presentation as distichiasis (Halliwell 1967, Helper & Magrane 1970, Lawson 1973).

2.5.2.1 ECTOPIC CILIA

Ectopic cilia are a rarer than distichiasis, but in many ways similar in appearance (DSVO/b 2014). The aberrant cilia arise in or near the meibomian glands, but instead of using the meibomian gland orifices as the route of least resistance the hairs protrude through the palpebral mucosa on the back of the eyelid (**Figure 8c**). The cilia are most often located in the middle of the eyelid, approximately 3 mm from the upper palpebral margin and will almost inevitably cause considerable discomfort (Helper & Magrane 1970, DSVO/b 2014). Ectopic cilia are often seen together with distichiasis and a common genetic link has been suggested (Barnett 1976, Barnett 1988). In a survey on inherited eye diseases in the dog and cat, Barnett (1976) found that 9 of 19 dogs examined for ectopic cilia also presented with distichiasis. The condition is considered congenital, but the clinical signs do not apply until later, when the cilia penetrate the palpebral conjunctiva (Helper & Magrane 1970).

2.5.2.2 TRICHIASIS

Trichiasis is a congenital and acquired condition, in which normally located lashes becomes distorted or misdirected and impinge on the eye (**Figure 7d**). The distorted cilia are usually located near the medial canthus of the lower eyelid, and may be a result of mild cases of entropion or a consequence of prominent nasal folds (Halliwell 1967, Bedford 1971, Maggs 2013). The typical clinical signs are epiphora, blepharospasm and photophobia, occasionally accompanied by superficial vascularization of the cornea (Bedford 1971). Trichiasis is predominantly seen in the Pekingese, Cocker Spaniel and Labrador retriever (Bedford 1971).

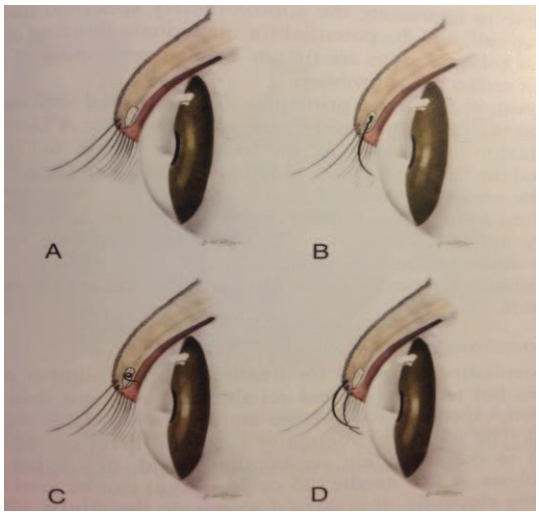

Figure 7 Schematic presentation of the most prevalent disorders of the cilia. Normal eyelid of the dog **(a)**, Distichiasis **(b)**, Ectopic cilium **(c)** and trichiasis **(d)** (Maggs 2013).

2.5.2.3 PSEUDO-DISTICHIASIS

Pseudo-distichiasis occurs when the normal hair cover of the eyelids is misdirected and the hairs emerge on the free margin of the eyelids, as with distichiasis. The abnormally placed hairs protrude between the meibomian glands or more commonly outside the lining of the meibomian gland orifices. As pseudo-distichiasis and distichiasis show many similarities, both in point of emergence and clinical presentation, the two can only be differentiated with adequate magnification (Lawson 1973).

2.5.3 THE ECVO-CERTIFICATE

The ECVO-certificate is an international document based on the Hereditary Eye Disease Scheme (HEDS) established by the European College of Veterinary Ophthalmologists (ECVO).The objective of the scheme is to standardize the diagnose and control of presumed inherited eye diseases (PIED) in predisposed animals (ECVO Scheme 2014). Diseases related to the PIED are either painful, disabling or disturbing for the animal's wellbeing, or a condition that necessitates surgical intervention or lifelong medical treatment (ECVO Scheme 2014). The ECVO-certificate can only be issued by specifically trained veterinary European eye specialists, the diplomats or panelists, or by the European eye scheme examiners, both of which are approved by the ECVO Association (ECVO Scheme 2014). In Denmark, all members of the Danish Veterinary Association's Eye Panel are approved by the ECVO (DSVO/a 2014).

The certificate is issued based on a general examination of the eye and its adnexa and is valid for 12 months (ECVO Scheme 2014). Animals used for breeding should be examined annually, and the examination can either be of the animal individually or by litter screening. In the litter screening, whole litters under the age of 12 weeks are examined for the specific congenital PIED related to their breed, but it is advised though that separate certificates are issued for each individual examined (ECVO Scheme 2014). The certificate is divided into five sections, comprising the data on the animal (i.e. name, breed and previous examinations) and the person responsible for the animal (i.e. owner, agent or breeder), identification number (microchip number or tattoo), method of examination and the results (DSVO/a 2014).
ECVO-Certificates are available in the public domain and are sent to the respective national kennel club for publication (ECVO Manual 2012). The ECVO-Certificate can only be issued for animals with permanent identification, such as microchip number or tattoo, which are statutory for all purebred dogs (ECVO Manual 2012).

2.6 THERAPY

The treatment of choice depends largely on the severity of the condition. In general, treatment is only considered necessary when clinical signs are present or when the dog seems otherwise affected by the condition (Bedford 1999). Simple methods may be used to temporarily control the condition, but permanent relief from the clinical signs associated with distichiasis is necessitated by the complete destruction of the aberrant hair follicles and often involves surgery (Bedford 1988, Maggs *et al* 2013). None of the methods available today is completely satisfactory and recurrence of disease is likely (Bedford 1988, DSVO/b 2014).

2.6.1 NON-INVASIVE TREATMENT

In mild cases simple procedures can be attained to control distichiasis. Cutting of hairs and manual epilation are the preferred methods of choice when only a few distichiae are present (Halliwell 1967, Bedford 1988). The methods are also preferred in patients where the eyelids are so thin that radical treatment would cause too many side effects, which is often the case in the smaller patients (Bedford 1971, 1988). As the distichiae are removed the dog is provided with immediate relief as the cause of corneal irritation is eliminated, and clinical signs are often diminished instantaneously. However, the effect of the treatment is only temporary as the distichiae rather quickly grow back and the clinical signs recur within short time after the treatment (Bedford 1971, Lawson 1973). In order to control distichiasis repeated treatments are required (Halliwell 1967). The procedures can be performed with or without sedating the dog, depending on its compliance (Halliwell 1967).

2.6.2 SURGICAL MANAGEMENT

Surgical management is preferred when large amounts of distichiae are present, or with frequent recurrence of clinical signs (Bedford 1973, 1999). Over time, several methods have been advocated when treating moderate to severe cases of distichiasis. The lid splitting technique (Halliwell 1967), entropion operation (Bedford 1971) and the partial tarsal plate excision technique (Bedford 1973, 1979) are no longer the preferred methods of choice, due to the many side effects associated with these procedures (Bedford 1971, Chambers & Slatter 1984, Maggs *et al* 2013). The most common side effects are cicatricial distortion of the eyelids, scarring and destruction of the meibomian glands with subsequent loss of function, especially if too much tissue is removed (Bedford 1971, Bedford 1988). The methods require an experienced surgeon and delicate equipment and are often

time consuming (Chambers & Slatter 1984). Currently, cryotherapy (Chambers & Slatter 1984) and electroepilation (Lawson 1973) are the most frequently used procedures, as they are easy to perform and cause less damage to the surrounding tissue than earlier techniques (Chambers & Slatter 1984, Maggs *et al* 2013).

2.6.2.1 ELECTROEPILATION

Electroepilation is effective when smaller amounts of distichiae are to be removed. An electro epilator is passed along the cilium 3 mm into the hair follicle using magnification, and destroys the hair follicle by electrolysis (Lawson 1973). A low current of approximately 1 to 5 mA is applied until bubbles of heated meibom (meibomian secretion) are seen at the gland orifice. On average the epilation needle is applied for 15 to 30 seconds (Lawson 1973, Maggs *et al* 2013). The distichiae usually adhere to the epilation needle when the hair follicle is destroyed but is otherwise removed afterwards by manual epilation (Lawson 1973, Maggs *et al* 2013). The low current used limits distortion and scarring of the eyelids, as it reduces twitching of the orbicularis muscles and facilitates correct placement of the epilation needle (Lawson 1973). High magnification is advocated to ensure that all aberrant hair follicles are destroyed, especially when multiple hairs emerges from the same gland, in which several hair follicles are involved (Maggs *et al* 2013). Recurrence of disease is possible if hair follicles are not completely destroyed, and some degree of distortion may occur when many hairs are removed or if repeated treatments are applied (Lawson 1973, Bedford 1973).

2.6.2.2 CRYOTHERAPY

Cryotherapy is preferred where several distichiae are to be removed (Chambers & Slatter 1984, Maggs *et al* 2013). Hair follicles show marked susceptibility to cellular damage by freezing compared to the surrounding structures in the eyelids, which enables selective destruction of the aberrant hair follicle without damaging the surrounding tissue (Chambers & Slatter 1984). The method is performed under general anesthesia and uses liquid nitrogen (N_2O) to destroy the aberrant hair follicles by cryonecrosis when frozen at -89^{o} C (Chambers & Slatter 1984, Maggs *et al* 2013). The tissue is fixated using a chalazion clamp and a cryoprobe is applied to the conjunctival surface directly above the hair follicle, approximately 2 mm from the eyelid margin (**Figure 8**). The duration of freezing varies, and is determined by observing the created “ice-ball” advance from the probe to the meibomian gland orifice at the lid margin. This is preferably performed under an

operating microscope (Chambers & Slatter 1984, Maggs *et al* 2013). The method is often performed as a double freeze-thaw cycle, and the average duration of freezing is 30 seconds for the first application, then the hair follicle is thawed and the cryoprobe is applied for another 15 seconds. Remaining loose lashes are removed by manual epilation (Chambers & Slatter 1984).

In an experimental study on 8 dogs of 4 different breeds, Chambers & Slatter (1984) reviewed the clinical effect and histological changes related to cryotherapy. A glaucoma probe was used as it increases the surface area treated compared to other probes (Chambers & Slatter 1984), and in conjunction with the haemostatic properties of the chalazion clamp, the duration and the amount of N_2O were reduced, which then shortened the anesthetic time for the dog (Chambers & Slatter 1984, Maggs *et al* 2013). Cryotherapy often leads to momentary depigmentation of the eyelids due to loss of melanin granules in the basal layer of the conjunctiva when frozen; this is only visible if the eyelid is elevated. Normal pigmentation is regained within 1 to 2 months after treatment (Chambers & Slatter 1984). The affected glands are not permanently altered though and regain normal function within 4 weeks, typically without the cilia (Chambers & Slatter 1984, Maggs *et al* 2013). If the duration is insufficient the hair follicle might not be completely destroyed, and recurrence may occur (Chambers & Slatter 1984). However, in 8 of 8 cases examined no re-growth of distichiae was observed in the 6 months follow up period (Chambers & Slatter 1984). The procedure causes a minimum of discomfort to the patient post operatively. NSAIDS or systemic corticosteroids are used to reduce swelling and as post operative analgesia. An antibiotic-corticosteroid ophthalmic ointment is applied to diminish swelling and prevent secondary bacterial infection (Chambers & Slatter 1984, Maggs *et al* 2013).

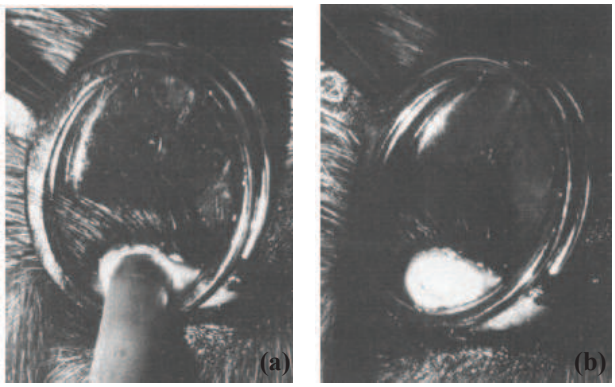

Figure 8 Cryotherapy performed on the eyelid of a dog. The application of the cryoprobe on top of the hair follicle **(a)** and the "ice-ball" at the eyelid margin **(b)** (Chambers & Slatter 1984).

2.7 PREVENTIVE MEASURES

The European College of Veterinary Ophthalmologists and other associations introduced a recommendation that two dogs with the same ophthalmic disease should not be mated (DSVO/b 2014, Hundeweb/a 2014, Hundeweb/b 2014). As this may present a problem in e.g. the cocker spaniel breeds, due to the high incidences of distichiasis, a new scheme was introduced from the 1st of July 2013 as a supplement to the registrations of distichiasis. Initially the scheme is carried out for a 5 years trial period (from the 1st of July 2013 to 31st of July 2018), and includes grading distichiasis into three different degrees (light, medium and severe) (Hundeweb/b 2014). The scheme is relatively new, and no standardization has been issued on how to graduate distichiasis, which is therefore performed solely on the subjective opinion from the examining vet (Hardon 2014, personal communication). Additionally the scheme includes that ECVO-certificates for dogs intended for breeding may be no more than 12 month old when the dogs are mated, and, in Denmark, that the grading of distichiasis must be registered in the Danish Kennel Club, if distichiasis is diagnosed. If a dog is diagnosed with a medium degree of distichiasis, the breeding partner is not allowed to have more than a light degree of distichiasis, at most (Hundeweb/b 2014). Since ophthalmic examination isn't mandatory in all countries, breeding a Danish dog with a foreign breeding partner without graduation, the Danish dog is maximally allowed to have a light degree of distichiasis (Hundeweb/b 2014). American and English cocker spaniels with a severe degree of distichiasis is excluded from breeding according to current breeding restrictions (Hundeweb/a 2014, Hundeweb/b 2014).

Another measure that have been introduced in order to reduce the high incidences of distichiasis and other PIED, e.g. in the cocker spaniels, is the introduction of two different stud books that the dogs can get when they are registered in the Danish Kennel Club (Dansk Kennel Klub 2014). The difference between the two stud books, Basic and Basic Plus, is that the Basic stud book follows the breeding restrictions for the respective breed, whereas the Basic Plus stud book also fulfill the breeding recommendations (Dansk Kennel Klub 2014). Thus, in the cocker spaniels the Basic Plus stud books would be issued for offspring of one or two healthy parents or two affected parents with different degrees of affection (light and medium), if they also fulfill the other breeding recommendations for the specific breed.

PART II – THE EXPERIMENTAL STUDY

3 MATERIALS AND METHODS

3.1 THE STUDY DESIGN

This thesis was conducted as a retrospective cohort study processed from March 2014 to June 2014. Examination of whole litters and the apparent prevalence were conducted as cross-sectional studies.

3.2 INCLUSION CRITERIA

American and English cocker spaniels were included based on the availability of a valid ECVO-certificate issued during the 1st of January 2004 to the 31st of December 2013. Furthermore, in order to determine the connection between the occurrences of distichiasis within the breeds in relation to the breeding combinations of the parents, animals included were additionally limited to dogs where both parents have at least one available ECVO-certificate issued at any point of their lives. As many of the dogs may have foreign parents from countries where ophthalmic examination isn't compulsory, all individuals that fulfill the above mentioned criteria are included. To determine the prevalence, all dogs of the American and English cocker spaniels breeds with a registered ECVO eye examination during the 1st of January 2004 to the 31st of December 2013 was included, regardless of the examination of the parents. For the study of the inheritance of distichiasis only whole litters were included. Litters were additionally limited to sets of offspring in which both parents have been examined for distichiasis.

3.3 DATA COLLECTION

Data was collected from *Hundedatabasen* and *DKK Update* at the dog registration website, *Hundeweb*, from the Danish Kennel Club. All data was collected retrospectively from a ten years period extending from the 1st of January 2004 to the 31st of December 2013. Dogs were sorted according to breed and disease status (Appendices I – VI).

During the time span of this thesis it was only possible to find whole litters at the appropriate age from one kennel, all of which were American cocker spaniels (Appendix V).

3.4 THE POPULATION

The dataset of this thesis included 1,051 dogs from the two cocker spaniel breeds. A total of 228 American cocker spaniels and 799 English cocker spaniels were investigated based on data retrieved from *Hundeweb*. Unfortunately, ECVO-certificates were not available for one or both parents of 358 of the dogs, therefore, these dogs were only partly included in this study. Thus, for the main purpose of this study 669 dogs were included, comprising 120 American cocker spaniels and 549 English cocker spaniels. The dogs were born between 1995 and 2012 and ranged from 2 months to 16 years of age. 112 dogs were deceased at some point during the study. The age of first examination ranged from 3 months to 8 years, and the dogs had been examined between 1 and 10 times. 87 dogs (13 %) had other ophthalmic conditions in addition to distichiasis.
The litter study consisted of 24 American cocker spaniels from 5 litters. All puppies were bred at Kennel Truly Yours.

3.5 DATA ANALYSIS FROM HUNDEWEB FOR STUDY OF HEREDITY

American and English cocker spaniels with a valid ECVO-certificate issued during the 2004-2014 were arranged in two excel sheets according to breed using Microsoft Excel 2007. Initially dogs were organized according to registration number and name, and sorted so that each dogs was only represented once. All ECVO certificates for each individual dog were examined, and the dogs were divided into two groups according to whether or not they were diagnosed with distichiasis. Dogs characterized as *Affected* by distichiasis in this study have been diagnosed with distichiasis at least one time during their life, from time of birth and until the end of the study period, or have a remark about detected distichiae in their ECVO-certificate. Thus, included dogs in which distichiasis was diagnosed during or prior to the studied period were considered to be *Affected*, regardless of the diagnoses made on later occasions. Dogs without any diagnoses or remarks about distichiasis were considered *Not Affected*. In order to determine a potential hereditary predisposition of distichiasis within the two breeds, dogs were limited to include those with a valid ECVO-certificate for both of their parents (Appendices I – IV). The distichiasis status of the parents and their offspring were investigated in order to describe the connection between the occurrences of distichiasis in offspring in relation to the breeding combinations. Appendices I-IV includes information regarding the ECVO examinations. For the American and English cocker spaniels *Not Affected* by distichiasis, the age at first ECVO examination, total amount of ECVO examinations and other registered ocular diseases (yes/no) are listed (Appendices II and IV). For the dogs *Affected* by distichiasis additional

information regarding, the severity of disease were listed, if available (Appendices I and III). Data on the sires used to produce the study population were also noted, including number of litters and puppies produced in total and on average for comparison of the two breeds (Appendix V).

3.6 THE OPHTHALMIC EXAMINATION

Puppies were observed for apparent clinical symptoms such as epiphora and corneal irritation, and the general appearance of the eye and its adnexa was evaluated. The ophthalmological examination was conducted using a Kowa SL-14 handheld slit lamp with 10X and 16X magnification. Eyelids were gently elevated exposing the *margo-intermarignalis* and systematically examined for the presence of abnormally located cilia (**Figure 9**). The presence of distichiae emerging from the meibomian gland orifices (distichiasis yes/no) was noted. The presence of other adventitious cilia (trichiasis/pseudo-distichiasis/ectopic cilia) was additionally noted. 8 puppies were reexamined on later occasion to see if results were consistent (Appendix V).

Figure 9 The ophthalmic examination. Eyelid examination of the lower palpebrae performed by the author on one of the puppies from Kennel Truly Yours.

3.7 STATISTICS

Data were sorted and analyzed using Microsoft Excel 2007, R 3.1.0 statistical software program (R Studio 2014) and online genetic calculation applets (Genetic Calculation Applet/a 2014).

The apparent prevalence of distichiasis within the general population of the two breeds was calculated as the proportion of diseased animals at a given point in time. The 95 % confidence intervals (CI) of the proportions were given. Formulas from Introduction to Veterinary Epidemiology, Chapter 6 were used (Toft *et al*/a 2004).

The hypothesis that there is a higher possibility that the offspring will get distichiasis if both of their parents are affected by distichiasis compared to offspring in which one or both parents are unaffected was tested using 2 x 2 tables and χ^2-test with 1 degree of freedom (df = 1). Results were given as χ^2-values, p-values and 95 % confidence interval (CI) for the difference between the variables being compared. Differences in the outcome for the offspring related to the different breeding combinations were considered significant if $\chi^2 > 3.84$ and $p < 0.05$. The Relative Risk (RR) was calculated to measure the association between the different levels of exposure and the risk of getting distichiasis. The χ^2-test was additionally used to compare potential differences in the prevalence of distichiasis between males and females.

The heritability of distichiasis as a threshold trait was calculated based on data from the English cocker spaniel. The *Applet for calculating heritability for threshold traits (diseases)* was used (Genetic Calculation Aapplet/a 2014).

In the litter study, proportion of distichiasis within the litters and in total was given. As the amount of submitted puppies was not considered sufficient to get statistically significant results, the χ^2-test, p-value and 95 % CI were not calculated.

4. RESULTS

The aim of this study was to examine the heredity and exact mode of inheritance of canine distichiasis and to determine the prevalence within the two cocker spaniel breeds. In order to test the stated hypothesis that there is a higher possibility that the offspring will get distichiasis if both of their parents are affected by distichiasis compared to offspring in which one or both parents are unaffected, the three different breeding combinations of the parents were analyzed.

4.1 THE APPARENT PREVALENCE OF DISTICHIASIS

All American and English cocker spaniels with a valid ECVO-certificate issued during the 1st of January 2004 and the 31st of December 2013 were used to calculate the apparent prevalence of distichiasis in the general populations of the two breeds. Diagnoses were made according to all issued ECVO-certificates for the individual dog. In total, 228 American cocker spaniels and 799 English cocker spaniels were included (**Table 1**). The apparent prevalence of distichiasis was found to be 71.93 % within the American cocker spaniel breed and 48.81 % within the English cocker spaniel breed, as illustrated in **Figure 10**.

Table 1 The apparent prevalence of distichiasis in the cocker spaniel breeds

Breed	*Affected*	*Not Affected*	*Total*	*Prevalence of Dist. (%)*	*95 % CI*
American cocker spaniels	164 dogs	64 dogs	228 dogs	71.93 %	[71.87 ; 71.99]
English cocker spaniels	390 dogs	409 dogs	799 dogs	48.81 %	[48.78 ; 48.84]

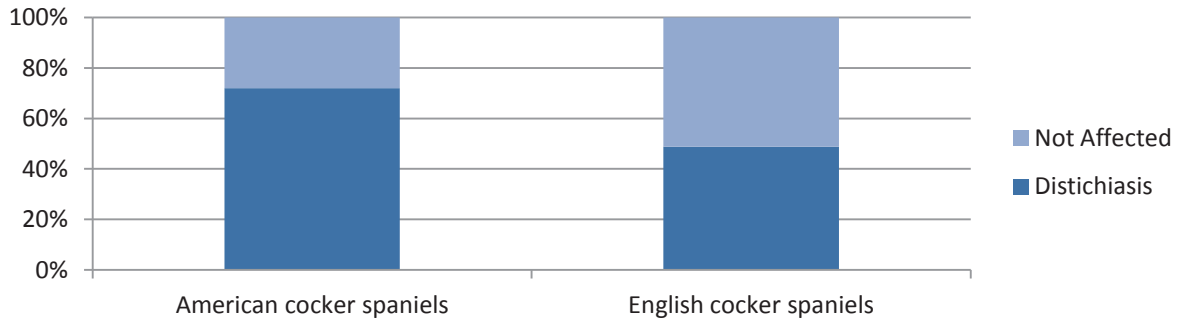

Figure 10 Overview of cocker spaniels with an ECVO-certificate issued during 2004-2014. The prevalence of distichiasis is 71.93 % in the American cocker spaniel and 48.81 % in the English cocker spaniel

4.2 STUDY OF HEREDITY

In order to examine the heredity of distichiasis in the American and English cocker spaniels, a total of 669 dogs were included (Appendices I-IV). Breeding combinations of parents with different distichiasis status were examined for statistical significance of the outcome. Results of the ECVO examinations, incl. the appointed degrees are given in Appendices I-IV.

4.2.1 THE AMERICAN COCKER SPANIEL

In total, 120 American cocker spaniels were included for further evaluation of distichiasis within the breed, based on available ECVO-certificates of both of their parents. The most frequently used breeding combination was between two affected dogs (59 %), and the least frequent was between two unaffected dogs (12 %). The breeding combination between one affected and one unaffected parent was used in 29 % of the cases (**Table 2**).

Table 2 The association of the distichiasis status of the parents and their offspring in the American cocker spaniels

Breeding Combinations	*Offspring with Distichiasis*	*Offspring without Distichiasis*
Affected X Affected	52 dogs	19 dogs
Affected X Not Affected	28 dogs	7 dogs
Not Affected X Not Affected	12 dogs	2 dogs
Total	92 dogs	28 dogs

Breeding combinations were tested against each other to see if the different distichiasis status in the parents had any significant influence on the outcome in the offspring (**Table 3**).

Table 3 Statistical significance of association

Compared Breeding Combinations	χ^2*-test*	*p-value*	*95 % CI*	*RR*
Affected x Unaffected vs. Both parents unaffected	0.0034	0.9535	[-0.333 ; 0.219]	0.933
Affected x Affected vs. Affected x Unaffected	0.0271	0.6025	[-0.257 ; 0.122]	0.915
Affected X Affected vs. Both parents unaffected	0.4226	0.5156	[-0.378 ; 0.128]	0.854

When comparing breeding combinations in which one or both parents were unaffected by distichiasis there was no statistical significance (p-value > 0.05) that the offspring after one affected and one unaffected parent would be more likely to be affected by distichiasis, than offspring after two unaffected parents. The same was asserted when comparing the breeding combination with two affected parents to breeding combinations including either one or two unaffected dogs (p-value > 0.05). The relative risk (RR) that the offspring would be affected by distichiasis is not higher if one or both parents are affected by distichiasis than if both parents are unaffected. Thus, for the American cocker spaniel it could not be confirmed that there is any difference in the occurrence of distichiasis in the offspring whether the parents are affected or unaffected by distichiasis.

4.2.2 THE ENGLISH COCKER SPANIEL

In total, 549 English cocker spaniels were included for the further evaluation of distichiasis within the breed, based on available ECVO-certificates on both of their parents. The most frequently used breeding combination was between one affected and one unaffected parent (41.7 %), and the breeding combinations including two affected (28.6 %) and two unaffected (29.7 %) parents were almost equally distributed (**Table 4**).

Table 4 The association of the distichiasis status of the parents and their offspring in the English cocker spaniels

Breeding Combinations	*Offspring with Distichiasis*	*Offspring without Distichiasis*
Affected X Affected	105 dogs	52 dogs
Affected X Not Affected	106 dogs	123 dogs
Not Affected X Not Affected	60 dogs	103 dogs
Total	271 dogs	278 dogs

When comparing breeding combinations in which one or both parents were unaffected by distichiasis there was no statistical significance (p-value > 0.05) that the offspring would be more likely to have distichiasis when only one parent were affected in relation to both parents being unaffected. However, when comparing the breeding combination in which both parents were affected by distichiasis to breeding combinations in which one or both parents were unaffected, there was a distinct statistical significance that the offspring is more likely to have distichiasis in both cases (p-value < 0.001) (**Table 5**). When both parents are affected by distichiasis, the relative risk (RR) that the offspring will get distichiasis is 1.4 times higher in relation to only one parent

being affected, and 1.8 times higher in relation to both parents being unaffected. Thus, in the English cocker spaniel it is confirmed that there is a marked increase of getting offspring with distichiasis when mating two dogs both affected by distichiasis compared to using one or two unaffected dogs, and the risk is almost 2 times higher when using two affected parents compared to two unaffected parents. Statistically significant results are marked in red.

Table 5 Statistical significance of association

Compared Breeding Combinations	*χ^2-test*	*p-value*	*95 % CI*	*RR*
Affected X Unaffected vs. Both parents unaffected	3.1265	0.0770	[-0.009 ; 0.200]	1.3
Affected x Affected vs. Affected x Unaffected	15.1148	0.0001012	[0.1026 ; 0.3092]	1.4
Affected X Affected vs. Both parents unaffected	27.7596	0.0000001374	[0.1900 ; 0.4114]	1.8

4.2.3 HERITABILITY

The heritability of distichiasis in the English cocker spaniel breed was calculated using information on the difference in mean liability within the population and within the related individuals to an affected animal, respectively (Falconer & Macay 1996). Based on the information presented in **Table 6** the heritability of distichiasis is 0.51 when the estimate is calculated on the basis of offspring from two affected parents and 0.22 when the estimate is based on one affected parent. Thus, distichiasis may be considered a hereditary disease with a high genetic influence. The difference between incidences of distichiasis in the population and in the ones that are related to both on and two affected parents are statistically significant (Two Affected: χ^2 28.95, p-value < 0.0001. One Affected: χ^2 3.50, p-value < 0.005).

Table 6 The heritability of distichiasis as a threshold trait

	Two affected parents			*One affected parent*		
	Not affected	*Affected*	*Frequency*	*Not affected*	*Affected*	*Frequency*
Population	155	165	0.52	226	166	0.42
Relatives to affected	52	105	0.67	123	106	0.46
Remaining population*	103	60		103	60	

*used for calculation of χ^2

4.2.4 GENDER PREDISPOSITION

In the American cocker spaniel 28 males and 64 bitches were diagnosed with distichiasis, and 7 males and 21 bitches were unaffected (Appendices I-II). In the English cocker spaniel 94 males and 184 bitches were affected by distichiasis and 74 males and 197 bitches were unaffected (Appendices III-IV) (**Figure 11**). Dogs were pooled together according to gender. Difference in the occurrence in relation to gender is not statistically significant (The χ^2-tests 2.4363, $p > 0.1186$)

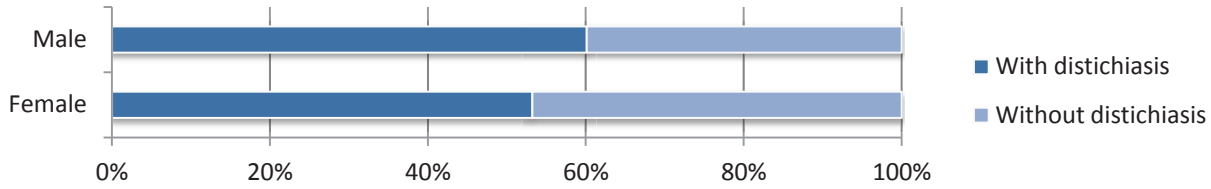

Figure 11 Distribution of distichiasis in relation to gender

4.2.5 SIRES

192 sires fathered the study population. **Table 7** shows the breeding information of the sires in accordance to breed and distichiasis status (Appendices VI-IX). ACS sires are allowed to produce 15 puppies according to the ethical recommendations of the Danish Kennel Club, and the ECS sires are allowed to produce 105 puppies. The limit is set according to the specific population size in the respective breed (Dansk Kennel Klub 2012). In the ACS breed 68 % (n = 23/34) of the sires with distichiasis and 37.5% (n = 3/8) of the sires without distichiasis exceeds the limit for number of offspring from 'popular sires' (LNOPPS). As illustrated in **Table 7** the mean of puppies produced by the ACS sires exceeds the limitations, additionally indicating that the majority of the sires produce more offspring that they are allowed. Exceeding values are marked in red. In the ECS breed 11 % (n = 6/55) of the sires with distichiasis and 4 % (n = 4/95) of the sires without distichiasis exceeds the breed limitations. The exceeding amount of offspring ranges from 2 to 64 (mean 32.9) in the ACS breed and from 5 to 69 (mean 35.4) in the ECS breed.

Table 7 Breeding information on American and English cocker spaniel sires

	American cocker spaniels		*English cocker spaniels*	
	With dist.	*Not affected*	*With dist.*	*Not affected*
Sires	34	8	55	95
Puppies from study population:				
With distichiasis	75 dogs	17 dogs	153 dogs	162 dogs
Without distichiasis	26 dogs	2 dogs	116 dogs	118 dogs
Litters / mean	231 / 6.8	39 / 4.8	509 / 9.3	756 / 8.0
Puppies / mean	983 / 28.9	162 / 20.25	2,708 / 49.2	3,820 / 40.2

4.3 THE LITTER STUDY

A total of 24 puppies from 5 litters were examined for distichiasis (Appendix V). **Figure 12** illustrates the distribution of distichiasis diagnoses. The prevalence within each litter is shown in **Table 8**. Although inconclusive, the results from the litter study are contradictory to the assumption of a simple autosomal dominant inheritance (Halliwell 1967), as the segregation found in the ophthalmic examination of whole litters doesn't support this mode of inheritance. Results deviating from the expected segregation of a simple dominant inheritance are marked in blue.

Table 8 The prevalence of distichiasis within the litters

Litter	*Breeding combination*	*Distichiasis (%)*	*Nothing detected (%)*	*Litter size*
1	Affected x Not Affected	50 %	50 %	4
2	Affected x Affected	33.3 %	66.6 %	3
3	Affected x Not Affected	28.6 %	71.4 %	7
4	Affected x Affected	14.3 %	85.7 %	7
5	Affected x Not Affected	33.3 %	66.6 %	3
Total		**29 %**	**71 %**	**24 puppies (100 %)**

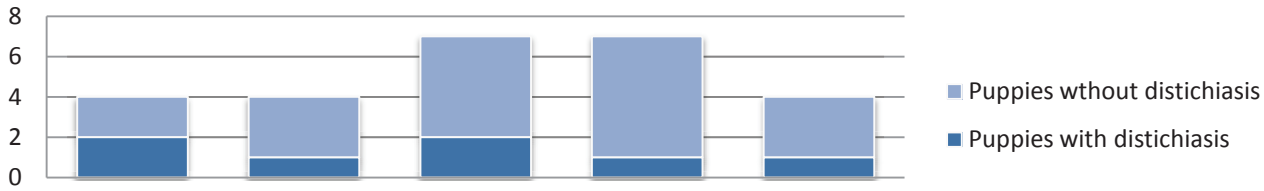

Figure 12 The distribution of distichiasis within the litters. The litters show a high amount of puppies without distichiasis

4.4 THE GRADING SCHEME

From the two populations of cocker spaniels, a total of 164 American cocker spaniels and 390 English cocker spaniels were diagnosed with distichiasis. In the ACS breed, 45 % of the dogs were graduated (n = 74/164). The majority of these dogs (73 %) had a light degree of affection (n = 54/74) and the remaining (27 %) were appointed a medium degree (n = 20/74). None of the ACSs were diagnosed with severe degree. In the ECS, 135 of 390 dogs were graduated (34.6 %). 116 dogs had a light degree (86 %), 15 dogs had a medium degree (11 %) and 4 dogs were diagnosed with a severe degree of affection (3 %). **Figure 13** illustrates the distribution.

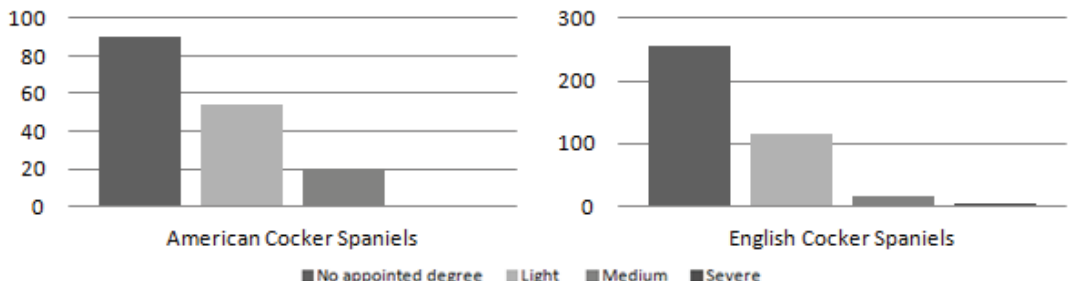

Figure 13 The distribution of the different levels of affection registered for the American and English cocker spaniels

5 DISCUSSION

5.1 THE APPARENT PREVALENCE OF DISTICHIASIS

From the 1,027 investigated dogs, the apparent prevalence was found to be 71.93 % in the American cocker spaniel and 48.81 % in the English cocker spaniel. The prevalences are comparable to that of other studies on ophthalmic diseases and distichiasis in the cocker spaniels. Additionally, the prevalences for the cocker spaniels are considerably higher than the incidences found in the general dog population which is consistent with the existing literature as well.

In a study on the incidence, age incidence and breed incidence of canine distichiasis, a series of 58 dogs were examined. The last 22 cases were retrieved from a population of 2,919 dogs referred to the University of Glasgow, thus demonstrating an incidence of 0.75 % (1:133) (Lawson 1973). Furthermore, a review on canine hereditary eye diseases investigated 2,374 dogs referred to the University of Cambridge, England, and found an incidence of distichiasis of 3.54 % (n = 84/2,374) within all breeds (Barnett 1976). These incidences are considerably lower than the prevalences given for the cocker spaniels in this study. Recent counts from Agria Pet Insurance in Sweden support the theory that the American and English cocker spaniels are highly overrepresented compared to other breeds in relation to ophthalmic diseases, with a relative risk (RR) of more than 4 (Agria Insurance Data 2011). In the above mentioned studies, the cocker spaniels constituted the majority of distichiasis cases, as the two breeds comprised over ¼ of all cases diagnosed with distichiasis (26 %). Earlier investigations on the Danish population of cocker spaniels reveled prevalences of 80 % in the American cocker spaniel and 47 % in the English cocker spaniel (Proschowsky 2014). As these data are retrieved from Hundeweb at the Danish Kennel Club, results are consistent with results given in this thesis.

In this thesis the apparent prevalence in the American and English cocker spaniels were found to be 71.93 % (n = 164/228) and 48.81 % (n = 390/799), respectively. The apparent prevalence is an estimate of the prevalence within the two breeds on the assumption that the included dogs are representative of the general populations of the two breeds. Since the inclusion criteria of this thesis comprised all cocker spaniels with a valid ECVO-certificate issued during a ten years period, an adequate sample size was not quantified in advance. However, the 95% CI's of the results given in section 4.1 are very narrow, demonstrating that the sample sizes of the two populations may be considered sufficient, and that the prevalences are representative for the two populations. It has not

been possible to find any literature that considers the grounds for why distichiasis has become so common within these two breeds, or any other breeds for that matter.

A problem presented in this thesis was the credibility of the diagnoses registered in the ECVO-certificates. During the investigation of the dogs, inconclusive diagnoses were detected, as some dogs that were registered as unaffected had a remark, that distichiae were detected during the examination. In this study all diagnoses were checked and taken into account, but misleading diagnoses may result in a general underestimation of the extent of the disease in the populations.

5.2STUDY OF HEREDITY

In the study of the heredity of distichiasis, the two cocker spaniel breeds were investigated under the assumption that two affected parent would be more likely to get affected offspring compared to breeding combinations in which one or both parents are unaffected.

In the American cocker spaniel (ACS) breed, a total of 120 dogs were investigated based on their distichiasis status. 92 dogs (76.7 %) were diagnosed with distichiasis and 28 dogs (23.3 %) were unaffected. The most frequently used breeding combination of American cocker spaniels are between two affected individuals, and the combination of two unaffected individuals was the least frequently used. When comparing the three breeding combinations to each other, there was no statistical significant difference on the outcome, which deviates from the expected hypothesis.

In the English cocker spaniels (ECS), a total of 549 dogs were investigated. 271 dogs (49 %) were diagnosed with distichiasis and 278 dogs (51 %) were unaffected. The most frequently used breeding combination was between one affected and one unaffected individual, whereas combinations in which the parents have similar distichiasis status were almost equally distributed. In this thesis there was no significant difference in the occurrence of distichiasis in the offspring where one or both parents were unaffected. However, the relative risk (RR) that offspring will be affected by distichiasis is higher if one of the parents are affected compared to two unaffected parents (RR = 1.3). When comparing the outcome of the breeding combination in which both parents are affected by distichiasis to the outcome of combinations in which one or both parents are unaffected, there is a pronounced statistical significant difference in the outcome. The RR that two affected parents will get offspring with distichiasis is 1.4 times higher compared to one affected and one unaffected parent, and 1.8 times higher compared to two unaffected parents. Thus, the chance of producing affected offspring is almost 2 times higher when mating two affected dogs compared to two unaffected dogs. These results are consistent with the stated hypothesis that there is a higher

possibility that the offspring will get distichiasis if both of their parents are affected by distichiasis compared to offspring in which one or both parents are unaffected. However, the results are contradictory to the mode of inheritance listed in the literature. Instead they indicate that distichiasis is inherited as a polygenic trait with threshold properties.

A threshold trait is inherited as a quantitative trait characterized as an either or trait and it is based on an assumed continuous distribution of factors that contribute to the trait (underlying liability). The underlying liability can be either predisposing genes or environmental factors (Falconer & Macay 1996, Genetic Calculation Applet/a 2014). Thus, when a breeding combination includes two individuals with predisposing genes (affected), the offspring are more likely to get a sufficient amount of 'unfortunate' genes to exceed the threshold and develop the condition. If distichiasis was inherited as a dominant trait, as earlier proposed (Halliwell 1967, Bedford 1973), the expected segregation would statistically have been as follows: Two affected parents would produce 2/3 affected offspring and 1/3 unaffected offspring. Affected and unaffected offspring would be evenly distributed when one parent is affected and the other one not, and two unaffected dogs would always produce offspring without the disease (Genetic Calculation Applet /b 2014). This segregation markedly deviates from the results presented in this thesis, especially considering the many affected offspring produced by two unaffected dogs. This thesis showed no difference in the incidence between sexes, which is consistent with that found in the literature (Lawson 1973, Barnett 1976).

The inconclusive results seen in the ACS should be interpreted with reservations to the limited dataset on ACS included in the study on heredity. As many ACS have foreign parents from countries where ophthalmic examination isn't compulsory, all dogs that fulfilled the criteria were included to get as large a sample size as possible. When calculating an adequate sample size based on the apparent prevalence (Toft *et al*/b 2004) presented in this thesis, the appropriate number of investigated dogs would have been 310 dogs. Therefore, the delimitation of this study may have presented a problem for the significance of the results in this breed. As the general population in the breed at this point in time only consists of approximately 640 dogs in total (Dansk Kennel Klub 2013), it would, in the authors opinion, be quite difficult to find this many individuals, especially since many dogs are not examined. Extending the study period is not assumed to make a significant difference for the results, and including other ophthalmic examination may cause bias due to inconsistent diagnostics. The amount of ECS included was considered sufficient.

As the exact mode of inheritance for distichiasis has been demonstrated to be polygenic with threshold properties, breeders need to pay extra attention to the future usage of affected sires within the breeding, as many of them at the time being produce far more offspring than the allowable limit. As illustrated in **Table 7** in section 4.2.5, the study population descended from a total of 42 ACS sires and 150 ECS sires. On average, both ACS and ECS sires affected by distichiasis produces more litters and hence more offspring than sires not affected by distichiasis. The majority of the ACS from the study population (84 %) were produced by sires diagnosed with distichiasis, whereas the ECS from the study population primarily were produced by unaffected sires (51 %). ACS sires are allowed to produce 15 offspring according to the ethical recommendations presented by the Danish Kennel Club. The mean number of offspring produced in this breed exceeds the limit for number of offspring produced by 'popular sires' (LNOPPS), which is attributed to the fact that more than half (62 %) of the ACS sires exceeds this limitation with up to 64 offspring above the LNOPPS (n = 79 puppies); this is more than 4 times the permitted number. The problem is not as extensive in the ECS breed, where only 10 of 150 (7 %) of the sires exceeds the LNOPPS of 105 offspring. The sire that exceeds the limit with the highest amount produced 174 offspring, thus 69 more than LNOPPS (> 0.6 times the permitted number).One of the major problems with sires exceeding the LNOPPS in relation to distichiasis, and any other inherited disease for that matter, is that it becomes very difficult to control the disease within the population and to measure the effects of preventive actions.

5.2.1 HERITABILITY

As the results presented in this thesis demonstrated that distichiasis is inherited as a polygenic threshold trait, the heritability could be calculated. The heritability of distichiasis in the English cocker spaniels is 0.51 when the estimate is calculated based on two affected parents, and 0.22 when the estimate is calculated based on one affected parent. The deviation between the two estimates of heritability might be explained by a higher amount of inbreeding in one of the breeding combinations. In both cases the difference between the incidence of distichiasis within the population and the incidence within the ones that are related to both one and two affected parents are statistically significant and it can be concluded that the heritability is between 0.22 and 0.51 The heritability of distichiasis in this study was considerably higher than the heritability estimated for distichiasis in the Tibetan terrier, in which the heritability was 0.043 (Ketteritzsch *et al* 2004). Distichiasis is considered genetically influenced in both breeds.

As distichiasis shows a high heritability, hence a high genetic influence, it indicates that selective breeding, excluding breeding combinations of two individuals with predisposing genes (affected), would likely have a positive effect on the incidence within the breed. Currently the most frequently used breeding combination, especially in the ACS, is between two affected parents, which in the light of the results presented in this thesis, may explain the high prevalence in this breed. Whether it is sufficient to exclude this breeding combination or if all affected individuals should be excluded from breeding is yet to be assigned, even though this would pose a major problem in the cocker spaniel breeds as so many individuals are affected.

5.3 THE LITTER STUDY

The examinations of whole litters were performed in order to examine the mode of inheritance based on the segregation of distichiasis within whole litters of one or two affected parents. A total of 24 puppies from 5 litters were screened for distichiasis, but unfortunately the sample was not sufficient to determine statistically significant results. The results are at some point additionally invalidated due to selection bias, since all litters were breed at the same kennel, and the sample may not be representative of the target population. However, when considering the segregation found in these litters, the results are far from the expected, as there are seemingly produced more affected offspring from litters of one affected and one unaffected parent than from two affected parents. As for a dominant inheritance, the expected segregations of either 66.6 % (both parents affected) or 50 % (affected/not affected) affected offspring are likewise contradicted by these results, as less than 1/3 of the puppies in total were affected. In a large scale study on 1,306 dogs with a similar objective, Ketteritzsch *et al* (2004) demonstrated that there was a considerable variation on the outcome in the offspring, but on average the prevalence was found to be 11.4 % within litters.

Earlier studies emphasized that distichiae may be hard to detect as they most frequently are small, has a fine texture and lacks pigmentation (Bedford 1973). Lawson (1973) demonstrated that half of the distichiae presented in his study was not detected until dogs were sedated and examined under an operating microscope. Additionally the age of onset is variable (Barnett, 1976, Bedford 1979, Williams *et al* 1979). In this thesis eyelid examination was performed on puppies ranging from 7 to 12 weeks of age using a handheld slit lamp biomicroscope without previous sedation. On later occasion 8 unaffected puppies were reexamined and one was then diagnosed with distichiasis. Whether the method or the age of the puppies interfered with the results was not confirmed in this thesis. A predicament with the age of onset and the examination of whole litters is that the puppies

usually leave the breeder when they are about 8 weeks old, which is around the time distichiasis becomes visible. Examination of whole litters including puppies older than 8 weeks of age may therefore present a problem as the dogs probably are distributed to new families in different parts of the country.

Even though the results are inconclusive they show some similarities to the results stated in the study on heredity. The conclusion, although not statistically significant, can only be, that distichiasis is inherited as a complex trait.

5.4THE GRADING SCHEME

The grading scheme was introduced in July 2013 and is carried out for a 5 years trial period. As the scheme is relatively new there are no publications on how the different levels of affection are distributed within the affected or predisposed breeds. In this thesis, the majority of dogs that were graduated were diagnosed with a light degree of distichiasis (> 73 %). Severe cases are rare and are only seen in 4 of the 554 graduated dogs. In the ECS breed all levels of affection are represented, which may be attributed to the fact that more dogs are investigated in this breed. The results are consistent with the expectations based on literature stating that distichiasis in the two cocker spaniel breeds are not always associated with disease, as distichiae regularly are small and soft (Bedford 1973, 1979). Furthermore, severe cases that cause corneal ulceration are quite rare in the cocker spaniel breeds, and are most likely associated with short and rigid distichiae or many distichiae located in clusters, which are not that frequent in these breeds (Lawson 1973, Bedford 1988). The lack of standardization may pose a problem in relation to the distribution of the results, as diagnoses may be biased since they are solely made on the subjective opinion of the examining veterinarian. The three grades of distichiasis may be characterized on different grounds which may cause inconsistence in the distribution, hence causing a misleading impression of the severity of the disease within the respective breeds. If the results are considered consistent, the scheme will be very applicable for identifying the general appearance of the disease within a given population.

6 CONCLUSION

The objective of this thesis was to analyze the heredity and mode of inheritance of canine distichiasis within the cocker spaniel breeds, and to determine the prevalence of distichiasis within the two populations. In the study of the heredity and mode of inheritance of canine distichiasis it was demonstrated that distichiasis is a hereditary disease that is inherited as an autosomal polygenic trait with threshold properties, and not as a dominant trait as earlier assumed. When diagnosed with distichiasis, cocker spaniels are predominantly mildly affected by the disease, demonstrated by the majority of dogs diagnosed with a light degree of affection. The stated hypothesis that there should be a higher possibility that the offspring will get distichiasis if both of their parents are affected by distichiasis compared to offspring in which one or both parents are unaffected was proved to be statistically significant, hence there is a significant difference in the outcome of distichiasis based on the breeding combinations of the parents. The relative risk that two affected parents will produce offspring with distichiasis was determined to be 1.4 times higher compared to the combination of one affected and one unaffected parent, and 1.8 times higher compared to two unaffected parents. Thus, the chance of producing affected offspring is almost 2 times higher when mating two affected dogs compared to two unaffected dogs. The heritability of distichiasis is between 0.22 and 0.51, which indicates that the condition is hereditary and is genetically influenced.
To answer the question stated in the beginning of this thesis, on whether the detection of the exact mode of inheritance would make a difference in preventing distichiasis, when the incidences within the two breeds are so high, the answer would be “Yes”. That distichiasis has a high heritability and is inherited as a polygenic threshold trait indicates that selective breeding, predominantly using unaffected individuals, would have a positive effect on the high incidences within the two breeds. The apparent prevalence demonstrated in this thesis was found to be 71.93 % in the American cocker spaniels and 48.81 % in the English cocker spaniels, and is considered to be equivalent to the true prevalence.

The study on the inheritance based on the examination of whole litters proved to be inconclusive, although indicating that distichiasis is inherited in a complex manner, as seen in a polygenic disease. All results attained in this thesis stresses the importance of selecting appropriate animals for breeding in order to reduce the extend of distichiasis in the future generations of our beloved pets and companions.

7 PERSPECTIVE

The results presented in this thesis outlined that distichiasis is inherited as a polygenic trait with threshold properties and a high heritability. The selection of suitable breeding animals for reducing the incidence of distichiasis in the future becomes much easier when the disease is inherited in this manner. Since there was a statistical significant difference in the occurrence of distichiasis in offspring descending from two affected parents compared to other breeding combinations is can only be stressed so much, that this breeding combination has a negative effect on the incidence within the population and is therefore not recommended.

It would be desirable to determine the heritability of distichiasis is other breeds as well and perhaps detect the specific predisposing genes. An interesting study would be to determine whether the different levels of affection bears a distinct proportion of predisposing genes, which may be clarified by comparing the degrees in parents and that of their offspring. The importance of this should be seen in the light of the fact that even though it is recommended that two dogs with the same ophthalmic diagnoses should not be paired, two dogs with distichiasis is still allowed to be paired according to the current breeding recommendations as long as they don't have the same degree. This study demonstrated that offspring of two affected parents have a higher risk of being affected regardless of the degree of affection in the parents, so whether it should merely be a recommendation and not a restriction may need to be reevaluated if the incidences are to be reduced in a near future.

Additional investigations may include a comparison of the sensitivity of the diagnostic tools for identifying distichiasis, as it was earlier stated that there might be a difference in the number of distichiae detected using a handheld slit lamp on a non-sedated dog, which is often the preferred method of choice, compared to the examination of a sedated dog using an operation microscope. A larger scale study on whole litters including no less than 3-5 generations may enhance our understanding of the genetic mechanisms underlying the disease and aid in the detection of genetic and/or environmental factors influencing the outcome of the disease.

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